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Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2017

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Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2017

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Cincinnati, OH 45268

Office of Research and Development
Homeland Security Research Program

Disclaimer

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Use of This Document

The information contained in this document represents the latest step in an ongoing effort of the Environmental Protection Agency's (EPA's) Homeland Security Research Program (HSRP) to provide selected analytical methods to laboratories tasked with analyzing environmental samples in support of EPA remediation and recovery efforts following an intentional or accidental homeland security-related contamination incident. The information is intended for use by EPA and EPA-contracted and -subcontracted laboratories; it also can be used by other agencies and laboratory networks, such as the Integrated Consortium of Laboratory Networks (ICLN). The information also can be found on the *Selected Analytical Methods for Environmental Remediation and Recovery* (SAM) website (https://www.epa.gov/homeland-security-research/sam), which provides searchable links to supporting information regarding selected methods.

At this time, not all of the methods that have been selected have been validated for the listed analyte and sample type. These methods, however, are considered to contain the most appropriate currently available techniques, based on expert judgment. Method usability tiers are assigned to provide an indication of the applicability of the methods that have been selected to address the chemistry, pathogen and biotoxin analytes (i.e., the extent to which the methods have been tested and applied for analysis of the specific analyte and sample type for which they have been selected). Unless a method states specific applicability to the analyte/sample type for which it has been selected, it should be assumed that method evaluation is needed, and adjustments may be required to accurately account for variations in analyte/sample type characteristics, environmental samples, analytical interferences, and data quality objectives (DQOs).

EPA will strive to continue development and evaluation of analytical methods and protocols, including optimization of procedures for measuring target analytes or agents in specific sample types, as appropriate. In those cases where method procedures are determined to be insufficient for a particular situation, HSRP will continue to provide technical support regarding appropriate actions. HSRP has also compiled information and published documents regarding sample collection, rapid screening/ preliminary identification equipment, and disposal of samples corresponding to the analytes and sample types addressed in this document. This information is available at https://www.epa.gov/homeland-security-research/sam-companion-documents-and-sample-collection-procedures.

Abbreviations and Acronyms

ACS	American Chemical Society
AOAC	AOAC International (formerly the Association of Official Analytical Chemists)
APCI	Atmospheric Pressure Chemical Ionization
APHA	American Public Health Association
APHL	Association of Public Health Laboratories
ASM	American Society for Microbiology
ASR	Analytical Service Requests
ASTM	ASTM International (formerly the American Society for Testing and Materials)
ATP	Alternate test procedure
ATSDR	Agency of Toxic Substances & Disease Registry
AWWA	American Water Works Association
BA	*Bacillus anthracis*
BAM	Bacteriological Analytical Manual
BCYE	Buffered charcoal yeast extract
BCYE GPCV	Buffered charcoal yeast extract with glycine, polymyxin B, cycloheximide and vancomycin
BCYE PCV	Buffered charcoal yeast extract with polymyxin B, cycloheximide and vancomycin
BEH	Ethylene-bridged hybrid
BGMK	Buffalo green monkey kidney
BHT	Butylated hydroxytoluene
BMBL	*Biosafety in Microbiological and Biomedical Laboratories*
BoNT	Botulinum neurotoxin
BSL	Biosafety level
BTX	Brevetoxin
BZ	Quinuclidinyl benzilate
°C	Degree Celsius
CAS RN®	Chemical Abstracts Service Registry Number
CBR	Chemical, biological and/or radiological
CCD	Charge-coupled device
CCID	Coordinating Center for Infectious Diseases
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFSAN	Center for Food Safety and Applied Nutrition (U.S. Food and Drug Administration)
CHCA	α-cyano-4-hydroxycinnamic acid
CLLE	Continuous liquid-liquid extraction
CLP	Contract Laboratory Program
CPE	Cytopathic effect
cps	Counts per second
C_T	Cycle threshold
CVAA	2-Chlorovinylarsonous acid
CVAFS	Cold vapor atomic fluorescence spectrometry
CVAOA	2-Chlorovinylarsonic acid
CWA	Chemical Warfare Agent
2,4-D	2,4-Dichlorophenoxyacetic acid
DA	Domoic acid
DAI	Direct aqueous injection
DAPI	4',6-Diamidino-2-phenylindole
DAS	Diacetoxyscirpenol
DAS-HG-HSA	Diacetoxyscirpenol hemiglutarate human serum albumin
DAS-HS-HRP	Diacetoxyscirpenol hemisuccinate horseradish peroxidase conjugate

DB-1	100% Dimethylpolysiloxane
DBPR	Division of Bioterrorism Preparedness and Response
dcNEOSTX	Decarbamoylneosaxitoxin
dcSTX	Decarbamoylsaxitoxin
DHHS	U.S. Department of Health and Human Services
DHS	U.S. Department of Homeland Security
DIC	Differential interference contrast
DIMP	Diisopropyl methylphosphonate
DL	Detection limit
DNA	Deoxyribonucleic acid
2,4-DNPH	2,4-Dinitrophenylhydrazine
DOC	U.S. Department of Commerce
DoD	U.S. Department of Defense
DOE	U.S. Department of Energy
DOT	U.S. Department of Transportation
DPD	N,N-Diethyl-p-phenylenediamine
DQO	Data quality objective
DTPA	Diethylenetriamine-pentaacetate
DVL	Detection verification level
EA2192	S-2-(Diisopropylamino)ethyl methylphosphonothioic acid
EC	*Escherichia coli*
ECD	Electron capture detector
e-CFR	Electronic Code of Federal Regulations
ECL	Electrochemiluminescence
ED	Ethyldichloroarsine
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
EDEA	N-Ethyldiethanolamine
EDL	Estimated detection limit
EDTA	Ethylenediaminetetraacetic acid
EDXA	Energy dispersive X-ray analysis
ELFA	Enzyme-linked fluorescent immunoassay
ELISA	Enzyme-linked immunosorbent assay
EMC	Emission Measurement Center
EML	Environmental Measurements Laboratory
EMMI	Environmental Monitoring Methods Index
EMPA	Ethyl methylphosphonic acid
EMSL	Environmental Monitoring and Support Laboratory
EPA	U.S. Environmental Protection Agency
EQL	Estimated quantitation limit
ERLN	Environmental Response Laboratory Network
ESI	Electrospray ionization
ESI-MS-MS	Electrospray ionization - tandem mass spectrometry
ETV	Environmental Technology Verification
FA	immunofluorescence assay
FBI	U.S. Federal Bureau of Investigation
FDA	U.S. Food and Drug Administration
FEMS	Federation of European Microbiological Societies
FGC-ECD	Fast gas chromatography with electron capture detection
FID	Flame ionization detector
FL	Fluorescence detector
FPD	Flame photometric detector
FRET	Forster resonance energy transfer

FRhK-4	Fetal rhesus monkey kidney
FRMAC	Federal Radiological Monitoring and Assessment Center
FSIS	Food Safety and Inspection Service
GA	Tabun
GB	Sarin
GC	Gas chromatograph or Gas chromatography
GC-ECD	Gas chromatography-electron capture detector
GC-FID	Gas chromatography-flame ionization detector
GC-FPD	Gas chromatography-flame photometric detector
GC-MS	Gas chromatography-mass spectrometry
GC-MS-TOF	Gas chromatography-mass spectrometry-time of flight
GC-NPD	Gas chromatography-nitrogen-phosphorus detector
GD	Soman
GE	1-Methylethyl ester ethylphosphonofluoridic acid
Ge	Germanium
Ge(Li)	Germanium (Lithium)
GF	Cyclohexyl sarin
GFAA	Graphite furnace atomic absorption spectrophotometer or Graphite furnace atomic absorption spectrophotometry
GTX	Gonyautoxins
HASL	Health and Safety Laboratory, currently known as National Urban Security Technology Laboratory (NUSTL)
HAV	Hepatitis A virus
HCoV	Human coronavirus
HD	Sulfur mustard / mustard gas; bis(2-chloroethyl) sulfide
HEPA	High-efficiency particulate air
HEV	Hepatitis E virus
HFBA	Heptafluorobutyric anhydride
HFBI	Heptafluorobutyrylimidazole
HHS	U.S. Health and Human Services
HILIC	Hydrophilic interaction liquid chromatography
HILIC-MS-MS	Hydrophilic interaction liquid chromatography-tandem mass spectrometry
HLB	Hydrophilic-lipophilic-balanced
HMTD	Hexamethylenetriperoxidediamine
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
HN-1	Nitrogen mustard 1; bis(2-chloroethyl)ethylamine
HN-2	Nitrogen mustard 2; 2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine
HN-3	Nitrogen mustard 3; tris(2-chloroethyl)amine
HPGe	High purity germanium
HPLC	High performance liquid chromatography
HPLC-FL	High performance liquid chromatography-fluorescence
HPLC-MS	High performance liquid chromatography-mass spectrometry
HPLC-MS-MS	High performance liquid chromatography tandem mass spectrometry
HPLC-UV	High performance liquid chromatography-ultraviolet
HPLC-vis	High performance liquid chromatography-visible
HRP	Horseradish peroxidase
HSRP	Homeland Security Research Program
HTO	Tritiated water
HV	High volume
IC	Ion chromatograph or Ion chromatography
ICLN	Integrated Consortium of Laboratory Networks

ICP	Intestinal contents preparation (pathogens); Inductively coupled plasma (chemistry)
ICP-AES	Inductively coupled plasma – atomic emission spectrometry
ICP-MS	Inductively coupled plasma – mass spectrometry
I.D.	Inner diameter
IDL	Instrument detection limit
IMPA	Isopropyl methylphosphonic acid
IMS	Immunomagnetic separation
IO	Inorganic
IRIS	Integrated Risk Information System (EPA)
ISE	Ion specific electrode
ISG	Impregnated silica gel
ISM02.3	Inorganic Superfund Methods Multi-Media, Multi-Concentration ISM02.3
ISO	International Organization for Standardization
KHP	Potassium hydrogen phthalate
L-1	Lewisite 1; 2-Chlorovinyldichloroarsine
L-2	Lewisite 2; bis(2-Chlorovinyl)chloroarsine
L-3	Lewisite 3; tris(2-Chlorovinyl)arsine
LC	Liquid chromatograph or Liquid chromatography
LC-APCI-MS	Liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry
LC-ESI-MS	Liquid chromatography-electrospray ionization-mass spectrometry
LC-ESI-MS-MS	Liquid chromatography-electrospray ionization-tandem mass spectrometry
LCMRL	Lowest common minimum reporting level
LC-MS	Liquid chromatography-mass spectrometry
LC-MS-MS	Liquid chromatography tandem mass spectrometry
LC-PIM-MS	Liquid chromatography-product ion monitoring-mass spectrometry
LC-UV	Liquid chromatography-ultraviolet
LD50	Median lethal dose
LFA	Lateral flow immunoassay
LFD	Lateral flow device
LLD	Lower limit of detection
LLOQ	Lower limit of quantitation
LOD	Limit of detection
LOQ	Limit of quantitation
LRN	Laboratory Response Network
LSC	Liquid scintillation counter
LSE	Liquid-solid extraction
M	Molar
mAbs	Monoclonal antibodies
MAE	Microwave-assisted extraction
MALDI	Matrix-assisted laser-desorption ionization
MALDI-TOF-MS	Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry
MARLAP	*Multi-Agency Radiological Laboratory Analytical Protocols*
MC	Microcystin
MDC	Minimum detectable concentration
MDCK	Madin-Darby canine kidney cells
MDEA	N-Methyldiethanolamine
MDL	Method detection limit
MFA	Monofluoroacetate
MIC	Methyl isocyanate
mLD_{50}	Mouse lethal dose
MPA	Methylphosphonic acid
MRL	Minimum reporting level

MRM	Multiple reaction monitoring
mRNA	Messenger ribonucleic acid
MS	Mass spectrometer or Mass spectrometry
MS-MS	Tandem mass spectrometry
MS/MSD	Matrix spike/Matrix spike duplicate
MSE	Microscale solvent extraction
MTBE	Methyl *tert*-butyl ether
MW	Molecular weight
MWCO	Molecular weight cut-off
NA	Not applicable
NaI(Tl)	Thallium-activated sodium iodide
NAREL	National Air and Radiation Environmental Laboratory
NBD chloride	7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole
NCPDCID	National Center for the Prevention, Detection, and Control of Infectious Diseases
NCRP	National Council on Radiation Protection and Measurements
NEMI	National Environmental Methods Index
NEO	Neosaxitoxins
NERL	National Exposure Research Laboratory (EPA)
NHSRC	National Homeland Security Research Center (EPA)
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
nM	Nanomolar
NMAM	NIOSH Manual of Analytical Methods
NNSA	National Nuclear Security Administration
NOD	Nodularins
NPD	Nitrogen-phosphorus detector
NRC	U.S. Nuclear Regulatory Commission
NRMRL	National Risk Management Research Laboratory (EPA)
nS	Nano siemens
NTIS	National Technical Information Service
NTU	Nephelometric turbidity units
OAQPS	Office of Air Quality Planning and Standards (EPA)
OAR	Office of Air and Radiation (EPA)
OGWDW	Office of Water, Office of Ground Water and Drinking Water (EPA)
OLEM	Office of Land and Emergency Management (EPA)
ORAU	Oak Ridge Associated Universities
ORD	Office of Research and Development (EPA)
ORIA	Office of Radiation and Indoor Air (EPA)
ORISE	Oak Ridge Institute for Science and Education
OSHA	Occupational Safety and Health Administration
OVS	OSHA versatile sampler
OW	Office of Water (EPA)
PCDDs	Polychlorinated dibenzo-p-dioxins
PCDFs	Polychlorinated dibenzofurans
PCR	Polymerase chain reaction
PEL	Permissible exposure limit
PETN	Pentaerythritol tetranitrate
PFBHA	O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine
PFE	Pressurized fluid extraction
PHILIS	Portable High Throughput Integrated Laboratory Identification Systems
PIM	Product ion monitoring
PLOS	Public Library of Science

PLRP-S	Polymeric reversed phase
PMPA	Pinacolyl methyl phosphonic acid
1,2-PP	1-(2-Pyridyl)piperazine
ppbv	Parts per billion by volume
pptv	Parts per trillion by volume
PST	Paralytic shellfish toxin
PTFE	Polytetrafluoroethylene
PubMED	U.S. National Library of Medicine (http://www.pubmed.gov)
PUF	Polyurethane foam
PVC	Polyvinyl chloride
PVDF	Polyvinylidene fluoride
QA	Quality assurance
QAP	Quality assessment program
QC	Quality control
QL	Quantitation limit
qPCR	Quantitative polymerase chain reaction
®	Registered trademark
R 33	Methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester (VR)
RBA	Receptor binding assay
RCRA	Resource Conservation and Recovery Act
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
RESL	Radiological and Environmental Sciences Laboratory
RFV	Relative fluorescence value
RLAB	Regional laboratory method
RNA	Ribonucleic acid
RNAse	Ribonuclease
rRNA	Ribosomal ribonucleic acid
RTECS	Registry of Toxic Effects of Chemical Substances
RTG	Radioisotope thermoelectric generator
RT-PCR	Reverse transcription polymerase chain reaction
RT-qPCR	Quantitative reverse transcription polymerase chain reaction
RV-PCR	Rapid viability-polymerase chain reaction
SAED	Select area electron diffraction
SAM	*Selected Analytical Methods for Environmental Remediation and Recovery*
SARS	Severe acute respiratory syndrome
SEA	Staphylococcal enterotoxin type A
SEB	Staphylococcal enterotoxin type B
SEC	Staphylococcal enterotoxin type C
SED	Staphylococcal enterotoxin type D
SEE	Staphylococcal enterotoxin type E
SET	Staphylococcal enterotoxin
SIM	Selective ion monitoring
SIS	Selected ion storage
SM	*Standard Methods for the Examination of Water and Wastewater*
SOP	Standard operating procedure
SPE	Solid-phase extraction
SPR	Solid-phase receptacle
SRC	Syracuse Research Corporation
SRM	Single reaction monitoring
SRS	Savannah River National Laboratory, Savannah River Site
STEC	Shiga-toxigenic *E. coli*
STEL	Short term exposure limit

STS	Sample test source
STX	Saxitoxin
Stx	Shiga toxin
Stx-1	Shiga toxin Type 1
Stx-2	Shiga toxin Type 2
SW	Solid waste
T_0	Time zero
T_2O	Tritium oxide
TBD	To be determined
TCLP	Toxicity Characteristic Leaching Procedure
TDG	Thiodiglycol
TEA	Triethanolamine
TEM	Transmission electron microscope or Transmission electron microscopy
TEPP	Tetraethyl pyrophosphate
TETS	Tetramethylenedisulfotetramine or tetramine
T_f	Time final
THF	Tetrahydrofuran
TIC	Total ion chromatogram
TIOA	Tri-isooctylamine
™	Unregistered trademark
1,3,5-TNB	1,3,5-Trinitrobenzene
2,4,6-TNT	2,4,6-Trinitrotoluene
TO	Toxic Organic
TOF	Time-of-flight
TOF-MS	Time-of-flight mass spectrometry
TOPO	Trioctylphosphine oxide
TOXNET	Toxicology Data Network
TRU	Transuranic
TTX	Tetrodotoxin
UF	Ultrafiltration
U.S.	United States
USDA	U.S. Department of Agriculture
USGS	U.S. Geological Survey
UV	Ultraviolet
VBNC	Viable but non-culturable
VC	*Vibrio cholerae*
VCSB	Voluntary Consensus Standard Body
VE	Phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester
VG	Phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester
vis	Visible detector
VM	Phosphonothioic acid, methyl-,S-(2-(diethylamino)ethyl) O-ethyl ester
VOA	Volatile organic analysis
VOC	Volatile organic compound
VR	Methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester (R 33)
VX	O-Ethyl-S-(2-diisopropylaminoethyl)methylphosphonothiolate
WCIT	Water Contaminant Information Tool
WEF	Water Environment Federation
WHO	World Health Organization
WLA	Water Laboratory Alliance
WSD	Water Security Division (EPA, Office of Water)
YP	*Yersinia pestis*

Dedication

This publication is dedicated to the memory of our friend and colleague, Terry Smith. Terry provided critical support both in initiating the efforts resulting in this document, and in putting in countless hours providing chemistry expertise beginning in 2004. Throughout the many updates to this document and its information, Terry provided valuable input as a work group member, as well as an alternate lead during the third revision. Terry was a friend and mentor to many chemists at the U.S. Environmental Protection Agency (EPA). His contributions to the EPA Homeland Security Research Program's Sampling and Analysis Methods program were always provided with devotion and camaraderie, all while keeping up with his duties within the Office of Land and Emergency Management and with the Environmental Response Laboratory Network. He left many friends throughout EPA and the chemistry community, and will be greatly missed.

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Contents

Disclaimer ii
Use of This Document iii
Abbreviations and Acronyms iv
Dedication xi
Acknowledgments xi
Section 1.0: Introduction 1
Section 2.0: Background 4
Section 3.0: Scope and Application 7
Section 4.0: Points of Contact 10
Section 5.0: Selected Chemical Methods 11
5.1 General Guidelines 12
5.1.1 Standard Operating Procedures for Identifying Chemical Methods 12
5.1.2 General QC Guidelines for Chemical Methods 27
5.1.3 Safety and Waste Management 29
5.2 Method Summaries 30
5.2.1 EPA Method 200.7: Determination of Metals and Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry 30
5.2.2 EPA Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry 31
5.2.3 EPA Method 245.1: Determination of Mercury in Water by Cold Vapor Atomic Absorption Spectrometry 32
5.2.4 EPA Method 300.1, Revision 1.0: Determination of Inorganic Anions in Drinking Water by Ion Chromatography 33
5.2.5 EPA Method 335.4: Determination of Total Cyanide by Semi-Automated Colorimetry 33
5.2.6 EPA Method 350.1: Nitrogen, Ammonia (Colorimetric, Automated Phenate) 34
5.2.7 EPA Method 524.2: Measurement of Purgeable Organic Compounds in Water by Capillary Column Gas Chromatography / Mass Spectrometry 35
5.2.8 EPA Method 525.2: Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography / Mass Spectrometry 36
5.2.9 EPA Method 525.3: Determination of Semivolatile Organic Chemicals in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/ Mass Spectrometry (GC/MS) 37
5.2.10 EPA Method 531.2: Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC With Postcolumn Derivatization 38
5.2.11 EPA Method 538: Determination of Selected Organic Contaminants in Drinking Water by Direct Aqueous Injection-Liquid Chromatography/Tandem Mass Spectrometry (DAI-LC/MS/MS) 38
5.2.12 EPA Method 540: Determination of Selected Organic Chemicals in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) 39

5.2.13 EPA Method 549.2: Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography With Ultraviolet Detection 40
5.2.14 EPA Method 551.1: Determination of Chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogenated Pesticides/Herbicides in Drinking Water by Liquid-Liquid Extraction and Gas Chromatography With Electron-Capture Detection 40
5.2.15 EPA Method 556.1: Determination of Carbonyl Compounds in Drinking Water by Fast Gas Chromatography 41
5.2.16 EPA Method 3015A (SW-846): Microwave Assisted Acid Digestion of Aqueous Samples and Extracts 42
5.2.17 EPA Method 3050B (SW-846): Acid Digestion of Sediments, Sludges, and Soils 43
5.2.18 EPA Method 3051A (SW-846): Microwave Assisted Acid Digestion of Sediments, Sludges, and Oils 44
5.2.19 EPA Method 3511 (SW-846): Organic Compounds in Water by Microextraction 45
5.2.20 EPA Method 3520C (SW-846): Continuous Liquid-Liquid Extraction 46
5.2.21 EPA Method 3535A (SW-846): Solid-Phase Extraction 46
5.2.22 EPA Method 3541 (SW-846): Automated Soxhlet Extraction 48
5.2.23 EPA Method 3545A (SW-846): Pressurized Fluid Extraction (PFE) 49
5.2.24 EPA Method 3570 (SW-846): Microscale Solvent Extraction (MSE) 50
5.2.25 EPA Method 5030C (SW-846): Purge-and-Trap for Aqueous Samples 51
5.2.26 EPA Method 5035A (SW-846): Closed-System Purge-and-Trap and Extraction for Volatile Organics in Soil and Waste Samples 52
5.2.27 EPA Method 6010D (SW-846): Inductively Coupled Plasma - Optical Emission Spectrometry 53
5.2.28 EPA Method 6020B (SW-846): Inductively Coupled Plasma - Mass Spectrometry 54
5.2.29 EPA Method 7470A (SW-846): Mercury in Liquid Wastes (Manual Cold-Vapor Technique) 55
5.2.30 EPA Method 7471B (SW-846): Mercury in Solid or Semisolid Wastes (Manual Cold-Vapor Technique) 56
5.2.31 EPA Method 7473 (SW-846): Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry 56
5.2.32 EPA Method 7580 (SW-846): White Phosphorus (P_4) by Solvent Extraction and Gas Chromatography 57
5.2.33 EPA Method 8015D (SW-846): Nonhalogenated Organics Using GC/FID 58
5.2.34 EPA Method 8260D (SW-846): Volatile Organic Compounds by Gas Chromatography-Mass Spectrometry (GC/MS) 59
5.2.35 EPA Method 8270E (SW-846): Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC-MS) 60
5.2.36 EPA Method 8290A, Appendix A (SW-846): Procedure for the Collection, Handling, Analysis, and Reporting of Wipe Tests Performed Within the Laboratory 61
5.2.37 EPA Method 8315A (SW-846): Determination of Carbonyl Compounds by High Performance Liquid Chromatography (HPLC) 62
5.2.38 EPA Method 8316 (SW-846): Acrylamide, Acrylonitrile and Acrolein by High Performance Liquid Chromatography (HPLC) 63
5.2.39 EPA Method 8318A (SW-846): *N*-Methylcarbamates by High Performance Liquid Chromatography (HPLC) 63
5.2.40 EPA Method 8330B (SW-846): Nitroaromatics, Nitramines, and Nitrate Esters by High Performance Liquid Chromatography (HPLC) 64
5.2.41 EPA ISM02.3 Cyanide: Analytical Methods for Total Cyanide Analysis 65
5.2.42 EPA Method 3135.2I: Cyanide, Total and Amenable in Aqueous and Solid Samples Automated Colorimetric With Manual Digestion 65

5.2.43 EPA IO [Inorganic] Compendium Method IO-3.1: Selection, Preparation, and Extraction of Filter Material 66
5.2.44 EPA IO [Inorganic] Compendium Method IO-3.4: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma (ICP) Spectroscopy 67
5.2.45 EPA IO [Inorganic] Compendium Method IO-3.5: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma/Mass Spectrometry (ICP-MS) 68
5.2.46 EPA IO [Inorganic] Compendium Method IO-5: Sampling and Analysis for Vapor and Particle Phase Mercury in Ambient Air Utilizing Cold Vapor Atomic Fluorescence Spectrometry (CVAFS) 69
5.2.47 EPA Air Method, Toxic Organics - 10A (TO-10 A): Determination of Pesticides and Polychlorinated Biphenyls in Ambient Air Using Low Volume Polyurethane Foam (PUF) Sampling Followed by Gas Chromatographic/Multi-Detector Detection (GC/MD) 70
5.2.48 EPA Air Method, Toxic Organics - 15 (TO-15): Determination of Volatile Organic Compounds (VOCs) in Air Collected in Specially-Prepared Canisters and Analyzed by Gas Chromatography/Mass Spectrometry (GC/MS) 71
5.2.49 EPA Air Method, Toxic Organics – 17 (TO-17): Determination of Volatile Organic Compounds in Ambient Air Using Active Sampling Onto Sorbent Tubes 72
5.2.50 EPA/600/R-11/143: Surface Analysis Using Wipes for the Determination of Nitrogen Mustard Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) 73
5.2.51 EPA/600/R-12/653: Verification of Methods for Selected Chemical Warfare Agents (CWAs) 74
5.2.52 EPA/600/R-13/224: Surface Analysis of Nerve Agent Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) 75
5.2.53 EPA/600/R-15/097: Adaptation of the Conditions of U.S. EPA Method 538 for the Analysis of a Toxic Degradation Product of Nerve Agent VX (EA2192) in Water by Direct Aqueous Injection- Liquid Chromatography/Tandem Mass Spectrometry 76
5.2.54 EPA/600/R-15/258: Extraction and Analysis of Lewisite 1, by its Degradation Products, Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) 77
5.2.55 EPA/600/R-16/114: Analytical Protocol for Measurement of Extractable Semivolatile Organic Compounds Using Gas Chromatography/Mass Spectrometry 78
5.2.56 EPA/600/R-16/115: Analytical Protocol for Cyclohexyl Sarin, Sarin, Soman and Sulfur Mustard Using Gas Chromatography/Mass Spectrometry 80
5.2.57 EPA/600/R-16/116: Analytical Protocol for VX Using Gas Chromatography/Mass Spectrometry (GC/MS) 80
5.2.58 EPA-821-B-01-009: Method Kelada-01: Kelada Automated Test Methods for Total Cyanide, Acid Dissociable Cyanide, and Thiocyanate 81
5.2.59 NIOSH Method 1612: Propylene Oxide 82
5.2.60 NIOSH Method 2016: Formaldehyde 82
5.2.61 NIOSH Method 2513: Ethylene Chlorohydrin 83
5.2.62 NIOSH Method 3509: Aminoethanol Compounds II 83
5.2.63 NIOSH Method 3510: Monomethylhydrazine 84
5.2.64 NIOSH Method 5600: Organophosphorus Pesticides 84
5.2.65 NIOSH Method 5601: Organonitrogen Pesticides 85
5.2.66 NIOSH Method 6001: Arsine 86
5.2.67 NIOSH Method 6002: Phosphine 86
5.2.68 NIOSH Method 6010: Hydrogen Cyanide 87
5.2.69 NIOSH Method 6013: Hydrogen Sulfide 88
5.2.70 NIOSH Method 6015: Ammonia 88
5.2.71 NIOSH Method 6402: Phosphorus Trichloride 88

5.2.72 NIOSH Method 7905: Phosphorus ... 89
5.2.73 NIOSH Method 7906: Particulate Fluorides and Hydrofluoric Acid by Ion Chromatography ... 90
5.2.74 NIOSH Method 7907: Volatile Acids by Ion Chromatography (Hydrogen Chloride, Hydrogen Bromide, Nitric Acid) ... 90
5.2.75 NIOSH Method 9102: Elements on Wipes ... 91
5.2.76 NIOSH Method 9106: Methamphetamine and Illicit Drugs, Precursors and Adulterants on Wipes by Liquid-Liquid Extraction ... 92
5.2.77 NIOSH Method 9109: Methamphetamine and Illicit Drugs, Precursors, and Adulterants on Wipes by Solid Phase Extraction ... 93
5.2.78 NIOSH Method S301-1: Fluoroacetate Anion ... 93
5.2.79 OSHA Method 40: Methylamine ... 94
5.2.80 OSHA Method 54: Methyl Isocyanate (MIC) ... 95
5.2.81 OSHA Method 61: Phosgene ... 95
5.2.82 OSHA Method ID-211: Sodium Azide and Hydrazoic Acid in Workplace Atmospheres ... 96
5.2.83 OSHA Method ID216SG: Boron Trifluoride (BF_3) ... 96
5.2.84 OSHA Method PV2004: Acrylamide ... 97
5.2.85 OSHA Method PV2103: Chloropicrin ... 97
5.2.86 ASTM Method D5755-09: Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Surface Loading ... 98
5.2.87 ASTM Method D6480-05: Standard Test Method for Wipe Sampling of Surfaces, Indirect Preparation, and Analysis for Asbestos Structure Number Concentration by Transmission Electron Microscopy ... 98
5.2.88 ASTM Method D7597-16: Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Hydrogen Dimethylamidophosphate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Water by Liquid Chromatography/Tandem Mass Spectrometry ... 99
5.2.89 ASTM Method D7598-16: Standard Test Method for Determination of Thiodiglycol in Water by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry ... 100
5.2.90 ASTM Method D7599-16: Standard Test Method for Determination of Diethanolamine, Triethanolamine, *N*-Methyldiethanolamine and *N*-Ethyldiethanolamine in Water by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) ... 100
5.2.91 ASTM Method D7644-16: Standard Test Method for Determination of Bromadiolone, Brodifacoum, Diphacinone and Warfarin in Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) ... 101
5.2.92 ASTM Method D7645-16: Standard Test Method for Determination of Aldicarb, Aldicarb Sulfone, Aldicarb Sulfoxide, Carbofuran, Methomyl, Oxamyl and Thiofanox in Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) ... 102
5.2.93 ASTM Method E2787-11: Standard Test Method for Determination of Thiodiglycol in Soil Using Pressurized Fluid Extraction Followed by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) ... 102
5.2.94 ASTM Method E2838-11: Standard Test Method for Determination of Thiodiglycol on Wipes by Solvent Extraction Followed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) ... 103
5.2.95 ASTM Method E2866-12: Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Soil by

Pressurized Fluid Extraction and Analyzed by Liquid Chromatography/Tandem Mass Spectrometry 104
5.2.96 ISO Method 10312:1995: Ambient Air - Determination of Asbestos Fibres - Direct-Transfer Transmission Electron Microscopy Method 105
5.2.97 Standard Method 4500-CN G: Cyanides Amenable to Chlorination after Distillation 105
5.2.98 Standard Method 4500-NH_3 B: Nitrogen (Ammonia) Preliminary Distillation Step 106
5.2.99 Standard Method 4500-NH_3 G: Nitrogen (Ammonia) Automated Phenate Method 107
5.2.100 Standard Method 4500-Cl G: Chlorine (Residual) DPD Colorimetric Method 107
5.2.101 Literature Reference for Chlorine in Air (Analyst, 1999. 124(12): 1853-1857) 108
5.2.102 Literature Reference for Hexamethylenetriperoxidediamine (HMTD) (Analyst, 2001. 126:1689-1693) 108
5.2.103 Literature Reference for Methamidophos (Chromatographia. 2006. 63(5/6): 233-237) 109
5.2.104 Literature Reference for Cyanogen Chloride (Encyclopedia of Anal. Chem. 2006 DOI: 10.1002/9780470027318.a0809) 109
5.2.105 Literature Reference for 3-Chloro-1,2-propanediol (Eur. J. Lipid Sci. Technol. 2011. 113: 345-355) 110
5.2.106 Literature Reference for Methyl Hydrazine (Journal of Chromatography. 1993. 617(1): 157-162) 111
5.2.107 Literature Reference for 3-Chloro-1,2-propanediol (Journal of Chromatography A. 2000. 866(1): 65-77) 112
5.2.108 Literature Reference for Fluoroacetic Acid/Fluoroacetate Salts/Methyl Fluoroacetate (Journal of Chromatography A. 2007. 1139: 271-278) 112
5.2.109 Literature Reference for Methamidophos (Journal of Chromatography A. 2007. 1154: 3-25) 113
5.2.110 Literature Reference for Paraquat (Journal of Chromatography A. 2008, 1196-1197, 110-116) 114
5.2.111 Literature Reference for Fentanyl (Journal of Chromatography A. 2011. 1218: 1620-1649) 114
5.2.112 Literature Reference for BZ (Journal of Chromatography B. 2008. 874: 42–50 115
5.2.113 Literature Reference for Fluoroacetamide (Journal of Chromatography B. 2008. 876(1): 103-108) 116
5.2.114 Literature Reference for Fluoroacetic Acid/Fluoroacetate Salts/Methyl Fluoroacetate (Journal of Chromatography B. 2010. 878: 1045-1050) 117
5.2.115 Literature Reference for Carfentanil and 3-Methyl Fentanyl (J. Chromatogr. B. 2014. 962: 52-58 117
5.2.116 Literature Reference for Sodium Azide (Journal of Forensic Sciences. 1998. 43(1): 200-202) 118

Section 6.0: Selected Radiochemical Methods 120

6.1 General Guidelines 121
6.1.1 Standard Operating Procedures for Identifying Radiochemical Methods 122
6.1.2 General QC Guidelines for Radiochemical Methods 128
6.1.3 Safety and Waste Management 129
6.2 Method Summaries (Environmental Samples) 131
6.2.1 EPA Method 111: Determination of Polonium-210 Emissions from Stationary Sources 131
6.2.2 EPA Method 900.0: Gross Alpha and Gross Beta Radioactivity in Drinking Water 131
6.2.3 EPA Method 901.1: Gamma Emitting Radionuclides in Drinking Water 132
6.2.4 EPA Method 903.1: Radium-226 in Drinking Water – Radon Emanation Technique 134
6.2.5 EPA Method 905.0: Radioactive Strontium in Drinking Water 134
6.2.6 EPA Method 906.0: Tritium in Drinking Water 135
6.2.7 EPA Method 907.0: Actinide Elements in Drinking Water - Thorium, Uranium, Neptunium, Plutonium, Americium and Curium 136

6.2.8 EPA Method EMSL-33: Isotopic Determination of Plutonium, Uranium, and Thorium in Water, Soil, Air, and Biological Tissue ... 137
6.2.9 EPA Method Rapid Radiochemical Method for Phosphorus-32 in Water for Environmental Restoration Following Homeland Security Events ... 138
6.2.10 EPA Method R4-73-014: Radioactive Phosphorus ... 138
6.2.11 EPA Method: Determination of Radiostrontium in Food and Bioenvironmental Samples ... 139
6.2.12 EPA Method: Rapid Radiochemical Method for Americium-241 in Water for Environmental Restoration Following Homeland Security Events ... 140
6.2.13 EPA Method: Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Water for Environmental Restoration Following Homeland Security Events ... 141
6.2.14 EPA Method: Rapid Radiochemical Method for Radium-226 in Water for Environmental Restoration Following Homeland Security Events ... 141
6.2.15 EPA Method: Rapid Radiochemical Method for Radiostrontium in Water for Environmental Restoration Following Homeland Security Events ... 142
6.2.16 EPA Method: Rapid Radiochemical Method for Isotopic Uranium in Water for Environmental Restoration Following Homeland Security Events ... 143
6.2.17 EPA Method: Rapid Method for Acid Digestion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events ... 144
6.2.18 EPA Method: Rapid Method for Sodium Carbonate Fusion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events ... 146
6.2.19 Rapid Method for Radium in Soil Incorporating the Fusion of Soil and Soil-Related Matrices with the Radioanalytical Counting Method for Environmental Remediation Following Radiological Incidents ... 147
6.2.20 Rapid Method for Fusion of Soil and Soil-Related Matrices Prior to Americium, Plutonium, and Uranium Analyses for Environmental Remediation Following Radiological Incidents ... 148
6.2.21 Rapid Method for Sodium Carbonate Fusion of Soil and Soil-Related Matrices Prior to Strontium-90 Analyses for Environmental Remediation Following Radiological Incidents ... 149
6.2.22 Rapid Method for Sodium Hydroxide/Sodium Peroxide Fusion of Radioisotope Thermoelectric Generator Materials in Water and Air Filter Matrices Prior to Plutonium Analyses for Environmental Remediation Following Radiological Incidents ... 150
6.2.23 EPA Method: Rapid Radiochemical Method for Californium-252 in Water, Air Particulate Filters, Swipes and Soil for Environmental Restoration Following Homeland Security Events ... 151
6.2.24 EPA Method: Rapid Radiochemical Method for Curium-244 in Water Samples for Environmental Remediation Following Radiological Incidents ... 153
6.2.25 EPA Method: Rapid Radiochemical Method for Curium-244 in Air Particulate Filters, Swipes and Soil ... 154
6.2.26 Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents ... 156
6.2.27 EPA Method: NAREL Standard Operating Procedure for Actinides in Environmental Matrices by Extraction Chromatography ... 157
6.2.28 EML HASL-300 Method Am-01-RC: Americium in Soil ... 159
6.2.29 EML HASL-300 Method Am-04-RC: Americium in QAP Water and Air Filters - Eichrom's TRU Resin ... 159

6.2.30 EML HASL-300 Method Am-06-RC: Americium and/or Plutonium in Vegetation 160
6.2.31 EML HASL-300 Method Ga-01-R: Gamma Radioassay 161
6.2.32 EML HASL-300 Method Po-02-RC: Polonium in Water, Vegetation, Soil, and Air Filters 162
6.2.33 EML HASL-300 Method Pu-12-RC: Plutonium and/or Americium in Soil or Sediments 162
6.2.34 EML HASL-300 Method Ra-03-RC: Radium-226 in Soil, Vegetable Ash, and Ion Exchange Resin 163
6.2.35 EML HASL-300 Method Sr-03-RC: Strontium-90 in Environmental Samples 164
6.2.36 EML HASL-300 Method Tc-01-RC: Technetium-99 in Water and Vegetation 164
6.2.37 EML HASL-300 Method Tc-02-RC: Technetium-99 in Water – TEVA® Resin 165
6.2.38 EML HASL-300 Method U-02-RC: Isotopic Uranium in Biological and Environmental Materials 165
6.2.39 DOE FRMAC Method Volume 2, Page 33: Gross Alpha and Beta in Air 166
6.2.40 DOE RESL Method P-2: P-32 Fish, Vegetation, Dry Ash, Ion Exchange 167
6.2.41 DOE SRS Actinides and Sr-89/90 in Soil Samples 168
6.2.42 DOE SRS Actinides and Sr-89/90 in Vegetation: Fusion Method 168
6.2.43 ORISE Method AP1: Gross Alpha and Beta for Various Matrices 169
6.2.44 ORISE Method AP2: Determination of Tritium 170
6.2.45 ORISE Method AP5: Determination of Technetium-99 171
6.2.46 ORISE Method AP7: Determination of Radium-226 in Water and Soil Samples Using Alpha Spectroscopy 171
6.2.47 ORISE Method AP11: Sequential Determination of the Actinides in Environmental Samples Using Total Sample Dissolution and Extraction Chromatography 172
6.2.48 ORISE Method Procedure #9: Determination of I-125 in Environmental Samples 173
6.2.49 ASTM Method D3084-05 (2012): Standard Practice for Alpha Spectrometry in Water 174
6.2.50 ASTM Method D3972-09 (2015): Standard Test Method for Isotopic Uranium in Water by Radiochemistry 175
6.2.51 ASTM Method D5811-08 (2013): Standard Test Method for Strontium-90 in Water 175
6.2.52 ASTM Method D7168-16: Standard Test Method for Technetium-99 in Water by Solid Phase Extraction Disk 176
6.2.53 Standard Method 7110 B: Gross Alpha and Gross Beta Radioactivity (Total, Suspended, and Dissolved) 177
6.2.54 Standard Method 7120: Gamma-Emitting Radionuclides 178
6.2.55 Standard Method 7500-Ra B: Radium: Precipitation Method 179
6.2.56 Standard Method 7500-Ra C: Radium: Emanation Method 179
6.2.57 Standard Method 7500-U B: Uranium: Radiochemical Method 180
6.2.58 Standard Method 7500-U C: Uranium: Isotopic Method 181
6.2.59 Y-12 (DOE) Preparation of Samples for Total Activity Screening 182
6.3 Method Summaries (Outdoor Infrastructure and Building Material Samples) 183
6.3.1 Rapid Radiochemical Method for Total Radiostrontium (Sr-90) In Building Materials for Environmental Remediation Following Radiological Incidents 183
6.3.2 Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents 184
6.3.3 Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses for Environmental Remediation Following Radiological Incidents 185
6.3.4 Rapid Radiochemical Method for Isotopic Uranium in Building Materials for Environmental Remediation Following Radiological Incidents 187
6.3.5 Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Building Materials for Environmental Remediation Following Radiological Incidents 188

6.3.6 Rapid Radiochemical Method for Americium-241 in Building Materials for Environmental Remediation Following Radiological Incidents 189
6.3.7 Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses 190
6.3.8 Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Material Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses 191

Section 7.0: Selected Pathogen Methods 194

7.1 General Guidelines 198
7.1.1 Standard Operating Procedures for Identifying Pathogen Methods 198
7.1.2 General QC Guidelines for Pathogen Methods 201
7.1.3 Safety and Waste Management 202
7.1.4 Laboratory Response Network (LRN) 203

7.2 Method Summaries for Bacteria 205
7.2.1 *Bacillus anthracis* [Anthrax] – BSL-3 205
7.2.2 *Brucella* spp. [Brucellosis] – BSL-3 208
7.2.3 *Burkholderia mallei* [Glanders] – BSL-3 and *Burkholderia pseudomallei* [Melioidosis] – BSL-3 211
7.2.4 *Campylobacter jejuni* [Campylobacteriosis] – BSL-2 213
7.2.5 *Chlamydophila psittaci* [Psittacosis] (formerly known as *Chlamydia psittaci*) – BSL-2; BSL-3 for Aerosols and Large Volumes 216
7.2.6 *Coxiella burnetii* [Q-fever] – BSL-3 218
7.2.7 *Escherichia coli* O157:H7 – BSL-2 221
7.2.8 *Francisella tularensis* [Tularemia] – BSL-3 223
7.2.9 *Legionella pneumophila* – BSL-2 226
7.2.10 *Leptospira interrogans* [Leptospirosis] – BSL-2 228
7.2.11 *Listeria monocytogenes* [Listeriosis] – BSL-2 230
7.2.12 Non-typhoidal *Salmonella* (Not applicable to *S*. Typhi) [Salmonellosis] – BSL-2 233
7.2.13 *Salmonella enterica* serovar Typhi (*S*. Typhi) [Typhoid fever] – BSL-2; BSL-3 for Aerosol Release 235
7.2.14 *Shigella* spp. [Shigellosis] – BSL-2 238
7.2.15 *Staphylococcus aureus* – BSL-2 240
7.2.16 *Vibrio cholerae* [Cholera] – BSL-2 242
7.2.17 *Yersinia pestis* [Plague] – BSL-3 245

7.3 Method Summaries for Viruses 248
7.3.1 Adenoviruses: Enteric and Non-enteric (A-F) – BSL-2 248
7.3.2 Astroviruses – BSL not specified 251
7.3.3 Caliciviruses: Noroviruses – BSL-2 254
7.3.4 Caliciviruses: Sapovirus – BSL-2 255
7.3.5 Coronaviruses: Severe Acute Respiratory Syndrome (SARS) -associated Human Coronavirus (HCoV) – BSL-2; BSL-3 for Propagation 258
7.3.6 Hepatitis E Virus (HEV) – BSL-2 261
7.3.7 Influenza H5N1 virus – BSL-3 264
7.3.8 Picornaviruses: Enteroviruses – BSL-2 267
7.3.9 Picornaviruses: Hepatitis A Virus (HAV) – BSL-2 270
7.3.10 Reoviruses: Rotavirus (Group A) – BSL-2 272

7.4 Method Summaries for Protozoa 276
7.4.1 *Cryptosporidium* spp. [Cryptosporidiosis] – BSL-2 276
7.4.2 *Entamoeba histolytica* – BSL-2 282
7.4.3 *Giardia* spp. [Giardiasis] – BSL-2 284
7.4.4 *Naegleria fowleri* [Naegleriasis] – BSL-2 288

7.4.5 *Toxoplasma gondii* [Toxoplasmosis] – BSL-2 291
7.5 Method Summaries for Helminths 294
7.5.1 *Baylisascaris procyonis* [Raccoon roundworm fever] – BSL-2 294
Section 8.0: Selected Biotoxin Methods 298
8.1 General Guidelines 300
8.1.1 Standard Operating Procedures for Identifying Biotoxin Methods 300
8.1.2 General QC Guidelines for Biotoxin Methods 303
8.1.3 Safety and Waste Management 304
8.2 Method Summaries 305
8.2.1 Abrin / Abrine 305
8.2.2 Aflatoxin 308
8.2.3 α-Amanitin 309
8.2.4 Anatoxin-a 311
8.2.5 Botulinum Neurotoxins (BoNTs) 312
8.2.6 Brevetoxins (BTX) 317
8.2.7 Cylindrospermopsin 318
8.2.8 Diacetoxyscirpenol (DAS) 320
8.2.9 Domoic Acid (DA) 321
8.2.10 Microcystins 323
8.2.11 Picrotoxin 325
8.2.12 Ricin (Ricinine) 326
8.2.13 Saxitoxins 330
8.2.14 Shiga and Shiga-like Toxins (Stx) 333
8.2.15 Staphylococcal Enterotoxins (SETs) 335
8.2.16 T-2 Mycotoxin 337
8.2.17 Tetrodotoxin (TTX) 339
Section 9.0: Conclusions 341

Figures

Figure 1-1. Environmental Evaluation Analytical Process Roadmap for Homeland Security Incidents 3
Figure 2-1. Method Selection Process 6
Figure 7-1. Sample Analysis During Site Characterization and Post Decontamination Phases Following a Biological Contamination Event 195

Tables

Table 5-1. Chemical Methods and Corresponding Section Numbers 13
Table 5-2. Sources of Chemical Methods 26
Table 6-1. Radiochemical Methods and Corresponding Section Numbers 122
Table 6-2. Sources of Radiochemical Methods 127
Table 7-1. Sources of Pathogen Methods 199
Table 8-1. Sources of Biotoxin Methods 301

Appendices

Appendix A: Selected Chemical Methods A-1
Appendix B1: Selected Radiochemical Methods for Environmental Samples B-1
Appendix B2: Selected Radiochemical Methods for Outdoor Building and Infrastructure Materials B-2
Appendix C: Selected Pathogen Methods C-1
Appendix D: Selected Biotoxin Methods D-1

Attachments

Attachment 1: Supporting Documents 1-1

Section 1.0: Introduction

After the terrorist attacks of September 11, 2001 and the anthrax attacks in the fall of 2001, federal and state personnel provided response, recovery and remediation under trying circumstances, including unprecedented demand on laboratory capabilities to analyze environmental samples. In reviewing these events, the Environmental Protection Agency (EPA) identified several areas to enhance the resiliency of the nation following homeland security-related incidents resulting in intentional or accidental contamination. The need to improve the nation's laboratory capacity and capability to analyze environmental samples following such incidents (i.e., chemical, biological and/or radiological [CBR] contamination) was one of the most important areas identified. This need has proven to be an on-going concern as the nation faces continued contamination incidents, such as the environmental contamination introduced by the 2014 microcystins contamination of drinking water in Toledo, Ohio; the 2013 ricin letter incident in Washington, D.C.; and the 2011 Fukushima power plant accident which threatened contamination of the western coast of the United States.

To address this need, EPA formed the Homeland Security Laboratory Capacity Work Group to identify and implement opportunities for near-term improvements and to develop recommendations for addressing longer-term laboratory issues. The EPA Homeland Security Laboratory Capacity Work Group consisted of representatives from the EPA's Office of Research and Development (ORD), Office of Air and Radiation (OAR), Office of Water (OW), Office of Land and Emergency Management (OLEM, formerly Office of Solid Waste and Emergency Management), Office of Environmental Information, Office of Chemical Safety and Pollution Prevention, and several EPA regional offices.

A critical area identified by the Homeland Security Laboratory Capacity Work Group was the need for a list of selected analytical methods to be used by all laboratories when analyzing contamination incident samples and, in particular, when analysis of many samples is required over a short period of time. EPA's Environmental Response Laboratory Network (ERLN) is a national network of laboratories that can be accessed as needed to support responses to large-scale environmental contamination incidents by providing analytical capabilities and data of consistent quality in a systematic, coordinated response. This network includes the Water Laboratory Alliance (WLA), which can be accessed specifically for responses pertaining to the nation's water sector.[1] Using the same selected methods should reduce confusion, permit sharing of sample load between laboratories, improve data comparability, and simplify the task of outsourcing analytical support to the commercial laboratory sector. Use of such methods would also improve follow-up activities, including validating results, evaluating data and making decisions. To this end, work group members formed an Analytical Methods Subteam to address homeland security methods issues.

The Analytical Methods Subteam recognized that widely different analytical methods are required for various phases of environmental sample analyses in support of homeland security preparedness and response: (1) ongoing surveillance and monitoring; (2) response and rapid screening for determining whether an incident has occurred; (3) preliminary site characterizations to determine the extent and type of contamination; and (4) confirmatory laboratory analyses to plan, implement, and evaluate the effectiveness of site remediation. **Figure 1-1** represents these analytical phases. EPA's *Selected Analytical Methods for Environmental Remediation and Recovery* (SAM)[2] provides information for analytical methods to be applied during the "Site Remediation" phase. Methods have been selected to support activities related to site assessment (including preliminary, qualitative analyses to characterize the

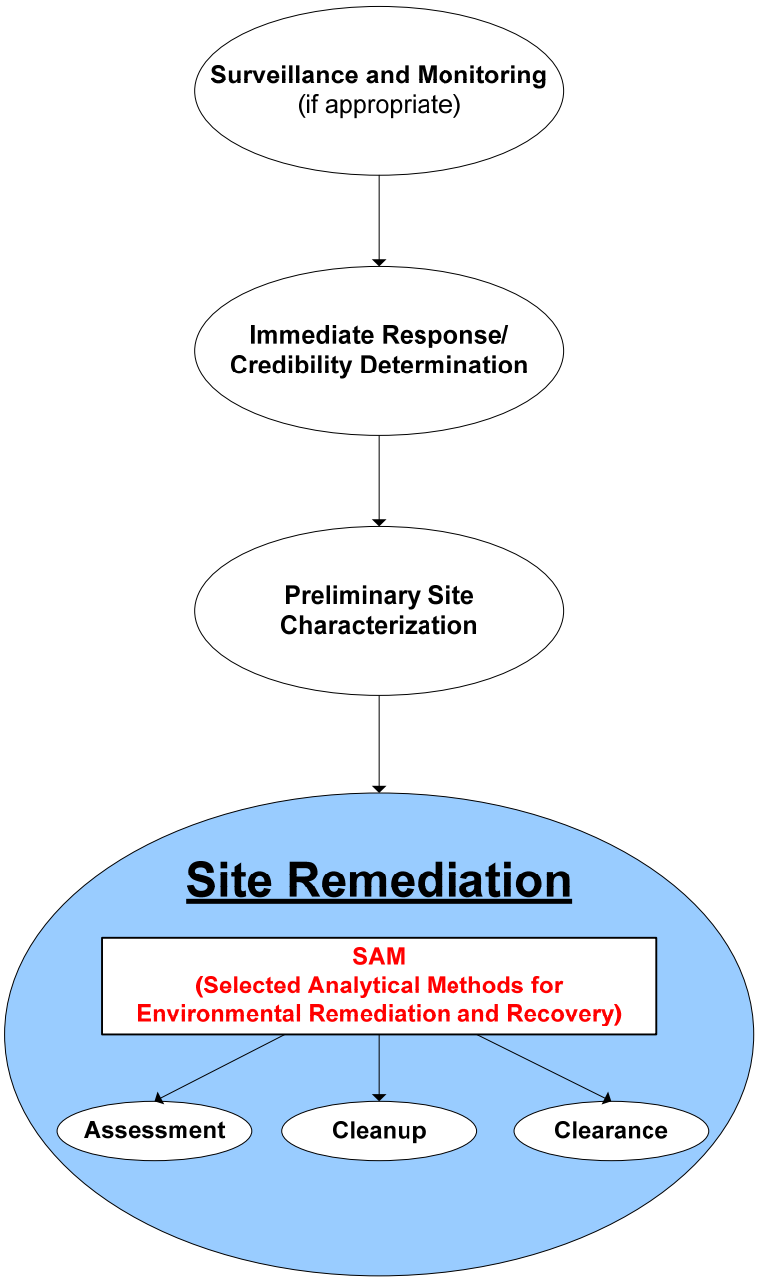
[1] Information regarding EPA's Environmental Response Laboratory Network is available at: https://www.epa.gov/emergency-response/environmental-response-laboratory-network.
Information regarding the Water Laboratory Alliance (WLA) is available at: https://www.epa.gov/waterlabnetwork/learn-about-water-laboratory-alliance

[2] Formerly EPA's *Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events*. SAM and its methods are available at: https://www.epa.gov/homeland-security-research/sam

extent of contamination), site cleanup (to evaluate the efficacy of remediation efforts), and site clearance (releasing a site, including water and wastewater systems, for its intended use) decisions.

In support of this document, EPA periodically assembles methods experts from within EPA, as well as other federal, state and local agencies; and public utilities, to review and update SAM. Workgroup members revise the methods listed or select new methods as appropriate, determine method tier classifications, provide input into special considerations, and add analytes of interest as needed. Analytes are included based on selection criteria that address the needs and priorities of EPA as well as other federal agencies (e.g., environmental persistence, half lives, availability, and toxicity). The sample types listed are specific to each technical section and have been determined by technical work groups to be a concern during site remediation. Work groups identify a single method or method group per analyte/sample type to ensure a consistent analytical approach across multiple laboratories when analyzing environmental samples.

Figure 1-1. Environmental Evaluation Analytical Process Roadmap for Homeland Security Incidents



Section 2.0: Background

Method selection is based on consideration of specific criteria that emphasize method performance and include existing laboratory capabilities, laboratory capacity, method applicability to multiple sample types, and method applicability to multiple analytes. For some analytes, the preferred method is a clear choice; for others, competing criteria make the choice more difficult. Final method selections are based on technical recommendations from the work groups under the direction of EPA's Homeland Security Research Program (HSRP). For analytes where limited laboratory capability exists, methods are selected based on their applicability to similar analytes. In these cases, laboratory studies to evaluate the ability of the selected method to measure the target analyte(s) are either underway or needed. Method usability tiers are assigned to provide an indication of the applicability of the methods that have been selected to address the chemical, pathogen and biotoxin analytes (i.e., the extent to which the methods have been tested and applied for analysis of the specific analyte and sample type(s) for which they have been selected).

Figure 2-1 summarizes steps and provides the criteria used during the method selection process. It is important to note that the method selection criteria included in this figure are listed in non-hierarchical order and, in some cases, only a subset of the criteria was considered when selecting methods.

Since 2004, the National Homeland Security Research Center (NHSRC) has brought together experts from across EPA and other interested agencies to develop this compendium of analytical methods to be used when analyzing environmental samples, and to address site characterization, remediation and clearance following homeland security-related contamination incidents. Participants have included representatives from EPA program offices, regions, and laboratories; U.S. Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), Department of Homeland Security (DHS), Federal Bureau of Investigation (FBI), Department of Defense (DoD), Department of Energy (DOE), Department of Agriculture (USDA), Geological Survey (USGS) and Department of Commerce (DOC); and numerous state agencies, municipalities and universities. Methods and protocols are considered for chemical, radiochemical, biological and biotoxin agents of concern in the types of environmental samples that would be anticipated, including outdoor building and infrastructure materials containing radiochemical contamination. Work groups have also begun considering methods that might be needed to address waste generated during site decontamination and the analytical impacts of decontamination agents.

Surveys of available analytical methods are conducted using existing resources including the following:

- National Environmental Methods Index (NEMI) and NEMI for Chemical, Biological and Radiological Methods (NEMI-CBR)
- Environmental Monitoring Method Index (EMMI)
- EPA Test Methods Index
- EPA Office of Water Methods
- EPA Office of Solid Waste SW-846 Methods
- EPA Microbiological Methods
- National Institute for Occupational Safety and Health (NIOSH) Manual of Analytical Methods (NMAM)
- Occupational Safety and Health Administration (OSHA) Index of Sampling and Analytical Methods
- AOAC International
- ASTM International
- International Organization for Standardization (ISO) methods
- *Standard Methods for the Examination of Water and Wastewater* (SM)
- Scientific Literature

The primary objective of this effort is to support EPA's ERLN and WLA by identifying appropriate

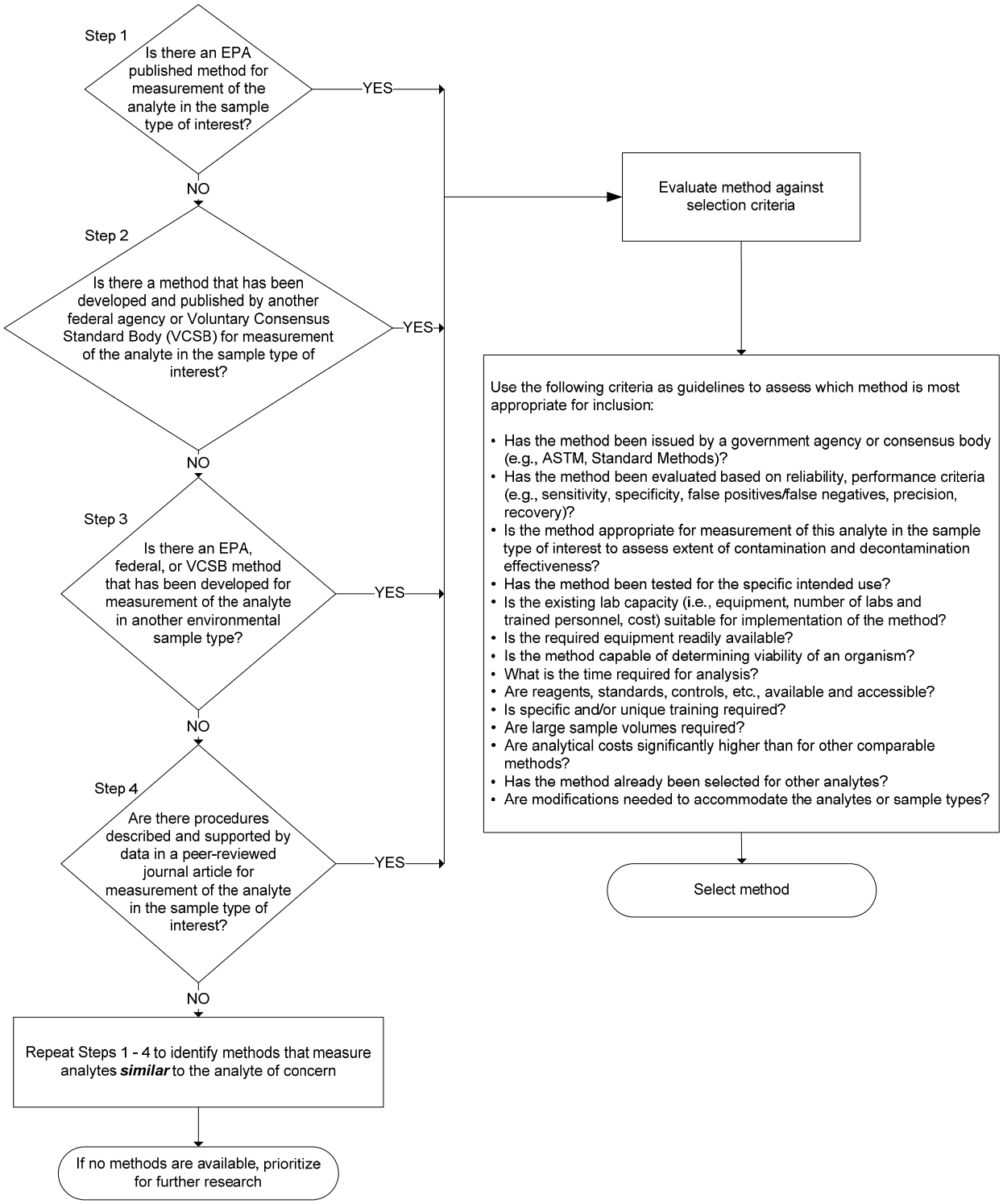
methods that represent a balance between providing existing, documented techniques and providing consistent and analytical results of known quality.

Since publication of Revision 1.0 of this document in September 2004, NHSRC has continued to convene technical work groups to evaluate and update the analytes and methods that are listed. Details regarding changes that have been incorporated into each revision are provided in Attachment 1. The 2012 revision also reflects a title change agreed to by stakeholders (i.e., EPA's HSRP; Office of Groundwater and Drinking Water [OGWDW], Water Security Division [WSD] and WLA; Office of Emergency Management and ERLN; Office of Radiation and Indoor Air [ORIA]; and Regional Offices) during a 2010 Summit, to better reflect its focus on providing selected analytical methods for use across multiple laboratories during environmental remediation and recovery. The current revision (2017) includes the addition of outdoor building and infrastructure materials as sample types in the radiochemistry sections; the addition of soil as a sample type in the pathogens sections; the assignment of method applicability tiers for methods selected for pathogen and biotoxin analytes; several new methods added or replaced for chemical, radiochemical, pathogen and biotoxin analytes; the addition of several new analytes to each of these technical sections (chemistry, radiochemistry, pathogens and biotoxins); and considerations regarding the potential impacts of decontamination agents on the analytical performance of selected radiochemical methods.

In addition to updating analytes and methods, work groups identified areas for development of companion documents to provide information regarding sample collection, rapid screening and preliminary analysis equipment, and sample disposal to supplement the selected analytical methods. The information listed in the companion documents generally corresponds to the analytes and methods in this document, and are updated as needed and as resources allow. Currently available companion documents are listed below and, with content descriptions, in Attachment 1.

- *Sample Collection Information Document for Chemicals, Radiochemicals and Biotoxins – Companion to SAM 2017*
- *Sample Collection Information Document for Pathogens – Companion to SAM 2017*
- *Rapid Screening and Preliminary Identification Techniques and Methods – Companion to SAM Revision 5.0*
- *Laboratory Environmental Sample Disposal Information Document – Companion to SAM Revision 5.0*
- *Guide for Development of Sample Collection Plans for Radiochemical Analytes in Environmental Matrices Following Homeland Security Events*
- *Sample Collection Procedures for Radiochemistry Analytes in Environmental Matrices.*
- *Sample Collection Procedures for Radiochemistry Analytes in Outdoor Building and Infrastructure Materials*

Figure 2-1. Method Selection Process



Section 3.0: Scope and Application

The premise and purpose of this document is to identify the analytical methods that will be used in cases when multiple laboratories are called on to analyze environmental samples following a homeland security-related intentional or accidental contamination incident. The document is intended to support the ERLN and WLA, and also can be used as a tool to assist state and local laboratories in planning for and analyzing environmental samples and outdoor building material samples containing radiochemical contamination following such incidents. The methods presented in this document should be used to:

- Determine the extent of site contamination (assumes early responders have identified contaminants prior to EPA's remediation effort);
- Evaluate the efficacy of remediation efforts during site cleanup; and
- Confirm effectiveness of decontamination in support of site clearance decisions.

The methods provided are limited to those that would be used to determine, to the extent possible within analytical limitations, the presence of chemical, radiochemical, pathogen and biotoxin analytes of concern and their concentrations and viability, when applicable, in environmental media and in outdoor building materials containing radiochemical contamination. The majority of methods include detailed laboratory procedures for confirming the identification of analytes and determining their concentrations in samples and, therefore, are not designed to be used for rapid or immediate response or for conducting an initial evaluation (triage or screening) of suspected material to determine if it poses an immediate danger. This document also is not intended to provide information regarding sample collection activities or equipment. Instead, NHSRC has developed companion documents that are intended to provide information regarding sample collection, laboratory rapid screening/preliminary identification equipment, and sample disposal. Currently available companion documents are listed in Attachment 1.

Methods are provided in this document as corresponding to specific analyte/sample type combinations that are listed in Appendices A (chemical), B (radiochemical), C (pathogen) and D (biotoxin). Summaries of each method are provided throughout Sections 5.2 (chemical methods), 6.2 and 6.3 (radiochemical methods), 7.2 (pathogen methods) and 8.2 (biotoxin methods).

The information contained in this document represents the latest step in an ongoing effort by EPA's NHSRC to provide selected analytical methods for use by those laboratories tasked with performing confirmatory analyses of environmental samples and outdoor building material samples in support of EPA remediation and recovery efforts following a homeland security-related contamination incident. The information is intended for use by EPA and EPA-contracted and-subcontracted laboratories; it also can be used by other agencies and laboratory networks, such as the Integrated Consortium of Laboratory Networks (ICLN). The information also can be found on the SAM website (https://www.epa.gov/homeland-security-research/sam), which provides searchable links to supporting information based on the analytes and analytical methods listed.

At this time, only some of the methods selected have been validated for the analyte and sample type. However, the methods are considered to contain the most appropriate currently available techniques based on expert judgment. Method usability tiers are assigned to provide an indication of the applicability of the methods that have been selected to address the chemical, pathogen and biotoxin analytes (i.e., the extent to which the methods have been tested and applied for analysis of the specific analyte and sample type for which they have been selected). Unless a published method states specific applicability to the analyte/sample type for which it has been selected, it should be assumed that method evaluation is needed, and adjustments to the procedures described in the method may be required to accurately account for variations in analyte/sample type characteristics, environmental samples, analytical interferences, and data quality objectives (DQOs). Where further development and testing are necessary, EPA is continuing to develop and evaluate analytical techniques based on the methods and protocols that are listed in this

document and based on current EPA policies for validating analytical methods. Once validation is complete, data regarding the resulting method performance and DQOs will be available.

EPA recognizes that selection of a single method might limit laboratory capability and affect laboratory capacity when techniques different from those provided in the methods are required for the analysis of difficult samples. In those cases where method procedures are determined to be insufficient for a particular situation, NHSRC will continue to provide technical support regarding appropriate actions (see list of contacts in Section 4.0). Additional information is also provided in the Agency Policy Directive Number FEM-2010-01.[3] NHSRC has also compiled information and published documents regarding field screening equipment, sample collection materials, rapid screening/preliminary identification equipment, and disposal of samples. These documents are available at https://www.epa.gov/homeland-security-research/sam-companion-documents-and-sample-collection-procedures.

EPA plans to continue to update the document as appropriate to address the needs of homeland security, to reflect improvements in analytical methodology and new technologies, and to incorporate changes in analytes based on needs. EPA also periodically provides addenda to provide updates regarding methods, information and issues that are not addressed by the most current versions of this document. Information regarding the use of deviations from the selected methods is provided in Section 4.0.

Participants in the chemical, radiochemical, pathogen and biotoxin work groups evaluated the suitability of existing methodologies and selected this set of methods for use by those laboratories that support EPA environmental remediation efforts following an intentional or unintentional contamination incident. EPA recognizes that this advanced selection of such methods may pose potential risks, including the following:

- Selecting technologies that may not be the most cost-effective technologies currently available for addressing the particular situation at hand;
- Selecting methodologies that may not be appropriate for use in responding to a particular incident because EPA did not anticipate having to analyze for a particular analyte or analyte/sample type combination; and
- Discouraging development and adoption of new and better measurement technologies.

To address these potential risks, the following measures are taken:

- Using an established method selection process (Figure 2-1) to help ensure that the analytical methods listed provide results that are consistent with and support their intended use;
- Collaborating with the ERLN, which includes the WLA and is part of the Integrated Consortium of Laboratory Networks (ICLN), to ensure that the methods selected meet the network's needs for consistent analytical capabilities, to address capacity, and to provide quality data to inform remediation decisions; and
- Continuing to work with multiple agencies and stakeholders to update methods and protocols, as needed.

Public officials need to accurately assess and characterize site contamination following an emergency situation. This assessment includes initial characterization of potential site contamination for determination of immediate public and environmental risk, determination of the extent of contamination, and effective approaches for site remediation. EPA recognizes that having data of known and documented quality is critical in making proper decisions during each of these activities, and strives to establish site-

[3] U.S. EPA, Forum on Environmental Measurements, July 21, 2010, *Ensuring the Validity of Agency Methods Validation and Peer Review Guidelines: Methods of Analysis Developed for Emergency Response Situations,* Agency Policy Directive Number FEM-2010-01. https://www.epa.gov/sites/production/files/2015-01/documents/emergency_response_validity_policy.pdf

specific DQOs for each response activity.[4] These DQOs are based upon needs for both quality and response time. During initial assessments, time is of utmost importance and DQOs must be established that weigh the need for rapid analytical response (e.g., using screening methods) against the need for very high quality data. Many of the methods listed in this document include quality control (QC) requirements for collecting and analyzing samples. EPA will assess these QC requirements to ensure analytical data quality supports decisions concerning site remediation and release. These QC requirements may be adjusted as necessary to maximize data and decision quality. Specific QC considerations and recommendations for analysis of samples for chemical, radiochemical, pathogen and biotoxin analytes are provided in each corresponding section of this document (i.e., Sections 5.1.2, 6.1.2, 7.1.2 and 8.1.2, respectively). EPA's ERLN, which is tasked with providing laboratory support following intentional or unintentional contamination incidents, also has established data reporting procedures. Requirements for receiving, tracking, storing, preparing, analyzing and reporting data are specified in the U.S. EPA (2011) *Environmental Response Laboratory Network Laboratory Requirements Document* at: https://www.epa.gov/sites/production/files/documents/erln_lab_requirements.pdf; project-specific requirements also are included in individual Analytical Service Requests (ASRs).

[4] Information regarding EPA's DQO process, considerations, and planning is available at: http://www.epa.gov/QUALITY/dqos.html.

Section 4.0: Points of Contact

Questions concerning this document, or the methods identified in this document, should be addressed to the appropriate point(s) of contact identified below. EPA recommends that these contacts be consulted regarding any method deviations or modifications, sample problems or interferences, QC requirements, the use of potential alternative methods, or the need to address analytes or sample types other than those listed. As previously indicated, any deviations from the recommended method(s) should be reported immediately to ensure data comparability is maintained when responding to intentional or unintentional contamination incidents. In cases where laboratories are specifically tasked by EPA to use these methods following an incident, method deviations or modifications must be approved by the Analytical Service Requestor (as defined by ERLN) prior to use. In addition, general questions and comments can be submitted via the SAM website (https://www.epa.gov/homeland-security-research/sam).

General Romy Campisano - *Primary* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 569-7016 campisano.romy@epa.gov	Kathy Hall - *Alternate* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 379-5260 hall.kathy@epa.gov
Chemical Methods Steve Reimer - *Primary* U.S. EPA Region 10 - Manchester Laboratory 7411 Beach Drive East Port Orchard, WA 98366 (360) 871-8718 reimer.steve@epa.gov	Stuart Willison - *Alternate* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 569-7253 willison.stuart@epa.gov
Radiochemical Methods John Griggs - *Primary* U.S. EPA National Analytical Radiation Environmental Laboratory, ORIA 540 South Morris Avenue Montgomery, AL 36115-2601 (334) 270-3401 griggs.john@epa.gov	Kathy Hall - *Alternate* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 379-5260 hall.kathy@epa.gov
Pathogen Methods Laura Boczek - *Primary* National Risk Management Research Laboratory U.S. EPA ORD (681) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 569-7282 boczek.laura@epa.gov	Erin Silvestri – *Alternate* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 569-7619 silvestri.erin@epa.gov
Biotoxins Methods Matthew Magnuson - *Primary* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 569-7321 magnuson.matthew@epa.gov	Heath Mash - *Alternate* National Risk Management Research Laboratory U.S. EPA ORD (681) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 569-7713 mash.heath@epa.gov

Section 5.0: Selected Chemical Methods

Appendix A provides a list of methods to be used in analyzing environmental samples for chemical contaminants during remediation activities that result from a contamination incident. Methods are listed for each analyte and for each sample type that potentially may need to be measured and analyzed when responding to an environmental contamination incident. In some cases, procedures from peer-reviewed journal articles or provisional methods are listed for those analyte-sample type combinations where validated methods are unavailable. In these instances, the best available procedure was selected based on its environmental application and on data quality objectives (DQOs). Appendix A includes method usability tiers that have been assigned to each method to indicate its applicability to the specific analyte-sample type combination(s) for which it has been selected. These tiers are described in Section 5.1.1 below, and are defined on the first page of Appendix A. As appropriate, when fully validated methods become available, the literature references and alternative methods will be replaced.

Please note: This section provides guidance for selecting chemical methods that have a high likelihood of assuring analytical consistency when laboratories are faced with a large scale environmental restoration crisis. Not all methods have been verified for the analyte/sample type combination listed in Appendix A. Please refer to the specified method to identify analyte/sample type combinations that have been verified. Any questions regarding information discussed in this section should be addressed to the appropriate contact(s) listed in Section 4.0.

Appendix A is sorted alphabetically by analyte and includes the following information:

- **Analyte(s).** The component, contaminant or constituent of interest.
- **Chemical Abstracts Service Registry Number (CAS RN®** [Chemical Abstracts Service, Columbus, OH]**).** A unique identifier for chemical substances that provides an unambiguous way to identify a chemical or molecular structure when there are many possible systematic, generic or trivial names.
- **Determinative technique.** An analytical instrument or technique used to determine the quantity and identification of compounds or components in a sample.
- **Method type.** Two method types (sample preparation and determinative) are used to complete sample analysis. In some cases, a single method contains information for both sample preparation and determinative procedures. In most instances, however, two separate methods may need to be used in conjunction.
- **Solid samples.** The recommended method / procedure to identify and measure the analyte of interest in solid phase samples.
- **Non-drinking water samples.** The recommended method / procedure to identify and measure the analyte of interest in aqueous liquid phase samples other than drinking water.
- **Drinking water samples.** The recommended method / procedure to identify and measure the analyte of interest in drinking water samples.
- **Air samples.** The recommended method / procedure to identify and measure the analyte of interest in air samples.
- **Wipe samples.** The recommended method / procedure to identify and measure the analyte of interest in wipes used to collect a sample from a surface.

Following an environmental contamination incident, it is assumed that only those areas with contamination greater than pre-existing / naturally prevalent levels commonly found in the environment would be subject to remediation. Dependent on site- and incident-specific goals, investigation of background levels using methods listed in Appendix A is recommended.

5.1 General Guidelines

This section provides a general overview of how to identify the appropriate chemical method(s) for a given analyte-sample type combination, as well as recommendations for quality control (QC) procedures.

For additional information on the properties of the chemicals listed in Appendix A, Toxicology Data Network (TOXNET) (http://toxnet.nlm.nih.gov/index.html), a cluster of databases on toxicology, hazardous chemicals, and related areas maintained by the National Library of Medicine, is an excellent resource. Additional resources include:

- Syracuse Research Corporation's (SRC) PHYSPROP (http://www.srcinc.com/what-we-do/environmental/scientific-databases.html) contains information pertaining to chemical structures, names, physical properties and persistence. PHYSPROP is sponsored by the U.S. Environmental Protection Agency (EPA).
- INCHEM (http://www.inchem.org/) contains both chemical and toxicity information.
- The Registry of Toxic Effects of Chemical Substances (RTECS) database can be accessed via the National Institute for Occupational Safety and Health (NIOSH) website (http://www.cdc.gov/niosh/rtecs/default.html) for toxicity information.
- EPA's Integrated Risk Information System (IRIS) (http://www.epa.gov/iris/) contains toxicity information (searchable on TOXNET).
- EPA's Water Contaminant Information Tool (WCIT) (https://www.epa.gov/waterdata/water-contaminant-information-tool-wcit) can be accessed by registered users.
- *Forensic Science and Communications* (http://www.fbi.gov/about-us/lab/forensic-science-communications) is published by the Laboratory Division of the Federal Bureau of Investigation (FBI).
- Joint Research Centre / Institute for Health & Consumer Protection (https://ec.europa.eu/jrc/en) contains information regarding European Directive 67/548/EEC and Annex V.
- Agency of Toxic Substances & Disease Registry (ATSDR) Toxic Substances Portal (http://www.atsdr.cdc.gov/toxprofiles/index.asp) provides Toxicological Profiles.
- Chemical Safety Data Sheets (http://www.ilpi.com/msds/).

Additional research on chemical contaminants is ongoing within EPA. Databases to manage this information are currently under development and will be updated when additional information becomes available.

5.1.1 Standard Operating Procedures for Identifying Chemical Methods

To determine the appropriate method to be used on an environmental sample, locate the analyte of concern under the "Analyte(s)" column in Appendix A: Selected Chemical Methods. After locating the analyte of concern, continue across the table to identify the appropriate determinative technique (e.g., high performance liquid chromatography [HPLC], gas chromatography-mass spectrometry [GC-MS]), then identify the appropriate sample preparation and determinative method(s) for the sample type of interest (solid, water, air or wipe). In some cases, two methods (sample preparation and determinative) are needed to complete sample analysis.

The fitness of a method for an intended use is related to site-specific DQOs for a particular environmental remediation activity. These selected chemical methods have been assigned tiers (below) to indicate a level

of method usability for the specific analyte and sample type. The assigned tiers reflect the conservative view for DQOs involving timely implementation of methods for analysis of a high number of samples (such that multiple laboratories are necessary), low limits of identification and quantification, and appropriate QC:

Tier I: Analyte/sample type is a target of the method(s). Data are available for all aspects of method performance and QC measures supporting its use for analysis of environmental samples following a contamination event. Evaluation and/or use of the method(s) in multiple laboratories indicate that the method can be implemented with no additional modifications for the analyte/sample type.

Tier II: (1) The analyte/sample type is a target of the method(s) and the method(s) has been evaluated for the analyte/sample type by one or more laboratories, or (2) the analyte/sample type is not a target of the method(s), but the method has been used by laboratories to address the analyte/sample type. In either case, available data and/or information indicate that modifications will likely be needed for use of the method(s) to address the analyte/sample type (e.g., due to potential interferences, alternate matrices, the need to address different DQOs).

Tier III: The analyte/sample type is not a target of the method(s), and/or no reliable data supporting the method's fitness for its intended use are available. Data from other analytes or sample types, however, suggest that the method(s), with significant modification, may be applicable.

Once a method has been identified in Appendix A, **Table 5-1** can be used to locate the method summary. Sections 5.2.1 through 5.2.116 below provide summaries of the sample preparation and determinative methods listed in Appendix A.

Table 5-1. Chemical Methods and Corresponding Section Numbers

Analyte	CAS RN	Method	Section
Acephate	30560-19-1	538 (EPA OW)	5.2.11
		Chromatographia (2006) 63(5/6): 233–237	5.2.103
		J. Chromatogr. A (2007) 1154(1): 3–25	5.2.109
Acrylamide	79-06-1	3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8316 (EPA SW-846)	5.2.38
		PV2004 (OSHA)	5.2.84
Acrylonitrile	107-13-1	524.2 (EPA OW)	5.2.7
		3570 (EPA SW-846)	5.2.24
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		8290A Appendix A (EPA SW-846)	5.2.36
		PV2004 (OSHA)	5.2.84
Aldicarb (Temik)	116-06-3	531.2 (EPA OW)	5.2.10
		3570 (EPA SW-846)	5.2.24
Aldicarb sulfone	1646-88-4	8290A Appendix A (EPA SW-846)	5.2.36
		8318A (EPA SW-846)	5.2.39
Aldicarb sulfoxide	1646-87-3	5601 (NIOSH)	5.2.65
		D7645-16 (ASTM)	5.2.92

Analyte	CAS RN	Method	Section
Allyl alcohol	107-18-6	5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		TO-15 (EPA ORD)	5.2.48
4-Aminopyridine	504-24-5	3535A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8330B (EPA SW-846)	5.2.40
Ammonia	7664-41-7	350.1 (EPA OW)	5.2.6
		6015 (NIOSH)	5.2.70
		4500-NH_3 B (SM)	5.2.98
		4500-NH_3 G (SM)	5.2.99
Ammonium metavanadate (analyze as total vanadium) Arsenic, Total Arsenic trioxide (analyze as total arsenic)	7803-55-6 7440-38-2 1327-53-3	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.75
Arsine (analyze as total arsenic in non-air samples)	7784-42-1	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		6001 (NIOSH)	5.2.66
		9102 (NIOSH)	5.2.75
Asbestos	1332-21-4	D5755-09 (ASTM)	5.2.86
		D6480-05 (ASTM)	5.2.87
		10312:1995 (ISO)	5.2.96
Boron trifluoride	7637-07-2	ID216SG (OSHA)	5.2.83
Brodifacoum Bromadiolone	56073-10-0 28772-56-7	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		D7644-16 (ASTM)	5.2.91

Analyte	CAS RN	Method	Section
BZ [Quinuclidinyl benzilate]	6581-06-2	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.47
		J. Chromatogr. B (2008) 874: 42–50	5.2.112
Calcium arsenate (analyze as total arsenic)	7778-44-1	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.75
Carbofuran (Furadan)	1563-66-2	531.2 (EPA OW)	5.2.10
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8318A (EPA SW-846)	5.2.39
		5601 (NIOSH)	5.2.65
		D7645-16 (ASTM)	5.2.92
Carbon disulfide	75-15-0	524.2 (EPA OW)	5.2.7
		5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		TO-15 (EPA ORD)	5.2.48
Carfentanil	59708-52-0	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		J. Chromatogr. B (2014) 962: 52–58	5.2.115
Chlorfenvinphos	470-90-6	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Chlorine	7782-50-5	4500-Cl G (SM)	5.2.100
		Analyst (1999) 124(12): 1853–1857	5.2.101

Analyte	CAS RN	Method	Section
2-Chloroethanol	107-07-3	5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		2513 (NIOSH)	5.2.61
3-Chloro-1,2-propanediol	96-24-2	TO-10A (EPA ORD)	5.2.47
		Eur. J. Lipid Sci. Technol. (2011) 113: 345–355	5.2.105
		J. Chromatogr. A (2000) 866: 65–77	5.2.107
Chloropicrin	76-06-2	551.1 (EPA OW)	5.2.14
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
		PV2103 (OSHA)	5.2.85
Chlorosarin	1445-76-7	TO-17 (EPA ORD)	5.2.49
Chlorosoman	7040-57-5	EPA/600/R-16/115	5.2.56
2-Chlorovinylarsonic acid (CVAOA) 2-Chlorovinylarsonous acid (CVAA)	64038-44-4 85090-33-1	**Analyze as CVAA and CVAOA**	
		EPA/600/R-15/258 (EPA NHSRC)	5.2.54
		Analyze as total arsenic	
		200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.75
Chlorpyrifos	2921-88-2	525.2 (EPA OW)	5.2.8
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Chlorpyrifos oxon	5598-15-2	540 (EPA OW)	5.2.12
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Crimidine	535-89-7	EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Cyanide, Amenable to chlorination	NA	RLAB Method 3135.2I (EPA Region 7)	5.2.42
		4500-CN G (SM)	5.2.97
Cyanide, Total	57-12-5	335.4 (EPA OW)	5.2.5
		ISM02.3 CN (EPA CLP)	5.2.41
		6010 (NIOSH)	5.2.68
Cyanogen chloride	506-77-4	TO-15 (EPA ORD)	5.2.48
		Encyclopedia of Anal. Chem. (2006) DOI:10.1002/9780470027318.a0809	5.2.104
Cyclohexyl sarin (GF)	329-99-7	TO-17 (EPA ORD)	5.2.49
		EPA/600/R-16/115 (EPA NHSRC)	5.2.56

Analyte	CAS RN	Method	Section
1,2-Dichloroethane	107-06-2	524.2 (EPA OW)	5.2.7
		5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		TO-15 (EPA ORD)	5.2.48
Dichlorvos	62-73-7	525.2 (EPA OW)	5.2.8
		3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Dicrotophos	141-66-2	3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Diesel range organics	NA	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8015D (EPA SW-846)	5.2.33
		8290A Appendix A (EPA SW-846)	5.2.36
Diisopropyl methylphosphonate (DIMP)	1445-75-6	538 (EPA OW)	5.2.11
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-13/224 (EPA NHSRC)	5.2.52
		D7597-16 (ASTM)	5.2.88
		E-2866-12 (ASTM)	5.2.95
Dimethylphosphite	868-85-9	EPA/600/R-16/114 (EPA NHSRC)	5.2.55
		TO-10A (EPA ORD)	5.2.47
Dimethylphosphoramidic acid	33876-51-6	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-13/224 (EPA NHSRC)	5.2.52
		D7597-16 (ASTM)	5.2.88
		E-2866-12 (ASTM)	5.2.95
Diphacinone	82-66-6	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		D7644-16 (ASTM)	5.2.91
Disulfoton	298-04-4	525.2 (EPA OW)	5.2.8
Disulfoton sulfone oxon	2496-91-5	EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Disulfoton sulfoxide	2497-07-6	5600 (NIOSH)	5.2.64
Disulfoton sulfoxide oxon	2496-92-6		
1,4-Dithiane	505-29-3	EPA/600/R-16/114 (EPA NHSRC)	5.2.55

Analyte	CAS RN	Method	Section
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-15/097 (EPA NHSRC)	5.2.53
Ethyl methylphosphonic acid (EMPA)	1832-53-7	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-13/224 (EPA NHSRC)	5.2.52
		D7597-16 (ASTM)	5.2.88
		E-2866-12 (ASTM)	5.2.95
Ethyldichloroarsine (ED)	598-14-1	3535A (EPA SW-846)	5.2.21
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		8270E (EPA SW-846)	5.2.35
		TO-15 (EPA ORD)	5.2.48
		9102 (NIOSH)	5.2.75
N-Ethyldiethanolamine (EDEA)	139-87-7	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		EPA/600/R-11/143 (EPA NHSRC / CDC)	5.2.50
		3509 (NIOSH)	5.2.62
		D7599-16 (ASTM)	5.2.90
Ethylene oxide	75-21-8	5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		TO-15 (EPA ORD)	5.2.48
Fenamiphos	22224-92-6	525.2 (EPA OW)	5.2.8
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Fentanyl	437-38-7	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		J. Chromatogr. A (2011) 1218: 1620–1649	5.2.111
Fluoride	16984-48-8	300.1, Rev 1.0 (EPA OW)	5.2.4
Fluoroacetamide	640-19-7	J. Chromatogr. B (2008) 876(1): 103–108	5.2.113
Fluoroacetic acid and fluoroacetate salts	NA	S301-1 (NIOSH)	5.2.78
		J. Chromatogr. A (2007) 1139: 271–278	5.2.108
		J. Chromatogr. B. (2010) 878: 1045–1050	5.2.114
2-Fluoroethanol	371-62-0	5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		2513 (NIOSH)	5.2.61
Fluorosilicic acid (analyze as fluoride)	16961-83-4	300.1, Rev 1.0 (EPA OW)	5.2.4

Analyte	CAS RN	Method	Section
Formaldehyde	50-00-0	556.1 (EPA OW)	5.2.15
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8315A (EPA SW-846)	5.2.37
		2016 (NIOSH)	5.2.60
Gasoline range organics	NA	3570 (EPA SW-846)	5.2.24
		5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8015D (EPA SW-846)	5.2.33
		8290A Appendix A (EPA SW-846)	5.2.36
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4	3535A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8330B (EPA SW-846)	5.2.40
Hexamethylenetriperoxidediamine (HMTD)	283-66-9	3535A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8330B (EPA SW-846)	5.2.40
		Analyst (2001) 126: 1689–1693	5.2.102
Hydrogen bromide Hydrogen chloride	10035-10-6 7647-01-0	7907 (NIOSH)	5.2.74
Hydrogen cyanide	74-90-8	6010 (NIOSH)	5.2.68
Hydrogen fluoride	7664-39-3	7906 (NIOSH)	5.2.73
Hydrogen sulfide	2148878	6013 (NIOSH)	5.2.69
Isopropyl methylphosphonic acid (IMPA)	1832-54-8	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-13/224 (EPA NHSRC)	5.2.52
		D7597-16 (ASTM)	5.2.88
		E-2866-12 (ASTM)	5.2.95
Kerosene	64742-81-0	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8015D (EPA SW-846)	5.2.33
		8290A Appendix A (EPA SW-846)	5.2.36
Lead arsenate (analyze as total arsenic)	7645-25-2	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.75

Analyte	CAS RN	Method	Section
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] Lewisite oxide	541-25-3 40334-69-8 40334-70-1 1306-02-1	**Analyze as lewisite I, 2, 3 or lewisite oxide**	
		EPA/600/R-15/258 (EPA NHSRC)	5.2.54
		Analyze as total arsenic	
		200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.75
Mercuric chloride (analyze as total mercury)	7487-94-7	245.1 (EPA OW)	5.2.3
		7473 (EPA SW-846)	5.2.31
		9102 (NIOSH)	5.2.75
Mercury, Total	7439-97-6	245.1 (EPA OW)	5.2.3
		7473 (EPA SW-846)	5.2.31
		IO-5 (EPA ORD)	5.2.46
		9102 (NIOSH)	5.2.75
Methamidophos	10265-92-6	538 (EPA OW)	5.2.11
		Chromatographia (2006) 63(5/6): 233–237	5.2.103
		J. Chromatogr. A (2007) 1154(1): 3–25	5.2.109
Methomyl	16752-77-5	531.2 (EPA OW)	5.2.10
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8318A (EPA SW-846)	5.2.39
		5601 (NIOSH)	5.2.65
		D7645-16 (ASTM)	5.2.92
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2	245.1 (EPA OW)	5.2.3
		7473 (EPA SW-846)	5.2.31
		IO-5 (EPA ORD)	5.2.46
		9102 (NIOSH)	5.2.75
Methyl acrylonitrile	126-98-7	524.2 (EPA OW)	5.2.7
		3570 (EPA SW-846)	5.2.24
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		8290A Appendix A (EPA SW-846)	5.2.36
		PV2004 (OSHA)	5.2.84

Analyte	CAS RN	Method	Section
3-Methyl fentanyl	42045-87-4	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		J. Chromatogr. B (2014) 962: 52–58	5.2.115
Methyl fluoroacetate (analyze as fluoroacetate ion)	453-18-9	S301-1 (NIOSH)	5.2.78
		J. Chromatogr. A (2007) 1139: 271–278	5.2.108
		J. Chromatogr. B (2010) 878: 1045–1050	5.2.114
Methyl hydrazine	60-34-4	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		3510 (NIOSH)	5.2.63
		J. Chromatogr. (1993) 617: 157–162	5.2.106
Methyl isocyanate	624-83-9	OSHA 54	5.2.80
Methyl paraoxon Methyl parathion	950-35-6 298-00-0	3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Methylamine	74-89-5	OSHA 40	5.2.79
N-Methyldiethanolamine (MDEA)	105-59-9	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		EPA/600/R-11/143 (EPA NHSRC / CDC)	5.2.50
		3509 (NIOSH)	5.2.62
		D7599-16 (ASTM)	5.2.90
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3	TO-17 (EPA ORD)	5.2.49
		EPA/600/R-16/115 (EPA NHSRC)	5.2.56
Methylphosphonic acid (MPA)	993-13-5	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-13/224 (EPA NHSRC)	5.2.52
		D7597-16 (ASTM)	5.2.88
		E-2866-12 (ASTM)	5.2.95
Mevinphos	7786-34-7	525.2 (EPA OW)	5.2.8
		3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Monocrotophos	6923-22-4	3535A (EPA SW-846)	5.2.21
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8270E (EPA SW-846)	5.2.35
		8290A Appendix A (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.47

Analyte	CAS RN	Method	Section
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)-ethylamine] Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)-methylamine] Mustard, nitrogen (HN-3) [tris(2-chloroethyl)-amine]	538-07-8 51-75-2 555-77-1	TO-17 (EPA ORD)	5.2.49
		EPA/600/R-12/653 (EPA NHSRC)	5.2.51
Mustard, sulfur / Mustard gas (HD)	505-60-2	TO-17 (EPA ORD)	5.2.49
		EPA/600/R-16/115 (EPA NHSRC)	5.2.56
Nicotine compounds	54-11-5	3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0	3535A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8330B (EPA SW-846)	5.2.40
Osmium tetroxide (analyze as total osmium)	20816-12-0	3015A (EPA SW-846)	5.2.16
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
Oxamyl	23135-22-0	531.2 (EPA OW)	5.2.10
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8318A (EPA SW-846)	5.2.39
		5601 (NIOSH)	5.2.65
		D7645-16 (ASTM)	5.2.92
Paraoxon	311-45-5	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Paraquat	4685-14-7	549.2 (EPA OW)	5.2.13
		J. Chromatogr. A (2008) 1196-97: 110–116	5.2.110
Parathion	56-38-2	3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Pentaerythritol tetranitrate (PETN)	78-11-5	3535A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8330B (EPA SW-846)	5.2.40

Analyte	CAS RN	Method	Section
Phencyclidine	77-10-1	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
		9106 (NIOSH)	5.2.76
		9109 (NIOSH)	5.2.77
Phorate	298-02-2	3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Phorate sulfone Phorate sulfone oxon	2588-04-7 2588-06-9	540 (EPA OW)	5.2.12
		TO-10A (EPA ORD)	5.2.47
Phorate sulfoxide Phorate sulfoxide oxon	2588-03-6 2588-05-8	EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Phosgene	75-44-5	OSHA 61	5.2.81
Phosphamidon	13171-21-6	525.3 (EPA OW)	5.2.9
		3520C (EPA SW-846)	5.2.20
		3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
		TO-10A (EPA ORD)	5.2.47
Phosphine	7803-51-2	6002 (NIOSH)	5.2.67
Phosphorus trichloride	2125683	6402 (NIOSH)	5.2.71
Pinacolyl methyl phosphonic acid (PMPA)	616-52-4	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-13/224 (EPA NHSRC)	5.2.52
		D7597-16 (ASTM)	5.2.88
		E-2866-12 (ASTM)	5.2.95
Propylene oxide	75-56-9	5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34
		1612 (NIOSH)	5.2.59
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4	TO-17 (EPA ORD)	5.2.49
		EPA/600/R-12/653 (EPA NHSRC)	5.2.51
Sarin (GB)	107-44-8	TO-17 (EPA ORD)	5.2.49
Soman (GD)	96-64-0	EPA/600/R-16/115 (EPA NHSRC)	5.2.56
Sodium arsenite (analyze as total arsenic)	7784-46-5	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.75

Analyte	CAS RN	Method	Section
Sodium azide (analyze as azide ion)	26628-22-8	300.1, Rev 1.0 (EPA OW)	5.2.4
		ID-211 (OSHA)	5.2.82
		J. Forensic Sci. (1998) 43(1): 200–202	5.2.116
Strychnine	57-24-9	3535A (EPA SW-846)	5.2.21
		8270E (EPA SW-846)	5.2.35
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Tabun (GA)	77-81-6	TO-17 (EPA ORD)	5.2.49
		EPA/600/R-12/653 (EPA NHSRC)	5.2.51
Tetraethyl pyrophosphate (TEPP)	107-49-3	3511 (EPA SW-846)	5.2.19
		8270E (EPA SW-846)	5.2.35
		TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Tetramethylenedisulfotetramine (TETS)	80-12-6	TO-10A (EPA ORD)	5.2.47
		EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Thallium sulfate (analyze as total thallium)	10031-59-1	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.75
Thiodiglycol (TDG)	111-48-8	TO-10A (EPA ORD)	5.2.47
		D7598-16 (ASTM)	5.2.89
		E2787-11 (ASTM)	5.2.93
		E2838-11 (ASTM)	5.2.94
Thiofanox	39196-18-4	538 (EPA OW)	5.2.11
		3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		5601 (NIOSH)	5.2.65
		D7645-16 (ASTM)	5.2.92
1,4-Thioxane	15980-15-1	EPA/600/R-16/114 (EPA NHSRC)	5.2.55
Titanium tetrachloride (analyze as total titanium)	7550-45-0	3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
Triethanolamine (TEA)	102-71-6	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		EPA/600/R-11/143 (EPA NHSRC / CDC)	5.2.50
		3509 (NIOSH)	5.2.62
		D7599-16 (ASTM)	5.2.90

Analyte	CAS RN	Method	Section
Trimethyl phosphite	121-45-9	3541 (EPA SW-846)	5.2.22
		3545A (EPA SW-846)	5.2.23
		3570 (EPA SW-846)	5.2.24
		8270E (EPA SW-846)	5.2.35
		8290A Appendix A (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.47
1,3,5-Trinitrobenzene (1,3,5-TNB) 2,4,6-Trinitrotoluene (2,4,6-TNT)	99-35-4 118-96-7	3535A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.36
		8330B (EPA SW-846)	5.2.40
Vanadium pentoxide (analyze as total vanadium)	1314-62-1	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3015A (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		3051A (EPA SW-846)	5.2.18
		6010D (EPA SW-846)	5.2.27
		6020B (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.43
		IO-3.4 (EPA ORD)	5.2.44
		IO-3.5 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.75
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester] VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	21738-25-0 78-53-5	TO-17 (EPA ORD)	5.2.49
VM [phosphonothioic acid, methyl-,S-(2-(diethylamino)ethyl) O-ethyl ester] VX [O-ethyl-S-(2-diisopropylaminoethyl) methyl-phosphonothiolate]	21770-86-5 50782-69-9	EPA/600/R-16/116 (EPA NHSRC)	5.2.57
White phosphorus	12185-10-3	3570 (EPA SW-846)	5.2.24
		7580 (EPA SW-846)	5.2.32
		8290A Appendix A (EPA SW-846)	5.2.36
		7905 (NIOSH)	5.2.72
The following analytes should be prepared and/or analyzed by the following methods only if problems (e.g., insufficient recovery, interferences) occur when using the sample preparation / determinative techniques identified for these analytes in Appendix A.			
Allyl alcohol	107-18-6	TO-10A (EPA ORD)	5.2.47
3-Chloro-1,2-propanediol	96-24-2	TO-15 (EPA ORD)	5.2.48
Chlorosarin Chlorosoman	1445-76-7 7040-57-5	TO-15 (EPA ORD)	5.2.48
Diisopropyl methylphosphonate (DIMP)	1445-75-6	TO-15 (EPA ORD)	5.2.48

Analyte	CAS RN	Method	Section
Mercuric chloride (analyze as total mercury)	7487-94-7	7470A (EPA SW-846)	5.2.29
Mercury, Total	7439-97-6	7471B (EPA SW-846)	5.2.30
Methamidophos	10265-92-6	5600 (NIOSH)	5.2.64
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2	7470A (EPA SW-846)	5.2.29
		7471B (EPA SW-846)	5.2.30
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3	TO-15 (EPA ORD)	5.2.48
Sarin (GB)	107-44-8	TO-15 (EPA ORD)	5.2.48
Soman (GD)	96-64-0		
1,4-Thioxane	15980-15-1	5030C (EPA SW-846)	5.2.25
		5035A (EPA SW-846)	5.2.26
		8260D (EPA SW-846)	5.2.34

Method summaries are listed in order of method selection hierarchy (see Figure 2-1), starting with EPA methods, followed by methods from other federal agencies, voluntary consensus standard bodies (VCSBs), and literature references. Methods are listed in numerical order under each publisher. Where available, a direct link to the full text of the method is provided in the method summary. For additional information on preparation procedures and methods available through consensus standards organizations, please use the contact information provided in **Table 5-2**.

Table 5-2. Sources of Chemical Methods

Name	Publisher	Reference
National Environmental Methods Index (NEMI)	EPA, U.S. Geological Survey (USGS)	http://www.nemi.gov
EPA Contract Laboratory Program (CLP) Methods	EPA, CLP	http://www.epa.gov/superfund/programs/clp/
EPA Office of Water (OW) Methods	EPA OW	https://www.epa.gov/dwanalyticalmethods
EPA Solid Waste (SW)-846 Methods	EPA Office of Land and Emergency Management (OLEM)	https://www.epa.gov/hw-sw846/sw-846-compendium
EPA Office of Research and Development (ORD) Methods	EPA ORD	https://www.epa.gov/aboutepa/about-office-research-and-development-ord
EPA Air Toxics Methods	EPA Office of Air and Radiation (OAR)	http://www.epa.gov/ttn/amtic/airtox.html
EPA Analytical Protocols	EPA National Homeland Security Research Center (NHSRC)	https://www.epa.gov/homeland-security-research/forms/contact-us-about-homeland-security-research
Occupational Safety and Health Administration (OSHA) Methods	OSHA	http://www.osha.gov/dts/sltc/methods/index.html
NIOSH Methods	NIOSH	http://www.cdc.gov/niosh/nmam/
Standard Methods for the Examination of Water and Wastewater (SM), 22nd Edition, 2005*	American Public Health Association (APHA)	http://www.standardmethods.org
*Annual Book of ASTM Standards**	ASTM International	http://www.astm.org
International Organization for Standardization (ISO) Methods*	ISO	http://www.iso.org
Official Methods of Analysis of AOAC International*	AOAC International	http://www.aoac.org

Name	Publisher	Reference
Analyst*	Royal Society of Chemistry	http://www.rsc.org/Publishing/Journals/AN/
Journal of Chromatography A and B*	Elsevier Science Publishers	http://www.journals.elsevier.com/journal-of-chromatography-a/
Journal of Forensic Sciences*	ASTM International	https://www.astm.org/DIGITAL_LIBRARY/JOURNALS/FORENSIC/index.html
Chromatographia*	Vieweg+Teubner	http://www.springer.com/chemistry/analytical+chemistry/journal/10337
Encyclopedia of Analytical Chemistry*	Wiley	http://www.wiley.com/WileyCDA/WileyTitle/productCd-0470973331.html
European Journal of Lipid Science and Technology*	Wiley	http://www.wiley-vch.de/publish/en/journals/alphabeticIndex/2114/
EPA WCIT	EPA OW Water Security Division (WSD)	https://www.epa.gov/waterdata/water-contaminant-information-tool-wcit
Analytical Chemistry*	American Chemical Society(ACS)	http://pubs.acs.org/journal/ancham

* Subscription and/or purchase required.

5.1.2 General QC Guidelines for Chemical Methods

Having analytical data of appropriate quality requires that laboratories: (1) conduct the necessary QC activities to ensure that measurement systems are in control and operating correctly; (2) properly document results of the analyses; and (3) properly document measurement system evaluation of the analysis-specific QC, including corrective actions.[5] In addition to the laboratories being capable of generating accurate and precise data during site remediation, they must be able to deliver results in a timely and efficient manner. Therefore, laboratories must be prepared with calibrated instruments, the proper standards, standard analytical procedures, standard operating procedures, and qualified and trained staff. Moreover, laboratories also must be capable of providing rapid turnaround of sample analyses and data reporting.

The level or amount of QC needed during sample analysis and reporting depends on the intended purpose of the data that are generated (e.g., the decision(s) to be made). The specific needs for data generation should be identified. QC requirements and DQOs should be derived based on those needs, and should be applied consistently across laboratories when multiple laboratories are used. For almost all of the chemical warfare agents (CWAs), most laboratories will not have access to analytical standards for calibration and QC. Use of these agents is strictly controlled by the Department of Defense (DoD) and access is limited. For information regarding laboratory analysis of samples containing CWAs or laboratory requirements to possess and use ultra-dilute agent standards, please use the contact information provided on the Environmental Response Laboratory Network (ERLN) website at: https://www.epa.gov/emergency-response/environmental-response-laboratory-network.

A minimum set of analytical QC procedures should be planned, documented and conducted for all chemical testing. Some method-specific QC requirements are described in many of the individual methods that are cited in this document and will be referenced in any analytical protocols developed to address specific analytes and sample types of concern. Individual methods, sampling and analysis protocols or contractual statements of work should also be consulted to determine if any additional QC might be needed. Analytical QC requirements generally consist of analysis of laboratory control samples to document whether the analytical system is in control; matrix spikes to identify and quantify

[5] Information regarding EPA's DQO process, considerations, and planning is available at: https://www.epa.gov/quality.

measurement system accuracy for the media of concern and, at the levels of concern, various blanks as a measure of freedom from contamination; as well as matrix spike duplicates or sample replicates to assess data precision.

In general, for measurement of chemical analytes, appropriate QC includes an initial demonstration of measurement system capability, as well as ongoing analysis of standards and other samples to ensure the continued reliability of the analytical results. Examples of appropriate QC include:

- Demonstration that the measurement system is operating properly
  - Initial calibration
  - Method blanks
- Demonstration of analytical method suitability for intended use
  - Detection and quantitation limits
  - Precision and recovery (verify measurement system has adequate accuracy)
  - Analyte / matrix / level of concern-specific QC samples (verify that measurement system has adequate sensitivity at levels of concern)
- Demonstration of continued analytical method reliability
  - Matrix spike / matrix spike duplicates (MS/MSDs) recovery and precision
  - QC samples (system accuracy and sensitivity at levels of concern)
  - Surrogate spikes (where appropriate)
  - Continuing calibration verification
  - Method blanks

QC tests should be consistent with EPA's Good Laboratory Practice Standards (https://www.epa.gov/compliance/good-laboratory-practices-standards-compliance-monitoring-program) and be run as frequently as necessary to ensure the reliability of analytical results. Additional guidance can be found at: https://www.epa.gov/quality; in Chapter 1 of EPA SW-846 "Test Methods for Evaluating Solid Waste, Physical/Chemical Methods" (https://www.epa.gov/sites/production/files/2015-10/documents/chap1_1.pdf); and in EPA's 2005 "Manual for the Certification of Laboratories Analyzing Drinking Water" (EPA 815-R-05-004) (https://nepis.epa.gov/Exe/ZyPDF.cgi/30006MXP.PDF?Dockey=30006MXP.PDF). As with the identification of needed QC samples, the frequency of QC sampling should be established based on an evaluation of DQOs. The type and frequency of QC tests can be refined over time.

Ensuring data quality also requires that laboratory results are properly assessed and documented. The results of the data quality assessment are included within the data report when transmitted to decision makers. This evaluation is as important as the data for ensuring informed and effective decisions. While some degree of data evaluation is necessary in order to be able to confirm data quality, 100% verification and/or validation is neither necessary nor conducive to efficient decision making in emergency situations. The level of such reviews should be determined based on the specific situation being assessed and on the corresponding DQOs. In every case, the levels of QC and data review necessary to support decision making should be determined as much in advance of data collection as possible.

Please note: The appropriate point of contact identified in Section 4.0 should be consulted regarding appropriate quality assurance (QA) and QC procedures prior to sample analysis. These contacts will consult with the EPA ERLN or Water Laboratory Alliance (WLA) coordinator responsible for laboratory activities during the specific incident to ensure QA/QC procedures are performed consistently across laboratories. EPA program offices will be responsible for ensuring that the QA/QC practices are implemented.

5.1.3 Safety and Waste Management

It is imperative that safety precautions are used during collection, processing and analysis of environmental samples. Laboratories should have a documented health and safety plan for handling samples that may contain the target chemical, biological and/or radiological (CBR) contaminants. Laboratory staff should be trained in, and need to implement, the safety procedures included in the plan. In addition, many of the methods summarized or cited in Section 5.2 contain some specific requirements, guidelines or information regarding safety precautions that should be followed when handling or processing environmental samples and reagents.

These methods also provide information regarding waste management. Other resources that can be consulted for additional information include the following:

- Centers for Disease Control and Prevention (CDC) – Title 42 of the Code of Federal Regulations part 72 (42 CFR 72). Interstate Shipment of Etiologic Agents
- CDC – 42 CFR part 73. Select Agents and Toxins
- Department of Transportation (DOT) – 49 CFR part 172. Hazardous Materials Table, Special Provisions, Hazardous Materials Communications, Emergency Response Information, and Training Requirements
- EPA – 40 CFR part 260. Hazardous Waste Management System: General
- EPA – 40 CFR part 270. EPA Administered Permit Programs: The Hazardous Waste Permit Program
- OSHA – 29 CFR part 1910.1450. Occupational Exposure to Hazardous Chemicals in Laboratories
- OSHA – 29 CFR part 1910.120. Hazardous Waste Operations and Emergency Response

Please note that the Electronic Code of Federal Regulations (e-CFR) is available at: http://www.ecfr.gov/cgi-bin/ECFR?page=browse

5.2 Method Summaries

Summaries for the analytical methods listed in Appendix A are provided in Sections 5.2.1 through 5.2.116. These sections contain summary information extracted from the selected methods. Each method summary contains a table identifying the contaminants listed in Appendix A to which the method applies, a brief description of the analytical method, and a link to, or source for, obtaining a full version of the method. Summaries are provided for informational use. Tiers that have been assigned to each method/analyte pair (see Section 5.1.1) can be found in Appendix A. The full version of the method should be consulted prior to sample analysis. For information regarding sample collection considerations for samples to be analyzed by these methods, see the companion *Sample Collection Information Document for Chemicals, Radiochemicals and Biotoxins – Companion to SAM 2017*.[6]

5.2.1 EPA Method 200.7: Determination of Metals and Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA) (analyze as total arsenic)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54)

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Acid digestion
Determinative Technique: Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES)

Method Developed for: Determination of metals in aqueous and solid samples.
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address the analytes listed in the table above as total arsenic, thallium or vanadium. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Method detection limits (MDLs) in aqueous samples are reported for arsenic (8 µg/L), vanadium (3 µg/L) and thallium (1 µg/L).

Description of Method: This method will determine metal-containing compounds only as the total metal (e.g., total arsenic) in aqueous samples. An aliquot of a well-mixed, homogeneous sample is accurately weighed or measured for sample processing. For total recoverable analysis of a sample containing

[6] EPA's *Sample Collection Information Document for Chemicals, Radiochemicals and Biotoxins – Companion to SAM 2017* can be found at: https://www.epa.gov/homeland-security-research/sam-companion-documents-and-sample-collection-procedures

undissolved material, analytes are first solubilized by gentle refluxing with nitric and hydrochloric acids. After cooling, the sample is made up to volume, mixed, and centrifuged or allowed to settle overnight prior to analysis. For determination of dissolved analytes in a filtered aqueous sample aliquot, or for the "direct analysis" total recoverable determination of analytes in drinking water where sample turbidity is < 1 nephelometric turbidity units (NTU), the sample is made ready for analysis by the addition of nitric acid, and then diluted to a predetermined volume and mixed before analysis. The prepared sample is analyzed using ICP-AES. Specific analytes targeted by Method 200.7 are listed in Section 1.1 of the method.

Special Considerations: If laboratories are approved for storing and handling the appropriate standards, lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Source: Martin, T.D., Brockhoff, C.A., Creed, J.T. and EMMC Methods Work Group. 1994. "Method 200.7: Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry," Revision 4.4. Cincinnati, OH: U.S. EPA.
https://www.epa.gov/sites/production/files/2015-06/documents/epa-200.7.pdf

5.2.2 EPA Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA) (analyze as total arsenic)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54)

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Acid digestion
Determinative Technique: Inductively coupled plasma-mass spectrometry (ICP-MS)

Method Developed for: Dissolved and total elements in ground water, surface water, drinking water, wastewater, sludges and soils
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address the analytes listed in the table above as total arsenic, thallium or vanadium. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: MDLs for arsenic, thallium and vanadium in aqueous samples are reported as 1.4, 0.3 and 2.5 µg/L, respectively (in scanning mode) and 0.4, 0.02 and 0.9 µg/L, respectively (in

selected ion monitoring (SIM) mode). The recommended calibration range is 10–200 µg/L (scanning mode) and may be lower depending on the sensitivity of the instrument.

Description of Method: This method will determine metal-containing compounds only as the total metal (e.g., total arsenic). An aliquot of a well-mixed, homogeneous sample is accurately weighed or measured for sample processing. For total recoverable analysis of a sample containing undissolved material, analytes are first solubilized by gentle refluxing with nitric and hydrochloric acids. After cooling, the sample is made up to volume, mixed, and centrifuged or allowed to settle overnight prior to analysis. For determination of dissolved analytes in a filtered aqueous sample aliquot, or for the "direct analysis" total recoverable determination of analytes in drinking water where sample turbidity is < 1 NTU, the sample is made ready for analysis by the addition of nitric acid, and then diluted to a predetermined volume and mixed before analysis. The prepared sample is analyzed using ICP-MS. Specific analytes targeted by Method 200.8 are listed in Section 1.1 of the method.

Special Considerations: If laboratories are approved for storing and handling the appropriate standards, lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Source: Creed, J.T., Brockhoff, C.A. and Martin, T.D. 1994. "Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry," Revision 5.4. Cincinnati, OH: U.S. EPA. https://www.epa.gov/sites/production/files/2015-06/documents/epa-200.8.pdf

5.2.3 EPA Method 245.1: Determination of Mercury in Water by Cold Vapor Atomic Absorption Spectrometry

Analyte(s)	CAS RN
Mercuric chloride (analyze as total mercury)	7487-94-7
Mercury, Total	7439-97-6
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Acid digestion
Determinative Technique: Cold vapor atomic absorption

Method Developed for: Mercury in surface waters. It may be applicable to saline waters, wastewaters, effluents, and domestic sewages providing potential interferences are not present.
Method Selected for: This method has been selected for preparation and analysis of water samples to address the analytes listed in the table above as total mercury. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Applicable concentration range is 0.2–10.0 µg Hg/L. The detection limit for this method is 0.2 µg Hg/L.

Description of Method: This method will determine mercuric chloride and methoxyethylmercuric acetate as total mercury. If dissolved mercury is targeted, the sample is filtered prior to acidification. To detect total mercury (inorganic and organic mercury), the sample is treated with potassium permanganate and potassium persulfate to oxidize organic mercury compounds prior to analysis. Inorganic mercury is reduced to the elemental state (using stannous chloride) and aerated from solution. The mercury vapor passes through a cell positioned in the light path of a cold vapor atomic absorption spectrophotometer. The concentration of mercury is measured using the spectrophotometer.

Special Considerations: If problems occur during analysis of aqueous samples other than drinking water, refer to Method 7470A (EPA SW-846).

Source: O'Dell, J.W., Potter, B.B., Lobring, L.B. and Martin, T.D. 1994. "Method 245.1: Determination of Mercury in Water by Cold Vapor Atomic Absorption Spectrometry," Revision 3.0. Cincinnati, OH: U.S. EPA. https://www.epa.gov/sites/production/files/2015-06/documents/epa-245.1.pdf

5.2.4 EPA Method 300.1, Revision 1.0: Determination of Inorganic Anions in Drinking Water by Ion Chromatography

Analyte(s)	CAS RN
Fluoride	16984-48-8
Fluorosilicic acid (analyze as fluoride)	16961-83-4
Sodium azide (analyze as azide ion)	26628-22-8

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: For fluoride and fluorosilicic acid, use direct injection. For sodium azide in water and solid samples, use water extraction, filtration and acidification steps from the Journal of Forensic Science, 1998. 43(1): 200-202 (Section 5.2.116).
Determinative Technique: Ion chromatography (IC) with conductivity detection

Method Developed for: Inorganic anions in reagent water, surface water, ground water and finished drinking water
Method Selected for: This method has been selected for preparation and analysis of water samples for fluoride and fluorosilicic acid (as fluoride). It also has been selected for analysis of prepared solid samples for sodium azide (as azide ion). See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit for fluoride in reagent water is 0.009 mg/L. The MDL varies depending upon the nature of the sample and the specific instrumentation employed. The estimated calibration range should not extend over more than 2 orders of magnitude in concentration over the expected concentration range of the samples.

Description of Method: This method will determine fluoride ion, fluorosilicic acids as fluoride ion, and sodium azide as azide ion. It was developed for analysis of aqueous samples, and can be adapted for analysis of sodium azide in solid and air samples when appropriate sample preparation techniques have been applied (see Appendix A). A small volume of a water sample (10 µL or 50 µL) is introduced into an ion chromatograph. The volume selected depends on the concentration of fluoride or azide ion in the sample. The anions of interest are separated and measured, using a system comprising a guard column, analytical column, suppressor device and conductivity detector. The separator columns and guard columns, as well as eluent conditions, are identical. To achieve comparable detection limits, an ion chromatographic system must use suppressed conductivity detection, be properly maintained, and be capable of yielding a baseline with no more than 5 nano siemens (nS) noise/drift per minute of monitored response over the background conductivity.

Special Considerations: For sodium azide, if analyses are problematic, refer to column manufacturer for alternate conditions.

Source: Hautman, D.P. and Munch, D.J. 1997. "Method 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography," Revision 1.0. Cincinnati, OH: U.S. EPA. https://www.epa.gov/sites/production/files/2015-06/documents/epa-300.1.pdf

5.2.5 EPA Method 335.4: Determination of Total Cyanide by Semi-Automated Colorimetry

Analyte(s)	CAS RN
Cyanide, Total	57-12-5

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Reflux-distillation
Determinative Technique: Visible spectrophotometry

Method Developed for: Cyanide in drinking, ground, surface and saline waters, and domestic and industrial wastes
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address total cyanide. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The applicable range is 5–500 µg/L.

Description of Method: Cyanide is released from cyanide complexes as hydrocyanic acid by manual reflux-distillation, and absorbed in a scrubber containing sodium hydroxide solution. The cyanide ion in the absorbing solution is converted to cyanogen chloride by reaction with chloramine-T, which subsequently reacts with pyridine and barbituric acid to give a red-colored complex.

Special Considerations: Interferences include aldehydes, nitrate-nitrite and oxidizing agents, such as chlorine, thiocyanate, thiosulfate and sulfide. These interferences can be eliminated or reduced by distillation.

Source: U.S. EPA. 1993. "Method 335.4: Determination of Total Cyanide by Semi-automated Colorimetry," Revision 1.0. Cincinnati, OH: U.S. EPA. https://www.epa.gov/sites/production/files/2015-06/documents/epa-335.4.pdf

5.2.6 EPA Method 350.1: Nitrogen, Ammonia (Colorimetric, Automated Phenate)

Analyte(s)	CAS RN
Ammonia	7664-41-7

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Distillation
Determinative Technique: Visible spectrophotometry

Method Developed for: Ammonia in drinking, ground, surface and saline waters, and domestic and industrial wastes
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address ammonia. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range for ammonia is 0.01–2.0 mg/L.

Description of Method: This method identifies and determines the concentration of ammonia in drinking water samples by spectrophotometry. Samples are buffered at a pH of 9.5 with borate buffer to decrease hydrolysis of cyanates and organic nitrogen compounds, and are distilled into a solution of boric acid. Alkaline phenol and hypochlorite react with ammonia to form indophenol blue that is proportional to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside and measured spectrophotometrically.

Special Considerations: Reduced volume distillation techniques, such as midi-distillation or micro-

distillation, can be used in place of traditional macro-distillation techniques.

Source: U.S. EPA. 1993. "Method 350.1: Nitrogen, Ammonia (Colorimetric, Automated Phenate)," Revision 2.0. Cincinnati, OH: U.S. EPA. https://www.epa.gov/sites/production/files/2015-06/documents/epa-350.1.pdf

5.2.7 EPA Method 524.2: Measurement of Purgeable Organic Compounds in Water by Capillary Column Gas Chromatography / Mass Spectrometry

Analyte(s)	CAS RN
Acrylonitrile	107-13-1
Carbon disulfide	75-15-0
1,2-Dichloroethane	107-06-2
Methyl acrylonitrile	126-98-7

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Purge-and-trap
Determinative Technique: GC-MS

Method Developed for: Purgeable volatile organic compounds (VOCs) in surface water, ground water and drinking water in any stage of treatment
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address carbon disulfide and 1,2-dichloroethane, and preparation and analysis of drinking and non-drinking water samples to address acrylonitrile and methyl acrylonitrile. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The method reports detection levels for acrylonitrile, carbon disulfide, 1,2-dichloroethane and methyl acrylonitrile in reagent water of 0.22, 0.093, 0.02 and 0.11 µg/L, respectively. The applicable concentration range of this method is primarily column and matrix dependent, and is approximately 0.02–200 µg/L when a wide-bore thick-film capillary column is used. Narrow-bore thin-film columns may have a lower capacity, which limits the range to approximately 0.02–20 µg/L.

Description of Method: VOCs and surrogates with low water solubility are extracted (purged) from the sample matrix by bubbling an inert gas through the sample. Purged sample components are trapped in a tube containing suitable sorbent materials. When purging is complete, the sorbent tube is heated and backflushed with helium to desorb the trapped sample components into a capillary gas chromatography (GC) column interfaced to a mass spectrometer (MS). The column is temperature programmed to facilitate the separation of the method analytes, which are then detected with the MS. Specific analytes targeted by Method 524.2 are listed in Section 1.1 of the method.

Special Considerations: The more recent versions of this method (Methods 524.3 or 524.4) may be used in place of Method 524.2, provided the laboratory has the necessary equipment (e.g., cryogenic auto samplers).

Source: Eichelberger, J.W., Munch, J.W. and Bellar, T.A. 1995. "Method 524.2: Measurement of Purgeable Organic Compounds in Water by Capillary Column Gas Chromatography/Mass Spectrometry," Revision 4.1. Cincinnati, OH: U.S. EPA. https://www.epa.gov/sites/production/files/2015-06/documents/epa-524.2.pdf

Additional Resources:

Prakash, A.D., Zaffiro, A.D., Zimmerman, M., Munch, D.J. and Pepich, B.V. 2009. "Method 524.3:

Measurement of Purgeable Organic Compounds in Water by Capillary Column Gas Chromatography/Mass Spectrometry," Revision 1.0. Cincinnati, OH: U.S. EPA. EPA 815-B-09-009. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J75C.PDF?Dockey=P100J75C.PDF

U.S. EPA. 2013. "Method 524.4: Measurement of Purgeable Organic Compounds in Water by Gas Chromatography/Mass Spectrometry," Revision 1. Cincinnati, OH: U.S. EPA. EPA 815-R-13-002. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7EE.PDF?Dockey=P100J7EE.PDF

5.2.8 EPA Method 525.2: Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography / Mass Spectrometry

Analyte(s)	CAS RN
Chlorpyrifos	2921-88-2
Dichlorvos	62-73-7
Disulfoton*	298-04-4
Disulfoton sulfone oxon*	2496-91-5
Disulfoton sulfoxide*	2497-07-6
Disulfoton sulfoxide oxon*	2496-92-6
Fenamiphos	22224-92-6
Mevinphos	7786-34-7

* If problems occur when using this method for measurement of oxon compounds, analysts should consider use of procedures included in "Oxidation of Selected Organophosphate Pesticides During Chlorination of Simulated Drinking Water." Water Research. 2009. 43(2): 522–534.http://www.sciencedirect.com/science/article/pii/S0043135408004995

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Liquid-solid extraction (LSE) or solid-phase extraction (SPE)
Determinative Technique: GC-MS

Method Developed for: Organic compounds in finished drinking water, source water or drinking water in any treatment stage
Method Selected for: This method has been selected for preparation and analysis of water samples to address the analytes listed in the table above. ***Note***:

- EPA NHSRC EPA/600/R-16/114 (Section 5.2.55) has been selected for preparation and analysis of non-drinking water samples to address chlorpyrifos and fenamiphos.
- SW-846 Method 3535A (Section 5.2.21) and Method 8270E (Section 5.2.35) have been selected for preparation and analysis of non-drinking water samples to address dichlorvos and mevinphos.

See Appendix A for corresponding method usability tiers.

Detection and Quantitation: The applicable concentration range for most analytes is 0.1–10 µg/L.

Description of Method: Organic compounds, internal standards and surrogates are extracted from a water sample by passing 1 L of sample through a cartridge or disk containing a solid matrix with chemically bonded C_{18} organic phase (LSE or SPE). The organic compounds are eluted from the LSE (SPE) cartridge or disk with small quantities of ethyl acetate followed by methylene chloride. The resulting extract is concentrated further by evaporation of some of the solvent. Sample components are separated, identified, and measured by injecting an aliquot of the concentrated extract into a high resolution fused silica capillary column of a GC-MS system. Specific analytes targeted by Method 525.2 are listed in Section 1.1 of the method.

Special Considerations: Refer to footnote provided in analyte table above for special considerations that should be applied when measuring specific analytes. SPE using C_{18} resin may not work for certain compounds having high water solubility. In these cases, other sample preparation techniques or different SPE resins may be required. The more recent version of this method (Method 525.3) may be used in place of Method 525.2, provided the laboratory has the necessary equipment and expertise.

Source: Munch, J.W. 1995. "Method 525.2: Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry," Revision 2.0. Cincinnati, OH: U.S. EPA. https://www.epa.gov/sites/production/files/2015-06/documents/epa-525.2.pdf

Additional Resource: Munch, J.W., Grimmett, P.E., Munch, D.J., Wendelken, S.C., Domino, M.M., Zaffiro, A.D. and Zimmerman, M.L. 2012. "Method 525.3: Determination of Semivolatile Organic Chemicals in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/ Mass Spectrometry (GC/MS)," Revision 1.0. Cincinnati, OH: U.S. EPA. EPA/600/R-12/010. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=241188

5.2.9 EPA Method 525.3: Determination of Semivolatile Organic Chemicals in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/ Mass Spectrometry (GC/MS)

Analyte(s)	CAS RN
Phosphamidon	13171-21-6

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: SPE
Determinative Technique: GC-MS

Method Developed for: Semivolatile organic compounds in drinking water
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address phosphamidon. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The applicable concentration range for most analytes is 0.1–10 µg/L.

Description of Method: A 1-L sample is fortified with surrogates and passed through an SPE apparatus. Phosphamidon and surrogates are eluted from the solid phase with a small amount of two or more organic solvents. The solvent extract is dried by passing it through a column of anhydrous sodium sulfate, concentrated by nitrogen gas blow-down, and adjusted to a 1-mL volume with ethyl acetate after adding internal standards. A splitless injection is made into a GC equipped with a high-resolution fused silica capillary column interfaced to an MS. Analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical GC-MS conditions. The GC-MS can be operated in the full scan, SIM, or selected ion storage (SIS) mode. Analyte concentrations are calculated using their integrated peak area and the internal standard technique.

Special Considerations: Phosphamidon was observed to exhibit matrix induced chromatographic response enhancement during method development, determined by comparing the peak area response of a standard prepared in solvent compared to a matrix-matched standard, both at a concentration of 0.2 ng/µL. The method includes use of matrix-matched standards as an option to evaluated matrix interferences. If the peak area of a matrix-matched standard is ≥ 130% of the of the peak area produced by the solvent-prepared standard, matrix enhancement is likely to be present.

Source: Munch, J.W., Grimmett, P.E., Munch, D.J., Wendelken, S.C., Domino, M.M., Zaffiro, A.D.

and Zimmerman, M.L. 2012. "Method 525.3 Determination of Semivolatile Organic Chemicals in Drinking Water by Solid Phase Extraction and Capillary Column Gas Chromatography/ Mass Spectrometry (GC/MS)," Version 1.0. Cincinnati, OH: U.S. EPA. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=241188

5.2.10 EPA Method 531.2: Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC With Postcolumn Derivatization

Analyte(s)	CAS RN
Aldicarb (Temik)	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Carbofuran (Furadan)	1563-66-2
Methomyl	16752-77-5
Oxamyl	23135-22-0

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Direct injection
Determinative Technique: High performance liquid chromatography (HPLC)-fluorescence (FL)

Method Developed for: N-methylcarbamoyloximes and N-methylcarbamates in finished drinking water
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Detection limits range from 0.026 to 0.115 µg/L. The concentration range for target analytes in this method was evaluated between 0.2 µg/L and 10 µg/L.

Description of Method: An aliquot of sample is measured in a volumetric flask. Samples are preserved, spiked with appropriate surrogates and then filtered. Analytes are chromatographically separated by injecting a sample aliquot (up to 1000 µL) into a HPLC system equipped with a reverse phase (C_{18}) column. After elution from the column, the analytes are hydrolyzed in a post column reaction to form methylamine, which is in turn reacted to form a fluorescent isoindole that is detected by an FL detector. Analytes also are quantitated using the external standard technique.

Source: Bassett, S.C., Wendelken, S.C., Pepich, B.V., Munch, D.J. and Henry, L. 2001. "Method 531.2: Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC With Postcolumn Derivatization," Revision 1.0. Cincinnati, OH: U.S. EPA. EPA/815/B-01/002. https://www.epa.gov/sites/production/files/2015-06/documents/epa-531.2.pdf

5.2.11 EPA Method 538: Determination of Selected Organic Contaminants in Drinking Water by Direct Aqueous Injection-Liquid Chromatography/Tandem Mass Spectrometry (DAI-LC/MS/MS)

Analyte(s)	CAS RN
Acephate	30560-19-1
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Methamidophos	10265-92-6
Thiofanox	39196-18-4

Analysis Purpose: Sample preparation, and analyte determination and measurement

Sample Preparation Technique: Direct injection
Determinative Technique: Liquid Chromatography Tandem Mass Spectrometry (LC-MS-MS)

Method Developed for: Acephate, DIMP, methamidophos and thiofanox in drinking water samples
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDLs for acephate, DIMP, methamidophos and thiofanox in reagent water were calculated to be 0.019, 0.014, 0.017 and 0.090 µg/L, respectively. The Lowest Concentration Minimum Reporting Levels (LCMRLs) in reagent water were calculated to be 0.044, 0.022, 0.032 and 0.18 µg/L, respectively.

Description of Method: A 40-mL water sample is collected in a bottle containing sodium omadine and ammonium acetate. An aliquot of the sample is placed in an autosampler vial and internal standards are added. A 50-µL or larger injection is made into a liquid chromatograph (LC) equipped with a C_{18} column that is interfaced to an MS-MS operated in the electrospray ionization (ESI) mode. The analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined by internal standard calibration using procedural standards.

Source: Shoemaker, J.A. 2009. "Method 538: Determination of Selected Organic Contaminants in Drinking Water by Direct Aqueous Injection-Liquid Chromatography/Tandem Mass Spectrometry (DAI-LC/MS/MS)," Revision 1.0. Cincinnati, OH: U.S. EPA. EPA/600/R-09/149.
https://www.epa.gov/sites/production/files/2015-06/documents/epa-538.pdf

5.2.12 EPA Method 540: Determination of Selected Organic Chemicals in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
Chlorpyrifos oxon	5598-15-2
Phorate sulfone	2588-04-7
Phorate sulfone oxon	2588-06-9
Phorate sulfoxide	2588-03-6
Phorate sulfoxide oxon	2588-05-8

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: SPE
Determinative Technique: LC-MS-MS

Method Developed for: Chlorpyrifos oxon, phorate sulfone and phorate sulfoxide in drinking water samples
Method Selected for: This method has been selected for preparation and analysis of water samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Depending on the SPE cartridge used, detection limits were calculated to be 0.77 and 1.0 ng/L (chlorpyrifos oxon), 0.32 and 0.57 ng/L (phorate sulfone) and 0.46 and 0.70 ng/L (phorate sulfoxide). The LCMRLs in reagent water were calculated to be 2.0 and 2.7 ng/L (chlorpyrifos oxon), 0.86 and 1.0 ng/L (phorate sulfone) and 0.99 and 1.1 ng/L (phorate sulfoxide).

Description of Method: A 250-mL water sample is preserved with Trizma® (Millipore Sigma, St. Louis, MO, or equivalent), 2-chloroacetamide and ascorbic acid. The sample is fortified with surrogates and

passed through a SPE cartridge. Compounds are eluted from the solid phase with a small amount of methanol, and the extract is concentrated by evaporation with nitrogen in a heated water bath, internal standards are added, and the volume is adjusted to 1 mL with methanol. A 10-µL injection is made into an LC equipped with a C_{18} column that is interfaced to an MS-MS. Analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined using the internal standard technique.

Source: Shoemaker, J.A. and Tettenhorst, D.R. 2013. "Method 540: Determination of Selected Organic Chemicals in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry," Revision 1.0. Cincinnati, OH: U.S. EPA. EPA/600/R-13/119.
https://nepis.epa.gov/Exe/ZyPDF.cgi?Dockey=P100H0Z5.txt

5.2.13 EPA Method 549.2: Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography With Ultraviolet Detection

Analyte(s)	CAS RN
Paraquat	4685-14-7

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: LSE or SPE
Determinative Technique: HPLC-ultraviolet (UV)

Method Developed for: Diquat and paraquat in drinking water sources and finished drinking water
Method Selected for: This method has been selected for preparation and analysis of water samples to address paraquat. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDL for paraquat is 0.68 µg/L. The analytical range depends on the sample matrix and the instrumentation used.

Description of Method: A 250-mL sample is extracted using a C_8 LSE cartridge or a C_8 disk that has been specially prepared for the reversed-phase, ion-pair mode. The LSE disk or cartridge is eluted with acidic aqueous solvent to yield the eluate/extract. An ion-pair reagent is added to the eluate/extract. The concentrations of paraquat in the eluate/extract are measured using a HPLC system equipped with a UV absorbance detector. A photodiode array detector is used to provide simultaneous detection and confirmation of the method analytes.

Source: Munch, J.W. and Bashe, W.J. 1997. "Method 549.2: Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography With Ultraviolet Detection," Revision 1.0. Cincinnati, OH: U.S. EPA.
https://www.epa.gov/sites/production/files/2015-06/documents/epa-549.2.pdf

5.2.14 EPA Method 551.1: Determination of Chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogenated Pesticides/Herbicides in Drinking Water by Liquid-Liquid Extraction and Gas Chromatography With Electron-Capture Detection

Analyte(s)	CAS RN
Chloropicrin	76-06-2

Analysis Purpose: Sample preparation, and analyte determination and measurement

Sample Preparation Technique: Solvent extraction
Determinative Technique: GC-electron capture detector (ECD)

Method Developed for: Chlorination disinfection byproducts, chlorinated solvents and halogenated pesticides/herbicides in finished drinking water, drinking water during intermediate stages of treatment and raw source water
Method Selected for: This method has been selected for preparation and analysis of water samples to address chloropicrin. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The estimated detection limit (EDL) using methyl *tert*-butyl ether (MTBE) and ammonium chloride-preserved reagent water on a 100% dimethylpolysiloxane (DB-1) column has been found to be 0.014 µg/L.

Description of Method: This is a GC-ECD method applicable to the determination of halogenated analytes in finished drinking water, drinking water during intermediate stages of treatment and raw source water. A 50-mL sample aliquot is extracted with 3 mL of MTBE or 5 mL of pentane. Two µL of the extract is then injected into a GC equipped with a fused silica capillary column and linearized ECD for separation and analysis. This liquid/liquid extraction technique efficiently extracts a wide boiling range of non-polar and polar organic components of the sample. Thus, confirmation is quite important, particularly at lower analyte concentrations. A confirmatory column is suggested for this purpose.

Special Considerations: The presence of chloropicrin should be confirmed using either a secondary GC column or an MS.

Source: Munch, D.J. and Hautman, D.P. 1995. "Method 551.1: Determination of Chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogenated Pesticides/Herbicides in Drinking Water by Liquid-Liquid Extraction and Gas Chromatography With Electron-Capture Detection," Revision 1.0. Cincinnati, OH: U.S. EPA. http://www.epa.gov/sites/production/files/2015-06/documents/epa-551.1.pdf

5.2.15 EPA Method 556.1: Determination of Carbonyl Compounds in Drinking Water by Fast Gas Chromatography

Analyte(s)	CAS RN
Formaldehyde	50-00-0

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Liquid-liquid extraction with hexane
Determinative Technique: Fast gas chromatography with electron capture detection (FGC-ECD)

Method Developed for: Formaldehyde in drinking water samples
Method Selected for: This method has been selected for preparation and analysis of drinking water samples to address formaldehyde. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: MDLs for formaldehyde in reagent water were calculated as 0.09 and 0.08 µg/L for primary and secondary columns, respectively. The applicable concentration range is approximately 5–40 µg/L.

Description of Method: A 20-mL volume of water sample is adjusted to pH 4 with potassium hydrogen phthalate (KHP) and the analytes are derivatized at 35 ºC for 2 hours with 15 mg of O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA) reagent. The oxime derivatives are extracted from the water with 4 mL of hexane. The extract is processed through an acidic wash step, and analyzed by FGC-ECD. The target analytes are identified and quantified by comparison to a procedural standard. Two chromatographic peaks will be observed for many of the target analytes. Both (E) and (Z) isomers are formed for carbonyl compounds that are asymmetrical, and that are not sterically hindered. The (E) and

(Z) isomers may not be chromatographically resolved in a few cases. Compounds with two carbonyl groups, such as glyoxal and methyl glyoxal, can produce even more isomers. Chromatographic peaks used for analyte identification are provided in Section 17, Table 1 and Figure 1 of the method.

Special Considerations: All results should be confirmed on a second, dissimilar capillary GC column.

Source: Wendelken, S.C., Pepich, B.V. and Munch, D.J. 1999. "Method 556.1: Determination of Carbonyl Compounds in Drinking Water by Fast Gas Chromatography," Revision 1.0. Cincinnati, OH: U.S. EPA. http://www.epa.gov/sites/production/files/2015-06/documents/epa-556.1.pdf

5.2.16 EPA Method 3015A (SW-846): Microwave Assisted Acid Digestion of Aqueous Samples and Extracts

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Osmium tetroxide (analyze as total osmium)	20816-12-0
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54)

Analysis Purpose: Sample preparation
Sample Preparation Technique: Microwave assisted acid digestion
Determinative Technique: ICP-AES / ICP-MS
Determinative Method: EPA SW-846 Method 6010D (Section 5.2.27) or Method 6020B (Section 5.2.28). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Metals in water, mobility-procedure extracts, and wastes that contain suspended solids
Method Selected for: This method has been selected for preparation of non-drinking water samples to address the analytes listed in the table above as total arsenic, osmium, thallium or vanadium. See Appendix A for corresponding method usability tiers.

Description of Method: This method is used to prepare samples for the determination of arsenic trioxide, arsine, lewisite, lewisite degradation products, calcium and lead arsenate and sodium arsenite as total arsenic; thallium sulfate as total thallium; osmium tetroxide as osmium; and ammonium metavanadate and vanadium pentoxide as total vanadium. A 45-mL aliquot of a well-shaken, homogenized sample is transferred to a fluorocarbon polymer or quartz microwave vessel or vessel liner, equipped with a controlled pressure relief mechanism. 5 mL of concentrated nitric acid or 4 mL of

concentrated nitric acid plus 1 mL of concentration hydrochloric acid are added to the vessel. The vessel is sealed, placed into the microwave system, and heated. The temperature of each sample should rise to 170 ± 5°C in approximately 10 minutes and remain at that temperature for 10 minutes, or for the remainder of the 20-minute digestion period. After cooling, the vessel contents are filtered, centrifuged, or allowed to settle and then diluted to volume. Samples are analyzed for total arsenic, total thallium, or total vanadium by SW-846 Method 6010D (Section 5.2.27) or 6020B (Section 5.2.28); use Method 6010D for total osmium.

Special Considerations: Digestion of samples that contain organics may create high pressures due to the evolution of gaseous digestion products. This may cause venting of the vessels with potential loss of sample components and/or analytes. In these cases, a smaller sample size should be used, but the volume prior to addition of acid(s) should be adjusted to 45 mL with deionized water. Highly reactive samples may also require pre-digestion in a hood to minimize the danger of thermal runaway and excessively vigorous reactions. Concerns have been raised regarding the use of nitric acid when analyzing samples for osmium tetroxide; hydrochloric acid should be considered and evaluated as a possible alternative. However, the addition of hydrochloric acid can limit the quantitation techniques when using some ICP-MS instruments. If laboratories are approved for storing and handling the appropriate standards, lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (Section 5.2.54).

Source: U.S. EPA. 2007. "Method 3015A (SW-846): Microwave Assisted Acid Digestion of Aqueous Samples and Extracts," Revision 1. Washington, DC: U.S. EPA.
https://www.epa.gov/sites/production/files/2015-12/documents/3015a.pdf

5.2.17 EPA Method 3050B (SW-846): Acid Digestion of Sediments, Sludges, and Soils

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54)

Analysis Purpose: Sample preparation
Sample Preparation Technique: Acid digestion
Determinative Technique: ICP-AES / ICP-MS
Determinative Method: EPA SW-846 Method 6010D (Section 5.2.27) or Method 6020B (Section 5.2.28). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Metals in sediments, sludges, and soil samples
Method Selected for: This method has been selected for preparation of solid samples to address the analytes listed in the table above as total arsenic, thallium or vanadium. See Appendix A for corresponding method usability tiers.

Description of Method: This method is used to prepare samples for the determination of arsenic trioxide, arsine, lewisite, lewisite degradation products, calcium and lead arsenate and sodium arsenite as total arsenic; thallium sulfate as total thallium; and ammonium metavanadate and vanadium pentoxide as total vanadium. A 1-g to 2-g sample is digested with nitric acid and hydrogen peroxide. Sample volumes are reduced, then brought up to a final volume of 100 mL. Samples are analyzed for total arsenic, total thallium, total titanium or total vanadium by Method 6010D (Section 5.2.27) or 6020B (Section 5.2.28); use Method 6010D for total osmium.

Special Considerations: If laboratories are approved for storing and handling the appropriate standards, lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (Section 5.2.54).

Source: U.S. EPA. 1996. "Method 3050B (SW-846): Acid Digestion of Sediments, Sludges, and Soils," Revision 2. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf

5.2.18 EPA Method 3051A (SW-846): Microwave Assisted Acid Digestion of Sediments, Sludges, and Oils

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Osmium tetroxide (analyze as total osmium)	20816-12-0
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Titanium tetrachloride (analyze as total titanium)	7550-45-0
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54)

Analysis Purpose: Sample preparation
Sample Preparation Technique: Microwave assisted acid digestion
Determinative Technique: ICP-AES / ICP-MS
Determinative Method: EPA SW-846 Method 6010D (Section 5.2.27) or Method 6020B (Section 5.2.28). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Metals in sediments, sludges, soils, and oils
Method Selected for: This method has been selected for:

- Preparation of solid samples to be analyzed for the total arsenic component of arsenic trioxide arsine, lewisite, lewisite degradation products, calcium and lead arsenate and sodium arsenite
- Preparation of solid samples to be analyzed for the total thallium component of thallium sulfate
- Preparation of solid samples to be analyzed for the total vanadium component of ammonium metavanadate and vanadium pentoxide
- Preparation of solid and wipe samples to be analyzed for the total osmium component of osmium tetroxide
- Preparation of solid and wipe samples to be analyzed for the total titanium component of titanium tetrachloride

NIOSH Method 9102 (see Section 5.2.75) should be used for the preparation of wipes to be analyzed for all other analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Description of Method: A well-mixed sample (no more than 0.500 g for soils, sediments and sludges, and no more than 0.250 g for oil or oil contaminated soil) to the nearest 0.001 g is weighed into a fluorocarbon polymer or quartz microwave vessel or vessel liner equipped with a controlled pressure relief mechanism. 10 mL of concentrated nitric acid or 9 mL of concentrated nitric acid plus 3 mL of concentration hydrochloric acid are added to the vessel, and the vessel is sealed, placed into the microwave system, and heated. After cooling, the vessel contents are filtered, centrifuged, or allowed to settle and then diluted to volume. Samples are analyzed for total arsenic, total thallium, total titanium or total vanadium by Method 6010D or 6020B (SW-846); use Method 6010D (SW-846) for total osmium.

Special Considerations: Digestion of samples that contain organics or carbonates can create high pressures due to the evolution of gaseous digestion products. This can cause venting of the vessels with potential loss of sample components and/or analytes. Samples that are highly reactive or contaminated might require dilution or pre-digestion in a hood to minimize the danger of thermal runaway and excessively vigorous reactions. Concerns have been raised regarding the use of nitric acid when analyzing samples for osmium tetroxide; hydrochloric acid should be considered and evaluated as a possible alternative. However, the addition of hydrochloric acid can limit the quantitation techniques when using some ICP-MS instruments. If laboratories are approved for storing and handling the appropriate standards, lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Source: U.S. EPA. 2007. "Method 3051A (SW-846): Microwave Assisted Acid Digestion of Sediments, Sludges, and Oils," Revision 1. Washington, DC: U.S. EPA.
https://www.epa.gov/sites/production/files/2015-12/documents/3051a.pdf

5.2.19 EPA Method 3511 (SW-846): Organic Compounds in Water by Microextraction

Analyte(s)	CAS RN
Tetraethyl pyrophosphate (TEPP)	107-49-3

Analysis Purpose: Sample preparation
Sample Preparation Technique: Microextraction
Determinative Technique: GC-MS
Determinative Method: EPA SW-846 Method 8270E (Section 5.2.35)

Method Developed for: Organic compounds in aqueous samples
Method Selected for: This method has been selected for preparation of water samples to address TEPP. See Appendix A for corresponding method usability tiers.

Description of Method: A measured volume of water sample, usually 35 mL, is placed into a 40-mL volatile organic analysis (VOA) vial. Surrogates (10 µg of each), 2 mL of methylene chloride, and 12 g of sodium chloride are added to the vial, and the vial is capped and shaken vigorously for 5 minutes or until the sodium chloride is completely dissolved. After the contents are allowed to settle (centrifuging if necessary), 1.5 mL of the lower (methylene chloride) layer is transferred to a 2-mL vial. The extract is then dried with sodium sulfate and a 1-mL aliquot is transferred to a GC vial. Samples are analyzed for TEPP by SW-846 Method 8270E (Section 5.2.35).

Source: U.S. EPA. 2014. "Method 3511 (SW-846): Organic Compounds in Water by Microextraction," Revision 1. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-12/documents/3511.pdf

5.2.20 EPA Method 3520C (SW-846): Continuous Liquid-Liquid Extraction

Analyte(s)	CAS RN
Carfentanil	59708-52-0
Chlorfenvinphos	470-90-6
Diesel range organics	NA
Fentanyl	437-38-7
Kerosene	64742-81-0
3-Methyl fentanyl	42045-87-4
Paraoxon	311-45-5
Parathion	56-38-2
Phosphamidon	13171-21-6

Analysis Purpose: Sample preparation
Sample Preparation Technique: Continuous liquid-liquid extraction (CLLE)
Determinative Technique: GC-flame ionization detector (FID) / GC-MS / LC-MS-MS
Determinative Method: EPA SW-846 Method 8015D (Section 5.2.33), Method 8270E (Section 5.2.35) , adapted from J. Chromatogr. B, 962: 52-58 (Section 5.2.115), or adapted from J. Chromatogr. A, 1218: 1620-1649 (Section 5.2.111). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Organic compounds in aqueous samples
Method Selected for: This method has been selected for preparation of water samples to address the analytes listed in the table above. ***Note***: Drinking water samples to be analyzed for phosphamidon should be prepared and analyzed using EPA Method 525.3 (Section 5.2.9). See Appendix A for corresponding method usability tiers.

Description of Method: This method is applicable to the isolation and concentration of water-insoluble and slightly soluble organics in preparation for a variety of chromatographic procedures. A measured volume of sample, usually 1 L, is placed into a continuous liquid-liquid extractor, adjusted, if necessary, to a specific pH and extracted with organic solvent for 18 to 24 hours. The extract is filtered through sodium sulfate to remove residual moisture, concentrated, and exchanged as necessary into a solvent compatible with the cleanup or determinative procedure used for analysis.

Special Considerations: Some of the target compounds will hydrolyze in water, with hydrolysis rates dependent on various factors such as sample pH and temperature.

Source: U.S. EPA. 1996. "Method 3520C (SW-846): Continuous Liquid-Liquid Extraction." Revision 3. http://www.epa.gov/sites/production/files/2015-06/documents/epa-3520c.pdf

5.2.21 EPA Method 3535A (SW-846): Solid-Phase Extraction

Analyte(s)	CAS RN
4-Aminopyridine	504-24-5
Carfentanil	59708-52-0
Chlorfenvinphos	470-90-6
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diesel range organics	NA
Ethyldichloroarsine (ED)	598-14-1
Fentanyl	437-38-7
Hexamethylenetriperoxidediamine (HMTD)	283-66-9
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4
Kerosene	64742-81-0
3-Methyl fentanyl	42045-87-4
Methyl paraoxon	950-35-6
Methyl parathion	298-00-0
Mevinphos	7786-34-7
Monocrotophos	6923-22-4
Nicotine compounds	54-11-5
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0
Paraoxon	311-45-5
Parathion	56-38-2
Pentaerythritol tetranitrate (PETN)	78-11-5
Phorate	298-02-2
Phosphamidon	13171-21-6
Strychnine	57-24-9
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7

Analysis Purpose: Sample preparation
Sample Preparation Technique: SPE
Determinative Technique: GC-FID / GC-MS / LC-MS-MS / HPLC
Determinative Method: EPA SW-846 Method 8015D (Section 5.2.33), Method 8270E (Section 5.2.35), Method 8330B (Section 5.2.40), adapted from Analyst, 126:1689-1693 (Section 5.2.102), adapted from J. Chromatogr. B, 962: 52-58 (Section 5.2.115), or adapted from J. Chromatogr. A, 1218: 1620-1649 (Section 5.2.111). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Organic compounds in ground water, wastewater and Toxicity Characteristic Leaching Procedure (TCLP, Method 1311) leachates
Method Selected for: This method has been selected for preparation of water samples to address the analytes listed in the table above. ***Note***:

- EPA Method 525.2 (Section 5.2.8) has been selected for preparation and analysis of drinking water samples to address dichlorvos and mevinphos.
- EPA Method 525.3 (Section 5.2.9) has been selected for preparation and analysis of drinking water samples to address phosphamidon.

All other drinking water samples and all non-drinking water samples should be prepared using this method (SW-846 Method 3535A). See Appendix A for corresponding method usability tiers.

Description of Method: This method describes a procedure for isolating target organic analytes from

samples using SPE media. Sample preparation procedures vary by analyte group. Following pH adjustment, a measured volume of sample is extracted by passing it through the SPE medium (disks or cartridges), which is held in an extraction device designed for vacuum filtration of the sample. Target analytes are eluted from the solid-phase media using an appropriate solvent which is collected in a receiving vessel. The resulting solvent extract is dried using sodium sulfate and concentrated, as needed.

Special Considerations: Some of the target compounds will hydrolyze in water, with hydrolysis rates dependent on various factors such as sample pH and temperature.

Source: U.S. EPA. 2007. "Method 3535A (SW-846): Solid-Phase Extraction (SPE)," Revision 1. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-12/documents/3535a.pdf

5.2.22 EPA Method 3541 (SW-846): Automated Soxhlet Extraction

Analyte(s)	CAS RN
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
BZ [Quinuclidinyl benzilate]	6581-06-2
Carfentanil	59708-52-0
Diesel range organics	NA
Diphacinone	82-66-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4
Ethyldichloroarsine (ED)	598-14-1
N-Ethyldiethanolamine (EDEA)	139-87-7
Fentanyl	437-38-7
Kerosene	64742-81-0
3-Methyl fentanyl	42045-87-4
Methyl hydrazine	60-34-4
N-Methyldiethanolamine (MDEA)	105-59-9
Monocrotophos	6923-22-4
Thiofanox	39196-18-4
Triethanolamine (TEA)	102-71-6
Trimethyl phosphite	121-45-9

Analysis Purpose: Sample preparation
Sample Preparation Technique: Automated Soxhlet extraction
Determinative Technique: GC-FID / GC-MS / LC-MS-MS
Determinative Method: EPA SW-846 Methods 8015D (Section 5.2.33) and 8270E (Section 5.2.35), EPA/600/R-15/097 (Section 5.2.53), EPA/600/R-11/143 (Section 5.2.50), ASTM Methods D7644 (Section 5.2.91) and D7645 (Section 5.2.92), adapted from J. Chromatogr. 617: 157-162 (Section 5.2.106), adapted from J. Chromatogr. B, 874: 42-50 (Section 5.2.112), adapted from J. Chromatogr. A, 1218: 1620-1649 (Section 5.2.111), or adapted from J. Chromatogr. B, 962: 52-58 (Section 5.2.115). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Organic compounds in soil, sediment, sludges and waste solids
Method Selected for: This method has been selected for preparation of solid samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Description of Method: Approximately 10 g of solid sample is mixed with an equal amount of anhydrous sodium sulfate and placed in an extraction thimble or between two plugs of glass wool. After adding the appropriate surrogate amount, the sample is extracted using an appropriate solvent in an automated Soxhlet extractor. The extract is dried with sodium sulfate to remove residual moisture,

concentrated and exchanged, as necessary, into a solvent compatible with the cleanup or determinative procedure for analysis.

Special Considerations: Some of the target compounds will hydrolyze in water, with hydrolysis rates dependent on various factors such as sample pH and temperature.

Source: U.S. EPA. 1994. "Method 3541 (SW-846): Automated Soxhlet Extraction," Revision 0. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-06/documents/epa-3541.pdf

5.2.23 EPA Method 3545A (SW-846): Pressurized Fluid Extraction (PFE)

Analyte(s)	CAS RN
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
BZ [Quinuclidinyl benzilate]	6581-06-2
Carfentanil	59708-52-0
Diesel range organics	NA
Diphacinone	82-66-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4
Ethyldichloroarsine (ED)	598-14-1
N-Ethyldiethanolamine (EDEA)	139-87-7
Fentanyl	437-38-7
Kerosene	64742-81-0
3-Methyl fentanyl	42045-87-4
Methyl hydrazine	60-34-4
N-Methyldiethanolamine (MDEA)	105-59-9
Monocrotophos	6923-22-4
Thiofanox	39196-18-4
Triethanolamine (TEA)	102-71-6
Trimethyl phosphite	121-45-9

Analysis Purpose: Sample preparation
Sample Preparation Technique: Pressurized fluid extraction (PFE)
Determinative Technique: GC-FID / GC-MS / LC-MS-MS / HPLC-UV
Determinative Method: EPA SW-846 Methods 8015D (Section 5.2.33) and 8270E (Section 5.2.35), EPA/600/R-15/097 (Section 5.2.53), EPA/600/R-11/143 (Section 5.2.50), ASTM Method D7644 (Section 5.2.91) and D7645 (Section 5.2.92), adapted from J. Chromatogr. 617: 157-162 (Section 5.2.106), adapted from J. Chromatogr. B, 874: 42-50 (Section 5.2.112), adapted from J. Chromatogr. A, 1218: 1620-1649 (Section 5.2.111), or adapted from J. Chromatogr. B, 962: 52-58 (Section 5.2.115). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Organic compounds in soils, clays, sediments, sludges and waste solids
Method Selected for: This method has been selected for preparation of solid samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: This method has been validated for solid matrices containing 250 to 12,500 µg/kg of semivolatile organic compounds, 250 to 2500 µg/kg of organophosphorus pesticides, 5 to 250 µg/kg of organochlorine pesticides, and 50 to 5000 µg/kg of chlorinated herbicides.

Description of Method: Approximately 10 to 30 g of soil sample is prepared for extraction either by air drying the sample, or by mixing the sample with anhydrous sodium sulfate or pelletized diatomaceous earth. The sample is then ground and loaded into the extraction cell. The extraction cell containing the sample is heated to the extraction temperature, pressurized with the appropriate solvent system, and

extracted for 5 minutes (or as recommended by the instrument manufacturer). The extract may be concentrated, if necessary, and exchanged into a solvent compatible with the cleanup or determinative step being employed.

Special Considerations: Sodium sulfate can cause clogging, and air-drying or pelletized diatomaceous earth may be preferred for drying samples. Some of the target compounds will hydrolyze in water, with hydrolysis rates dependent on various factors such as sample pH and temperature.

Source: U.S. EPA. 2007. "Method 3545A (SW-846): Pressurized Fluid Extraction (PFE)," Revision 1. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-12/documents/3545a.pdf

5.2.24 EPA Method 3570 (SW-846): Microscale Solvent Extraction (MSE)

Analyte(s)	CAS RN
Acrylamide	79-06-1
Acrylonitrile	107-13-1
Aldicarb (Temik)	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
4-Aminopyridine	504-24-5
BZ [Quinuclidinyl benzilate]	6581-06-2
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
Carbofuran (Furadan)	1563-66-2
Carfentanil	59708-52-0
Diesel range organics	NA
Diphacinone	82-66-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4
Fentanyl	437-38-7
Formaldehyde	50-00-0
Gasoline range organics	NA
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4
Hexamethylenetriperoxidediamine (HMTD)	283-66-9
Kerosene	64742-81-0
Methomyl	16752-77-5
Methyl acrylonitrile	126-98-7
3-Methyl fentanyl	42045-87-4
Methyl hydrazine	60-34-4
Monocrotophos	6923-22-4
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0
Oxamyl	23135-22-0
Pentaerythritol tetranitrate (PETN)	78-11-5
Thiofanox	39196-18-4
Trimethyl phosphite	121-45-9
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7
White phosphorus	12185-10-3

Analysis Purpose: Sample preparation
Sample Preparation Technique: Microscale solvent extraction (MSE)
Determinative Technique: GC – nitrogen-phosphorus detector (NPD) / GC-FID / GC-MS / HPLC-UV / LC-MS-MS

Determinative Method: EPA SW-846 Methods 7580 (Section 5.2.32), 8015D (Section 5.2.33), 8260D (Section 5.2.34), 8270E (Section 5.2.35), 8315A (Section 5.2.37), 8316 (Section 5.2.38), 8318A (Section 5.2.39), and 8330B (Section 5.2.40); EPA/600/R-15/097 (Section 5.2.53); ASTM Methods D7644-16 (Section 5.2.91) and D7645-16 (Section 5.2.92); adapted from Analyst, 126:1689-1693 (Section 5.2.102); adapted from J. Chromatogr. 617: 157-162 (Section 5.2.106); adapted from J. Chromatogr. B, 874: 42-50 (Section 5.2.112); adapted from J. Chromatogr. A, 1218: 1620-1649 (Section 5.2.111); or adapted from J. Chromatogr. B, 962: 52-58 (Section 5.2.115). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Extracting volatile, semivolatile and nonvolatile organic compounds from solids such as soils, sludges and wastes
Method Selected for: This method has been selected for preparation of wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Description of Method: Samples are prepared by shake extraction with an organic solvent in sealed extraction tubes. Careful manipulation of the sample, solvent, drying agent and spiking solutions minimizes loss of volatile compounds while maximizing extraction of volatile, semivolatile and nonvolatile compounds. Sample extracts are collected, dried, and concentrated using a modification of the Kuderna-Danish concentration method or other appropriate technique. By increasing the number of theoretical plates and reducing the distillation temperature, extracts are concentrated without loss of volatile constituents. Samples should be prepared one at a time to the point of solvent addition (i.e., do not pre-weigh a number of samples then add the solvent). Samples should be extracted as soon after collection as possible, and exposure to air before sample extraction is minimized as much as possible.

Special Considerations: Method 3570 is not amenable for analysis of samples that have been preserved in the field using methanol.

Source: U.S. EPA. 2002. "Method 3570 (SW-846): Microscale Solvent Extraction (MSE)," Revision 0. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-3570.pdf

5.2.25 EPA Method 5030C (SW-846): Purge-and-Trap for Aqueous Samples

Analyte(s)	CAS RN
Allyl alcohol	107-18-6
Carbon disulfide	75-15-0
2-Chloroethanol	107-07-3
1,2-Dichloroethane	107-06-2
Ethylene oxide	75-21-8
2-Fluoroethanol	371-62-0
Gasoline range organics	NA
Propylene oxide	75-56-9
The following analyte should be prepared by this method (and determined by the corresponding SW-846 Method 8260D) **only** if problems (e.g., insufficient recovery, interferences) occur when using the sample preparation/determinative techniques identified for these analytes in Appendix A.	
1,4-Thioxane	15980-15-1

Analysis Purpose: Sample preparation
Sample Preparation Technique: Purge-and-trap
Determinative Technique: GC-FID / GC-MS
Determinative Method: EPA SW-846 Method 8015D (Section 5.2.33) or Method 8260D (Section 5.2.34). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: VOCs in aqueous and water miscible liquid samples
Method Selected for: This method has been selected for preparation of water samples to address the analytes listed in the table above. **Note**: For carbon disulfide and 1,2-dichloroethane, EPA Method 524.2 (Section 5.2.7) should be used for preparation of drinking water samples. See Appendix A for corresponding method usability tiers.

Description of Method: This method describes a purge-and-trap procedure for the analysis of VOCs in aqueous liquid samples and water miscible liquid samples. An inert gas is bubbled through a portion of the aqueous liquid sample at ambient temperature, and the volatile components are transferred from the aqueous liquid phase to the vapor phase. The vapor is swept through a sorbent column where the volatile components are adsorbed. After purging is completed, the sorbent column is heated and backflushed with inert gas to desorb the components onto a GC column.

Special Considerations: Heated purge may be required for poor-purging analytes.

Source: U.S. EPA. 2003. "Method 5030C (SW-846): Purge-and-Trap for Aqueous Samples," Revision 3. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-5030c.pdf

5.2.26 EPA Method 5035A (SW-846): Closed-System Purge-and-Trap and Extraction for Volatile Organics in Soil and Waste Samples

Analyte(s)	CAS RN
Acrylonitrile	107-13-1
Allyl alcohol	107-18-6
Carbon disulfide	75-15-0
2-Chloroethanol	107-07-3
1,2-Dichloroethane	107-06-2
Ethylene oxide	75-21-8
2-Fluoroethanol	371-62-0
Gasoline range organics	NA
Methyl acrylonitrile	126-98-7
Propylene oxide	75-56-9
The following analyte should be prepared by this method (and determined by the corresponding SW-846 Method 8260D) **only** if problems (e.g., insufficient recovery, interferences) occur when using the sample preparation/determinative techniques identified for these analytes in Appendix A.	
1,4-Thioxane	15980-15-1

Analysis Purpose: Sample preparation
Sample Preparation Technique: Purge-and-trap
Determinative Technique: GC-FID / GC-MS
Determinative Method: EPA SW-846 Method 8015D (Section 5.2.33) or Method 8260D (Section 5.2.34). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: VOCs in solid materials (e.g., soils, sediments and solid waste) and oily wastes
Method Selected for: This method has been selected for preparation of solid samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Description of Method: This method describes a closed-system purge-and-trap process for analysis of VOCs in solid samples containing low levels (0.5 to 200 µg/kg) of VOCs. The method also provides specific procedures for preparation of samples containing high levels (>200 µg/kg) of VOCs. For low-

level VOCs, a 5-g sample is collected into a vial that is placed into an autosampler device. Reagent water, surrogates and internal standards are added automatically, and the vial is heated to 40°C. The volatiles are purged into an appropriate trap using an inert gas combined with sample agitation. When purging is complete, the trap is heated and backflushed with helium to desorb the trapped sample components into a GC for analysis. For high-level VOCs, samples are collected into a vial that contains a water-miscible organic solvent or a portion of sample is removed from the vial and dispersed in a water-miscible solvent. An aliquot of the solvent is added to reagent water, along with surrogates and internal standards, then purged and analyzed using an appropriate determinative method [e.g., SW-846 Methods 8015D (Section 5.2.33) or 8260D (Section 5.2.34)].

Source: U.S. EPA. 2002. "Method 5035A (SW-846): Closed-System Purge-and-Trap and Extraction for Volatile Organics in Soil and Waste Samples," Draft Revision 1. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-5035a.pdf

5.2.27 EPA Method 6010D (SW-846): Inductively Coupled Plasma - Optical Emission Spectrometry

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA) (analyze as total arsenic)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Osmium tetroxide (analyze as total osmium)	20816-12-0
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Titanium tetrachloride (analyze as total titanium)	7550-45-0
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54)

Analysis Purpose: Analyte determination and measurement
Determinative Technique: ICP-AES
Sample Preparation Method: EPA SW-846 Methods 3015A (Section 5.2.16) for non-drinking water samples, EPA SW-846 Methods 3050B (Section 5.2.17) and 3051A (Section 5.2.18) for solid samples, and NIOSH Method 9102 (Section 5.2.75) for wipe samples
Sample Preparation Technique: Acid digestion

Method Developed for: Trace elements in solution
Method Selected for: This method has been selected for analysis of non-drinking water, solid and wipe samples to address the analytes listed in the table above as total arsenic, osmium, thallium, titanium or vanadium. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Detection limits vary with each analyte and the specific instrument used. Instrument manufacturer documentation should be consulted for appropriate wavelengths, estimated instrument detection limits (IDLs) and analytical ranges. The upper end of the analytical range may be

extended by sample dilution.

Description of Method: This method determines arsenic trioxide, lewisite, lewisite degradation products, calcium and lead arsenate and sodium arsenite as total arsenic; osmium tetroxide as osmium; thallium sulfate as thallium; titanium tetrachloride as titanium; and ammonium metavanadate and vanadium pentoxide as total vanadium. Non-drinking water samples (prepared using SW-846 Method 3015A [Section 5.2.16]), soil samples (prepared using SW-846 Method 3050B [Section 5.2.17] or 3051A [Section 5.2.18]) and wipe samples (prepared using NIOSH Method 9102 [Section 5.2.75] or SW-846 Method 3051A [Section 5.2.18]) are analyzed by ICP-AES.

Special Considerations: This method uses hydrofluoric acid, which is highly toxic and penetrates the skin and tissues deeply if not treated immediately. Boric acid and/or other complexing reagents and appropriate treatment agents (e.g., benzalkonium chloride or calcium gluconate) should be administered immediately.[7] Concerns also have been raised regarding the use of nitric acid when analyzing samples for osmium tetroxide; hydrochloric acid should be considered and evaluated as a possible alternative. However, the addition of hydrochloric acid can limit quantitation techniques when samples are analyzed using some ICP-MS instruments. If laboratories are approved for storing and handling the appropriate standards, then lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (Section 5.2.54).

Source: U.S. EPA. 2014. "Method 6010D (SW-846): Inductively Coupled Plasma-Atomic Emission Spectrometry," Revision 4. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-12/documents/6010d.pdf

5.2.28 EPA Method 6020B (SW-846): Inductively Coupled Plasma - Mass Spectrometry

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA) (analyze a total arsenic)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Osmium tetroxide (analyze as total osmium)	20816-12-0
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Titanium tetrachloride (analyze as total titanium)	7550-45-0
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54)

Analysis Purpose: Analyte determination and measurement
Determinative Technique: ICP-MS

[7] Medical management guidelines for hydrofluoric acid exposure are provided on the U.S. Centers for Disease and Prevention (CDC) National Institute for Occupational Safety and Health (NIOSH) Emergency Response Safety and Health Database at: https://www.cdc.gov/niosh/ershdb/emergencyresponsecard_29750030.html

Sample Preparation Method: EPA SW-846 Methods 3015A (Section 5.2.16) for non-drinking water samples, EPA SW-846 Methods 3050B (Section 5.2.17) and 3051A (Section 5.2.18) for solid samples, and NIOSH Method 9102 (Section 5.2.75) for wipe samples
Sample Preparation Technique: Acid digestion

Method Developed for: Elements in water samples and in waste extracts or digests
Method Selected for: This method has been selected for analysis of non-drinking water, solid and wipe samples to address the analytes listed in the table above as total arsenic, osmium, thallium, titanium or vanadium. It has also been selected for analysis of osmium tetroxide in drinking water. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: IDLs, sensitivities and linear ranges vary with sample type, instrumentation and operation conditions. In relatively simple sample types, detection limits will generally be below 0.1 µg/L. Less sensitive elements, such as arsenic, may have detection limits of 1.0 µg/L or higher. The upper end of the analytical range may be extended by sample dilution.
Description of Method: This method will determine arsenic trioxide, lewisite, lewisite degradation products, calcium and lead arsenate and sodium arsenite as total arsenic. The method also will determine osmium tetroxide as total osmium, thallium sulfate as total thallium, titanium tetrachloride as titanium, and ammonium metavanadate and vanadium pentoxide as total vanadium. Water samples (prepared using SW-846 Method 3015A [Section 5.2.16]), soil samples (prepared using SW-846 Method 3050B [Section 5.2.17] or 3051A [Section 5.2.18) and wipe samples (prepared using NIOSH Method 9102 [Section 5.2.75] or SW-846 Method 3051A [Section 5.2.18]) are analyzed by ICP-MS.

Special Considerations: If laboratories are approved for storing and handling the appropriate standards, then lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (see Section 5.2.54).

Source: U.S. EPA. 2014. "Method 6020B (SW-846): Inductively Coupled Plasma-Mass Spectrometry," Revision 2. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-12/documents/6020b.pdf

5.2.29 EPA Method 7470A (SW-846): Mercury in Liquid Wastes (Manual Cold-Vapor Technique)

Analyte(s)	CAS RN
This method has been selected to address the following analytes as total mercury if problems occur when using EPA Method 245.1 for preparation and analysis of non-drinking water samples.	
Mercuric chloride (analyze as total mercury)	7487-94-7
Mercury, Total	7439-97-6
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Acid digestion (solid and non-drinking water samples) and acid digestion by NIOSH Method 9102 (wipe samples)
Determinative Technique: Cold vapor atomic absorption spectrophotometry

Method Developed for: Mercury in mobility-procedure extracts, aqueous wastes and ground waters
Method Selected for: This method has been selected for use if problems occur when using EPA Method 245.1 for preparation and analysis of non-drinking water samples to address the analytes listed in the table above as total mercury. (See Footnote 11 of Appendix A.)
Detection and Quantitation: The detection limit for total mercury is 0.2 µg/L.

Description of Method: A 100-mL aqueous sample is digested with acids, permanganate solution, persulfate solution and heat. The sample is cooled and reduced with hydroxylamine-sodium chloride

solution. Just prior to analysis, the sample is treated with Sn(II), reducing the mercury to Hg(0). The reduced sample is sparged and the mercury vapor is analyzed by cold vapor atomic absorption spectrometry.

Special Considerations: Chloride and copper are potential interferences.

Source: U.S. EPA. 1994. "Method 7470A (SW-846): Mercury in Liquid Waste (Manual Cold-Vapor Technique)," Revision 1. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-7470a.pdf

5.2.30 EPA Method 7471B (SW-846): Mercury in Solid or Semisolid Wastes (Manual Cold-Vapor Technique)

Analyte(s)	CAS RN
This method has been selected to address the following analytes as total mercury if problems occur when using EPA SW-846 Method 7473 for preparation and analysis of solid and wipe samples.	
Mercuric chloride (analyze as total mercury)	7487-94-7
Mercury, Total	7439-97-6
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Acid digestion for solid and non-drinking water samples, and acid digestion by NIOSH Method 9102 (Section 5.2.75) for wipe samples
Determinative Technique: Cold vapor atomic absorption spectrophotometry

Method Developed for: Total mercury in soils, sediments, bottom deposits and sludge-type materials
Method Selected for: This method has been selected for use if problems occur when using EPA SW-846 Method 7473 (Section 5.2.31) during preparation and analysis of solid and wipe samples to address the analytes listed in the table above as total mercury. (See Footnote 11 of Appendix A.)
Detection and Quantitation: Depending on the instrument used, the IDL for mercury is 0.2 µg/L.

Description of Method: A 0.5-g to 0.6-g sample is digested with aqua regia, permanganate solution and heat, then cooled and reduced with hydroxylamine-sodium chloride solution. Just prior to analysis, the sample is treated with Sn(II), reducing mercury to Hg(0). The reduced sample is sparged and the mercury vapor is analyzed by cold vapor atomic absorption spectrophotometry at a wavelength of 253.7 nm.

Special Considerations: Sulfides, chloride and copper are potential interferences.

Source: U.S. EPA. 2007. "Method 7471B (SW-846): Mercury in Solid or Semisolid Waste (Manual Cold-Vapor Technique)," Revision 2. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-12/documents/7471b.pdf

5.2.31 EPA Method 7473 (SW-846): Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry

Analyte(s)	CAS RN
Mercuric chloride (analyze as total mercury)	7487-94-7
Mercury, Total	7439-97-6
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Thermal decomposition (solid samples) and acid digestion by NIOSH

Method 9102 (wipe samples)
Determinative Technique: Visible spectrophotometry

Method Developed for: Total mercury in solids, aqueous samples and digested solutions
Method Selected for: This method has been selected for preparation of solid samples and for the analysis of prepared solid and wipe samples to address the analytes listed in the table above as total mercury. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The IDL for total mercury is 0.01 ng. The typical working range is 0.05–600 ng, depending on the instrument used.

Description of Method: Controlled heating in an oxygenated decomposition furnace is used to liberate mercury from samples. The sample is dried and then thermally and chemically decomposed within the furnace. The decomposition products are carried by flowing oxygen to the catalytic section of the furnace, where oxidation is completed and halogens and nitrogen/sulfur oxides are trapped. The remaining decomposition products are then carried to an amalgamator that selectively traps mercury. After the system is flushed with oxygen to remove any remaining gases or decomposition products, the amalgamator is rapidly heated, releasing mercury vapor. Flowing oxygen carries mercury vapor through absorbance cells positioned in the light path of a single wavelength atomic absorption spectrophotometer. Absorbance (peak height or peak area) is measured at 253.7 nanometers (nm) as a function of mercury concentration.

Special Considerations: If equipment is not available, use Method 7471B (EPA SW-846) for analysis of solid samples.

Source: U.S. EPA. 2007. "Method 7473 (SW-846): Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry," Revision 0. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-07/documents/epa-7473.pdf

5.2.32 EPA Method 7580 (SW-846): White Phosphorus (P_4) by Solvent Extraction and Gas Chromatography

Analyte(s)	CAS RN
White phosphorus	12185-10-3

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Solvent extraction (solid samples and water samples) and MSE / solvent extraction by EPA SW-846 Method 3570/8290A Appendix A (wipe samples)
Determinative Technique: GC-NPD

Method Developed for: White phosphorus in soil, sediment and water
Method Selected for: This method has been selected for preparation and analysis of solid, water and wipe samples to address white phosphorus. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: MDLs for reagent water, well water and pond water were calculated to be 0.008, 0.009, 0.008 µg/L, respectively. MDLs for sand, sandy loam soil, and soil from the Rocky Mountain Arsenal were calculated to be 0.02, 0.43, 0.07 µg/kg, respectively. This procedure provides sensitivity on the order of 0.01 µg/L for water samples and 1 µg/kg for soil samples.

Description of Method: Method 7580 can be used to determine the concentration of white phosphorus in soil, sediment and water samples using solvent extraction and GC. Water samples are extracted by one of two procedures, depending on the sensitivity required. For the more sensitive procedure, a 500-mL water sample is extracted with 50 mL of diethyl ether. The extract is concentrated by back extraction with reagent water, yielding a final extract volume of approximately 1.0 mL. A 1.0 µL aliquot of this extract is

injected into a GC equipped with an NPD. Wet soil or sediment samples are analyzed by extracting a 40 g wet-weight aliquot of the sample with a mixture of 10.0 mL degassed reagent water and 10.0 mL isooctane. The extraction is performed in a glass jar on a platform shaker for 18 hours. A 1.0-µL aliquot of the extract is analyzed by GC-NPD.

Special Considerations: The presence of white phosphorus should be confirmed using either a secondary GC column or an MS.

Source: U.S. EPA. 1996. "Method 7580 (SW-846): White Phosphorus (P_4) by Solvent Extraction and Gas Chromatography," Revision 0. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-7580.pdf

5.2.33 EPA Method 8015D (SW-846): Nonhalogenated Organics Using GC/FID

Analyte(s)	CAS RN
Diesel range organics	NA
Gasoline range organics	NA
Kerosene	64742-81-0

Analysis Purpose: Analyte determination and measurement
Determinative Technique: GC-FID
Sample Preparation Method: EPA SW-846 Methods 3541 (Section 5.2.22)/3545A (Section 5.2.23) or 5035A (Section 5.2.26) for solid samples, 3520C (Section 5.2.20)/3535A (Section 5.2.21) or 5030C (Section 5.2.25) for water samples, and 3570 (Section 5.2.24)/8290A Appendix A (Section 5.2.36) for wipe samples. Refer to Appendix A for which of these preparation methods should be used for a particular analyte/sample type combination.
Sample Preparation Technique: Automated Soxhlet extraction / PFE / purge-and-trap (solid samples), SPE / purge-and-trap (water samples), and MSE / solvent extraction (wipe samples).

Method Developed for: Nonhalogenated VOCs and semivolatile organic compounds in water and soil samples
Method Selected for: This method has been selected for analysis of solid, water and wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The method reports that estimated MDLs vary with each analyte and range from 2 to 48 µg/L for aqueous samples. MDLs in other matrices have not been evaluated. The analytical range depends on the target analyte(s) and the instrument used.

Description of Method: This method provides GC conditions for the detection of certain nonhalogenated volatile and semivolatile organic compounds. Depending on the analytes of interest, samples may be introduced into the GC by a variety of techniques including purge-and-trap, direct injection of aqueous samples, and solvent extraction. An appropriate GC column and temperature program is used to separate the organic compounds, and the compounds are detected and measured by an FID.

Special Considerations: The presence of the analytes listed in the table above should be confirmed using either a secondary GC column or an MS.

Source: U.S. EPA. 2003. "Method 8015D (SW-846): Nonhalogenated Organics Using GC/FID," Revision 4. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-12/documents/8015d_r4.pdf

5.2.34 EPA Method 8260D (SW-846): Volatile Organic Compounds by Gas Chromatography-Mass Spectrometry (GC/MS)

Analyte(s)	CAS RN
Acrylonitrile	107-13-1
Allyl alcohol	107-18-6
Carbon disulfide	75-15-0
2-Chloroethanol	107-07-3
1,2-Dichloroethane	107-06-2
Ethylene oxide	75-21-8
2-Fluoroethanol	371-62-0
Methyl acrylonitrile	126-98-7
Propylene oxide	75-56-9
The following analytes should be determined by this method (and corresponding sample preparation methods) **only** if problems (e.g., insufficient recovery, interferences) occur when using the sample preparation/determinative techniques identified for these analytes in Appendix A.	
1,4-Thioxane	15980-15-1

Analysis Purpose: Analyte determination and measurement
Determinative Technique: GC-MS
Sample Preparation Method: EPA SW-846 Methods 5035A (Section 5.2.26) for solid samples, 5030C (Section 5.2.25) for water samples, 3570 (Section 5.2.24)/8290A Appendix A (Section 5.2.36) for wipes.
Sample Preparation Technique: Purge-and-trap (solid samples and water samples) and MSE / solvent extraction (wipes).

Method Developed for: Applicable to nearly all types of samples, regardless of water content, including various air sampling trapping media, ground and surface water, aqueous sludges, caustic liquors, acid liquors, waste solvents, oily wastes, mousses (emulsified oil), tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils and sediments.
Method Selected for: This method has been selected for analysis of solid, water and/or wipe samples to address the analytes listed in the table above. ***Note***: EPA Method 524.2 (Section 5.2.7), rather than 8260D (Section 5.2.34) should be used for analysis of acrylonitrile, carbon disulfide, 1,2-dichloroethane and methyl acrylonitrile in drinking water samples. EPA Method 524.2 also should be used for analysis of acrylonitrile and methyl acrylonitrile in non-drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The method reports estimated quantitation limits (EQLs) of 5 µg/kg (wet weight) for soil/sediment samples and 5 µg/L for ground water, when using quadrupole instrumentation and purge-and-trap. The method also reports a lower limit of quantitation (LLOQ) of 0.02 µg/L for 1,2-dichloroethane. EQLs will be proportionately higher for sample extracts and samples that require dilution or when a reduced sample size is used to avoid saturation of the detector. The EQL for an individual analyte is dependent on the instrument as well as the choice of a sample preparation/introduction method.

Description of Method: Volatile compounds are introduced into a GC by purge-and-trap or other procedures (see Section 1.2 of this method). The analytes can be introduced directly to a wide-bore capillary column or cryofocused on a capillary pre-column before being flash evaporated to a narrow-bore capillary for analysis. Alternatively, the effluent from the trap is sent to an injection port operating in the split mode for injection to a narrow-bore capillary column. The column is temperature-programmed to separate the analytes, which are then detected with a MS interfaced to the GC. Analytes eluted from the capillary column are introduced into the MS via a jet separator or a direct connection.

Source: U.S. EPA. 2006. "Method 8260D (SW-846): Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)." Washington, DC: U.S. EPA.
https://www.epa.gov/sites/production/files/2017-04/documents/method_8260d_update_vi_final_03-13-2017.pdf

5.2.35 EPA Method 8270E (SW-846): Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC-MS)

Analyte(s)	CAS RN
Chlorfenvinphos	470-90-6
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Ethyldichloroarsine (ED)	598-14-1
Methyl paraoxon[1]	950-35-6
Methyl parathion	298-00-0
Mevinphos	7786-34-7
Monocrotophos	6923-22-4
Nicotine compounds	54-11-5
Paraoxon	311-45-5
Parathion	56-38-2
Phorate[1]	298-02-2
Phosphamidon	13171-21-6
Strychnine	57-24-9
Tetraethyl pyrophosphate (TEPP)	107-49-3
Trimethyl phosphite[2]	121-45-9

[1] If problems occur during measurement of oxon compounds, analysts should consider use of procedures included in Kamal, A. *et al.*, "Oxidation of selected organophosphate pesticides during chlorination of simulated drinking water." Water Research. 2009. 43(2): 522–534. http://www.sciencedirect.com/science/article/pii/S0043135408004995.

[2] If problems occur with analyses, lower the injection temperature.

Analysis Purpose: Analyte determination and measurement
Determinative Technique: GC-MS
Sample Preparation Method: EPA SW-846 Methods 3541/3545A/3570 (solid samples), 3511/3520C/3535A (water samples), and 3570/8290A Appendix A or NIOSH Method 9102 (wipe samples). Refer to Appendix A for which of these preparation methods should be used for a particular analyte/sample type combination.
Sample Preparation Technique: Automated Soxhlet extraction/PFE/MSE (solid samples), continuous liquid-liquid extraction/SPE/MSE (water samples), and MSE/solvent extraction/acid digestion (wipe samples).

Method Developed for: Semivolatile organic compounds in extracts prepared from many types of solid waste matrices, soils, air sampling media and water samples
Method Selected for: This method has been selected for analysis of solid, water and/or wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Note:

- EPA Method 525.2 (Section 5.2.8) should be used to prepare and analyze drinking water samples for dichlorvos, disulfoton, fenamiphos and mevinphos; it also should be used to prepare and analyze non-drinking water samples for disulfoton.
- EPA/600/R-16/114 (Section 5.2.55) should be used to prepare and analyze solid and wipe samples for chlorfenvinphos, dichlorvos, dicrotophos, methyl paraoxon, methyl parathion, mevinphos, nicotine compounds, paraoxon, parathion, phorate, phosphamidon, strychnine and TEPP.

Detection and Quantitation: The method reports LLOQs in water ranging from 10 to 100 µg/L, depending on the analyte. EQLs reported in the method vary with each analyte and range between 10 and 40 µg/L for aqueous samples. Ranges are not provided for these analytes in soil samples. The analytical range depends on the target analyte(s) and the instrument used.

Description of Method: Samples are prepared for analysis by GC-MS using the appropriate sample preparation and, if necessary, sample cleanup procedures. Semivolatile compounds are introduced into the GC-MS by injecting the sample extract into a GC with a narrow-bore fused-silica capillary column. The GC column is temperature-programmed to separate the analytes, which are then detected with a MS connected to the GC. Analytes eluted from the capillary column are introduced into the MS.

Special Considerations: Lower injection temperatures can alleviate problems that can occur with analysis of trimethyl phosphite.

Source: U.S. EPA. 2014. "Method 8270E (SW-846): Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)." Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2017-04/documents/method_8260d_update_vi_final_03-13-2017_0.pdf

5.2.36 EPA Method 8290A, Appendix A (SW-846): Procedure for the Collection, Handling, Analysis, and Reporting of Wipe Tests Performed Within the Laboratory

Analyte(s)	CAS RN
Acrylamide	79-06-1
Acrylonitrile	107-13-1
Aldicarb (Temik)	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
4-Aminopyridine	504-24-5
BZ [Quinuclidinyl benzilate]	6581-06-2
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
Carfentanil	59708-52-0
Carbofuran (Furadan)	1563-66-2
Diesel range organics	NA
Diphacinone	82-66-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4
Fentanyl	437-38-7
Formaldehyde	50-00-0
Gasoline range organics	NA
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4
Hexamethylenetriperoxidediamine (HMTD)	283-66-9
Kerosene	64742-81-0
Methomyl	16752-77-5
Methyl acrylonitrile	126-98-7
3-Methyl fentanyl	42045-87-4
Methyl hydrazine	60-34-4
Monocrotophos	6923-22-4
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0
Oxamyl	23135-22-0
Pentaerythritol tetranitrate (PETN)	78-11-5
Thiofanox	39196-18-4
Trimethyl phosphite	121-45-9
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7
White phosphorus	12185-10-3

Analysis Purpose: Sample preparation

Sample Preparation Technique: Solvent extraction
Determinative Technique: GC-NPD / GC-FID / GC-MS / HPLC
Determinative Method: EPA SW-846 Methods 7580 (Section 5.2.32), 8015D (Section 5.2.33), 8260D (Section 5.2.34), 8270E (Section 5.2.35), 8315A (Section 5.2.37), 8316 (Section 5.2.38), 8318A (Section 5.2.39), 8330B (Section 5.2.40); NHSRC EPA/600/R-15/097 (Section 5.2.53); ASTM Methods D7644-16 (Section 5.2.91) and D7645-16 (Section 5.2.92); adapted from Analyst, 126:1689-1693 (Section 5.2.102), adapted from J. Chromatogr. 617: 157-162 (Section 5.2.106); adapted from J. Chromatogr. A, 1218: 1620-1649 (Section 5.2.111); adapted from J. Chromatogr. B, 874 (Section 5.2.112): 42-50; or adapted from J. Chromatogr. B, 962: 52-58 (Section 5.2.115). Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Evaluation of surface contamination by 2,3,7,8-substituted polychlorinated dibenzodioxins (PCDD) and polychlorinated dibenzofurans (PCDF) congeners
Method Selected for: This method has been selected for preparation of wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Description of Method: A surface area of 2 inches by 1 foot is wiped with glass fiber paper saturated with distilled-in-glass acetone. One wipe is used per designated area. Wipes are combined into a single composite sample in an extraction jar and solvent extracted using a wrist action shaker.

Special Considerations: The solvent systems described in this method extraction have been evaluated for PCDD and PCDF congeners only. Other analytes may require different solvent systems for optimal sample extraction.

Source: U.S. EPA. 2007. "Method 8290A, Appendix A (SW-846): Procedure for the Collection, Handling, Analysis, and Reporting of Wipe Tests Performed Within the Laboratory," Revision 1. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-8290a.pdf

5.2.37 EPA Method 8315A (SW-846): Determination of Carbonyl Compounds by High Performance Liquid Chromatography (HPLC)

Analyte(s)	CAS RN
Formaldehyde	50-00-0

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Solvent extraction (solid and non-drinking water samples) and MSE / solvent extraction by EPA SW-846 Method 3570/8290A Appendix A (wipe samples)
Determinative Technique: HPLC

Method Developed for: Free carbonyl compounds in aqueous, soil, waste and stack samples
Method Selected for: This method has been selected for preparation and analysis of solid, non-drinking water and wipe samples to address formaldehyde. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDL for formaldehyde varies depending on sample conditions and instrumentation, but is approximately 6.2 µg/L for reagent water.

Description of Method: A measured volume of aqueous sample (approximately 100 mL), or 100 mL of extract from an appropriate amount of solids (approximately 25 g), is buffered to pH 5 for analysis of formaldehyde, and derivatized with 2,4-dinitrophenylhydrazine (2,4-DNPH). Using the appropriate technique, the derivatives are extracted using methylene chloride and the extracts are exchanged with acetonitrile prior to HPLC analysis. HPLC conditions are described permitting the separation and measurement of various carbonyl compounds by absorbance detection at 360 nm.

Source: U.S. EPA. 1996. "Method 8315A (SW-846): Determination of Carbonyl Compounds by High Performance Liquid Chromatography (HPLC)," Revision 1. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-8315a.pdf

5.2.38 EPA Method 8316 (SW-846): Acrylamide, Acrylonitrile and Acrolein by High Performance Liquid Chromatography (HPLC)

Analyte(s)	CAS RN
Acrylamide	79-06-1

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Direct injection (water samples), water extraction (solid), and MSE/solvent extraction by EPA SW-846 Method 3570/8290A Appendix A (wipe samples)
Determinative Technique: HPLC-UV

Method Developed for: Acrylamide, acrylonitrile and acrolein in water samples
Method Selected for: This method has been selected for preparation and analysis of water samples, and for analysis of prepared solid and wipe samples to address acrylamide. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDL for acrylamide is 10 µg/L.

Description of Method: Samples are analyzed by HPLC. A 200-µL aliquot is injected onto a C_{18} reverse-phase column, and compounds in the effluent are detected with a UV detector. Water samples can be injected directly into the HPLC; solid samples must be extracted in water prior to injection.

Special Considerations: For details on method modifications allowing for the use of LC-MS-MS detection, please refer to the points of contact in Section 4.0.

Source: U.S. EPA. 1994. "Method 8316 (SW-846): Acrylamide, Acrylonitrile and Acrolein by High Performance Liquid Chromatography (HPLC)," Revision 0. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-8316.pdf

5.2.39 EPA Method 8318A (SW-846): *N*-Methylcarbamates by High Performance Liquid Chromatography (HPLC)

Analyte(s)	CAS RN
Aldicarb (Temik)	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Carbofuran (Furadan)	1563-66-2
Methomyl	16752-77-5
Oxamyl	23135-22-0

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Solvent extraction (solid samples), and MSE / solvent extraction by EPA SW-846 Method 3570/8290A Appendix A (wipe samples)
Determinative Technique: HPLC-FL

Method Developed for: *N*-methylcarbamates in soil, water and waste matrices
Method Selected for: This method has been selected for preparation and/or analysis of solid and wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method

usability tiers.
Detection and Quantitation: The estimated MDLs vary with each analyte and range from 1.7 to 9.4 µg/L for aqueous samples and 10 to 50 µg/kg for soil samples.

Description of Method: *N*-methylcarbamates are extracted from aqueous samples with methylene chloride, and from soils, oily solid waste and oils with acetonitrile. The extract solvent is exchanged to methanol/ethylene glycol, and the extract is cleaned using a C_{18} cartridge, filtered, and eluted on a C_{18} analytical column. After separation, the target analytes are hydrolyzed and derivatized post-column, then quantified fluorometrically. The sensitivity of the method usually depends on the level of interferences present, rather than on instrument conditions. Waste samples with a high level of extractable fluorescent compounds are expected to yield significantly higher detection limits.

Special Considerations: Techniques for analysis of these compounds in soil have been moving towards the use of LC-MS. Laboratories that are routinely using LC-MS for analysis of these compounds should consult with an appropriate contact in Section 4.0 regarding its use.

Source: U.S. EPA. 2007. "Method 8318A (SW-846): N-Methylcarbamates by High Performance Liquid Chromatography (HPLC)," Revision 1. Washington, DC: U.S. EPA.
https://www.epa.gov/sites/production/files/2015-12/documents/8318a.pdf

5.2.40 EPA Method 8330B (SW-846): Nitroaromatics, Nitramines, and Nitrate Esters by High Performance Liquid Chromatography (HPLC)

Analyte(s)	CAS RN
4-Aminopyridine	504-24-5
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4
Hexamethylenetriperoxidediamine (HMTD)	283-66-9
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0
Pentaerythritol tetranitrate (PETN)	78-11-5
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Solvent extraction or direct injection (solid samples), SPE by EPA SW-846 Method 3535A (water samples), and MSE / solvent extraction by EPA SW-846 Method 3570/8290A Appendix A (wipe samples)
Determinative Technique: HPLC-UV

Method Developed for: Trace analysis of explosives and propellant residues in water, soil or sediment
Method Selected for: This method has been selected for preparation and/or analysis of solid, water and wipe samples to address the analytes listed in the table above. ***Note***: Methods 3535A (Section 5.2.21) and 8330B have been selected for preparation of water samples to address these analytes. For HMTD, procedures adapted from Analyst (2001) 126:1689-1693 (Section 5.2.102) have been selected for sample analysis. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limits, ranges and interferences depend on the target compound

Description of Method: This method is intended for the trace analysis of explosives and propellant residues by HPLC using a dual wavelength UV detector in a water, soil or sediment matrix. All of the compounds listed in this method either are used in the manufacture of explosives and propellants, or are the degradation products of compounds used for that purpose. Samples are prepared for analysis by HPLC-UV detection using the appropriate sample preparation technique (SPE by Method 3535A or

solvent extraction by Method 8330B) and, if necessary, sample cleanup procedures. Direct injection of diluted and filtered water samples can be used for water samples of higher concentration. Soil and sediment samples are extracted using acetonitrile in an ultrasonic bath, filtered and chromatographed.

Source: U.S. EPA. 2006. "Method 8330B (SW-846): Nitroaromatics, Nitramines, and Nitrate Esters by High Performance Liquid Chromatography (HPLC)," Revision 2. Washington, DC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-07/documents/epa-8330b.pdf

5.2.41 EPA ISM02.3 Cyanide: Analytical Methods for Total Cyanide Analysis

Analyte(s)	CAS RN
Cyanide, Total	57-12-5

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Midi- or micro-distillation
Determinative Technique: Visible spectrophotometry

Method Developed for: Total cyanide in water, sediment, sludge and soil
Method Selected for: This method has been selected for preparation and analysis of solid, non-drinking water and wipe samples to address total cyanide. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The method quantitation limits for total cyanide are 10 µg/L for aqueous samples and 0.5 mg/kg for solid samples.

Description of Method: Cyanide is released as hydrocyanic acid from cyanide complexes by means of reflux-distillation, using either a midi- or micro-distillation process, and absorbed in a scrubber containing sodium hydroxide solution. The cyanide ion in the absorbing solution is then determined spectrophotometrically. In the semi-automated spectrophotometric measurement, cyanide is converted to cyanogen chloride without hydrolyzing to cyanate, by reaction with chloramine-T, at a pH less than 8. After the reaction is complete, color is formed on the addition of pyridine-barbituric acid reagent, and absorbance is read between 570 and 580 nm. To obtain colors of comparable intensity, it is essential to have the same salt content in both the sample and the standards.

Special Considerations: Midi-distillation is recommended for soil samples to mitigate low analyte recoveries that can occur when analyzing these sample types. If the appropriate equipment is available, the in-line distillation procedure described in EPA-821-B-01-009 (Section 5.2.58) can be used when preparing and analyzing aqueous samples, to shorten analysis time and reduce matrix interferences.

Source: U.S. EPA. "ISM02.3: Exhibit D – Part D: Analytical Methods for Total Cyanide Analysis." Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2015-10/documents/ism23d.pdf

5.2.42 EPA Method 3135.2I: Cyanide, Total and Amenable in Aqueous and Solid Samples Automated Colorimetric With Manual Digestion

Analyte(s)	CAS RN
Cyanide, Amenable to chlorination	NA

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Acid digestion followed by distillation
Determinative Technique: Visible spectrophotometry

Method Developed for: Cyanide in drinking, ground and surface waters, domestic and industrial wastewaters, sediments and solid waste
Method Selected for: This method has been selected for preparation and analysis of solid, water and wipe samples to address cyanide amenable to chlorination. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The applicable range is 0.003–0.500 mg/L cyanide in the distillate. This range can be expanded either by using less sample for distillation or by diluting the distillate.

Description of Method: This method detects inorganic cyanides that are present as either simple soluble salts or complex radicals. It may be used to determine values for both total cyanide and cyanide amenable to chlorination (also known as available cyanide). Cyanide is released as hydrocyanic acid by refluxing a sample with strong acid. The hydrocyanic acid is distilled and collected in an absorber-scrubber containing sodium hydroxide solution. The cyanide ion in the absorbing solution is then determined by automated colorimetry. For determination of cyanide amenable to chlorination, a portion of the sample is chlorinated using sodium hypochlorite at a pH > 11 to decompose the cyanide. Cyanide levels are then determined in both the chlorinated sample portion of the sample and a portion of the sample that has not been chlorinated using the total cyanide method. Cyanides amenable to chlorination are then calculated by difference between unchlorinated and the chlorinated aliquots of the sample.

Special Considerations: Alternate cyanide analyzer equipment may be used, provided it is used according to the procedures described and the laboratory can demonstrate equivalent performance. If preferred, Standard Method 4500-CN-G (Section 5.2.97) can be used in place of this method for the analysis of cyanide amenable to chlorination in water samples.

Source: Greenlee, A. 2008. "RLAB Method 3135.2I: Cyanide, Total and Amenable in Aqueous and Soil Samples Automated Colorimetric With Manual Digestion." Lenexa, KS: U.S. EPA Region 7 Laboratory. http://www.epa.gov/sites/production/files/2015-07/documents/epa-3135.2i.pdf

5.2.43 EPA IO [Inorganic] Compendium Method IO-3.1: Selection, Preparation, and Extraction of Filter Material

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA) (analyze as total arsenic)	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)	40334-70-1
Lewisite oxide (analyze as total arsenic)	1306-02-1
Osmium tetroxide (analyze as total osmium)	20816-12-0
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

Analysis Purpose: Sample preparation
Sample Preparation Technique: Acid extraction
Determinative Technique: ICP-AES / ICP-MS
Determinative Method: EPA Method IO-3.4 (osmium tetroxide) or Methods IO3.4/IO-3.5 (all other

analytes)

Method Developed for: Particulate metals in air
Method Selected for: This method has been selected for preparation of air samples to address the analytes listed in the table above as total arsenic, osmium, thallium or vanadium. See Appendix A for corresponding method usability tiers.

Description of Method: This method supports determination of arsenic trioxide, lewisite, lewisite degradation products, calcium and lead arsenate, and sodium arsenite as total arsenic. Thallium sulfate is determined as total thallium, ammonium metavanadate and vanadium pentoxide are determined as total vanadium, and osmium tetroxide is determined as total osmium. A subsample (one-ninth of the overall filter) is obtained by cutting a strip from the filter used to collect the sample. The filter strip is extracted using a hydrochloric/nitric acid mix and microwave or hotplate heating. The extract is filtered, worked up to 20 mL, and analyzed using either Method IO-3.4 (Section 5.2.44) or Method IO-3.5 (Section 5.2.45).

Source: Mainey, A. and Winberry, W.T. 1999. "IO Compendium Method IO-3.1: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Selection, Preparation and Extraction of Filter Material." Cincinnati, OH: U.S. EPA. EPA/625/R-96/010a.
http://www.epa.gov/sites/production/files/2015-07/documents/epa-io-3.1.pdf

5.2.44 EPA IO [Inorganic] Compendium Method IO-3.4: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma (ICP) Spectroscopy

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA) (analyze as total arsenic)	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)	40334-70-1
Lewisite oxide (analyze as total arsenic)	1306-02-1
Osmium tetroxide (analyze as total osmium)	20816-12-0
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

Analysis Purpose: Analyte determination and measurement
Determinative Technique: ICP-AES
Sample Preparation Method: EPA Method IO-3.1
Sample Preparation Technique: Acid extraction

Method Developed for: Metals in ambient particulate matter
Method Selected for: This method has been selected for analysis of prepared air samples to address the analytes listed in the table above as total arsenic, osmium, thallium or vanadium. See Appendix A for corresponding method usability tiers.

Description of Method: This method determines arsenic trioxide, lewisite, lewisite degradation products, calcium and lead arsenate, and sodium arsenite as total arsenic. Osmium tetroxide is determined as total osmium, thallium sulfate is determined as total thallium, and ammonium metavanadate and vanadium pentoxide are determined as total vanadium. Ambient air is sampled by high-volume filters using Method IO-2.1 (a sampling method) and the filters are extracted by Method IO-3.1 (Section 5.2.43). Detection limits, ranges and interference corrections are dependent on the analyte and the instrument used.

Special Considerations: Concerns have been raised regarding the use of nitric acid when analyzing samples for osmium tetroxide; hydrochloric acid should be considered and evaluated as a possible alternative.

Sources: Winberry, W.T. 1999. "IO Compendium Method IO-3.4: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma (ICP) Spectroscopy." Cincinnati, OH: U.S. EPA. EPA/625/R-96/010a. http://www.epa.gov/sites/production/files/2015-07/documents/epa-io-3.4.pdf

Winberry, W.T. 1999. "IO Compendium Method IO-2.1: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Sampling of Ambient Air for Total Suspended Particulate Matter (SPM) and PM_{10} Using High Volume (HV) Sampler." Cincinnati, OH: U.S. EPA. EPA/625/R-96/010a. http://www.epa.gov/sites/production/files/2015-07/documents/epa-io-2.1.pdf

5.2.45 EPA IO [Inorganic] Compendium Method IO-3.5: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma/Mass Spectrometry (ICP-MS)

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonic acid (CVAOA) (analyze as total arsenic)	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)	85090-33-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)	40334-70-1
Lewisite oxide (analyze as total arsenic)	1306-02-1
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

Analysis Purpose: Analyte determination and measurement
Determinative Technique: ICP-MS
Sample Preparation Method: EPA Method IO-3.1
Sample Preparation Technique: Acid extraction

Method Developed for: Metals in ambient particulate matter
Method Selected for: This method has been selected for analysis of prepared air samples to address the analytes listed in the table above as total arsenic, thallium or vanadium. See Appendix A for corresponding method usability tiers.

Description of Method: This method determines arsenic trioxide, lewisite, lewisite degradation

products, calcium and lead arsenate, and sodium arsenite as total arsenic. Thallium sulfate is determined as total thallium, and ammonium metavanadate and vanadium pentoxide are determined as total vanadium. Ambient air is sampled by high-volume filters using Method IO-2.1 (a sampling method). The filters are extracted by Method IO-3.1 (see Section 5.2.43). Detection limits, ranges and interference corrections are dependent on the analyte and the instrument used.

Source: Winberry, W.T. 1999. "IO Compendium Method IO-3.5: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma/Mass Spectrometry (ICP/MS)." Cincinnati, OH: U.S. EPA. EPA/625/R-96/010a. http://www.epa.gov/sites/production/files/2015-07/documents/epa-io-3.5.pdf

Winberry, W.T. 1999. "IO Compendium Method IO-2.1: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Sampling of Ambient Air for Total Suspended Particulate Matter (SPM) and PM_{10} Using High Volume (HV) Sampler." Cincinnati, OH: U.S. EPA. EPA/625/R-96/010a. http://www.epa.gov/sites/production/files/2015-07/documents/epa-io-2.1.pdf

5.2.46 EPA IO [Inorganic] Compendium Method IO-5: Sampling and Analysis for Vapor and Particle Phase Mercury in Ambient Air Utilizing Cold Vapor Atomic Fluorescence Spectrometry (CVAFS)

Analyte(s)	CAS RN
Mercury, Total	7439-97-6
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Acid digestion for particulate mercury
Determinative Technique: Cold vapor atomic fluorescence spectrometry (CVAFS)

Method Developed for: Vapor and particle phase mercury in ambient air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above as total mercury. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limits are 30 pg/m^3 for particulate mercury and 45 pg/m^3 for vapor phase mercury. Detection limits, analytical range and interferences are dependent on the instrument used.

Description of Method: Vapor phase mercury is collected using gold-coated glass bead traps at a flow rate of 0.3 L/minute. The traps are directly desorbed onto a second (analytical) trap, and the desorbed mercury is determined by CVAFS. Particulate mercury is sampled on glass-fiber filters at a flow rate of 30 L/minute. The filters are extracted with nitric acid and microwave heating, and the extract is oxidized with bromine chloride, then reduced with stannous chloride and purged from solution onto a gold-coated glass bead trap. This trap is desorbed onto a second trap, the second trap is desorbed, and the mercury is determined by CVAFS.

Special Considerations: There are no known positive interferences at 253.7 nm wavelength. Water vapor will cause a negative interference.

Source: Keele, G. and Barres, J. 1999. "IO Compendium Method IO-5: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Sampling and Analysis for Vapor and Particle Phase Mercury in Ambient Air Utilizing Cold Vapor Atomic Fluorescence Spectrometry (CVAFS)." Cincinnati, OH: U.S. EPA. EPA/625/R-96/010a. http://www.epa.gov/sites/production/files/2015-07/documents/epa-io-5.pdf

5.2.47 EPA Air Method, Toxic Organics - 10A (TO-10 A): Determination of Pesticides and Polychlorinated Biphenyls in Ambient Air Using Low Volume Polyurethane Foam (PUF) Sampling Followed by Gas Chromatographic/Multi-Detector Detection (GC/MD)

Analyte(s)	CAS RN
BZ [Quinuclidinyl benzilate][1]	6581-06-2
Chlorfenvinphos	470-90-6
3-Chloro-1,2-propanediol[2]	96-24-2
Chlorpyrifos	2921-88-2
Chlorpyrifos oxon	5598-15-2
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diisopropyl methylphosphonate (DIMP)[2]	1445-75-6
Dimethylphosphite	868-85-9
Dimethylphosphoramidic acid[1]	33876-51-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid][1]	73207-98-4
Ethyl methylphosphonic acid (EMPA)[1]	1832-53-7
Fenamiphos	22224-92-6
Isopropyl methylphosphonic acid (IMPA)[1]	1832-54-8
Methyl paraoxon	950-35-6
Methyl parathion	298-00-0
Methylphosphonic acid (MPA)[1]	993-13-5
Mevinphos	7786-34-7
Monocrotophos	6923-22-4
Paraoxon	311-45-5
Parathion	56-38-2
Phencyclidine	77-10-1
Phorate	298-02-2
Phorate sulfone	2588-04-7
Phorate sulfone oxon	2588-06-9
Phorate sulfoxide	2588-03-6
Phorate sulfoxide oxon	2588-05-8
Phosphamidon	13171-21-6
Pinacolyl methyl phosphonic acid (PMPA)[1]	616-52-4
Tetraethyl pyrophosphate (TEPP)	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Thiodiglycol (TDG)	111-48-8
Trimethyl phosphite	121-45-9
The following analyte should be determined by this method **only** if problems (e.g., insufficient recovery, interferences) occur when using Method TO-15.	
Allyl alcohol	107-18-6

[1] For this analyte, HPLC is the preferred technique; however, if problems occur, Method TO-10A must be modified to include a derivatization step prior to analysis by GC-MS (see references listed under Special Considerations in Section 5.2.31).
[2] If problems occur when using this method, it is recommended that Method TO-15 be used.

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction
Determinative Technique: GC-MS or HPLC-UV

Method Developed for: Pesticides and polychlorinated biphenyls in ambient air
Method Selected for: This method has been selected for preparation and analysis of air samples to

address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The limit of detection (LOD) will depend on the specific compound measured, the concentration level, and the degree of specificity required. This method is applicable to multicomponent atmospheres, 0.001–50 µg/m^3 concentrations, and 4–24-hour sampling periods.

Description of Method: A low-volume sample collection rate (1–5 L/minute) is used to collect vapors on a sorbent cartridge containing polyurethane foam (PUF) in combination with another solid sorbent. Airborne particles also are collected, but the sampling efficiency for particulates is not known. Pesticides and other chemicals are extracted from the sorbent cartridge with 5% diethyl ether in hexane, and determined by GC-MS. For common pesticides, HPLC coupled with a UV detector is preferable. HPLC-UV is also the preferred technique for BZ, dimethylphosphoramidic acid, EA2192, EMPA, IMPA, MPA and PMPA. If problems occur when using HPLC-UV, these analytes can be analyzed by GC-MS but require derivatization with a trimethylsilyl agent prior to injection into the GC.

Special Considerations: Refer to footnotes provided in analyte table above for special considerations that should be applied when measuring specific analytes. Also see Special Considerations for EPA SW-846 8270E (Section 5.2.35) for information regarding derivatization of compounds.

Source: Lewis, R.G. 1999. "Air Method, Toxic Organics-10A (TO-10A): Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air: Determination of Pesticides and Polychlorinated Biphenyls in Ambient Air Using Low Volume Polyurethane Foam (PUF) Sampling Followed by Gas Chromatographic/Multi-Detector Detection (GC/MD)." Cincinnati, OH: U.S. EPA. EPA 625/R-96/010b. https://www.epa.gov/sites/production/files/2015-07/documents/epa-to-10a.pdf

Additional Resource: Karmel, A., Byrne, C., Bigo, C., Ferrario, J., Stafford, C., Verdin, G., Siegelman, F., Knizner, S. and Hetrick, J.. 2009. "Oxidation of Selected Organophosphate Pesticides During Chlorination of Simulated Drinking Water." Water Research. 43(2): 522-534. http://www.sciencedirect.com/science/article/pii/S0043135408004995

5.2.48 EPA Air Method, Toxic Organics - 15 (TO-15): Determination of Volatile Organic Compounds (VOCs) in Air Collected in Specially-Prepared Canisters and Analyzed by Gas Chromatography/Mass Spectrometry (GC/MS)

Analyte(s)	CAS RN
Allyl alcohol	107-18-6
Carbon disulfide	75-15-0
Cyanogen chloride	506-77-4
1,2-Dichloroethane	107-06-2
Ethyldichloroarsine (ED)	598-14-1
Ethylene oxide	75-21-8
The following analytes should be determined by this method **only** if problems (e.g., insufficient recovery, interferences) occur when using Method TO-10A or TO-17.	
3-Chloro-1,2-propanediol	96-24-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Diisopropyl methylphosphonate (DIMP)	1445-75-6
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Sarin (GB)	107-44-8
Soman (GD)	96-64-0

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Samples are collected using canisters.

Determinative Technique: GC-MS

Method Developed for: VOCs in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: This method applies to ambient concentrations of VOCs above 0.5 parts per billion by volume (ppbv) and typically requires VOC enrichment by concentrating up to 1 L of a sample volume; however, when using current technologies, quantifications of approximately 100 parts per trillion by volume (pptv) have been achieved with 0.5-L sample volumes.

Description of Method: The atmosphere is sampled by introduction of air into a specially prepared stainless steel canister (electropolished or silica-coated). A sample of air is drawn through a sampling train comprising components that regulate the rate and duration of sampling into the pre-evacuated and passivated canister. Grab samples also may be collected. After the air sample is collected, the canister valve is closed, an identification tag is attached to the canister, and the canister is transported to the laboratory for analysis. To analyze the sample, a known sample volume is directed from the canister through a solid multisorbent concentrator. Recovery of less volatile compounds may require heating the canister. After the concentration and drying steps are completed, VOCs are thermally desorbed, entrained in a carrier gas stream, and then focused in a small volume by trapping on a cryo-focusing (ultra-low temperature) trap or small volume multisorbent trap. The sample is then released by thermal desorption and analyzed by GC-MS.

Special Considerations: If problems occur when using this method for determination of allyl alcohol, it is recommended that Method TO-10A (Section 5.2.47) be used. In cases where lower detection levels are needed, use procedures included in the supplement to EPA Compendium Method TO-15: Reduction of Method Detection Limits to Meet Vapor Intrusion Monitoring Needs (http://www.epa.gov/ttnamti1/files/ambient/airtox/TO-15-Supplement.pdf).

Source: McClenny, W.A. and Holdren, M.W. 1999. "Air Method, Toxic Organics-15 (TO-15): Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air, Second Edition: Determination of Volatile Organic Compounds (VOCs) in Air Collected in Specially-Prepared Canisters and Analyzed by Gas Chromatography/Mass Spectrometry (GC/MS)." Cincinnati, OH: U.S. EPA. EPA 625/R-96/010b.
http://wipp.energy.gov/library/Information_Repository_A/Supplemental_Information/EPA%201999/TO-15.pdf

5.2.49 EPA Air Method, Toxic Organics – 17 (TO-17): Determination of Volatile Organic Compounds in Ambient Air Using Active Sampling Onto Sorbent Tubes

Analyte(s)	CAS RN
Chlorosarin*	1445-76-7
Chlorosoman*	7040-57-5
Cyclohexyl sarin (GF)	329-99-7
1-Methylethyl ester ethylphosphonofluoridic acid (GE)*	1189-87-3
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl) methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
Mustard sulfur / Mustard gas (HD)	505-60-2
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
Sarin (GB)*	107-44-8
Soman (GD)*	96-64-0
Tabun (GA)	77-81-6

Analyte(s)	CAS RN
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5
VX [O-ethyl-S-(2-diisopropylaminoethyl)methyl-phosphonothiolate]	50782-69-9

*If problems occur when using this method, it is recommended that Method TO-15 be used.

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Thermal desorption
Determinative Technique: GC-MS

Method Developed for: VOCs
Method Selected for: This method has been selected for preparation and analysis of CWAs in air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The LOD will depend on the specific compounds measured, the concentration level, and the degree of specificity required. This method is applicable to multicomponent atmospheres, 2.86 to 275 µg/m^3 concentrations, and 1 to 24-hour sampling periods.

Description of Method: A low-volume (10 to 200 mL/minute) sample collection rate is used to collect vapors on a sorbent tube. Airborne particles also are collected, but the sampling efficiency for particulates is not known. Compounds are then thermally desorbed from the sorbent tube and determined by GC-MS.

Special Considerations: Refer to the footnote provided in analyte table above for special considerations that should be applied when measuring specific analytes. Higher volume sampling flow rates can be used for high boiling materials such as the V-agents.

Source: Woolfenden, E.A. and McClenny, W.A. 1999. "Air Method, Toxic Organics-17 (TO-17): Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air: Determination of Volatile Organic Compounds in Ambient Air Using Active Sampling Onto Sorbent Tubes." Cincinnati, OH: U.S. EPA. EPA/625/R-96/010b.
https://www3.epa.gov/ttnamti1/files/ambient/airtox/to-17r.pdf

5.2.50 EPA/600/R-11/143: Surface Analysis Using Wipes for the Determination of Nitrogen Mustard Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
N-Ethyldiethanolamine (EDEA)	139-87-7
N-Methyldiethanolamine (MDEA)	105-59-9
Triethanolamine (TEA)	102-71-6

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Extracted using sonication, and filtered using a syringe-polyvinylidene fluoride (PVDF) filter unit
Determinative Technique: LC-MS-MS

Method Developed for: TEA, EDEA and MDEA in wipe surfaces
Method Selected for: This method has been selected for preparation and analysis of wipe samples and for the analysis of prepared solid samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers. ***Note***: SW-846 Methods 3541/3545A should be used for preparation of solid samples.

Detection and Quantitation: Detection limits (DL) for EDEA, MDEA and TEA are 0.06, 0.07 and 0.12 ng/cm^2, respectively. The limit of quantitations (LOQs) for EDEA, MDEA and TEA are 0.63, 0.69, and 1.23 ng/cm^2, respectively. The reporting range for all three target compounds is 0.1–5.0 ng/cm^2.

Description of Method: Samples are collected from surfaces with wipes and stored at 0–6°C if not analyzed within 24-hours. Samples are brought to ambient temperature, then spiked with a surrogate compound and solvent. Samples are then sonicated, extracted with a syringe filter unit, concentrated, and analyzed directly by LC-MS-MS in the positive electrospray ionization (ESI+) mode. Each target compound is separated and identified by retention time and by comparing the sample primary multiple reaction monitoring (MRM) transition to the known standard MRM transition from reference spectra under identical LC-MS-MS conditions. The retention time for the analytes in the sample must fall within ± 5% of the retention time of the analytes in standard solution. The concentration of each analyte is determined by the instrumentation software using external calibration.

Special Considerations: A more recent procedure based on this method is provided in the additional resource cited below. This procedure uses a lower calibration curve and modified LC-MS-MS instrument conditions.

Source: U.S. EPA and CDC. 2011. "Surface Analysis Using Wipes for the Determination of Nitrogen Mustard Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)." Cincinnati, OH: U.S. EPA. EPA/600/R-11/143.
https://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc%2F&dirEntryId=238641

Additional Resource: Dynamac Corporation. 2012. "Standard Operating Procedure for the Determination of Ethanolamines," Dynamac SOP L-A-303 Rev. 2. Copies of this analytical protocol may be requested from NHSRC at https://www.epa.gov/homeland-security-research/forms/contact-us-about-homeland-security-research

5.2.51 EPA/600/R-12/653: Verification of Methods for Selected Chemical Warfare Agents (CWAs)

Analyte(s)	CAS RN
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl) methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
Tabun (GA)	77-81-6

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Microscale extraction
Determinative Technique: GC-MS

Method Developed for: HN-1, HN-3, VR and GA in solid, water and/or wipe samples
Method Selected for: This method has been selected for preparation and analysis of solid, water and/or wipe samples to address the analytes listed in the table above.
Detection and Quantitation: IDLs for GA, HN-1, HN-3 and R-33, respectively, are: 0.8, 0.025, 0.025 and 0.025 ng/µL (GC-MS full scan); 0.1, 0.01, 0.01 and 0.01 ng/µL (GC-MS SIM); and 0.1, 0.025, 0.005 and 0.02 ng/µL (GC-MS time of flight [TOF]). IDLs for GC-MS full-scan using the total ion chromatogram (TIC) for GA, HN-1, HN-3, and R 33, respectively, are: 0.4, 0.05, 0.025 and 0.025 ng/µL.

- MDLs for GC-MS full-scan in soils ranged from: 8 to 106 µg/kg for GA, 35 to 81 µg/kg for HN-1, 57 to 243 µg/kg for HN-3, and 81 to 213 µg/kg for R 33.

- MDLs for GC-MS TOF in soils ranged from 0.33 to 0.39 µg/kg for GA, 0.57 to 2.3 µg/kg for HN-1, 1.6 to 12 µg/kg for HN-3, and 15 to 49 µg/kg for R 33.
- MDLs for GA, HN-1, HN-3 and R 33 in reagent water using GC-MS full-scan are 16, 1.8, 20 and 69 µg/L, respectively.
- MDLs for GA, HN-1, HN-3 and R 33 in reagent water using GC-MS TOF are 0.13, 0.084, 0.72 and 22 µg/L, respectively.
- MDLs for GA, HN-1, HN-3 and R 33 in wipes using GC-MS full-scan are 0.11, 0.023, 0.35, and 4.41 ng/cm^2, respectively.

Calibration ranges for GC-MS full scan are 0.025–1.0 ng/µL for GA, HN-1 and HN-3 and 0.8–3.0 ng/µL for R 33. Calibration ranges for GC-MS SIM are 0.01–0.25 ng/µL for GA, HN-1 and HN-3 and 0.1–1.0 ng/µL for R 33. Calibration ranges for GC-MS TOF are 0.01–1 for GA, HN-1 and HN-3 and 0.10–5 for R 33.

Description of Method: Water samples are extracted by adding ~8.8 g of sodium chloride to 35-mL of water sample. Surrogates and 2 mL of methylene chloride are added. The samples are extracted on a shaker table for 2 minutes and the layers are allowed to separate. The methylene chloride layer is collected, dried with anhydrous sodium sulfate, and 1 mL is transferred to an autosampler vial. Solid samples are extracted by mixing 10 g of solid, 2.5 g of anhydrous sodium sulfate, 5–10 glass beads, and 25 mL of a 25/50/25 (v/v/v) mixture of acetone/methylene chloride/ethyl acetate. The solid samples are then sonicated in a water bath for 1 hour. The extract is retained, and dried with an additional 1–2 g of sodium sulfate. The sample is then re-extracted by water bath sonication for an additional hour using 25 mL of 5% TEA/95% ethyl acetate. The second extract is retained and dried with 1–2 g of sodium sulfate. The first and second extracts are separately reduced in volume under nitrogen, and 1 mL of each extract is transferred to a separate autosampler vial. Internal standards are added to both extracts, and the extracts are analyzed by GC-MS.

Special Considerations: During method development studies for the analytical protocol described in the EPA/600/R-16/114 (Section 5.2.55), ethyl acetate and TEA were found to produce chromatographic interferences. In addition, ethyl acetate has a higher boiling point than other solvents (e.g., methylene chloride), resulting in a longer nitrogen blowdown step than if other solvents are used. Alternative solvent systems used for similar compounds (see methods in Sections 5.2.56 and 5.2.57) may result in improved chromatography. The method has been single-laboratory tested in reagent water, sand, soil and wipes. The procedures are specifically for use by laboratories with EPA approval for handling and analysis of samples and standards containing CWAs.

Source: U.S. EPA. 2013. "Verification of Methods for Selected Chemical Warfare Agents (CWAs)." Washington, DC: U.S. EPA. EPA/600/R-12/653.
https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=248575

5.2.52 EPA/600/R-13/224: Surface Analysis of Nerve Agent Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
Diisopropyl methylphosphonate (DIMP) (degradation product of GB)	1445-75-6
Dimethylphosphoramidic acid (degradation product of GA)	33876-51-6
Ethyl methylphosphonic acid (EMPA)	1832-53-7
Isopropyl methylphosphonic acid (IMPA)	1832-54-8
Methylphosphonic acid (MPA)	993-13-5
Pinacolyl methyl phosphonic acid (PMPA)	616-54-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Extracted using sonication and filtered using a syringe- PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: DIMP, EMPA, IMPA, MPA and PMPA in wipe samples
Method Selected for: This method has been selected for analysis of wipe samples to address the analytes listed in the table above.
Detection and Quantitation: The working range of this method is 0.10–3.0 ng/cm^2 for EMPA; 0.25–7.50 ng/cm^2 for IMPA and MPA; and 0.05–1.5 ng/cm^2 for DIMP and MPA. MDLs obtained from wiping a laminate surface for EMPA, IMPA, MPA and PMPA are reported as 0.05, 0.04, 0.07 and 0.02 ng/cm^2, respectively. Method reporting limits for EMPA, IMPA, MPA and PMPA are 0.1, 0.25, 0.25, 0.05 ng/cm^2, respectively.

Description of Method: Wipe samples are spiked with surrogates and 5-mL of LC-MS grade water. The sample solution is then sonicated and extracted with a syringe filter unit, and the extract is analyzed directly by LC-MS-MS operated simultaneously in positive and negative ESI modes. Each target compound is separated chromatographically and identified by retention time and by comparison of the primary MRM transition for the sample to the reference spectra of MRM transition for known standards. The concentration of each analyte is determined using external calibration. Surrogates are used to monitor extraction efficiency.

Special Considerations: This procedure uses cotton gauze wipes, which were determined to provide the highest analyte recoveries with the least interference. Other wipes, such as filter paper or glass fiber filters had comparable recoveries and could be appropriate alternatives, but are not as sturdy. Data described in this procedure refer to ESI- mode because some complications can occur in ESI+ mode. Recoveries of DIMP may be problematic due to the volatility or rapid decomposition. Because wood surfaces resulted in poor recoveries (likely due to surface porosity), the method recommends that it should not be used to identify these analytes on wood surfaces. The method does not include analysis of dimethylphosphoramidic acid, and method modifications (e.g., pH adjustment) may be needed when analyzing samples for this compound.

Source: U.S. EPA and CDC. 2013. "Surface Analysis of Nerve Agent Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)." Cincinnati, OH: U.S. EPA. EPA/600/R-13/224. https://www.hsdl.org/?abstract&did=746488

5.2.53 EPA/600/R-15/097: Adaptation of the Conditions of U.S. EPA Method 538 for the Analysis of a Toxic Degradation Product of Nerve Agent VX (EA2192) in Water by Direct Aqueous Injection- Liquid Chromatography/Tandem Mass Spectrometry

Analyte(s)	CAS RN
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Direct injection (water samples), EPA SW-846 Method 3541/3545A (solid samples) and EPA SW-846 Method 3570/8290A Appendix A (wipes)
Determinative Technique: LC-MS-MS

Method Developed for: EA2192 in water
Method Selected for: This method has been selected for preparation and analysis of water samples, and for analysis of prepared solid samples and wipes to address EA2192.
Detection and Quantitation: The detection limit for EA2192 in deionized water is 0.0130 µg/L. The

minimum reporting level in deionized water is 0.125 µg/L. The suggested calibration range is 0.05–20 µg/L.

Description of Method: A 40-mL water sample is collected in a bottle containing sodium omadine (antimicrobial agent) and ammonium acetate. An aliquot of sample is placed in an autosampler vial with the internal standard added. A 50-µL injection is made into an LC equipped with a C_{18} column interfaced to an MS-MS operated in ESI+ mode. Analytes are separated and identified by comparing the acquired mass spectra and retention times to reference spectra and retention times for calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined by internal standard calibration using procedural standards.

Special Considerations: The method has been tested in deionized water and various drinking waters, including chlorinated and chloraminated surface and ground waters. EA2192 is highly toxic; therefore, the procedures are specifically for use by laboratories with EPA approval for handling and analysis of samples and standards containing CWAs.

Source: U.S. EPA. 2016. "Adaptation of the Conditions of U.S. EPA Method 538 for the Analysis of a Toxic Degradation Product of Nerve Agent VX (EA2192) in Water by Direct Aqueous Injection- Liquid Chromatography/Tandem Mass Spectrometry." Cincinnati, OH: U.S. EPA. EPA/600/R-15/097. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=311259

5.2.54 EPA/600/R-15/258: Extraction and Analysis of Lewisite 1, by its Degradation Products, Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
2-Chlorovinylarsonic acid (CVAOA)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA)*	85090-33-1
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]*	40334-70-1
Lewisite oxide*	1306-02-1

* In cases where standards are not available or increased sample throughput is needed, these compounds also can be addressed by analyzing samples for total arsenic (see Appendix A for appropriate ICP-AES or -MS methods).

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction
Determinative Technique: LC-MS-MS

Method Developed for: Extraction and analysis of lewisite I by its degradation products (CVAA and CVAOA)
Method Selected for: This method has been selected for preparation and analysis of solid, water and wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: This method detects both CVAA and CVAOA as total CVAOA. Detection limits for CVAOA are 0.041 mg/L for water, 0.38 µg/wipe for wipes, 0.073 µg/g for sand, 0.032 µg/g for Nebraska soil, 0.028 µg/g for Virginia soil and 0.055 µg/g for Georgia soil. The suggested calibration range for CVAOA is 0.02–0.2 µg/mL.

Description of Method: Phenyl arsonous acid is added to all samples as a surrogate prior to sample extraction and analysis. Water samples are mixed thoroughly. An acidified (with hydrochloric acid) methanolic solution is added to soil samples, followed by agitation on a shaker table for 30 minutes. Soil samples are then allowed to settle by gravity before an aliquot of the extract (liquid layer) is taken. A

dilute aqueous solution of hydrochloric acid is added to wipe samples followed by agitation on a shaker table for 30 minutes. Prior to analysis by LC-MS-MS, hydrogen peroxide is added to all extracts to completely degrade target analytes to CVAOA and the surrogate to phenyl arsenic acid.

Special Considerations: In cases where standards are not available or increased sample throughput is needed, these compounds also can be addressed by analyzing samples for total arsenic (see Appendix A for appropriate ICP-AES or -MS methods and corresponding method usability tiers).

Source: U.S. EPA. 2015. "Extraction and Analysis of Lewisite 1, by its Degradation Products, Using Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)," Revision 1. Washington, DC: U.S. EPA. EPA/600/R-15/258. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=310272

5.2.55 EPA/600/R-16/114: Analytical Protocol for Measurement of Extractable Semivolatile Organic Compounds Using Gas Chromatography/Mass Spectrometry

Analyte(s)	CAS RN
Chlorfenvinphos	470-90-6
Chloropicrin[1]	76-06-2
Chlorpyrifos	2921-88-2
Chlorpyrifos oxon	5598-15-2
Crimidine	535-89-7
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Dimethylphosphite	868-85-9
Disulfoton	298-04-4
Disulfoton sulfone oxon	2496-91-5
Disulfoton sulfoxide	2497-07-6
Disulfoton sulfoxide oxon	2496-92-6
1,4-Dithiane	505-29-3
Fenamiphos	22224-92-6
Methyl paraoxon	950-35-6
Methyl parathion	298-00-0
Mevinphos	7786-34-7
Nicotine compounds	54-11-5
Paraoxon	311-45-5
Parathion	56-38-2
Phencyclidine	77-10-1
Phorate	298-02-2
Phorate sulfone	2588-04-7
Phorate sulfone oxon	2588-06-9
Phorate sulfoxide	2588-03-6
Phorate sulfoxide oxon	2588-05-8
Phosphamidon	13171-21-6
Strychnine	57-24-9
Tetraethyl pyrophosphate (TEPP)	107-49-3
Tetramethylenedisulfotetramine	80-12-6
1,4-Thioxane[2]	15980-15-1

[1] If problems occur with analyses, lower the injection temperature.
[2] If problems occur when using this method, it is recommended that SW-846 Method 8260D and appropriate corresponding sample preparation procedures (i.e., Method 5035A [Section 5.2.26] for solid samples and Method 5030C [Section 5.2.25] for water samples) be used.

Sample Preparation Technique: Microscale extraction
Determinative Technique: GC-MS

Method Developed for: Semivolatile organic compounds in extracts prepared from solid waste matrices, soils, air sampling media and water samples
Method Selected for: This method has been selected for analysis of solid, water and/or wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Note:

- EPA Method 525.2 (Section 5.2.8) has been selected for preparation and analysis of drinking water samples for chlorpyrifos, dichlorvos, fenamiphos and mevinphos.
- EPA Method 525.2 (Section 5.2.8) also has been selected for preparation and analysis of water samples for disulfoton, disulfoton sulfone oxon, disulfoton sulfoxide and disulfoton sulfoxide oxon.
- EPA Method 525.3 (Section 5.2.9) has been selected for preparation and analysis of drinking water samples for phosphamidon.
- EPA Method 540 (Section 5.2.12) has been selected for preparation and analysis of water samples for chlorpyrifos oxon, phorate sulfone, phorate sulfone oxon, phorate sulfoxide and phorate sulfoxide oxon.
- EPA Method 551.1 (Section 5.2.14) has been selected for preparation and analysis of water samples for chloropicrin.
- SW-846 Method 8270E (Section 5.2.35) has been selected for analysis of prepared water samples for chlorfenvinphos, dicrotophos, methyl paraoxon, methyl parathion, nicotine compounds, paraoxon, parathion, phorate, strychnine and TEPP (see Appendix A for the appropriate sample preparation methods).
- SW-846 Method 8270E (Section 5.2.35) also has been selected for analysis of prepared non-drinking water samples for dichlorvos, mevinphos and phosphamidon (see Appendix A for the appropriate sample preparation methods).

All other analyte/sample type combinations should be prepared and analyzed by this method.

Detection and Quantitation: MDL and Quantitation Limit (QL) ranges, when performing full-scan analysis of aqueous samples, are 0.79–4.0 and 28.6–286 µg/L, respectively; and 1.6–93.1 µg/L and 50–1200 µg/kg, respectively, for soil samples. MDL and QL ranges when performing SIM analysis of aqueous samples are 0.030–1.45 and 0.23–114 µg/L, respectively; and 0.047–15.4 and 0.4–80 µg/kg, respectively, for soil samples. The analytical range depends on the target analyte(s) and the mode of analysis used (i.e., full-scan or SIM).

Description of Method: Prior to analysis, surrogates, sodium chloride and methylene chloride are added to aqueous, soil and wipe samples and prepared by MSE. Extracts are dried by the addition of sodium sulfate, concentrated (if necessary in order to achieve appropriate detection and quantitation) by nitrogen evaporation, and then analyzed by GC-MS in full-scan or SIM mode.

Special Considerations: Laboratory results indicate that improved recovery of alkaline compounds (e.g., strychnine, nicotine compounds, crimidine, and phencyclidine) from water may result when extracting samples under acidic conditions (e.g., pH <2) during the first extraction, followed by back extraction under basic conditions. If problems occur with the analysis of chloropicrin, lower the injection temperature. If problems occur when analyzing for 1,4-thioxane, it is recommended that SW-846 Method 8260D and appropriate corresponding sample preparation procedures (i.e., Method 5035A [Section 5.2.26] for solid samples or Method 5030C [Section 5.2.25] for water samples) be used.

Source: U.S. EPA. 2016. "Analytical Protocol for Measurement of Extractable Semivolatile Organic Compounds Using Gas Chromatography/Mass Spectrometry." Cincinnati, OH: U.S. EPA. EPA/600/R-16/114. https://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=532353

5.2.56 EPA/600/R-16/115: Analytical Protocol for Cyclohexyl Sarin, Sarin, Soman and Sulfur Mustard Using Gas Chromatography/Mass Spectrometry

Analyte(s)	CAS RN
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Cyclohexyl sarin (GF)	329-99-7
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Mustard, sulfur / Mustard gas (HD)	505-60-2
Sarin (GB)	107-44-8
Soman (GD)	96-64-0

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Microscale extraction
Determinative Technique: GC-MS

Method Developed for: Determination of GF, GB, GD and HD in water, soil and wipes
Method Selected for: This method has been selected for preparation and analysis of water, solid and wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The calibration ranges in full scan mode are 11.4–114 µg/L (GB and GF) and 5.7–57 µg/L (GD and HD) for water samples; 20–200 µg/kg (GB and GF) and 10–100 µg/kg (GD and HD) for soil samples; and 0.02–0.2 µg/cm^2 (GB and GF) and 0.01–0.1 µg/cm^2 (GD and HD) for wipes.

Description of Method: The method involves solvent extraction of the sample followed by GC-MS analysis to determine cyclohexyl sarin, sarin, soman and HD in water, soil and wipes. Prior to analysis, samples must be prepared using sample preparation techniques appropriate for each sample type. Aqueous, solid and wipe samples are spiked with surrogates and extracted by microscale extraction, using methylene chloride. Water is removed from extracts with anhydrous sodium sulfate, and the extracts are concentrated (solids and wipe extracts only) by nitrogen evaporation, then analyzed by GC-MS using a mass selective detector in either full scan mode or TOF.

Special Considerations: This method has been tested in multiple laboratories for analysis of cyclohexyl sarin, sarin, soman and HD in reagent water, drinking water, groundwater, sand and wipes. The procedures are specifically for use by laboratories with EPA approval for handling and analysis of samples and standards containing CWAs.

Source: U.S. EPA. 2016. "Analytical Protocol for Cyclohexyl Sarin, Sarin, Soman and Sulfur Mustard Using Gas Chromatography/Mass Spectrometry." Cincinnati, OH: U.S. EPA. EPA/600/R-16/115. https://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=532354

5.2.57 EPA/600/R-16/116: Analytical Protocol for VX Using Gas Chromatography/Mass Spectrometry (GC/MS)

Analyte(s)	CAS RN
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5
VX [O-ethyl-S-(2-diisopropylaminoethyl)methyl-phosphonothiolate]	50782-69-9

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Microscale extraction
Determinative Technique: GC-MS

Method Developed for: Determination of VX in water, soil and wipes
Method Selected for: This method has been selected for preparation and analysis of water, solid and wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The calibration ranges for analysis of VX using full scan mode are 11.4–114 µg/L for water samples; 20–200 µg/kg for soil samples; and 0.02–0.2 µg/cm^2 for wipes.

Description of Method: The method involves micro-scale solvent extraction of samples followed by GC-MS analysis. Prior to analysis, samples must be prepared using sample preparation techniques appropriate for each sample type. Aqueous and wipe samples are spiked with surrogates and extracted using methylene chloride. Solid samples are spiked with surrogates, then extracted first using a Tris buffer solution, followed by extraction with methylene chloride. Water is removed from extracts using anhydrous sodium sulfate, and the extracts are concentrated (solids and wipe extracts only) by nitrogen evaporation, then analyzed by GC-MS using either a mass selective detector in full scan mode or TOF.

Special Considerations: The method has been tested in multiple laboratories for analysis of VX in reagent water, drinking water, groundwater, soil and wipes. Laboratory data indicate some difficulties with analyte recoveries in soil; modifications might be needed for application of the procedures to various soil types. The procedures are specifically for use by laboratories with EPA approval for handling and analysis of samples and standards containing CWAs.

Source: U.S. EPA. 2016. "Analytical Protocol for VX Using Gas Chromatography/Mass Spectrometry (GC/MS)." Cincinnati, OH: U.S. EPA. EPA/600/R-16/116.
https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=337633

5.2.58 EPA-821-B-01-009: Method Kelada-01: Kelada Automated Test Methods for Total Cyanide, Acid Dissociable Cyanide, and Thiocyanate

Analyte(s)	CAS RN
Cyanide, Total	57-12-5

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: On-line UV irradiation followed by flash distillation
Determinative Technique: Visible spectrophotometry

Method Developed for: Total cyanide, acid dissociable cyanide and thiocyanate in water, sediment, sludge and soil
Method Selected for: This method has been selected as an alternative to ISM02.3 for preparation and analysis of non-drinking water samples to address total cyanide.
Detection and Quantitation: The LOD is 0.5 µg/L. The working range is 0–100 µg/L.

Description of Method: The method uses a combined on-line UV irradiation and flash distillation system in place of manual cyanide distillation procedures to determine total cyanide. Strongly-bound cyanide complexes (excluding thiocyanate) are degraded into free cyanide by irradiating the sample in a glass coil. The free cyanide is distilled from the sample matrix and detected using an on-line colorimeter. The concentration of dissociable cyanide complexes is determined by omitting the UV-irradiation step. Thiocyanate can also be determined by using a glass irradiation coil instead of a quartz coil.

Special Considerations: The method was evaluated under EPA's Alternate Test Procedure (ATP) program and can be used in place of ISMO2.3 to prepare and analyze aqueous samples for total cyanide.

Source: U.S. EPA. 2001. "Method Kelada-01: Kelada Automated Test Methods for Total Cyanide, Acid Dissociable Cyanide, and Thiocyanate." Washington, DC: U.S. EPA. EPA-821-B-01-009. http://webapp1.dlib.indiana.edu/virtual_disk_library/index.cgi/5315321/FID2672/kelada.pdf

5.2.59 NIOSH Method 1612: Propylene Oxide

Analyte(s)	CAS RN
Propylene oxide	75-56-9

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Coconut shell charcoal solid sorbent tube
Determinative Technique: GC-FID

Method Developed for: Propylene oxide in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address propylene oxide. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range is 8–295 ppm for air samples of 5 L.

Description of Method: A sample tube containing coconut shell charcoal is used for sample collection with a flow rate of 0.01 to 0.2 L/minute. A 1-mL volume of carbon disulfide is added to the vial and allowed to sit for 30 minutes prior to analysis with occasional agitation. Analysis is performed on a GC-FID.

Special Considerations: No interferences have been found. The presence of propylene oxide should be confirmed using either a secondary GC column or an MS.

Source: NIOSH. 1994. "Method 1612: Propylene Oxide," Issue 2. *NIOSH Manual of Analytical Methods*, 4th Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-1612.pdf

5.2.60 NIOSH Method 2016: Formaldehyde

Analyte(s)	CAS RN
Formaldehyde	50-00-0

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction
Determinative Technique: HPLC-UV

Method Developed for: Formaldehyde in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address formaldehyde. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The detection limit for formaldehyde is 0.07 µg/sample. The working range is 0.015–to 2.5 mg/m^3 (0.012–2.0 ppm) for a 15-L sample.

Description of Method: This method can be used for the determination of formaldehyde using HPLC with a UV detector. Air is sampled onto a cartridge containing silica gel coated with 2,4-DNPH, at a rate of 0.03 to 1.5 L/minute. The cartridge is extracted with 10 mL of acetonitrile and analyzed by HPLC-UV at a wavelength of 360 nm.

Special Considerations: Ozone has been observed to consume the 2,4-DNPH reagent and to degrade the formaldehyde derivative. Ketones and other aldehydes can react with 2,4-DNPH; the derivatives produced, however, are separated chromatographically from the formaldehyde derivative.

Source: NIOSH. 2003. "Method 2016: Formaldehyde," Issue 2. *NIOSH Manual of Analytical Methods, Third Supplement*. DHHS (NIOSH) Publication No. 2003-154. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-2016.pdf

5.2.61 NIOSH Method 2513: Ethylene Chlorohydrin

Analyte(s)	CAS RN
2-Chloroethanol	107-07-3
2-Fluoroethanol	371-62-0

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: GC-FID

Method Developed for: Ethylene chlorohydrin (2-chloroethanol) in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range of the method is 0.5–15 ppm for a 20-L air sample.

Description of Method: Samples are drawn into a tube containing petroleum charcoal at a rate of 0.01 to 0.2 L/minute and transferred into vials containing eluent (carbon disulfide, 2-propanol and *n*-pentadiene as an internal standard). Vials must sit for 30 minutes prior to analysis by GC-FID.

Special Considerations: No interferences have been identified. Humidity may decrease the breakthrough volume during sample collection. The presence of 2-chloroethanol should be confirmed using either a secondary GC column or an MS.

Source: NIOSH. 1994. "Method 2513: Ethylene Chlorohydrin," Issue 2. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-2513.pdf

5.2.62 NIOSH Method 3509: Aminoethanol Compounds II

Analyte(s)	CAS RN
N-Ethyldiethanolamine (EDEA)	139-87-7
N-Methyldiethanolamine (MDEA)	105-59-9
Triethanolamine (TEA)	102-71-6

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Samples are collected with an impinger containing 15 mL of 2 mM hexanesulfonic acid
Determinative Technique: IC with conductivity detection

Method Developed for: Triethanolamine in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Detection and Quantitation: The LOD and LOQ for triethanolamine are 0.067 and 0.2 µg/L, respectively.

Description of Method: Samples are collected into an impinger containing 15 mL of 2 mM hexanesulfonic acid using a sampling pump, at a flow rate 0.5 to 1 L/minute for a total sample size of 5 to 300 L. After sampling, the impinger is filled to the 15-mL mark with distilled water and transferred to a vial for shipment. A portion of the sample is filtered through an in-line membrane filter into an autosampler vial. The autosampler injects 50 µL of sample into an ion chromatograph equipped with an ion-pairing guard, cation separator and cation suppressor. Conductivity is set at 3 µS full scale and the eluent used is 2 mM hexanesulfonic acid.

Special Considerations: If high sample throughput is needed, 2 mM hexanesulfonic acid/0.5% v/v acetonitrile can be used as the eluent to reduce run time.

Source: NIOSH. 1994. "Method 3509: Aminoethanol Compounds II," Issue 2. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH). https://www.cdc.gov/niosh/docs/2003-154/pdfs/3509.pdf

5.2.63 NIOSH Method 3510: Monomethylhydrazine

Analyte(s)	CAS RN
Methyl hydrazine (monomethylhydrazine)	60-34-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Samples are collected into a bubbler containing hydrochloric acid.
Determinative Technique: Visible spectrophotometry

Method Developed for: Monomethylhydrazine in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address methyl hydrazine. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range of the method is 0.027–2.7 ppm for a 20-L sample.

Description of Method: Samples are collected into a bubbler containing hydrochloric acid, using a flow rate of 0.5 to 1.5 L/minute, then transferred to a 25-mL flask, mixed with phosphomolybdic acid solution, diluted with 0.1 M hydrochloric acid, and transferred to a large test tube for spectrophotometric analysis.

Special Considerations: Positive interferences include other hydrazines, as well as stannous and ferrous ion, zinc, sulfur dioxide and hydrogen sulfide. Negative interferences may occur by oxidation of mono-methylhydrazine by halogens, oxygen (especially in the presence of copper (I) ions) and hydrogen dioxide.

Source: NIOSH. 1994. "Method 3510: Monomethylhydrazine," Issue 1. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-3510.pdf

5.2.64 NIOSH Method 5600: Organophosphorus Pesticides

Analyte(s)	CAS RN
Disulfoton	298-04-4
Disulfoton sulfone oxon	2496-91-5
Disulfoton sulfoxide	2497-07-6
Disulfoton sulfoxide oxon	2496-92-6

Analyte(s)	CAS RN
The following analyte should be prepared by this method **only** if problems (e.g., insufficient recovery, interferences) occur when using the sample preparation/determinative techniques identified for these analytes in Appendix A	
Methamidophos	10265-92-6

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: GC-flame photometric detector (FPD)

Method Developed for: Organophosphorus pesticides in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit depends on the compound being measured. The working range for each analyte is provided in Table 5 of the method. These ranges cover from 0.1 to 2 times the OSHA Permissible Exposure Limits (PELs).

Description of Method: This method is used for the detection of organophosphorus pesticides using a GC-FPD. Samples are prepared by desorbing the sampler resin with 2 mL of toluene/acetone (90/10 v/v) solution. The method also may be applicable to the determination of other organophosphorus compounds after evaluation for desorption efficiency, sample capacity, sample stability, and precision and accuracy. The method also is applicable to Short Term Exposure Limit (STEL) measurements using 12-L samples.

Special Considerations: Refer to footnote provided in analyte table above for special considerations that should be applied when measuring specific analytes. Several organophosphates may co-elute with either target analytes or internal standards causing integration errors. These include other pesticides, and the following: tributyl phosphate, tris-(2-butoxy ethyl) phosphate, tricresyl phosphate and triphenyl phosphate. The presence of the analytes listed in the table above should be confirmed using either a secondary GC column or an MS.

Source: NIOSH. 1994. "Method 5600: Organophosphorus Pesticides," Issue 1. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-5600.pdf

5.2.65 NIOSH Method 5601: Organonitrogen Pesticides

Analyte(s)	CAS RN
Aldicarb (Temik)	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Carbofuran (Furadan)	1563-66-2
Methomyl	16752-77-5
Oxamyl	23135-22-0
Thiofanox	39196-18-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: HPLC-UV

Method Developed for: Organonitrogen pesticides in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Detection and Quantitation: The method reports detection limits of 1.2 µg for aldicarb and 0.6 µg for carbofuran, methomyl and oxamyl for collected air sample volumes of 240 L. The working ranges for aldicarb, carbofuran and oxamyl are listed in Table 2 of the method, and range from 0.5 to 10 times the OSHA PEL.

Description of Method: This method can be used for the determination of organonitrogen pesticides using HPLC with a UV detector. Samples are prepared by desorbing the sampler resin with 2 mL of triethylamine-phosphate solution, rotating end-over-end for 45 minutes, and filtering. The method also may be applicable to the determination of other organonitrogen compounds and to a broad range of pesticides having UV chromophores, e.g., acetanilides, acid herbicides, organophosphates, phenols, pyrethroids, sulfonyl ureas, sulfonamides, triazines and uracil pesticides.

Special Considerations: The presence of analytes listed in the table above should be confirmed using either a secondary HPLC column or an MS. Because of the broad response of the UV detector at shorter wavelengths, there are many potential interferences. Those tested include solvents (chloroform and toluene), antioxidants (butylated hydroxytoluene [BHT]), plasticizers (dialkyl phthalates), nitrogen compounds (nicotine, caffeine), HPLC reagent impurities (e.g., in triethylamine), other pesticides (2,4-dichlorophenoxyacetic acid [2,4-D], atrazine, parathion), and pesticide hydrolysis products (1-naphthol).

Source: NIOSH. 1998. "Method 5601: Organonitrogen Pesticides," Issue 1. *NIOSH Manual of Analytical Methods, Second Supplement*. DHHS (NIOSH) Publication No. 98-119. Washington, DC: DHHS (NIOSH). http://www.cdc.gov/niosh/docs/2003-154/pdfs/5601.pdf

5.2.66 NIOSH Method 6001: Arsine

Analyte(s)	CAS RN
Arsine	7784-42-1

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Coconut shell charcoal solid sorbent tube
Determinative Technique: Graphite furnace atomic absorption **(**GFAA)

Method Developed for: Arsine in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address arsine. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range of the method is 0.001–0.2 mg/m^3 for a 10-L sample.

Description of Method: Arsine is determined as arsenic. 0.1 to 10 L of air is drawn through a sorbent tube containing activated charcoal. The sorbent is extracted with a nitric acid solution, and arsenic is determined by GFAA.

Special Considerations: The method is subject to interferences from other arsenic compounds.

Source: NIOSH. 1994. "Method 6001: Arsine," Issue 2. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-6001.pdf

5.2.67 NIOSH Method 6002: Phosphine

Analyte(s)	CAS RN
Phosphine	7803-51-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption with hot acidic potassium permanganate solution
Determinative Technique: Visible spectrophotometry

Method Developed for: Phosphine in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address phosphine. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range of the method is 0.02–0.9 mg/m^3 for a 16-L sample.

Description of Method: **P**hosphine is determined as phosphate. 1 to 16 L of air is drawn through a sorbent tube containing silica gel coated with mercuric cyanide. The sorbent is extracted with a potassium permanganate/sulfuric acid solution and washed with reagent water. Following treatment with the color agent and extraction into organic solvent, phosphate is determined by visible spectrometry.

Special Considerations: The method is subject to interferences from phosphorus trichloride, phosphorus pentachloride and organic phosphorus compounds.

Source: NIOSH. 1998. "Method 6002: Phosphine," Issue 2. *NIOSH Manual of Analytical Methods, Second Supplement*. DHHS (NIOSH) Publication No. 98-119. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-6002.pdf

5.2.68 NIOSH Method 6010: Hydrogen Cyanide

Analyte(s)	CAS RN
Cyanide, Total	57-12-5
Hydrogen cyanide	74-90-8

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: Visible spectrophotometry

Method Developed for: Hydrogen cyanide in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range of the method is 3–260 mg/m^3 for a 3-L sample.

Description of Method: Hydrogen cyanide is determined as a cyanide ion complex by this method. A volume of 0.6 to 90 L of air is drawn through a soda lime sorbent tube. A glass-fiber filter is used to remove particulate cyanides prior to the sorbent tube. Cyanide is extracted from the sorbent with reagent water treated with sodium hydroxide. The extract is pH adjusted with hydrochloric acid, oxidized with N-chlorosuccinimide/succinimide, and treated with the coupling-color agent (barbituric acid/pyridine). The cyanide ion is determined by visible spectrophotometry using a wavelength of 580 nm.

Special Considerations: The method is subject to interference from high concentrations of hydrogen sulfide. Two liters is the minimum volume required to measure concentration of 5 ppm.

Source: NIOSH. 1994. "Method 6010: Hydrogen Cyanide," Issue 2. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-6010.pdf

5.2.69 NIOSH Method 6013: Hydrogen Sulfide

Analyte(s)	CAS RN
Hydrogen sulfide	7783-06-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction
Determinative Technique: IC with conductivity detection

Method Developed for: Hydrogen sulfide in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address hydrogen sulfide. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range of the method is 0.9–20 mg/m^3 for a 20-L sample.

Description of Method: This method determines hydrogen sulfide as sulfate. 15 to 40 L of air is drawn through charcoal sorbent. A prefilter is used to remove particulates. The sorbent portions are extracted with an ammonium hydroxide/hydrogen peroxide solution and the extract is analyzed for sulfate by IC.

Special Considerations: The method is subject to interference from sulfur dioxide.

Source: NIOSH. 1994. "Method 6013: Hydrogen Sulfide," Issue 1. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH).
http://www.epa.gov/sites/production/files/2015-07/documents/niosh-6013.pdf

5.2.70 NIOSH Method 6015: Ammonia

Analyte(s)	CAS RN
Ammonia	7664-41-7

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Water extraction
Determinative Technique: Visible spectrophotometry

Method Developed for: Ammonia in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address ammonia. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range of the method is 0.15–300 mg/m^3 for a 10-L sample.

Description of Method: Ammonia is determined as indophenol blue by this method. A volume of 0.1 to 96 L of air is drawn through a sulfuric acid-treated silica gel sorbent. A prefilter is used to remove particulates. The sorbent is extracted with reagent water, the pH adjusted, and reagents are added to generate the indophenol blue compound in the presence of ammonium. The extract is analyzed by visible spectrophotometry. No interferences have been identified.

Source: NIOSH. 1994. "Method 6015: Ammonia," Issue 2. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH).
http://www.epa.gov/sites/production/files/2015-07/documents/niosh-6015.pdf

5.2.71 NIOSH Method 6402: Phosphorus Trichloride

Analyte(s)	CAS RN
Phosphorus trichloride	7719-12-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Add reagent to samples in bubbler solution and heat
Determinative Technique: Visible spectrophotometry

Method Developed for: Phosphorus trichloride in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address phosphorus trichloride. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The working range of the method is 1.2–80 mg/m^3 for a 25-L sample.

Description of Method: In this method, phosphorus trichloride is determined as phosphate. A volume of 11 to 100 L of air is drawn through a bubbler containing reagent water. The resulting phosphorus acid solution is oxidized with bromine to phosphoric acid and color agent (sodium molybdate) and reducing agent (hydrazine sulfate) are added. The solution is analyzed for the resulting molybdenum blue complex by visible spectrophotometry.

Special Considerations: Phosphorus (III) compounds can interfere with analysis of phosphorus trichloride, by increasing the amount of phosphorus that is measured. Phosphorus (V) compounds do not interfere.

Source: NIOSH. 1994. "Method 6402: Phosphorus Trichloride," Issue 2. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-6402.pdf

5.2.72 NIOSH Method 7905: Phosphorus

Analyte(s)	CAS RN
White phosphorus	12185-10-3

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: GC solid sorbent tube and solvent extracted (desorbed)
Determinative Technique: GC-FPD

Method Developed for: Phosphorus in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address white phosphorus. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The LOD for samples analyzed by GC-FPD is 0.005 µg per sample. The working range for samples analyzed by GC-FPD is 0.056–0.24 mg/m^3 for a 12-L sample.

Description of Method: This method identifies and determines the concentration of white phosphorus in air by using a GC-FPD. Five to 100 L of air is drawn through a GC solid sorbent tube, and the sorbent is extracted (desorbed) with xylene. The method is applicable to vapor-phase phosphorus only; if particulate phosphorus is expected, a filter can be used in the sampling train.

Special Considerations: The presence of white phosphorus should be confirmed using either a secondary GC column or and MS.

Source: NIOSH. 1994. "Method 7905: Phosphorus," Issue 2. *NIOSH Manual of Analytical Methods*, Fourth Edition. DHHS (NIOSH) Publication No. 94-113. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-7905.pdf

5.2.73 NIOSH Method 7906: Particulate Fluorides and Hydrofluoric Acid by Ion Chromatography

Analyte(s)	CAS RN
Hydrogen fluoride	7664-39-3

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Aqueous solution extraction
Determinative Technique: IC with conductivity detection

Method Developed for: Fluorides in aerosol and gas
Method Selected for: This method has been selected for preparation and analysis of air samples to address hydrogen fluoride. (See Footnote 10 of Appendix A.)
Detection and Quantitation: The working range of the method is 0.04–8 mg/m^3 for 250-L samples.

Description of Method: Hydrogen fluoride is determined as fluoride ion by this method. A volume of 15 to 1000 L of air is drawn through a 0.8-µm cellulose nitrate prefilter (to trap particulate fluorides) and a cellulose nitrate filter treated with sodium carbonate (to trap gaseous fluoride). The filter is extracted with an aqueous solution of 8 mM sodium carbonate /1 mM sodium bicarbonate and the extract is analyzed for fluoride by IC.

Special Considerations: If other aerosols are present, gaseous fluoride may be slightly underestimated due to adsorption onto or reaction with particles, with concurrent overestimation of particulate/gaseous fluoride ratio.

Source: NIOSH. 2014. "Method 7906: Particulate Fluorides and Hydrofluoric Acid 7906 by Ion Chromatography," Issue 2. *NIOSH Manual of Analytical Methods*, Fifth Edition. Washington, DC: DHHS (NIOSH). https://www.cdc.gov/niosh/docs/2003-154/pdfs/7906.pdf

5.2.74 NIOSH Method 7907: Volatile Acids by Ion Chromatography (Hydrogen Chloride, Hydrogen Bromide, Nitric Acid)

Analyte(s)	CAS RN
Hydrogen bromide	10035-10-6
Hydrogen chloride	7647-01-0

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Aqueous solution extraction
Determinative Technique: IC with conductivity detection

Method Developed for: Hydrogen bromide, hydrogen chloride and nitric acid in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address hydrogen bromide and hydrogen chloride.
Detection and Quantitation: The working range is 0.04–8 mg/m^3 for hydrogen bromide and hydrogen chloride in 240-L samples.

Description of Method: Hydrogen bromide and hydrogen chloride are determined as bromide and chloride ions, respectively. A volume of 30 to 600 L of air is drawn through a 37-mm diameter quartz fiber prefilter (to trap potentially interfering particulate chlorides) and a 37-mm diameter quartz fiber filter treated with sodium carbonate (to trap gaseous hydrogen bromide and hydrogen chloride). After discarding the prefilters, the filter is extracted with an aqueous solution of 3.1 mM sodium carbonate/0.35 mM sodium carbonate and the extract is analyzed for bromide and chloride by IC.

Special Considerations: Inorganic acids can react with co-sampled particulate matter on the pre-filter, leading to low results (e.g., zinc oxide reacting with hydrochloric acid). Potentially interfering particulate chlorides and nitrates removed by the pre-filter can react with the sampled acids and liberate hydrochloric acid that is subsequently collected on the sampling filter, leading to high results. Silica gel sorbent tubes can be used instead of treated filters, but each sorbent tube must be preceded by a pre-filter.

Source: NIOSH. 2014. "Method 7907: Volatile Acids by Ion Chromatography (Hydrogen Chloride, Hydrogen Bromide, Nitric Acid)," Issue 1. *NIOSH Manual of Analytical Methods*, Fifth Edition. Washington, DC: DHHS (NIOSH). https://www.cdc.gov/niosh/docs/2003-154/pdfs/7907.pdf

5.2.75 NIOSH Method 9102: Elements on Wipes

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
Chlorovinyl arsonic acid (CVAOA) (analyze as total arsenic)*	64038-44-4
2-Chlorovinylarsonous acid (CVAA) (analyze as total arsenic)*	85090-33-1
Ethyldichloroarsine (ED)	598-14-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)*	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)*	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)*	40334-70-1
Lewisite oxide (analyze as total arsenic)*	1306-02-1
Mercuric chloride (analyze as total mercury)	7487-94-7
Mercury, Total	7439-97-6
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

* If laboratories are approved for storing and handling the appropriate standards, these analytes can be detected and measured using EPA/600/R-15/258 (Section 5.2.54)

Analysis Purpose: Sample preparation
Sample Preparation Technique: Acid digestion
Determinative Technique: ICP-AES / ICP-MS / Spectrophotometry
Determinative Method: EPA SW-846 Methods 6010D, 6020B, 7473 and 8270E. Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Measurement of metals on wipe surfaces using ICP-AES
Method Selected for: This method has been selected for preparation of wipe samples to address the analytes listed in the table above as total arsenic, mercury, thallium or vanadium. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working ranges are: 0.261–105 µg/wipe (arsenic); 0.136–50.0 µg/wipe (thallium); and 0.0333–25.0 µg/wipe (vanadium). A working range is not provided for mercury.

Description of Method: Surface wipe samples are transferred to a clean beaker, followed by the addition of concentrated nitric and perchloric acids. The beaker contents are held at room temperature for 30 minutes, then heated at 150°C for 8 hours. Additional nitric acid is added until the wipe media is completely destroyed. The sample is then taken to near dryness and the residue dissolved and diluted

before being analyzed.

Special Considerations: ICP-MS may also be used for the analysis of wipe samples; however, at this time, this technique has not been evaluated for wipes. Nitric and perchloric acids are strong oxidizers and extremely corrosive. Perform all perchloric acid digestions in a perchloric acid hood. When working with acids, use gloves and avoid inhalation or contact with skin or clothing. If laboratories are approved for storing and handling the appropriate standards, lewisites 1, 2 and 3 and their degradation products (CVAOA, CVAA and lewisite oxide) can be detected and measured using EPA/600/R-15/258 (Section 5.2.54).

Source: NIOSH. 2003. "Method 9102, Issue 1: Elements on Wipes." *NIOSH Manual of Analytical Methods*, 3rd Supplement 2003-154. Washington, DC: DHHS (NIOSH). http://www.epa.gov/sites/production/files/2015-07/documents/niosh-9102.pdf

5.2.76 NIOSH Method 9106: Methamphetamine and Illicit Drugs, Precursors and Adulterants on Wipes by Liquid-Liquid Extraction

Analyte(s)	CAS RN
Phencyclidine	77-10-1

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Solvent extraction
Determinative Technique: GC-MS

Method Developed for: Phencyclidine in wipe samples
Method Selected for: This method has been selected for the preparation and analysis of wipe samples to address phencyclidine. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The MDLs for phencyclidine on spiked cotton gauze in full-scan and SIM mode are 0.3 and 0.2 ng/cm^2, respectively. The working range of the method is 1–100 ng/ cm^2 for both full-scan and SIM modes.

Description of Method: Internal standards and desorption solution (0.1M sulfuric acid) are added to each sample contained in a centrifuge tube. The tubes are capped and the samples and solution are mixed with a rotatory mixer at 10–30 rpm for at least one hour. If necessary, the pH is adjusted to ≤4 with 3M sulfuric acid. Sample extracts are transferred to a glass centrifuge tube and cleaned by adding 10 mL of hexane to 10 mL of extract. The contents are mixed by rotary mixer for at least one hour, then allowed to stand for 15–20 minutes. If an emulsion forms, extracts are centrifuged for a few minutes at 1500–2000 rpm. The upper organic layer is aspirated off as waste, and 1–2 drops of pH indicator (phenolphthalein and bromothymol blue) and 0.5 mL of 10M sodium hydroxide are added to the aqueous fraction, as needed, to turn the solution to purple or magenta. Once this color change is achieved, 10 mL of methylene chloride is added, the sample container is capped and the contents remixed on a rotary mixer for 1 hour. The mixture is allowed to stand for 15–30 minutes, and the centrifuge procedure is repeated if an emulsion forms. Any remaining water is removed using packed potassium carbonate-sodium sulfate drying columns. The methylene chloride is evaporated under nitrogen, and 100 µL of chlorodifluoroacetic anhydride is added followed by additional mixing. The tube is then placed into an oven at 70–75°C and heated for 20–30 minutes. After cooling, the extract is evaporated to dryness under nitrogen until a blue or violet color is visible. Reconstituting solution (1 mL) is added and the solution is transferred to an amber-colored GC vial containing 200–250 mg anhydrous sodium sulfate. Vials are capped and analyzed by GC-MS.

Special Considerations: If an oil-like residue or film persists, the sample may contain contaminants that were not removed during the cleanup step or were introduced following sample cleanup. In such cases, the cleanup step is repeated on another 10-mL sample aliquot, using methylene chloride instead of hexane as the cleanup solvent. Analyte losses have been experienced during the derivatization step if blowing is

continued for more than 2 minutes beyond the appearance of a blue or violet color.

Source: NIOSH. 2011. "Method 9106: Methamphetamine and Illicit Drugs, Precursors and Adulterants on Wipes by Liquid-Liquid Extraction," Issue 1. *NIOSH Manual of Analytical Methods*, Fifth Edition. Washington, DC: DHHS (NIOSH). https://www.cdc.gov/niosh/docs/2003-154/pdfs/9106.pdf

5.2.77 NIOSH Method 9109: Methamphetamine and Illicit Drugs, Precursors, and Adulterants on Wipes by Solid Phase Extraction

Analyte(s)	CAS RN
Phencyclidine	77-10-1

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Solvent extraction
Determinative Technique: GC-MS

Method Developed for: Phencyclidine in wipe samples
Method Selected for: This method has been selected for preparation and analysis of wipe samples to address phencyclidine. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The MDLs for phencyclidine on spiked cotton gauze and AlphaWipes® wipes (All-Spec, Wilmington, NC, or equivalent) are 1 and 5 ng/cm^2, respectively. The working range of the method is 3–300 ng/ cm^2.

Description of Method: Internal standards and desorption solution (0.1 M sulfuric acid) are added to wipe samples contained in a centrifuge tube. The tubes are capped and samples mixed with a rotatory mixer at 10–30 rpm for at least one hour. If necessary, the pH is adjusted to ≤4 with 2.5 to 3M sulfuric acid. An SPE column is attached to a vacuum capable of 25–30 psi pressure and conditioned with 3 mL of methanol, followed by 1 mL of ASTM Type II deionized water. The SPE column is loaded with 10 mL of sample and the sample is pulled through the column via vacuum. The column is then washed with 3 mL of 0.1M hydrochloric acid, followed by 3 mL of methanol, and all effluent is discarded. The vacuum is increased to remove all traces of water, and the analytes are eluted with 3 mL of 80:20:2 methylene chloride:isopropanol:concentrated ammonium hydroxide (v/v) into a collection tube. About 5 µL of crystal violet solution and 100 µL of 0.3M hydrochloric acid in methanol are added to the tube, and the samples are evaporated to dryness under nitrogen. Acetonitrile containing internal standard (100 µL) and derivatizing agents is added to the collection tube, and the tubes are capped, vortexed for 4–5 seconds, and a 300–500 µL aliquot is transferred to an autosampler vial for analysis by GC-MS.

Special Considerations: No chromatographic interferences were observed during method development; however, water, surfactants and polyols can inhibit derivatization. The color of the reconstituted solution should be deep blue to violet. If the color turns light blue or turquoise upon standing, moisture may be present. Such samples need to be reprocessed beginning at the SPE extraction step, since the derivatives are not stable in the presence of moisture.

Source: NIOSH. 2011. "Method 9109: Methamphetamine and Illicit Drugs, Precursors, and Adulterants on Wipes by Solid Phase Extraction," Issue 1. *NIOSH Manual of Analytical Methods*, Fifth Edition. Washington, DC: DHHS (NIOSH). http://www.cdc.gov/niosh/docs/2003-154/pdfs/9109.pdf

5.2.78 NIOSH Method S301-1: Fluoroacetate Anion

Analyte(s)	CAS RN
Fluoroacetic acid and fluoroacetate salts	NA
Methyl fluoroacetate	453-18-9

Analysis Purpose: Sample preparation
Sample Preparation Technique: Water extraction
Determinative Technique: LC-MS
Determinative Method: Adapted from J. Chromatogr. A, 1139 (2002) 271-278.

Method Developed for: Fluoroacetate anion in air
Method Selected for: This method has been selected for preparation of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit is estimated to be 20 ng of sodium fluoroacetate per injection, corresponding to a 100-µL aliquot of a 0.2-µg/mL standard. The analytical range of this method is estimated to be 0.01–0.16 mg/m^3.

Description of Method: This method was developed specifically for sodium fluoroacetate, but also may be applicable to other fluoroacetate salts. The method determines fluoroacetate salts as fluoroacetate anion. A known volume of air (e.g., 480 L was used in validation of this method) is drawn through a cellulose ester membrane filter to collect sodium fluoroacetate. Sodium fluoroacetate is extracted from the filter with 5 mL of deionized water, and the resulting sample is analyzed by LC-MS.

Special Considerations: When analyzing samples for methyl fluoroacetate (as fluoroacetate ion), addition of base is required to assist dissociation into fluoroacetate anion.

Source: NIOSH. 1977. "Method S301-1: Sodium Fluoroacetate." *NIOSH Manual of Analytical Methods*, Second Edition, Volume 5. Washington, DC: DHHS (NIOSH).
http://www.epa.gov/sites/production/files/2015-07/documents/niosh-s301-1.pdf

5.2.79 OSHA Method 40: Methylamine

Analyte(s)	CAS RN
Methylamine	74-89-5

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: HPLC-FL/vis

Method Developed for: Methylamine in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address methylamine. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The detection limit is 0.35 µg per sample (28 ppb or 35 µg/m^3). Quantitation limits of 28 ppb (35 µg/m^3) have been achieved. This is the smallest amount of methylamine that can be quantified within the requirements of a recovery of at least 75% and a precision (standard deviation of 1.96) of ± 25% or better.

Description of Method: This method is used for detection of methylamine using HPLC with a FL or visible (vis) detector. Samples are collected by drawing 10-L volumes of air at a rate of 0.2 L/minute through standard size sampling tubes containing sampler resin coated with 10% 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD chloride) by weight. Samples are desorbed with 5% (w/v) NBD chloride in tetrahydrofuran (with a small amount of sodium bicarbonate present), heated in a hot water bath, and analyzed by HPLC-FL or high performance liquid chromatography-visible (HPLC-vis).

Source: OSHA. 1982. "Method 40: Methylamine." Method originally obtained from https://www.osha.gov, but is provided here for reference. Salt Lake City, UT: OSHA.
http://www.epa.gov/sites/production/files/2015-07/documents/osha-method40.pdf

5.2.80 OSHA Method 54: Methyl Isocyanate (MIC)

Analyte(s)	CAS RN
Methyl isocyanate	624-83-9

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: HPLC

Method Developed for: Methyl isocyanate in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address methyl isocyanate. See Appendix A for the corresponding method usability tier.

Description of Method: This method determines the concentration of methyl isocyanate in air by using HPLC with a FL or UV detector. Samples are collected by drawing a known volume of air through sampler tubes containing resin coated with 0.3 mg of 1-(2-pyridyl)piperazine (1-2PP). Samples are desorbed with acetonitrile and analyzed by HPLC using a FL or UV detector.

Source: OSHA. 1985. "Method 54: Methyl Isocyanate (MIC)." Method originally obtained from https://www.osha.gov, but is provided here for reference. Sandy, UT: OSHA. https://www.osha.gov/dts/sltc/methods/organic/org054/org054.html

5.2.81 OSHA Method 61: Phosgene

Analyte(s)	CAS RN
Phosgene	75-44-5

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: GC-NPD

Method Developed for: Phosgene in air samples
Method Selected for: This method has been selected for preparation and analysis of air samples to address phosgene. See Appendix A for the corresponding method usability tier.

Description of Method: This method determines the concentration of phosgene in air by using GC with an NPD. Air samples are collected by drawing known volumes of air through sampling tubes containing resin adsorbent that has been coated with 2-(hydroxymethyl)piperidine. The samples are desorbed with toluene and then analyzed by GC using an NPD.

Special Considerations: The presence of phosgene should be confirmed using either a secondary GC column or an MS.

Source: OSHA. 1986. "Method 61: Phosgene." Method originally obtained from https://www.osha.gov, but is provided here for reference. Salt Lake City, UT: OSHA. http://www.epa.gov/sites/production/files/2015-07/documents/osha-method61.pdf

5.2.82 OSHA Method ID-211: Sodium Azide and Hydrazoic Acid in Workplace Atmospheres

Analyte(s)	CAS RN
Sodium azide (analyze as azide ion)	26628-22-8

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Buffer desorption
Determinative Technique: IC-UV

Method Developed for: Sodium azide and hydrazoic acid in workplace atmospheres
Method Selected for: This method has been selected for preparation and analysis of air and wipe samples to address sodium azide as azide ion. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit for sodium azide was found to be 0.003 mg/m^3 for a 5-L air sample. The quantitation limit was found to 0.011 mg/m^3, also for a 5-L air sample.

Description of Method: This method describes sample collection and analysis of airborne azides (as sodium azide and hydrazoic acid. Particulate sodium azide is collected on a polyvinyl chloride (PVC) filter or in the glass wool plug of the sampling tube. Gaseous hydrazoic acid is collected and converted to sodium azide by the impregnated silica gel (ISG) sorbent within the sampling tube. The collected azide on either media is desorbed in a weak buffer solution, and the resultant azide anion is analyzed by IC using a variable wavelength UV detector at 210 nm. A gravimetric conversion is used to calculate the amount of sodium azide or hydrazoic acid collected.

Source: OSHA. 1992. "Method ID-211: Sodium Azide and Hydrazoic Acid in Workplace Atmospheres." Sandy, UT: OSHA. http://www.epa.gov/sites/production/files/2015-07/documents/osha-id-211.pdf

5.2.83 OSHA Method ID216SG: Boron Trifluoride (BF_3)

Analyte(s)	CAS RN
Boron trifluoride	7637-07-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Sample collected in bubbler (no sample preparation required)
Determinative Technique: Ion specific electrode (ISE)

Method Developed for: Boron trifluoride in air samples
Method Selected for: This method has been selected for preparation and analysis of air samples to address boron trifluoride. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The detection limit is 10 µg in a 30-L sample.

Description of Method: Boron trifluoride is determined as fluoroborate. A volume of 30 to 480 L of air is drawn through a bubbler containing 0.1M ammonium fluoride. The bubbler solution is diluted and analyzed with a fluoroborate ISE.

Source: OSHA. 1989. "Method ID216SG: Boron Trifluoride (BF_3)." Method originally obtained from https://www.osha.gov, but is provided here for reference. Sandy, UT: OSHA. http://www.epa.gov/sites/production/files/2015-07/documents/osha-id216sg.pdf

5.2.84 OSHA Method PV2004: Acrylamide

Analyte(s)	CAS RN
Acrylamide	79-06-1
Acrylonitrile	107-13-1
Methyl acrylonitrile	126-98-7

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: HPLC-UV

Method Developed for: Acrylamide in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit was found to be 0.7 µg/mL (0.006 mg/m^3 for a 1-mL desorption volume) or 0.029 mg/m^3 (for a 5-mL desorption volume), based on a 120-L air sample). Applicable working ranges for 1-mL and 5-mL desorption volumes are 0.017–1.5 mg/m^3 and 0.083–7.5 mg/m^3, respectively.

Description of Method: This method determines the concentration of acrylamide in air by using HPLC with a UV detector. Samples are collected by drawing known volumes of air through OSHA versatile sampler (OVS-7) tubes, each containing a glass fiber filter and two sections of adsorbent. Samples are desorbed with a solution of 5% methanol/95% water, and analyzed by HPLC-UV.

Special Considerations: The presence of acrylamide, acrylonitrile and methyl acrylonitrile should be confirmed using either a secondary HPLC column or an MS.

Source: OSHA. 1991. "Method PV2004: Acrylamide." Sandy, UT: OSHA.
http://www.epa.gov/sites/production/files/2015-07/documents/osha-pv2004.pdf

5.2.85 OSHA Method PV2103: Chloropicrin

Analyte(s)	CAS RN
Chloropicrin	79-06-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent desorption
Determinative Technique: GC-ECD

Method Developed for: Chloropicrin in air
Method Selected for: This method has been selected for preparation and analysis of air samples to address chloropicrin. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The detection limit is 0.01 ng, with a 1-µL injection volume. This is the smallest amount that could be detected under normal operating conditions. The working range is 33.2–1330 µg/m^3.

Description of Method: This method determines the concentration of chloropicrin in air by GC-ECD. Samples are collected by drawing a known volume of air through two adsorbent tubes in series. Samples are desorbed with ethyl acetate and analyzed by GC-ECD.

Special Considerations: The presence of chloropicrin should be confirmed using either a secondary GC column or an MS. Chloropicrin is light sensitive, and samples should be protected from light.

Source: OSHA. 1991. "Method PV2103: Chloropicrin." Salt Lake City, UT: OSHA. http://www.epa.gov/sites/production/files/2015-07/documents/osha-pv2103.pdf

5.2.86 ASTM Method D5755-09: Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Surface Loading

Analyte(s)	CAS RN
Asbestos	1332-21-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Direct transfer
Determinative Technique: Transmission electron microscopy (TEM)

Method Developed for: Asbestos in dust
Method Selected for: This method has been selected for preparation and analysis of solid (e.g., soft surfaces-microvac) samples to address asbestos. See Appendix A for the corresponding method usability tier.

Description of Method: This method describes procedures to identify asbestos in dust and provide an estimate of surface loading reported as the number of asbestos structures per unit area of sampled surface. Samples are collected by vacuuming a known surface area with a standard 25- or 37-mm air sampling cassette using a plastic tube that is attached to the inlet orifice, which acts as a nozzle. Once collected, samples are transferred from inside the cassette to an aqueous suspension of known volume. Aliquots of the suspension are then filtered through a membrane, and a section of the membrane is prepared and transferred to a TEM grid using a direct transfer method. The asbestiform structures are identified, sized, and counted by TEM, using select area electron diffraction (SAED) and energy dispersive X-ray analysis (EDXA) at a magnification of 15,000 to 20,000X.

Source: ASTM. 2014. "Method D5755-09(e1): Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Surface Loading." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D5755.htm

5.2.87 ASTM Method D6480-05: Standard Test Method for Wipe Sampling of Surfaces, Indirect Preparation, and Analysis for Asbestos Structure Number Concentration by Transmission Electron Microscopy

Analyte(s)	CAS RN
Asbestos	1332-21-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Direct transfer
Determinative Technique: TEM

Method Developed for: Asbestos in samples wiped from surfaces
Method Selected for: This method has been selected for preparation and analysis of wipe (e.g., hard surfaces-wipes) samples to address asbestos. See Appendix A for the corresponding method usability tier.
Description of Method: This method describes a procedure to identify asbestos in samples wiped from surfaces and to provide an estimate of the concentration reported as the number of asbestos structures per unit area of sampled surface. Samples are collected by wiping a surface of known area with a wipe material. Once collected, samples are transferred from the wipe material to an aqueous suspension of

known volume. Aliquots of the suspension are then filtered through a membrane filter, and a section of the membrane filter is prepared and transferred to a TEM grid, using the direct transfer method. The asbestiform structures are identified, sized, and counted by TEM, using electron diffraction and EDXA at a magnification from 15,000 to 20,000X.

Source: ASTM. 2010. "Method D6480-05: Standard Test Method for Wipe Sampling of Surfaces, Indirect Preparation, and Analysis for Asbestos Structure Number Concentration by Transmission Electron Microscopy." West Conshohocken, PA: ASTM International.
http://www.astm.org/Standards/D6480.htm

5.2.88 ASTM Method D7597-16: Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Hydrogen Dimethylamidophosphate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Water by Liquid Chromatography/Tandem Mass Spectrometry

Analyte(s)	CAS RN
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Dimethylphosphoramidic acid	33876-51-6
Ethyl methylphosphonic acid (EMPA)	1832-53-7
Isopropyl methylphosphonic acid (IMPA)	1832-54-8
Methylphosphonic acid (MPA)	993-13-5
Pinacolyl methyl phosphonic acid (PMPA)	616-52-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Filtered using a syringe-driven Millex®-HV PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: DIMP, EMPA, IMPA, MPA and PMPA in surface water
Method Selected for: This method has been selected for preparation and analysis of water samples to address the analytes listed in the table above. ***Note***: EPA Method 538 (Section 5.2.11) has been selected for sample preparation and analysis of DIMP in drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection verification levels (DVLs) and reporting range vary for each analyte and range from 0.25 to 20 µg/L and 5 to 1500 µg/L, respectively.

Description of Method: Target compounds are analyzed by direct injection without derivatization by LC-MS-MS. Samples are shipped to the laboratory at 0 to 6°C, spiked with surrogates, filtered using a syringe-driven filter unit and analyzed directly by LC-MS-MS within 1 day. The target compounds are identified by comparing the sample single reaction monitoring (SRM) transitions to the known standard SRM transitions. The retention time for the analytes of interest must also fall within the retention time of the standard by ± 5%. Target compounds are quantitated using the SRM transition of the target compounds and external standard calibration.

Special Considerations: Method modifications (e.g., pH adjustment) may be needed when analyzing for dimethyphosphoramidic acid.

Source: ASTM. 2016. "Method D7597-16: Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Hydrogen Dimethylamidophosphate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Water by Liquid Chromatography/Tandem Mass Spectrometry." West Conshohocken, PA: ASTM International.
http://www.astm.org/Standards/D7597.htm

5.2.89 ASTM Method D7598-16: Standard Test Method for Determination of Thiodiglycol in Water by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry

Analyte(s)	CAS RN
Thiodiglycol	111-48-8

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Filtered using a syringe-driven Millex® HV PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: Thiodiglycol in surface water samples
Method Selected for: This method has been selected for preparation and analysis of water samples to address thiodiglycol. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The DVL for thiodiglycol is 20 µg/L; the reporting range is 100–10000 µg/L.

Description of Method: Thiodiglycol is analyzed by direct injection without derivatization by LC-MS-MS. Samples are shipped to the laboratory at 0 to 6°C, spiked with surrogates, filtered using a syringe-driven filter unit and analyzed directly by LC-MS-MS within 7 days. The target compound is identified by comparing the sample primary SRM transition to the known standard SRM transition. The retention time must fall within the retention time of the standard by ± 5%. Thiodiglycol is quantitated using the primary SRM transition and external standard calibration.

Source: ASTM. 2016. "Method D7598-16: Standard Test Method for Determination of Thiodiglycol in Water by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D7598.htm

5.2.90 ASTM Method D7599-16: Standard Test Method for Determination of Diethanolamine, Triethanolamine, *N*-Methyldiethanolamine and *N*-Ethyldiethanolamine in Water by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
N-Ethyldiethanolamine (EDEA)	139-87-7
N-Methyldiethanolamine (MDEA)	105-59-9
Triethanolamine (TEA)	102-71-6

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Filtered using a syringe-driven Millex® HV PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: Diethanolamine, triethanolamine, MDEA and EDEA in surface water samples
Method Selected for: This method has been selected for preparation and analysis of water samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The DVL and reporting range for EDEA and TEA are 5 µg/L and 25–500 µg/L, respectively. The DVL and reporting range for MDEA are 10 µg/L and 50–500 µg/L, respectively.
Description of Method: Target compounds are analyzed by direct injection without derivatization by LC-MS-MS. Samples are shipped to the laboratory at 0 to 6°C, spiked with surrogates, filtered using a syringe-driven filter unit and analyzed directly by LC-MS-MS within 7 days. Target compounds are identified by comparing sample SRM transitions to the known standard SRM transitions. The retention

time for the analytes of interest must also fall within the retention time of the standard by ± 5%. Target compounds are quantitated using the SRM transition and external standard calibration.

Source: ASTM. 2016. "Method D7599-16: Standard Test Method for Determination of Diethanolamine, Triethanolamine, *N*-Methyldiethanolamine and *N*-Ethyldiethanolamine in Water by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D7599.htm

5.2.91 ASTM Method D7644-16: Standard Test Method for Determination of Bromadiolone, Brodifacoum, Diphacinone and Warfarin in Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
Diphacinone	82-66-6

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Filtered using a syringe-driven PVDF filter unit for water samples; automated Soxhlet or pressured fluid extraction for solid samples, and solvent extraction for wipes.
Determinative Technique: LC-MS-MS

Method Developed for: Bromadiolone, brodifacoum and diphacinone in reagent, surface and drinking water
Method Selected for: This method has been selected for preparation and analysis of water samples, and for analysis of prepared solid and wipe samples to address the analytes listed in the table above. ***Note***: EPA SW-846 Methods 3541/3545A (Sections 5.2.22 and 5.2.23) and Methods 3570/8290A Appendix A (Sections 5.2.24 and 5.2.36) have been selected for preparation of solid and wipe samples, respectively. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The DVLs and reporting range for each analyte are 0.020 µg/L and 0.125–2.5 µg/L, respectively.

Description of Method: Target compounds are analyzed by direct injection without derivatization using LC-MS-MS. Samples are shipped to the laboratory at 0 to 6°C, spiked with surrogates, filtered using a syringe-driven filter unit, and analyzed directly by LC-MS-MS within 14 days. The target analytes are identified by retention time and two SRM transitions. The retention time for the analytes in the sample must fall within ± 5% of the retention time of the analytes in standard solution. Target analytes are measured using the primary SRM transition of the analytes and external standard calibration. Analytes are confirmed using the confirmatory SRM transitions.

Source: ASTM. 2016. "Method D7644-16: Standard Test Method for Determination of Bromadiolone, Brodifacoum, Diphacinone and Warfarin in Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D7644.htm.

5.2.92 ASTM Method D7645-16: Standard Test Method for Determination of Aldicarb, Aldicarb Sulfone, Aldicarb Sulfoxide, Carbofuran, Methomyl, Oxamyl and Thiofanox in Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
Aldicarb	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
Carbofuran	1563-66-2
Methomyl	16752-77-5
Oxamyl	23135-22-0
Thiofanox	39196-18-4

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Filtered using a syringe-driven PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: Aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbofuran, oxamyl, methomyl and thiofanox in water
Method Selected for: This method has been selected for preparation and analysis of non-drinking water samples to address aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbofuran, methomyl, oxamyl and thiofanox. It has also been selected for the analysis of prepared solid and wipe samples to address thiofanox. See Appendix A for corresponding method usability tiers. ***Note***:

- SW-846 Methods 3541 (see Section 5.2.22) or 3545A (see Section 5.2.23) have been selected for preparation of solid samples to be analyzed for thiofanox.
- SW-846 Methods 3570 (see Section 5.2.24) and 8290 (see Section 5.2.36) have been selected for preparation of wipe samples to be analyzed for thiofanox.

Detection and Quantitation: A DVL is reported as 250 ng/L for all compounds listed in the table above. The reporting range for these compounds is 1–100 µg/L.

Description of Method: Samples are spiked with surrogates, filtered using a syringe-driven filter unit, and analyzed directly by LC-MS-MS within 14 days. Target analytes are identified by comparing primary and confirmatory MRM transitions to known standard primary and confirmatory MRM transitions. The retention time for the analytes must fall within ± 5% of the retention time of the analytes in standard solution. Analytes are measured using the primary SRM transition and external standard calibration.

Source: ASTM. 2016. "Method D7645-16: Standard Test Method for Determination of Aldicarb, Aldicarb Sulfone, Aldicarb Sulfoxide, Carbofuran, Methomyl, Oxamyl and Thiofanox in Water by Liquid Chromatography/Tandem Mass Spectrometry." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D7645.htm.

5.2.93 ASTM Method E2787-11: Standard Test Method for Determination of Thiodiglycol in Soil Using Pressurized Fluid Extraction Followed by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
Thiodiglycol	111-48-8

Analysis Purpose: Sample preparation, and/or analyte determination and measurement

Sample Preparation Technique: Extracted using PFE, and filtered using a syringe-driven PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: Thiodiglycol in solid samples
Method Selected for: This method has been selected for preparation and analysis of solid samples to address thiodiglycol. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDL is 54 µg/kg. The reporting range is 200–16,000 µg/kg.

Description of Method: Approximately 5–30 g of soil is mixed with an appropriate amount (depending on the wetness of the soil) of drying agent (diatomaceous earth), spiked with a surrogate, and extracted in a PFE system using methanol. Extracts are filtered using a 0.2-micron filter and concentrated to a final volume of 0.4 mL using a nitrogen evaporation device. The volume of the extract is brought up to 2 mL with HPLC-grade water and analyzed by LC-MS-MS. The target analytes are identified by comparing the sample SRM transitions to the known standard SRM transitions. The retention time for the analytes in the sample must fall within ± 5% of the retention time of the analytes in standard solution. Target analytes are measured using the SRM transition and external standard calibration.

Source: ASTM. 2016. "Method E2787-11: Standard Test Method for Determination of Thiodiglycol in Soil Using Pressurized Fluid Extraction Followed by Single Reaction Monitoring Liquid Chromatography/ Tandem Mass Spectrometry." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/E2787.htm.

5.2.94 ASTM Method E2838-11: Standard Test Method for Determination of Thiodiglycol on Wipes by Solvent Extraction Followed by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
Thiodiglycol	111-48-8

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Extracted using sonication or PFE and filtered using a syringe-driven PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: Thiodiglycol in wipes
Method Selected for: This method has been selected for preparation and analysis of wipe samples to address thiodiglycol. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The MDL is 0.085 µg/wipe. The reporting range is 1–80 µg/wipe.

Description of Method: Wipe samples are shipped to the laboratory at 0 to 6°C, and must be extracted, concentrated, and analyzed by LC-MS-MS within 7 days. Extraction may be performed using sonication or PFE. Extracts are filtered using a 0.2-micron filter and concentrated to a final volume of 2 mL when using sonication or 4 mL when using PFE. If sample throughput is less of a concern, the PFE extracts can be concentrated down to 2 mL. Extracts are analyzed by LC-MS-MS. Thiodiglycol is identified by comparing the SRM transitions to the known standard SRM transitions. The retention time for the analytes in the sample must fall within ± 5% of the retention time of the analytes in standard solution. Target analytes are measured using the SRM transition and external standard calibration.

Source: ASTM. 2016. "Method E2838-11: Standard Test Method for Determination of Thiodiglycol on Wipes by Solvent Extraction Followed by Liquid Chromatography/Tandem Mass Spectrometry." West

Conshohocken, PA: ASTM International. http://www.astm.org/Standards/E2838.htm.

5.2.95 ASTM Method E2866-12: Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Soil by Pressurized Fluid Extraction and Analyzed by Liquid Chromatography/Tandem Mass Spectrometry

Analyte(s)	CAS RN
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Dimethylphosphoramidic acid	33876-51-6
Ethyl methylphosphonic acid (EMPA)	1832-53-7
Isopropyl methylphosphonic acid (IMPA)	1832-54-8
Methylphosphonic acid (MPA)	993-13-5
Pinacolyl methyl phosphonic acid (PMPA)	616-52-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Extracted using PFE and filtered using a syringe-driven Millex®-HV PVDF filter unit
Determinative Technique: LC-MS-MS

Method Developed for: DIMP, EMPA, IPMA, MPA and PMPA in soil
Method Selected for: This method has been selected for preparation and analysis of solid samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The reporting range for all analytes is 40–2000 µg/kg. MDLs range from 1.3 to 8.7 µg/kg.

Description of Method: Target compounds are analyzed by direct injection without derivatization by LC-MS-MS. Samples are shipped to the laboratory at 0 to 6°C and must be extracted, concentrated, and analyzed by LC-MS-MS within 7 days. Approximately 5–30 g of soil are mixed with an appropriate amount (depending on the wetness of the soil) of drying agent (diatomaceous earth), spiked with a surrogate, and extracted in a PFE system using water. Extracts are filtered using a 0.2-micron filter and analyzed by LC-MS-MS. The target compounds are identified by comparing the sample SRM transitions to the known standard SRM transitions. The retention time for the analytes of interest must also fall within the retention time of the standard by ± 5%. Target compounds are quantitated using the SRM transition of the target compounds and external standard calibration.

Special Considerations: Method modifications (e.g., pH adjustment) may be needed when analyzing for dimethyphosphoramidic acid.

Source: ASTM. 2016. "Method E2866-12: Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Soil by Pressurized Fluid Extraction and Analyzed by Liquid Chromatography/Tandem Mass Spectrometry." West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/E2866.htm.

5.2.96 ISO Method 10312:1995: Ambient Air - Determination of Asbestos Fibres - Direct-Transfer Transmission Electron Microscopy Method

Analyte(s)	CAS RN
Asbestos	1332-21-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Direct transfer
Determinative Technique: TEM

Method Developed for: Asbestos in ambient air
Method Selected for: This method has been selected for preparation and analysis of air samples to address asbestos. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: In a 4000-L air sample with approximately 10 pg/m^3 (typical of clean or rural atmospheres), an analytical sensitivity of 0.5 structure/L can be obtained. This is equivalent to a detection limit of 1.8 structure/L when an area of 0.195 mm of the TEM specimen is examined. The range of concentrations that can be determined is 50–7,000 structures/mm^2 on the filter.

Description of Method: This method determines the type(s) of asbestos fibers present, but cannot discriminate between individual fibers of the asbestos and non-asbestos analogues of the same amphibole mineral. The method is defined for polycarbonate capillan/pore filters or cellulose ester (either mixed esters of cellulose or cellulose nitrate) filters through which a known volume of air has been drawn. The method is suitable for determination of asbestos in both exterior and building atmospheres.

Source: ISO. 2005. "Method 10312: 1995: Ambient Air - Determination of Asbestos Fibres - Direct Transfer Transmission Electron Microscopy Method."
http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=18358

5.2.97 Standard Method 4500-CN G: Cyanides Amenable to Chlorination after Distillation

Analyte(s)	CAS RN
Cyanide, Amenable to chlorination	NA

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Acid digestion followed by distillation
Determinative Technique: Visible spectrophotometry, titrimetry or cyanide-selective electrode

Method Developed for: Cyanide in drinking water, groundwater, surface water, domestic and industrial wastewaters, and solid waste
Method Selected for: This method has been selected for preparation and analysis of water samples to address cyanide amenable to chlorination. The method has been selected as an alternative to EPA Regional Laboratory (RLAB) Method 3135.2I (see Section 5.2.42), for use by laboratories more familiar with its procedures.
Detection and Quantitation: The method has been evaluated in the ranges of 0.008–0.191 mg/L (colorimetric procedure) and 1–4 mg/L (titrimetric procedure). These ranges can be expanded by sample dilution, by either distilling less sample or diluting the distillate.

Description of Method: This method is applicable to the determination of cyanides amenable to chlorination (also known as available cyanide). After part of the sample is chlorinated to decompose the cyanides, both the chlorinated and the untreated sample are distilled as described in Standard Method (SM) 4500-CN C. The difference between the cyanide concentrations in the two samples is expressed as cyanides amenable to chlorination. The sample is divided into two equal portions of 500 mL (or equal portions diluted to 500 mL). Chlorinate one of the portions using the procedure in the next paragraph.

Both portions are analyzed for cyanide and the difference in determined concentrations is the cyanide amenable to chlorination.

One portion is placed in a 1-L beaker covered with aluminum foil or black paper. The beaker is kept covered with a wrapped watch glass during chlorination. Calcium hypochlorite solution is added dropwise to the sample while agitating and maintaining pH between 11 and 12 by adding sodium hydroxide solution. The sample is then tested for chlorine by placing a drop of treated sample on a strip of KI-starch paper. A distinct blue color indicates sufficient chlorine (approximately 50 to 100 mg chlorine/L). The sample is agitated for 1 hour, while adding more calcium hypochlorite if necessary to maintain the chlorine concentration. After agitating for 1 hour, residual chlorine is removed by the dropwise addition of sodium arsenite solution (2g/100 mL) or by the addition of 8 drops of hydrogen peroxide (3% solution) followed by 4 drops of sodium thiosulfate (500 g/L). The sample is tested with potassium iodide-starch paper by adding a drop or two of sample to the paper. The dechlorinating solutions are to be added until there is no color change. Both the chlorinated and unchlorinated samples are distilled as described in 4500-CN C. The samples are tested according to the procedures in SM 4500-CN D (titrimetric), E (colorimetric) or F (cyanide-selective electrode).

Special Considerations: Samples should be protected from exposure to UV radiation. All sample manipulations should be performed under incandescent light, to prevent photodecomposition of some metal-cyanide complexes by UV light. Some unidentified organic chemicals may oxidize or form breakdown products during chlorination, giving higher results for cyanide after chlorination than before chlorination. This may lead to a negative value for cyanides amenable to chlorination after distillation for wastes from, for example, the steel industry, petroleum refining, and pulp and paper processing. Where such interferences are encountered use SM 4500-CN I for determining dissociable cyanide.

Source: APHA, AWWA and WEF. 2011. "Method 4500-CN G: Cyanides Amenable to Chlorination after Distillation." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: APHA. http://www.standardmethods.org/

5.2.98 Standard Method 4500-NH_3 B: Nitrogen (Ammonia) Preliminary Distillation Step

Analyte(s)	CAS RN
Ammonia	7664-41-7

Analysis Purpose: Sample preparation
Sample Preparation Technique: Distillation
Determinative Technique: Visible spectrophotometry
Determinative Method: Standard Method 4500-NH_3 G

Method Developed for: Nitrogen (ammonia) in drinking waters, clean surface or groundwater, and good-quality nitrified wastewater effluent
Method Selected for: This method has been selected for preparation of non-drinking water samples to address ammonia. See Appendix A for the corresponding method usability tier.

Description of Method: A 0.5- to 1-L sample is dechlorinated, buffered, adjusted to pH 9.5, and distilled into a sulfuric acid solution. The distillate is brought up to volume, neutralized with sodium hydroxide, and analyzed by SM 4500-NH_3 G.

Source: APHA, AWWA and WEF. 2011. "Method 4500-NH3 B: Nitrogen (Ammonia) Preliminary Distillation Step." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: APHA. http://www.standardmethods.org/

5.2.99 Standard Method 4500-NH_3 G: Nitrogen (Ammonia) Automated Phenate Method

Analyte(s)	CAS RN
Ammonia	7664-41-7

Analysis Purpose: Analyte determination and measurement
Determinative Technique: Visible spectrophotometry
Sample Preparation Method: Standard Method 4500-NH_3 B
Sample Preparation Technique: Distillation

Method Developed for: Nitrogen (ammonia) in drinking waters, clean surface or groundwater, and good-quality nitrified wastewater effluent
Method Selected for: This method has been selected for analysis of non-drinking water samples to address ammonia. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: The range of the method in drinking water, surface, and domestic and industrial wastewaters is 0.02–2.0 mg/L.

Description of Method: Ammonia is determined as indophenol blue by this method. A portion of the neutralized sample distillate (from procedure 4500-NH_3 B [Section 5.2.98]) is run through a manifold. The ammonium in the distillate reacts with solutions of disodium ethylenediaminetetraacetic acid (EDTA), sodium phenate, sodium hypochlorite and sodium nitroprusside. The resulting indophenol blue is detected by colorimetry in a flow cell. Photometric measurement is made between the wavelengths of 630 and 660 nm.

Special Considerations: Remove interfering turbidity by filtration. Color in the samples that absorbs in the photometric range (630–660 nm) can interfere with analysis.

Source: APHA, AWWA and WEF. 2011. "Method 4500-NH_3 G: Nitrogen (Ammonia) Automated Phenate Method." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: APHA. http://www.standardmethods.org/

5.2.100 Standard Method 4500-Cl G: Chlorine (Residual) DPD Colorimetric Method

Analyte(s)	CAS RN
Chlorine	7782-50-5

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Water samples are buffered and colorimetric agent is added. Buffered water extraction by Analyst, 1999. 124: 1853-1857 is used for preparation of air samples.
Determinative Technique: Visible spectrophotometry

Method Developed for: Chlorine in water and wastewater
Method Selected for: This method has been selected for preparation and analysis of water samples to address chlorine. It also has been selected for analysis of air samples when appropriate sample preparation techniques have been applied. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The method can detect 10 µg/L chlorine.

Description of Method: A 10-mL portion of buffered aqueous sample is reacted with N,N-diethyl-*p*-phenylenediamine (DPD) color agent. The resulting free chlorine is determined by colorimetry. If total chlorine (including chloramines and nitrogen trichloride) is to be determined, potassium iodide crystals are added. Results for chromate and manganese are blank corrected using thioacetamide solution.

Special Considerations: Organic contaminants and strong oxidizers may cause interference. Color and

turbidity in the sample can cause interference and can be compensated for by first zeroing the photometer using the sample. Chromate interferences are minimized by using thioacetamide blank correction.

Source: APHA, AWWA and WEF. 2011. "Method 4500-Cl G: DPD Colorimetric Method." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: APHA. http://www.standardmethods.org/

5.2.101 Literature Reference for Chlorine in Air (Analyst, 1999. 124(12): 1853-1857)

Analyte(s)	CAS RN
Chlorine	7782-50-5

Analysis Purpose: Sample preparation
Sample Preparation Technique: Buffered water extraction
Determinative Technique: Visible spectrophotometry
Determinative Method: Standard Method 4500-Cl G

Method Developed for: Active chlorine in air
Method Selected for: This method has been selected for preparation of air samples to address chlorine. See Appendix A for the corresponding method usability tier.
Detection and Quantitation: Detection limit of 0.1 µg of chlorine; the collection efficiency was >90%; recovery of chlorine spikes from 0.05-g aliquots of the sorbent was not quantitative (~60%) but was reproducible.

Description of Method: A procedure is described for determination of total combined gas-phase active chlorine (i.e., molecular chlorine, hypochlorous acid, and chloramines) and is based on a sulfonamide-functionalized silica gel sorbent. For determination of the collected chlorine, a modified version of the DPD colorimetric procedure is used, which yielded a detection limit of 0.1 µg of chlorine. At flow rates ranging from 31 to 294 mL/minute, the collection efficiency was >90% based on breakthrough analysis. Recovery of chlorine spikes from 0.05-g aliquots of the sorbent was not quantitative (~60%) but was reproducible; the recovery is accounted for in samples by adding weighed amounts of sorbent to the standards.

Source: Johnson, B.J., Emerson, D.W., Song, L., Floyd, J. and Tadepalli, B. 1999. "Determination of Active Chlorine in Air by Bonded Phase Sorbent Collection and Spectrophotometric Analysis." Analyst. 124(12): 1853-1857. http://pubs.rsc.org/en/content/articlelanding/1999/an/a906305f

5.2.102 Literature Reference for Hexamethylenetriperoxidediamine (HMTD) (Analyst, 2001. 126:1689-1693)

Analyte(s)	CAS RN
Hexamethylenetriperoxidediamine (HMTD)	283-66-9

Analysis Purpose: Analyte determination and measurement
Sample Preparation Technique: SW-846 Methods 8330B/3535A (solid samples and water samples), and 3570/8290A Appendix A (wipe samples). Refer to Appendix A for which of these preparation methods should be used for a particular analyte/sample type combination.
Determinative Technique: LC-MS-MS

Method Developed for: Trace quantities of HMTD in explosives or explosive mixtures
Method Selected for: This method has been selected for analysis of solid, water and wipe samples to

address HMTD. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The LOD is 20 µg/L.

Description of Method: Prepared samples are analyzed by positive mode atmospheric pressure chemical ionization (APCI) LC-MS-MS using a C_{18} analytical column (150 mm × 2.0 mm inner diameter [I.D.], 5µm particle size) coupled with a C_{18} guard cartridge system (10 mm × 2.0 mm I.D.). Elution using a 95/5 water/methanol solution detects HMTD at m/z = 209 and a retention time of ~ 15.5 minutes.

Special Considerations: The procedure has been developed for the determination of HMTD in explosives or explosive mixtures; modifications will be needed for application to environmental samples such as soils, wipes and water samples. Until modifications can be developed and tested, it is recommended that the procedures described in SW-846 Methods 8330B and 3535A (Sections 5.2.40 and 5.2.21) be used to prepare solid and water samples, and the procedures described in SW-846 Methods 3570 and 8290A Appendix A (Sections 5.2.24 and 5.2.36) be used to prepare wipe samples.

Source: Crowson, A. and Berardah, M.S. 2001. "Development of an LC/MS Method for the Trace Analysis of Hexamethylenetriperoxidediamine (HMTD)." Analyst 126(10): 1689-1693. http://pubs.rsc.org/en/Content/ArticleLanding/2001/AN/b107354k

5.2.103 Literature Reference for Methamidophos (Chromatographia. 2006. 63(5/6): 233-237)

Analyte(s)	CAS RN
Acephate	30560-19-1
Methamidophos	10265-92-6

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: SPE
Determinative Technique: LC-MS-MS

Method Developed for: Pesticides (methamidophos) in water samples
Method Selected for: This method has been selected for preparation and analysis of non-drinking water samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The LOD is 30 µg/L.

Description of Method: A multi-residue analytical method is described for monitoring polar pesticides, such as acephate and methamidophos, in water with SPE and LC-MS-MS. Samples are analyzed using a C_{18} analytical column (150 mm × 3.2 mm I.D., 5µm particle size) coupled with a C_{18} guard cartridge system (4 mm × 3.0 mm I.D.).#

Source: Liu, F., Bischoff, G., Pestemer, W., Xu, W. and Kofoet, A. 2006. "Multi-residue Analysis of Some Polar Pesticides in Water Samples With SPE and LC/MS/MS." Chromatographia. 63(5/6): 233-237. http://www.springerlink.com/content/gg87150ll26390x6/

5.2.104 Literature Reference for Cyanogen Chloride (Encyclopedia of Anal. Chem. 2006 DOI: 10.1002/9780470027318.a0809)

Analyte(s)	CAS RN
Cyanogen chloride	506-77-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Purge-and-trap, headspace, liquid-liquid microextraction
Determinative Technique: GC-MS, GC-ECD

Method Developed for: Determination of cyanogen chloride in drinking water
Method Selected for: This method has been selected for preparation and analysis of water and solid samples to address cyanogen chloride. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: In drinking water, the MDL is 0.13 µg/L when using purge-and-trap GC-MS or liquid-liquid microextraction GC-ECD, and 0.04 µg/L when using headspace GC-ECD.

Description of Method: The method describes three different sample preparation techniques (purge-and-trap, headspace and micro liquid-liquid extraction) and two different determinative techniques (GC-MS and GC-ECD). Using the purge-and-trap technique, cyanogen chloride and an internal standard are extracted (purged) from the sample matrix by bubbling an inert gas through the sample. Purged sample components are trapped in a tube containing suitable sorbent materials. When purging is complete, the sorbent tube is heated. Simultaneously, a short piece of deactivated fused silica precolumn is cooled with liquid nitrogen to refocus the analytes. The cryotrap is heated to inject the sample onto a GC-MS.

For headspace GC-ECD analyses, a 40-mL vial is filled with sample without headspace. With the vial upside down, a volume of nitrogen is forced into the sample using a syringe, and an equivalent sample volume is dispelled through a second syringe. The sample is shaken by hand and, after settling, a volume of the headspace is sampled by syringe and injected into a split-mode GC-ECD. For liquid-liquid microextraction GC-ECD analyses, 30 mL of water sample is extracted in a 40-mL vial, with 10 g of sodium sulfate, 4 mL of MTBE and an internal standard. The sample is shaken by mechanical shaker or by hand. After allowing the phases to separate, the MTBE layer is transferred to another vial and injected into a GC-ECD.

Special Considerations: This procedure has been developed for water samples; modifications may be needed for application to environmental samples such as solid samples.

Source: Xie, Y. 2006. "Cyanogen Chloride and Cyanogen Bromide Analysis in Drinking Water." Encyclopedia of Analytical Chemistry. 1-11.
http://onlinelibrary.wiley.com/doi/10.1002/9780470027318.a0809/abstract

5.2.105 Literature Reference for 3-Chloro-1,2-propanediol (Eur. J. Lipid Sci. Technol. 2011. 113: 345-355)

Analyte(s)	CAS RN
3-Chloro-1,2-propanediol	96-24-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction, followed by SPE cleanup and derivatization
Determinative Technique: GC-MS

Method Developed for: Trace quantities of 3-chloro-1,2-propanediol in foodstuffs
Method Selected for: This method has been selected for preparation and analysis of solid and wipe samples to address 3-chloro-1,2-propanediol. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The low calibration standard is 5 µg/L. The MDL in food ranges from 4 to 16 µg/kg. The working range is 4–4,000 µg/kg.

Description of Method: Foodstuffs (olive oil, cereal and potato products) are solvent extracted with hexane/diethyl ether and centrifuged. The resulting organic layer is washed several times (by adding water, vortexing and then centrifuging), then dried with sodium sulfate. The extract is concentrated to dryness, and redissolved in tetrahydrofuran (THF) to which acidified methanol is added. The reaction mixture is neutralized with sodium bicarbonate and washed with 3 aliquots of hexane, and the residue is quantitatively transferred to a sodium chloride solution. This solution is mixed with the contents of a highly pure diatomaceous earth based solid phase refill sachet, transferred to a chromatography column, and then eluted with diethyl ether. The collected eluent is concentrated by rotary evaporation and derivatized with heptafluorobutyrylimidazole (HFBI) at 70°C for 15–20 minutes. After washing with water, the extracts are analyzed using a GC-MS.

Special Considerations: The procedure has been developed for the determination of 3-chloro-1,2-propanediol in foodstuffs only; modifications may be needed for application to environmental samples.

Source: Hamlet, C. G. and Asuncion, L. 2011. "Single-Laboratory Validation of a Method to Quantify Bound 2-Chloropropane-1,3-diol and 3-Chloropropane-1,2-diol in Foodstuffs Using Acid Catalysed Transesterification, HFBI Derivatisation and GC/MS Detection." Eur. J. Lipid Sci. Technol. 113(3): 345-355. http://onlinelibrary.wiley.com/doi/10.1002/ejlt.v113.3/issuetoc

5.2.106 Literature Reference for Methyl Hydrazine (Journal of Chromatography. 1993. 617(1): 157-162)

Analyte(s)	CAS RN
Methyl hydrazine	60-34-4

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: SW-846 Method 3541/3545 (for solids), SW-846 Methods 3570/8290 Appendix A (for wipes), filtration for water samples, followed by derivatization for all sample types
Determinative Technique: HPLC-UV

Method Developed for: Determination of hydrazine in human plasma
Method Selected for: This method has been selected for preparation and analysis of water samples, and for the analysis of solid and wipe samples to address methyl hydrazine. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Detection limit in pooled plasma is 1 µg/L. The reporting range is 5–1000 µg/L.

Description of Method: Samples are prepared in a single-step reaction by protein denaturation with trichloroacetic acid, and derivatization to a stable azine with 4-hydroxybenzaldehyde. Chromatographic separation is carried out on a reversed-phase (octadecylsilane) column with methanol:water (60:40) as the mobile phase and UV detection at 340 nm. Retention time of the azine derivative of methyl hydrazine is 3.5 minutes.

Special Considerations: This procedure has been developed for human plasma; modifications may be needed for application to environmental samples such as water, solid and wipe samples.

Source: Kircherr, H. 1993. "Determination of Hydrazine in Human Plasma by High Performance Liquid Chromatography." Journal of Chromatography B. 617(1): 157-162.
http://www.sciencedirect.com/science/article/pii/0378434793804368

5.2.107 Literature Reference for 3-Chloro-1,2-propanediol (Journal of Chromatography A. 2000. 866(1): 65-77)

Analyte(s)	CAS RN
3-Chloro-1,2-propanediol	96-24-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction followed by derivatization
Determinative Technique: GC-ECD

Method Developed for: Determination of 3-chloro-1,2-propanediol in water
Method Selected for: This method has been selected for preparation and analysis of water samples to address 3-chloro-1,2-propanediol. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDL is 0.73 µg/L. The reporting range is 11–169 µg/L.

Description of Method: Sodium sulfate, sodium bisulfate and a surrogate are added to a 5-mL sample and extracted twice with 5 mL of ethyl acetate. The two ethyl acetate extracts are combined and concentrated to 50 µL under nitrogen evaporation. Then 100 µL of acetonitrile is added, and the solution is mixed and transferred to a drying column containing sodium sulfate. An additional 100 µL of acetonitrile is used to rinse the sample vial and the rinse is transferred to the drying column. After letting the sample sit on the column for 10 minutes, it is eluted with 2 mL of acetonitrile. The dried extract is derivatized by adding 50 µL of heptafluorobutyric anhydride (HFBA) and heating at 75°C for 30 minutes. The derivatized sample is extracted with water, then hexane, followed by a saturated sodium bicarbonate solution. The aqueous layer is discarded, and the hexane layer is washed twice with sodium bicarbonate solution and shaken for 30 seconds. The hexane extract is then transferred to a GC vial and analyzed by GC-ECD with a DB5-MS column.

Special Considerations: The procedure has been tested for reagent grade water and seawater; modifications may be needed for application to environmental samples. The presence of 3-chloro-1,2-propanediol should be confirmed using either a secondary GC column or an MS.

Source: Matthew, B.M. and Anastasio, C. 2000. "Determination of Halogenated Mono-alcohols and Diols in Water by Gas Chromatography With Electron-Capture Detection." Journal of Chromatography A. 866(1): 65-77. http://www.sciencedirect.com/science/article/pii/S002196739901081X

5.2.108 Literature Reference for Fluoroacetic Acid/Fluoroacetate Salts/Methyl Fluoroacetate (Journal of Chromatography A. 2007. 1139: 271-278)

Analyte(s)	CAS RN
Fluoroacetic acid and fluoroacetate salts (analyze as fluoroacetate ion)	NA
Methyl fluoroacetate (analyze as fluoroacetate ion)	453-18-9

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Water extraction followed by SPE cleanup and derivatization for solid and wipe samples. Use NIOSH Method S301-1 for air samples.
Determinative Technique: LC-MS

Method Developed for: Determination of fluoroacetate in food
Method Selected for: This method has been selected for preparation and analysis of solids and wipes and for the analysis of air samples to address the analytes listed in the table above as fluoroacetate ion. See Appendix A for corresponding method usability tiers.

Detection and Quantitation: The LOD is 0.8 µg/L. The calibration range is 20–10,000 µg/L.

Description of Method: The method utilizes a water extraction, SPE cleanup, and LC-MS for determination of fluoroacetate as monofluoroacetate (MFA). SPE is performed using C_{18} cartridges. The LC-MS system utilizes a C_{18} column and the MS is operated in APCI negative mode. If significant interferences are observed, the method describes a qualitative procedure that can be used to confirm the presence of fluoroacetate. The sample is first prepared as described in the quantitative method. Then an aliquot is derivatized by adding 2-nitrophenylhydrazine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and pyridine buffer, and heating at 65°C for 15 minutes. The extract is then cleaned by putting it through a C_{18} cartridge. The extracts are then blown to dryness, reconstituted in 2 mL of water/methanol (20/80), and filtered through a 0.2 µm filter. Analysis of the cleaned extract is performed on an LC-MS using a C_8 column and gradient elution, beginning with 25% methanol for the first 3 minutes, followed by 80% methanol over the next 10 minutes. A post run equilibration (7 minutes) is used prior to the next injection.

Special Considerations: This procedure has been developed for food; modifications may be needed for application to environmental samples such as solid and wipe samples. In addition, the air filter extraction procedure (described in NIOSH Method S301-1) was not developed for the LC-MS-MS detector, and it may be necessary to alter the extraction method if interferences arising from the extraction are observed.

Source: Noonan, G.O., Begley, T.H. and Diachenko, G.W. 2007. "Rapid Quantitative and Qualitative Confirmatory Method for the Determination of Monofluoroacetic Acid in Foods by Liquid Chromatography–Mass Spectrometry." Journal of Chromatography A. 1139: 271-278. http://www.sciencedirect.com/science/article/pii/S0021967306021388

5.2.109 Literature Reference for Methamidophos (Journal of Chromatography A. 2007. 1154: 3-25)

Analyte(s)	CAS RN
Acephate	30560-19-1
Methamidophos	10265-92-6

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction
Determinative Technique: LC-MS-MS

Method Developed for: Pesticides (methamidophos) in crops
Method Selected for: This method has been selected for preparation and analysis of solid, air and wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The LOD for this method is 0.01 mg/kg.

Description of Method: An LC-MS-MS multi-residue method for the simultaneous target analysis of a wide range of pesticides and metabolites in fruit, vegetables and cereals is described. Gradient elution has been used in conjunction with ESI+ tandem mass spectrometry to detect up to 171 pesticides and/or metabolites in different crop matrices using a single chromatographic run. Pesticide residues are extracted/partitioned from the samples with acetone/dichloromethane/light petroleum. Samples are analyzed by LC-MS-MS using a C_{18} analytical column (150 mm × 3.2 mm I.D., 5µm particle size) coupled with a C_{18} guard cartridge system (4 mm × 3.0 mm I.D.).

Special Considerations: The procedure has been developed for the analysis of various pesticides (methamidophos) in crops using LC-MS-MS; modifications will be needed for application to

environmental samples such as soils, wipes and air samples collected on sorbent/filters. If problems occur when using this method to analyze for methamidophos in air samples, NIOSH Method 5600 (Section 5.2.64) should be used.

Source: Hiemstra, M. and de Kok, A. 2007. "Comprehensive Multi-residue Method for the Target Analysis of Pesticides in Crops Using Liquid Chromatography-Tandem Mass Spectrometry." Journal of Chromatography A. 1154(1): 3-25. http://www.sciencedirect.com/science/article/pii/S0021967307005845

5.2.110 Literature Reference for Paraquat (Journal of Chromatography A. 2008, 1196-1197, 110-116)

Analyte(s)	CAS RN
Paraquat	4685-14-7

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Extraction by digestion, shaking or microwave-assisted extraction (MAE) followed by SPE cleanup
Determinative Technique: LC-UV or LC-MS-MS

Method Developed for: Determination of quaternary ammonium herbicides in soil
Method Selected for: This method has been selected for preparation and analysis of solid and wipe samples to address paraquat. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Limits of detection are 10 µg/kg by digestion and 50 µg/kg by MAE when using LC-UV and 1.0 µg/kg by digestion and 3.0 µg/kg by MAE when using LC-MS-MS. EQLs are 20 µg/kg and 100 µg/kg when using LC-UV and 2.0 µg/kg by digestion and 7.5µg/kg by MAE when using LC-MS-MS.

Description of Method: Soil matrices can be extracted using one of the following three procedures: (1) digestion with an acidic methanol/EDTA solution, (2) shaking in an EDTA/ammonium formate solution, or (3) using a MAE system in a benzalkonium chloride/acid solution. Cleanup of extracts is performed by SPE using silica cartridges for all three extraction procedures. Detection of these herbicides is carried out by either LC-UV or LC-MS-MS.

Special Considerations: This procedure has been developed for soil samples; modifications may be needed for application to environmental samples such as wipes samples.

Source: Pateiro-Moure, M., Martínez-Carballo, E., Arias-Estévez, M. and Simal-Gándara, J. 2008. "Determination of Quaternary Ammonium Herbicides in Soils. Comparison of Digestion, Shaking and Microwave-Assisted Extractions." Journal of Chromatography A. 1196-1197, 110-116. http://www.sciencedirect.com/science/article/pii/S0021967308005335

5.2.111 Literature Reference for Fentanyl (Journal of Chromatography A. 2011. 1218: 1620-1649)

Analyte(s)	CAS RN
Fentanyl	437-38-7

Analysis Purpose: Analyte determination and measurement
Sample Preparation Technique: SW-846 Methods 3541 and 3545A (solid samples), 3520C and 3535A (water samples), and 3570 and 8290A Appendix A (wipes)
Determinative Technique: LC-MS-MS

Method Developed for: Fentanyl in wastewater and surface water
Method Selected for: This method has been selected for analysis of water, solid and wipe samples to address fentanyl. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: MDLs in surface water and wastewater (effluent and influent) are 0.05 and 0.2 ng/L, respectively. Method quantitation limits in surface water, wastewater effluent and wastewater influent are 0.10, 0.6 and 0.7 ng/L, respectively. The reportable range for fentanyl in surface water is 0.08–750 ng/L.

Description of Method: Water samples are vacuum filtered, first through two sequential glass fiber filters (2.7 µm, then 0.7 µm). After filtration, samples are acidified with 31 % hydrochloric acid to pH 1.8–1.9. The SPE cartridge (60 mg) is conditioned with 2 mL of methanol and equilibrated with 2% formic acid in water (2 mL, pH 2), both at a flow rate of 3 mL/minute. Acidified samples are spiked with 50 ng of each surrogate and internal standard and then passed through the cartridge at a rate of 6 mL/minute. Immediately following loading, cartridges are washed with 2% formic acid in water (2 mL at pH 2) at a flow rate of 3 mL/minute, then washed again with 2 mL of 0.6% formic acid in methanol (pH 2) at a flow rate of 3 mL/minute, followed by elution with 3 mL of 7% ammonium hydroxide in methanol at a flow rate of 1 mL/minute into silanized vials. Extracts are evaporated to dryness under nitrogen, reconstituted with 500 µL of 0.3% acetic acid/5% methanol in reagent-grade water, and filtered through 0.2 µm polytetrafluoroethylene (PTFE) filters before being transferred to deactivated maximum recovery vials with PTFE septa. Extracts are analyzed by LC-MS-MS equipped with an ethylene-bridged hybrid (BEH) column.

Source: Baker, D. and Kaxprzyk-Hordern, B. 2011. "Multi-residue analysis of drugs of abuse in wastewater and surface water by solid-phase extraction and liquid chromatography-positive electrospray ionisation tandem mass spectrometry." Journal of Chromatography A. 1218(12): 1620-1659. http://www.sciencedirect.com/science/article/pii/S0021967311001312

5.2.112 Literature Reference for BZ (Journal of Chromatography B. 2008. 874: 42–50.

Analyte(s)	CAS RN
BZ [Quinclidinyl benzylate]	6581-06-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Direct injection for water samples, SW-846 Methods 3541 or 3545 for solid samples, and SW-846 Methods 3570 and 8290A Appendix A for wipes.
Determinative Technique: LC-MS-MS

Method Developed for: Benzodiazepines in human plasma
Method Selected for: This method has been selected for preparation and analysis of water samples and for the analysis of prepared solid and wipe samples to address BZ. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The limits of detection for the benzodiazepines in human plasma ranged from 0.1 to 1 ng/mL, the LOQs ranged from 0.25 to 5 ng/mL, and the working range for the benzodiazepines in human plasma is 0.25–1000 ng/mL (depending on the analyte). This method was developed for compounds similar in structure to BZ; therefore, no detection or quantification information is available for BZ.

Description of Method: Water samples are filtered and directly injected into an LC-MS-MS for analysis. Soil samples are extracted by PFE or Soxhlet extraction using SW-846 Method 3541/3545A (Sections 5.2.22 and 5.2.23), prior to filtration and injection into the LC-MS-MS. Wipe samples are solvent extracted prior to filtration and injection into the LC-MS-MS. In all cases, an internal standard is

added prior to extraction or filtration. The triple quadrupole LC-MS-MS is equipped with a C_8 column and operated in positive ion mode with MRM. Instrument parameters specific for BZ can be found in Schaer, 2012 (see Additional Resource).

Special Considerations: The procedure has been developed for the analysis of benzodiazepines in human plasma; modifications will be needed for application to BZ in environmental samples. An overview of a strategy for detection and identification of BZ, including information regarding ESI-source, fragmentation, precursor-ion, collision energies, and scanning time, is provided in a poster presentation cited in the Additional Resource cited below.

Source: Abbara, C., Bardot, I., Cailleux, A., Lallement, G., Le Bouil, A., Turcant, A., Clair, P. and Diquet, B. 2008. "High-performance liquid chromatography coupled with electrospray tandem mass spectrometry (LC/MS/MS) method for the simultaneous determination of diazepam, atropine and pralidoxime in human plasma." Journal of Chromatography B. 874: 42-50. http://www.sciencedirect.com/science/article/pii/S1570023208006545

Additional Resource: Schaer, M. 2012. "Rapid Screening and Identification of Chemical Warfare Agents in Environmental Samples using LC/MS and a MS/MS-Library." Spiez Laboratory. https://www.labor-spiez.ch/pdf/en/dok/fas/LC-MS_Screening_and_Identification_of_CWA_in_Environmental_Samples_using_LCMS_and_a_MSMS-Library_2008.pdf

5.2.113 Literature Reference for Fluoroacetamide (Journal of Chromatography B. 2008. 876(1): 103-108)

Analyte(s)	CAS RN
Fluoroacetamide	640-19-7

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Water extraction
Determinative Technique: GC-MS

Method Developed for: Fluoroacetamide and tetramine in blood, urine and stomach contents
Method Selected for: This method has been selected for preparation and analysis of solid, water, air and wipe samples to address fluoroacetamide. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit of this method for fluoroacetamide is 0.01 µg/g.

Description of Method: Samples are extracted by microscale liquid-liquid extraction using acetonitrile, ENVI-Carb™ (Sigma-Aldrich, St. Louis, MO) and sodium chloride. Samples are analyzed by GC-MS using a 30-m DB-5MS capillary column (or equivalent) coupled with a 1.5 m Innowax capillary column (or equivalent) by a quartz capillary column connector. If analyzing for fluoroacetamide alone, only the Innowax capillary column is needed.

Special Considerations: The procedure has been developed for the analysis of fluoroacetamide and tetramine in blood, urine and stomach fluid samples; modifications will be needed for application to environmental samples.

Source: Xu, X., Song, G., Zhu, Y., Zhang, J., Zhao, Y., Shen, H., Cai, Z., Han, J. and Ren, Y. 2008. "Simultaneous Determination of Two Acute Poisoning Rodenticides Tetramine and Fluoroacetamide With a Coupled Column in Poisoning Cases." Journal of Chromatography B. 876(1): 103-108. http://www.sciencedirect.com/science/article/pii/S1570023208007757

5.2.114 Literature Reference for Fluoroacetic Acid/Fluoroacetate Salts/Methyl Fluoroacetate (Journal of Chromatography B. 2010. 878: 1045-1050)

Analyte(s)	CAS RN
Fluoroacetic acid and fluoroacetate salts (analyze as fluoroacetate ion)	NA
Methyl fluoroacetate (analyze as fluoroacetate ion)	453-18-9

Analysis Purpose: Sample preparation (water only), and analyte determination and measurement
Sample Preparation Technique: SPE using 96-well plates (see Special Considerations)
Determinative Technique: LC-MS-MS
Method Developed for: Determination of fluoroacetate in urine
Method Selected for: This method has been selected for preparation and analysis of water samples to address the analytes listed in the table above as fluoroacetate ion. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit for fluoroacetate in human urine is 0.9 µg/L. The reporting range is 50–5000 µg/L.

Description of Method: Fluoroacetate, in the form of MFA, is extracted from urine by placing aliquots onto a 96-well plate and performing SPE using hydrophilic-lipophilic-balanced (HLB) reverse-phase sorbent plates (or equivalent). The extracts are then separated with isocratic high-performance liquid chromatography with a reverse-phase analytical column with embedded basic ion-pairing groups. Target compounds are identified using ESI tandem mass spectrometry. The retention time, when using the conditions described in this journal article, is expected to be ~1.4 minutes.

Special Considerations: This procedure has been developed for urine samples; modifications may be needed for application to environmental samples. For drinking water samples and relatively clean aqueous samples, direct injection may be suitable. If the laboratory has the capability of performing IC-MS, they may consider using the following method, which has been developed specifically for water samples: http://www.dionex.com/en-us/webdocs/110767-AN276-IC-MS-Flouroacetate-Water-AN70478_E.pdf

Source: Hamelin, E., Mawhinney, D.B., Parry R. and Kobelski, R.J. 2010. "Quantification of Monofluoroacetate and Monochloroacetate in Human Urine by Isotope Dilution Liquid Chromatography Tandem Mass Spectrometry." Journal of Chromatography B. 878(15-16): 1045-1050. http://www.sciencedirect.com/science/article/pii/S157002321000142X

5.2.115 Literature Reference for Carfentanil and 3-Methyl Fentanyl (J. Chromatogr. B. 2014. 962: 52-58

Analyte(s)	CAS RN
Carfentanil	59708-52-0
3-Methyl fentanyl	42045-87-4

Analysis Purpose: Analyte determination and measurement
Sample Preparation Technique: SW-846 Methods 3541 and 3545A (solid samples), 3520C and 3535A (water samples), and 3570 and 8290A Appendix A (wipe samples)
Determinative Technique: LC-MS-MS

Method Developed for: Carfentanil, fentanyl and 3-methyl fentanyl in human urine
Method Selected for: This method has been selected for analysis of water, solid and wipe samples to address the analytes listed in the table above. See Appendix A for corresponding method usability tiers.

Detection and Quantitation: LODs for carfentanil, fentanyl and 3-methyl fentanyl in human urine when using off-line SPE are estimated to be 0.008, 0.007 and 0.020 ng/mL, respectively. The reportable range is 0.010–10 ng/mL for carfentanil and fentanyl, and 0.050–10 ng/mL for 3-methyl fentanyl.

Description of Method: Carfentanil, fentanyl and 3-methylfentanyl are extracted from samples by placing aliquots off-line on a 96-well plate or using an on-line SPE system. In both cases, internal standards are added to sample aliquots prior to loading onto the SPE cartridge. For on-line SPE, cartridges are conditioned with acetonitrile and aqueous ammonium hydroxide solutions. Sample aliquots are loaded onto the SPE unit, washed with aqueous ammonium hydroxide:acetonitrile solution and eluted with LC gradient onto the HPLC column. When using the 96-well plate, sample aliquots are diluted with aqueous ammonium hydroxide and loaded onto a plate conditioned with acetonitrile and aqueous ammonium hydroxide solutions, washed with a water:acetonitrile:ammonium hydroxide mixture, and eluted with acetonitrile. The extracts are evaporated to dryness, reconstituted in water and injected into the HPLC for analysis. Target compounds are identified using ESI tandem mass spectrometry. The retention time for all three compounds is expected to be ~3.4 minutes. The analytes can be measured as individual compounds due to the use of different monitoring ions.

Special Considerations: This procedure has been developed for urine samples; modifications may be needed for application to environmental samples.

Source: Shaner, R. L., Kaplan, P., Hamelin, E. I., William A. Bragg, W. A., Johnson, R. C. 2014. "Comparison of two automated solid phase extractions for the detection of ten fentanyl analogs and metabolites in human urine using liquid chromatography tandem mass spectrometry." Journal of Chromatography B. 962: 52-55.
http://www.sciencedirect.com/science/article/pii/S157002321400333X?via%3Dihub

5.2.116 Literature Reference for Sodium Azide (Journal of Forensic Sciences. 1998. 43(1): 200-202)

Analyte(s)	CAS RN
Sodium azide (analyze as azide ion)	26628-22-8

Analysis Purpose: Sample preparation
Sample Preparation Technique: Water extraction, filtration and/or acidification
Determinative Technique: IC with conductivity detection
Determinative Method: EPA Method 300.1, Revision 1.0

Method Developed for: Sodium azide in blood
Method Selected for: This method has been selected for preparation of solid, water samples to address sodium azide as azide ion. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: This method can routinely quantify to at least 100 µg/L, and the detection limit is estimated to be 30 µg/L.

Description of Method: Samples are analyzed by IC using suppressed conductivity detection. Water extraction and filtration steps should be used for the preparation of solid samples. Filtration steps should be used for preparation of aqueous liquid and drinking water samples.

Special Considerations: The procedure was developed for analysis of sodium azide in blood samples.

Source: Kruszyna, R., Smith, R.P. and Kruszyna, H. 1998. "Determining Sodium Azide Concentration in the Blood by Ion Chromatography." Journal of Forensic Sciences. 43(1): 200-202.
http://www.astm.org/DIGITAL_LIBRARY/JOURNALS/FORENSIC/PAGES/JFS16113J.htm

Section 6.0: Selected Radiochemical Methods

A list of analytical methods to be used in analyzing environmental and outdoor building and infrastructure material samples for radiochemical contaminants following a contamination incident is provided in Appendix B. Methods are listed for each isotope and for each sample type that potentially may need to be measured and analyzed when responding to a radiological or nuclear incident. The isotopes included are based on selection criteria that address the needs and priorities of EPA as well as other federal agencies (see Section 1.0).

Please note: This section provides guidance for selecting radiochemical methods that have a high likelihood of assuring analytical consistency when laboratories are tasked with analyzing samples following a large scale radiological or nuclear contamination incident. Although the majority of methods have been validated for the analyte/sample type combination for which they have been selected, validation is still needed for a few of the methods that have been selected for analysis of vegetation. Please refer to the specified method to identify analyte/sample type combinations that have been verified. Any questions regarding information discussed in this section should be addressed to the appropriate contact(s) listed in Section 4.0.

Appendix B1 (environmental samples) is sorted alphabetically by analyte and includes the following information:

- **Analyte(s).** The radionuclide(s) or contaminant(s) of interest.
- **Chemical Abstracts Service Registry Number (CAS RN).** A unique identifier for chemical substances that provides an unambiguous way to identify a chemical or molecular structure when there are many possible systematic, generic or trivial names. In this section (Section 6.0) and Appendix B, the CAS RNs correspond to the specific radionuclide identified.
- **Determinative technique.** An analytical instrument or technique used for qualitative and confirmatory determination of compounds or components in a sample.
- **Drinking water sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in drinking water samples. Methods have been identified for qualitative and confirmatory determination.
- **Aqueous and liquid phase sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in aqueous and/or non-aqueous liquid phase samples. Methods have been identified for qualitative and confirmatory determination.
- **Soil and sediment phase sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in soil and sediment samples. Methods have been identified for qualitative and confirmatory determination.
- **Surface wipe sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in surface wipe samples. Methods have been identified for qualitative and confirmatory determination.
- **Air filter sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in air filter samples. Methods have been identified for qualitative and confirmatory determination.
- **Vegetation sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in vegetation (i.e., grasses, leaves, trees, etc.) not intended for human consumption. Methods have been identified for qualitative and confirmatory determination.
- **Qualitative determination method identifier.** A unique identifier or number assigned to an analytical method by the method publisher. The identified method is intended to determine the

presence of a radionuclide. Although quantitative, these methods have been selected as qualitative methods since they can be utilized with shorter counting times, at greater uncertainty, when increased sample throughput and more rapid reporting of results are required.

- **Confirmatory method identifier.** A unique identifier or number assigned to an analytical method by the method publisher. The identified method is for measurement of the activity from a particular radionuclide per unit of mass, volume or area sampled.

Appendix B2 (outdoor building and infrastructure materials) is sorted alphabetically by analyte and includes the following information:

- **Analyte(s).** The radionuclide(s) or contaminant(s) of interest.
- **Chemical Abstracts Service Registry Number (CAS RN).** A unique identifier for chemical substances that provides an unambiguous way to identify a chemical or molecular structure when there are many possible systematic, generic or trivial names. In this section (Section 6.0) and Appendix B, the CAS RNs correspond to the specific radionuclide identified.
- **Determinative technique.** An analytical instrument or technique used for qualitative and confirmatory determination of compounds or components in a sample.
- **Asphalt shingle sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in asphalt roofing materials. Methods have been identified for sample preparation and confirmatory determination.
- **Asphalt matrices sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in asphalt paving material samples. Methods have been identified for sample preparation and confirmatory determination.
- **Concrete sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in concrete samples. Methods have been identified for sample preparation and confirmatory determination.
- **Brick sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in brick samples. Methods have been identified for qualitative and confirmatory determination.
- **Sample preparation method identifier.** A unique identifier or number assigned to an analytical method by the method publisher. The identified method is intended to digest solid samples into a liquid form suitable for analysis.
- **Confirmatory method identifier.** A unique identifier or number assigned to an analytical method by the method publisher. The identified method is for measurement of the activity from a particular radionuclide per unit of mass, volume or area sampled.

Following a contamination incident, it is assumed that only those areas with contamination greater than pre-existing/naturally prevalent levels (i.e., background) commonly found in the environment or on buildings and infrastructure would be subject to remediation. Dependent on site- and event-specific goals, investigation of background levels using methods listed in Appendix B is recommended.

6.1 General Guidelines

The guidelines summarized in this section provide a general overview of how to identify the appropriate radiochemical method(s) for a given analyte-sample type combination, as well as recommendations for quality control (QC) procedures.

For additional information on the properties of the radionuclides listed in Appendix B, Toxicology Data Network (TOXNET) (http://toxnet.nlm.nih.gov/index.html), a cluster of databases on toxicology, hazardous chemicals, and related areas maintained by the National Library of Medicine, is an excellent

resource. EPA's Radiation Protection Program (http://www.epa.gov/radiation/radionuclides/index.html) and the *Multi-Agency Radiological Laboratory Analytical Protocols Manual* (MARLAP) (https://www.epa.gov/radiation/marlap-manual-and-supporting-documents) (last accessed 07/2017) websites provide some additional information pertaining to radionuclides of interest and selection of radiochemical methods. Documents for emergency response operations for laboratories, recently developed by EPA's Office of Radiation and Indoor Air (ORIA), describe the likely analytical decision paths that would be required by personnel at a radioanalytical laboratory following a radiological or nuclear contamination incident. These documents may be found at https://www.epa.gov/radiation/radiation-protection-document-library (last accessed 07/2017; enter "incident guides" in the search bar for quick access).

6.1.1 Standard Operating Procedures for Identifying Radiochemical Methods

To determine the appropriate method to be used on an environmental sample, locate the analyte of concern in Appendix B1: Selected Radiochemical Methods under the "Analyte Class" or "Analyte(s)" column. After locating the analyte of concern, continue across the table to identify the appropriate determinative technique (e.g., alpha spectrometry), then identify the appropriate qualitative and/or confirmatory method for the sample type of interest (drinking water, aqueous and liquid phase, soil and sediment, surface wipes and air filters) for the particular analyte.

To determine the appropriate method to be used on an outdoor building or infrastructure material sample, locate the analyte of concern in Appendix B2: Selected Radiochemical Methods under the "Analyte Class" or "Analyte(s)" column. After locating the analyte of concern, continue across the table to identify the appropriate determinative technique (e.g., alpha spectrometry), then identify the appropriate sample preparation and/or confirmatory method for the sample type of interest (asphalt shingles, asphalt materials, concrete or brick).

Once a method has been identified in Appendix B1 or B2, **Table 6-1** can be used to locate the method summary. Sections 6.2.1 through 6.2.59 provide summaries of the qualitative and confirmatory methods listed in Appendix B1 for analysis of environmental samples. Sections 6.3.1 through 6.3.8 provide summaries of the sample preparation and confirmatory methods listed in Appendix B2 for analysis of outdoor building and infrastructure material samples.

Table 6-1. Radiochemical Methods and Corresponding Section Numbers

Analyte / Analyte Class	CAS RN	Method	Section
Gross Alpha Gross Beta	NA NA	900.0 (EPA)	6.2.2
		FRMAC, Vol 2, pg. 33 (DOE)	6.2.39
		AP1 (ORISE)	6.2.43
		7110 B (SM)	6.2.53
Gamma Select Mixed Fission Products	NA	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.31
Total Activity Screening	NA	Y-12 Preparation of Samples for Total Activity Screening (DOE)	6.2.59

Analyte / Analyte Class	CAS RN	Method	Section
Americium-241	14596-10-2	***Alpha Spectrometry:***	
		Rapid Radiochemical Method for Am-241 (EPA)	6.2.12
		Rapid methods for acid or fusion digestion (EPA)	6.2.17 and 6.2.18
		Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	6.2.20
		Am-01-RC (HASL-300)	6.2.28
		Am-04-RC (HASL-300)	6.2.29
		Am-06-RC (HASL-300)	6.2.30
		Pu-12-RC (HASL-300)	6.2.33
		Actinides and Sr-89/90 in Vegetation (SRS)	6.2.42
		AP11 (ORISE)	6.2.47
		D3084-05 (ASTM)	6.2.49
		Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	6.3.3
		Rapid Method for Americium-241 in Building Materials (EPA)	6.3.6
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	6.3.7
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	6.3.8
		Gamma Spectrometry:	
		901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.31
		7120 (SM)	6.2.54
Californium-252	13981-17-4	Rapid Radiochemical Method for Californium-252 (EPA)	6.2.23
		Am-06-RC (HASL-300)	6.2.30
		AP11 (ORISE)	6.2.47
		D3084-05 (ASTM)	6.2.49
Cesium-137	10045-97-3	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.31
Cobalt-60	10198-40-0	7120 (SM)	6.2.54
Curium-244	13981-15-2	Rapid Radiochemical Method for Curium-244 in Water (EPA)	6.2.24
		Rapid Radiochemical Method for Curium-244 in Air Particulate Filters, Swipes and Soil (EPA)	6.2.25
		Am-06-RC (HASL-300)	6.2.30
		AP11 (ORISE)	6.2.47
		D3084-05 (ASTM)	6.2.49
Europium-154	15585-10-1	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.31
		7120 (SM)	6.2.54
Indium-111	15750-15-9	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.31
Iodine-125	14158-31-7	Procedure #9 (ORISE)	6.2.48

Analyte / Analyte Class	CAS RN	Method	Section
Iodine-131	10043-66-0	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.31
Iridium-192	14694-69-0	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.31
		7120 (SM)	6.2.54
Molybdenum-99	14119-15-4	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.31
Neptunium-237	13994-20-2	907.0 (EPA)	6.2.7
		SOP for Actinides in Environmental Matrices (EPA-NAREL)	6.2.27
Neptunium-239	13968-59-7	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.31
		7120 (SM)	6.2.54
Phosphorus-32	14596-37-3	Rapid Radiochemical Method for P-32 (EPA)	6.2.9
		R4-73-014 (EPA)	6.2.10
		RESL P-2 (DOE)	6.2.40
Plutonium-238 Plutonium-239	13981-16-3 15117-48-3	EMSL-33 (EPA)	6.2.8
		Rapid Radiochemical Method for Pu-238 and -239/240 (EPA)	6.2.13
		Rapid methods for acid or fusion digestion (EPA)	6.2.17 and 6.2.18
		Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	6.2.20
		Rapid Method for Sodium Hydroxide/Sodium Peroxide Fusion of Radioisotope Thermoelectric Generator Materials in Water and Air Filter Matrices (EPA)	6.2.22
		SOP for Actinides in Environmental Matrices (EPA-NAREL)	6.2.27
		Am-06-RC (HASL-300)	6.2.30
		Actinides and Sr-89/90 in Vegetation (SRS)	6.2.42
		AP11 (ORISE)	6.2.47
		D3084-05 (ASTM)	6.2.49
		Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	6.3.3
		Rapid Method for Plutonium-238, -239/240 in Building Materials (EPA)	6.3.5
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	6.3.7
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	6.3.8
Polonium-210	13981-52-7	Method 111 (EPA)	6.2.1
		Po-02-RC (HASL-300)	6.2.32
Radium-223	15623-45-7	Rapid Radiochemical Method for Ra-226 in Water (EPA)	6.2.14

Analyte / Analyte Class	CAS RN	Method	Section
Radium-226	13982-63-3	903.1 (EPA)	6.2.4
		Rapid Radiochemical Method for Ra-226 in Water (EPA)	6.2.14
		Rapid methods for acid or fusion digestion (EPA)	6.2.17 and 6.2.18
		Rapid Method for Radium in Soil (EPA)	6.2.19
		Ra-03-RC (HASL-300)	6.2.34
		AP7 (ORISE)	6.2.46
		7500-Ra B (SM)	6.2.55
		7500-Ra C (SM)	6.2.56
		Rapid Radiochemical Method for Radium-226 in Building Materials (EPA)	6.2.26 and 6.3.2
		Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	6.3.3
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	6.3.7
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	6.3.8
Rhenium-188	14378-26-8	901.1 (EPA)	6.2.3
Rubidium-82	14391-63-0	Ga-01-R (HASL-300)	6.2.31
Ruthenium-103	13968-53-1	901.1 (EPA)	6.2.3
Ruthenium-106	13967-48-1	Ga-01-R (HASL-300)	6.2.31
Selenium-75	14265-71-5	7120 (SM)	6.2.54
Strontium-89	14158-27-1	905.0 (EPA)	6.2.5
		Strontium in Food and Bioenvironmental Samples (EPA)	6.2.11
		Actinides and Sr-89/90 in Soil Samples (SRS)	6.2.41
		Actinides and Sr-89/90 in Vegetation (SRS)	6.2.42
Strontium-90	10098-97-2	905.0 (EPA)	6.2.5
		Rapid Radiochemical Method for Radiostrontium (EPA)	6.2.15
		Rapid methods for acid or fusion digestion (EPA)	6.2.17 and 6.2.18
		Rapid Method for Sodium Carbonate Fusion of Soil and Soil-Related Matrices Prior to Strontium-90 Analyses (EPA)	6.2.21
		Sr-03-RC (HASL-300)	6.2.35
		Actinides and Sr-89/90 in Vegetation (SRS)	6.2.42
		D5811-08 (ASTM)	6.2.51
		Rapid Method for Total Radiostrontium in Building Materials (EPA)	6.3.1
		Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	6.3.3
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	6.3.7
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	6.3.8

Analyte / Analyte Class	CAS RN	Method	Section
Technetium-99	14133-76-7	Tc-01-RC (HASL-300)	6.2.36
		Tc-02-RC (HASL-300)	6.2.37
		AP5 (ORISE)	6.2.45
		D7168-05 (ASTM)	6.2.52
Technetium-99m	378784-45-3	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.31
Thorium-227 Thorium-228	15623-47-9 14274-82-9	907.0 (EPA)	6.2.7
Thorium-230 Thorium-232	14269-63-7 7440-29-1	SOP for Actinides in Environmental Matrices (EPA-NAREL)	6.2.27
Tritium (Hydrogen-3)	10028-17-8	906.0 (EPA)	6.2.6
		AP2 (ORISE)	6.2.44
Uranium-234 Uranium-235 Uranium-238	13966-29-5 15117-96-1 7440-61-1	Rapid Radiochemical Method for Isotopic Uranium in Water (EPA)	6.2.16
		Rapid methods for acid or fusion digestion (EPA)	6.2.17 and 6.2.18
		Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	6.2.20
		U-02-RC (HASL-300)	6.2.38
		Actinides and Sr-89/90 in Vegetation(SRS)	6.2.42
		AP11 (ORISE)	6.2.47
		D3972-02 (ASTM)	6.2.50
		7500-U B (SM)	6.2.57
		7500-U C (SM)	6.2.58
		SOP for Actinides in Environmental Matrices (EPA-NAREL)	6.2.27
		Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	6.3.3
		Rapid Method for Isotopic Uranium in Building Materials (EPA)	6.3.4
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	6.3.7
		Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	6.3.8

The method summaries are listed in order of method selection hierarchy (see Figure 2-1), starting with EPA methods, followed by methods from other federal agencies and voluntary consensus standard bodies (VCSBs). Methods are listed in numerical order under each publisher. Where available, a direct link to the full text of the selected analytical method is provided in the method summary. For additional information regarding sample preparation and analysis procedures and on methods available through consensus standards organizations, please use the contact information provided in **Table 6-2**.

Table 6-2. Sources of Radiochemical Methods

Name	Publisher	Reference
National Environmental Methods Index (NEMI)	EPA, U.S. Geological Survey (USGS)	http://www.nemi.gov
Code of Federal Regulations (CFR) Promulgated Test Methods	EPA, Emission Measurement Center (EMC)	https://www.epa.gov/emc/emc-promulgated-test-methods
Prescribed Procedures for Measurement of Radioactivity in Drinking Water (EPA-600 4-80-032, August 1980)	EPA, ORD, Environmental Monitoring and Support Laboratory (EMSL)	https://nepis.epa.gov/Exe/ZyPDF.cgi/30000QHM.PDF?Dockey=30000QHM.PDF Also available from National Technical Information Service (NTIS)*, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, (703) 605-6000.
Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events (EPA 402-R-10-001)	EPA, ORIA, National Analytical and Radiation Environmental Laboratory (NAREL)	https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides
Rapid Radiochemical Methods for Selected Radionuclides	EPA, ORIA, NAREL	https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides
Radiochemical Analytical Procedures for Analysis of Environmental Samples, March 1979. EMSL-LV-0539-17	EPA, EMSL	Available NTIS*, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, (703) 605-6000.
EML Procedures Manual, Health and Safety Laboratory (HASL-300), 28th Edition, February, 1997	Department of Energy (DOE), Environmental Measurements Laboratory (EML) / Now DHS	http://www.wipp.energy.gov/NAMP/EMLLegacy/ Also available from NTIS*, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, (703) 605-6000.
Federal Radiological Monitoring and Assessment Center (FRMAC) Laboratory Manual	DOE, National Nuclear Security Administration (NNSA)	https://www.epa.gov/sites/production/files/2015-06/documents/frmac-vol2-pg33.pdf
Y-12 National Security Complex (Y-12)	DOE, NNSA	http://www.y12.doe.gov/
Radiological and Environmental Sciences Laboratory (RESL) Analytical Chemistry Branch Procedures Manual	DOE, RESL	Available from NTIS, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, (703) 605-6000.
Savannah River Site (SRS) Methods	DOE, SRS	Savannah River National Laboratory Savannah River Site Aiken, SC 29808, (803) 725-6211
Annual Book of ASTM Standards, Vol. 11.02*	ASTM International	http://www.astm.org
Standard Methods for the Examination of Water and Wastewater, 22nd Edition, 2005*	American Public Health Association (APHA)	http://www.standardmethods.org

* Subscription and/or purchase required.

6.1.2 General QC Guidelines for Radiochemical Methods

Having data of known and documented quality is critical so that public officials can accurately assess the activities that may be needed in remediating a site and determine the effectiveness of those activities.[8] Having such data requires that laboratories: (1) conduct the necessary QC to ensure that measurement systems are in control and operating correctly; (2) properly document results of the analyses; and (3) properly document measurement system evaluation of the analysis-specific QC. Ensuring data quality also requires that laboratory results are properly evaluated and the results of the data quality evaluation are included within the data report when transmitted to decision makers.

The level or amount of QC needed often depends on the intended purpose of the data that are generated. Various levels of QC may be required if the data are generated during contaminant presence/absence qualitative determinations versus confirmatory analyses. The specific needs for data generation should be identified. QC requirements and data quality objectives (DQOs) should be derived based on those needs, and should be applied consistently across laboratories when multiple laboratories are used. For example, during rapid sample screening analyses, minimal QC samples (e.g., blanks, duplicates) and documentation might be used. Implementation of the analytical methods for evaluation of environmental and outdoor building and infrastructure material samples during site assessment through site clearance, such as those identified in this document, might require increased QC frequency and more stringent QC criteria.

Some method-specific QC requirements are described in many of the individual methods that are cited in this manual. QC requirements will be referenced in analytical protocols developed to address specific analytes and sample types of concern. Additional information regarding QC requirements specific to radiochemical methods is included in the MARLAP manual at: https://www.epa.gov/radiation/radiation-protection-document-library (last accessed 07/2017; enter "MARLAP" in the search for quicker access). Individual methods, sampling and analysis protocols or contractual statements of work should also be consulted to determine any additional QC that may be needed.

QC samples are required to assess the precision, bias and reliability of sample results. All QC results are tracked on control charts and reviewed for acceptability and trends in analysis or instrument operation. QC parameters are measured as required per method at the prescribed frequency. QC of laboratory analyses using radiochemical methods includes ongoing analysis of QC samples and tracking QC parameters including, but not limited to the following:

- Method blanks
- Calibration checks
- Sample and sample duplicates
- Laboratory control sample recoveries
- Matrix spike/matrix spike duplicate (MS/MSD) recoveries and precision
- Tracer and/or carrier yield

Please note: The appropriate point of contact identified in Section 4.0 should be consulted regarding appropriate QA/QC procedures prior to sample analysis. These contacts will consult with the EPA Environmental Response Laboratory Network (ERLN) or Water Laboratory Alliance (WLA) coordinator responsible for laboratory activities during the specific event to ensure QA/QC procedures are performed consistently across laboratories. EPA program offices will be responsible for ensuring that the QA/QC practices are implemented.

[8] Information regarding EPA's DQO process, considerations, and planning is available at: http://www.epa.gov/QUALITY/dqos.html (last accessed 09/2017).

6.1.3 Safety and Waste Management

It is imperative that safety precautions be used during collection, processing and analysis of environmental and outdoor building and infrastructure material samples. Laboratories should have a documented radiation safety plan or manual in addition to a health and safety plan for handling samples that may contain target chemical, biological and/or radiological (CBR) contaminants, and laboratory staff should be trained in and implement the safety procedures in the plan or manual. In addition, many of the methods summarized or cited in Section 6.2 and Section 6.3 contain specific requirements, guidelines or information regarding safety precautions that should be followed when handling or processing samples and reagents. These methods may also provide information regarding waste management. Laboratories should consult with the responsible government agencies prior to disposal of waste materials. Other resources that can be consulted for additional information include the following:

- Occupational Safety and Health Administration (OSHA) - 29 CFR part 1910.1450. Occupational Exposure to Hazardous Chemicals in Laboratories. Available at: http://www.access.gpo.gov/nara/cfr/waisidx_06/29cfr1910a_06.html (last accessed 9/2017)
- EPA - 40 CFR part 260. Hazardous Waste Management System: General. Available at: https://www.gpo.gov/fdsys/granule/CFR-2012-title40-vol27/CFR-2012-title40-vol27-part260 (last accessed 9/2017)
- EPA - 40 CFR part 270. EPA Administered Permit Programs: The Hazardous Waste Permit Program. Available at: https://www.gpo.gov/fdsys/granule/CFR-2012-title40-vol28/CFR-2012-title40-vol28-part270 (last accessed 9/2017)
- U.S. Nuclear Regulatory Commission (NRC) - 10 CFR part 20. Standards for Protection Against Radiation. Available at: http://www.access.gpo.gov/nara/cfr/waisidx_00/10cfr20_00.html (last accessed 9/2017)
- DOE. Order O 435.1: Radioactive Waste Management. January 1, 2007. Available at: https://www.directives.doe.gov/directives-documents/400-series/0435.1-BOrder-chg1 (last accessed 9/2017)
- DOE. M 435.1-1. *Radioactive Waste Management Manual*. Office of Environmental Management. June 8, 2011. Available at: https://www.directives.doe.gov/directives-documents/400-series/0435.1-DManual-1-chg2-AdmChg (last accessed 9/2017)
- DOE. *Compendium of EPA-Approved Analytical Methods for Measuring Radionuclides in Drinking Water*. Prepared by the Office of Environmental Policy and Assistance Air, Water and Radiation Division (EH-412). June 1998. Available at: http://www.orau.org/ptp/PTP%20Library/library/DOE/Misc/radmeth3.pdf (last accessed 9/2017)
- EPA. 1996. *Profile and Management Options for EPA Laboratory Generated Mixed Waste*. ORIA, Washington, DC. EPA 402-R-96-015. Available at: http://www.epa.gov/rpdweb00/docs/mixed-waste/402-r-96-015.pdf (last accessed 9/2017)
- EPA. 2001. Changes to 40 CFR 266 (Storage, Treatment, Transportation, and Disposal of Mixed Waste), *Federal Register* 66:27217-27266, May 16, 2001. Available at: https://www.federalregister.gov/documents/2001/05/16/01-11411/hazardous-waste-identification-rule-hwir-revisions-to-the-mixture-and-derived-from-rules (last accessed 9/2017)
- EPA. 2014. *Resource Conservation and Recovery Act* (*RCRA) Orientation Manual.* Office Of Resource Conservation And Recovery (ORCR), Washington, DC. EPA530-F-11-003. 242 pp. Available at: https://www.epa.gov/sites/production/files/2015-07/documents/rom.pdf (last accessed 09/2017)
- MARLAP Manual. 2004. Chapter 17. Waste Management in a Radioanalytical Laboratory. EPA 402-B-04-001B. Available at: https://www.epa.gov/sites/production/files/2015-05/documents/402-b-04-001b-17-final.pdf (last accessed 09/2017)

- National Research Council. 1995. *Prudent Practices in the Laboratory; Handling and Disposal of Chemicals,* National Academy Press, Washington, DC. Available at: http://books.nap.edu/openbook.php?isbn=0309052297 (last accessed 09/2017)
- National Council on Radiation Protection and Measurements (NCRP). 2002. *Risk-Based Classification of Radioactive and Hazardous Chemical Wastes*, Report Number 139. 7910 Woodmont Avenue, Suite 400, Bethesda, MD 20814–3095
- NRC / EPA. 1995. Joint Nuclear Regulatory Commission/Environmental Protection Agency Guidance on the Storage of Mixed Radioactive and Hazardous Waste. *Federal Register* 60:40204-40211

6.2 Method Summaries (Environmental Samples)

Summaries corresponding to the methods selected for analysis of environmental samples listed in Appendix B1 are provided in Sections 6.2.1 through 6.2.59. These summaries contain information that has been extracted from the selected methods. Each method summary contains a table identifying the contaminants listed in Appendix B1 to which the method applies, a brief description of the analytical method, and a link to the full version of the method or a source for obtaining a full version of the method. Summaries are provided for informational use. The full version of the method should be consulted prior to sample analysis. For information regarding sample collection considerations for samples to be analyzed by these methods, see the companion *Sample Collection Information Document for Chemicals, Radiochemicals and Biotoxins, Companion to SAM 2017*.

6.2.1 EPA Method 111: Determination of Polonium-210 Emissions from Stationary Sources

Analyte(s)	CAS RN
Polonium-210	13981-52-7

Analysis Purpose: Qualitative and confirmatory determination
Technique: Alpha spectrometry

Method Developed for: Polonium-210 in particulate matter samples collected from stationary source exhaust stacks
Method Selected for: This method has been selected for qualitative and confirmatory analysis of surface wipes and air filters.

Description of Method: This method covers the determination of polonium-210 in particulate matter samples collected from stationary sources such as exhaust stacks. Polonium-210 in the sample is put in solution, deposited on a metal disc, and the radioactive disintegration rate measured. Polonium in acid solution spontaneously deposits onto the surface of metals that are more electropositive than polonium. Polonium-209 tracers should be added to each sample to determine the chemical yield.

Special Considerations: Compounds, such as clays that are present in some decontamination agents, can contain iron, magnesium and/or calcium, which can potentially be released as ions via ion exchange in the presence of certain radionuclides, and cause analytical interferences. Although iron (III), a major interference in the analysis of polonium-210 by alpha spectrometry, is extracted from the concentrated hydrochloric solution using liquid-liquid extraction with diisopropylether, high concentrations of iron may not be completely removed. Chelators, also present in some decontamination agents, can tightly complex iron that may be present in the sample, preventing its removal.

Source: U.S. EPA. EMC, prepared by the Office of Air Quality Planning and Standards (OAQPS). 2000. "Method 111: Determination of Polonium-210 Emissions from Stationary Sources." Research Triangle Park, NC: U.S. EPA. http://www.epa.gov/sites/production/files/2015-06/documents/epa-111.pdf

6.2.2 EPA Method 900.0: Gross Alpha and Gross Beta Radioactivity in Drinking Water

Analysis Purpose: Gross alpha and gross beta determination
Technique: Alpha/Beta counting

Method Developed for: Gross alpha and gross beta particle activities in drinking water
Method Selected for: This method has been selected for gross alpha and gross beta determination in drinking water samples.

Description of Method: The method provides an indication of the presence of alpha and beta emitters, including the following analytes:

- Americium-241 (CAS RN 14596-10-2) Alpha emitter
- Californium-252 (CAS RN 13981-17-4) Alpha emitter
- Cesium-137 (CAS RN 10045-97-3) Beta emitter
- Cobalt-60 (CAS RN 10198-40-0) Beta emitter
- Curium-244 (CAS RN 13981-15-2) Alpha emitter
- Europium-154 (CAS RN 15585-10-1) Beta emitter
- Iridium-192 (CAS RN 14694-69-0) Beta emitter
- Plutonium-238 (CAS RN 13981-16-3) Alpha emitter
- Plutonium-239 (CAS RN 15117-48-3) Alpha emitter
- Polonium-210 (CAS RN 13981-52-7) Alpha emitter
- Radium-226 (CAS RN 13982-63-3) Alpha emitter
- Ruthenium-103 (CAS RN 13968-53-1) Beta emitter
- Ruthenium-106 (CAS RN 13967-48-1) Beta emitter
- Strontium-90 (CAS RN 10098-97-2) Beta emitter
- Uranium-234 (CAS RN 13966-29-5) Alpha emitter
- Uranium-235 (CAS RN 15117-96-1) Alpha emitter
- Uranium-238 (CAS RN 7440-16-1) Alpha emitter

An aliquot of a preserved drinking water sample is evaporated to a small volume (3 to 5 mL) and transferred quantitatively to a tared 2-inch planchet. The aliquot volume is determined based on a maximum total solids content of 100 mg. The sample aliquot is evaporated to dryness in the planchet to a constant weight, cooled, and counted using a gas proportional or scintillation counting system. The counting system is calibrated with thorium-230 for gross alpha, and with strontium-90 for gross beta analysis.[9] A traceable standards-based efficiency curve must be developed for each calibration nuclide (thorium-230 and strontium-90) based on a range of total solids content in the 2-inch planchet from 0 to 100 mg (see method for specific recommendations and requirements for the use of cesium-137).

Special Considerations: Long counting time and increased sample size may be required to meet detection limits. Sensitivity is limited by the concentration of solids in the sample. The method provides an overall measure of alpha and beta activity, including activity for the radionuclides listed above, but does not permit the specific identification of any alpha or beta emitting radionuclides. Compounds containing carbonate, fluoride, hydroxide, or phosphate, such as those present in some decontamination agents, can precipitate radionuclides out of solution prior to analysis. This precipitation can result in a lesser amount of radionuclides in cases where an aliquot of a water sample is transferred and analyzed separately from the entire sample.

Source: U.S. EPA, EMSL. 1980. "Method 900.0: Gross Alpha and Gross Beta Radioactivity in Drinking Water." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*. Cincinnati, OH: U.S. EPA. EPA/600/4/80/032. http://www.epa.gov/sites/production/files/2015-06/documents/epa-900.0.pdf

6.2.3 EPA Method 901.1: Gamma Emitting Radionuclides in Drinking Water

Analyte(s)	CAS RN
Americium-241	14596-10-2
Cesium-137	10045-97-3
Cobalt-60	10198-40-0

[9] EPA lists standards for use when analyzing drinking water in the table at 40 CFR 141.25 (Footnote 11).

Analyte(s)	CAS RN
Europium-154	15585-10-1
Gamma	NA
Iodine-131	10043-66-0
Indium-111	15750-15-9
Iridium-192	14694-69-0
Molybdenum-99	14119-15-4
Neptunium-239	13968-59-7
Rhenium-188	14378-26-8
Rubidium-82	14391-63-0
Ruthenium-103	13968-53-1
Ruthenium-106	13967-48-1
Selenium-75	14265-71-5
Select Mixed Fission Products	NA
Technetium-99m	378784-45-3

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Gamma spectrometry

Method Developed for: Gamma emitting radionuclides in drinking water
Method Selected for: This method has been selected for qualitative and confirmatory analysis of select gamma emitters in drinking water samples.

Description of Method: This method is applicable for analysis of water samples that contain radionuclides that emit gamma photons with energies ranging from approximately 60 to 2000 keV. The method uses gamma spectroscopy for measurement of gamma photons emitted from radionuclides without separating them from the sample matrix. A homogeneous aliquot of water is placed into a standard geometry (normally a Marinelli beaker) for gamma counting, typically using a high purity germanium detector. Detectors such as germanium (lithium) or thallium-activated sodium iodide also can be used. Sample aliquots are counted long enough to meet the required sensitivity of measurement. To reduce adsorbance of radionuclides on the walls of the counting container, the sample is acidified at collection time. Due to its poorer resolution, significant interference can occur using the thallium-activated sodium iodide detector when counting a sample containing radionuclides that emit gamma photons of similar energies. When using this method, shielding is needed to reduce background interference. Detection limits are, in general, dependent on analyte radionuclide gamma-ray abundance, sample volume, geometry (physical shape) and counting time.

Special Considerations: The presence of reducing agents, such as those contained in some decontamination agents, can convert radionuclides to an insoluble zero-valent state that can precipitate out of solution. Although the addition of nitric acid can prevent this precipitation from occurring, iridium, molybdenum and ruthenium would likely still precipitate in the presence of these agents. Compounds such as clays, which are also present in some decontamination agents, can sequester cesium-137, which would only be released upon complete dissolution when using this method. Compounds containing carbonate, fluoride, hydroxide or phosphate also can precipitate radionuclides out of solution. All of these are a concern in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample.

Source: U.S. EPA, EMSL. 1980. "Method 901.1: Gamma Emitting Radionuclides in Drinking Water." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*. Cincinnati, OH: U.S. EPA. EPA/600/4/80/032. http://www.epa.gov/sites/production/files/2015-06/documents/epa-901.1.pdf

6.2.4 EPA Method 903.1: Radium-226 in Drinking Water – Radon Emanation Technique

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha counting

Method Developed for: Radium-226 in drinking water
Method Selected for: This method has been selected for confirmatory analysis of drinking water samples.

Description of Method: This method is specific for radium-226, and is based on the emanation and scintillation counting of radon-222, the immediate decay product of radium-226. Radium-226 is concentrated and separated from the water sample by coprecipitation on barium sulfate. The precipitate is dissolved in ethylenediamine tetraacetic acid (EDTA) reagent, placed in a sealed bubbler and stored for ingrowth of radon-222. After ingrowth, the radon-222 gas is purged into a scintillation cell. When the short-lived radon-222 daughters are in equilibrium with the parent (after ~4h), the scintillation cell is counted for activity. The absolute measurement of radium-226 is effected by calibrating the scintillation cell system with a standard solution of the nuclide. There are no radioactive interferences in this method. Based on a 1000-mL sample and 100-minute counting time, the minimum detectable level for this method is 0.5 picocuries/liter (pCi/L).

Special Considerations: The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately form the entire sample. Such compounds include:

- Permanganate and permanganic acid can be reduced to insoluble manganese (IV) oxide which can remove radium.
- Certain chelating agents may compromise the collection of radionuclides prior to analysis, by preventing them from being precipitated out of solution. Dispersants and corrosion inhibitors can have this chelating ability as well.
- Clays contain iron, magnesium and calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause interference in the analysis of the water.
- Compounds containing sulfate, carbonate, oxalate, or phosphate can precipitate radium out of solution prior to analysis, resulting in a lesser amount of radium in cases where an aliquot of water sample is transferred prior to the precipitation step, and analyzed separately from the entire sample.

Source: U.S. EPA, EMSL. 1980. "Method 903.1: Radium-226 in Drinking Water – Radon Emanation Technique." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*. Cincinnati, OH: U.S. EPA. EPA/600/4/80/032. http://www.epa.gov/sites/production/files/2015-06/documents/epa-903.1.pdf

6.2.5 EPA Method 905.0: Radioactive Strontium in Drinking Water

Analyte(s)	CAS RN
Strontium-89	14158-27-1
Strontium-90	10098-97-2

Analysis Purpose: Qualitative and confirmatory analysis

Technique: Beta counting

Method Developed for: Strontium-89, strontium-90 and total strontium in drinking water
Method Selected for: This method has been selected for qualitative and confirmatory analysis of aqueous/liquid and drinking water samples for strontium-89 and confirmatory analysis of drinking water samples for strontium-90.

Description of Method: Stable strontium carrier is added to the water sample. Both strontium-89 and strontium-90 are precipitated from the solution as insoluble carbonates. Interferences from calcium and from some radionuclides are removed by one or more precipitations of the strontium carrier as strontium nitrate. Barium and radium are removed by precipitation as chromates. The yttrium-90 decay product of strontium-90 is removed by a hydroxide precipitation step. The separated strontium-89 and strontium-90 are precipitated as carbonates, weighed for determination of the chemical recovery, and counted for beta particle activity. The counting result, ascertained immediately after separation, represents the total strontium activity (strontium-89 and strontium-90) plus an insignificant fraction of the yttrium-90 that has grown into the separated strontium-90. The yttrium-90 decay product is allowed to in-grow for approximately two weeks and then is separated with stable yttrium carrier as hydroxide and finally precipitated as the oxalate, weighed for chemical recovery, and mounted for beta counting. The strontium-90 concentration is determined from the yttrium-90 activity; strontium-89 concentration is determined from the difference.

Special Considerations: Certain chelating compounds found in decontamination agents can tightly complex barium, iron, lead, magnesium and potassium, causing interference when analyzing for strontium-89 and -90. Compounds containing carbonate, fluoride, phosphate or sulfate, which are also present in some decontamination agents, can precipitate radionuclides out of solution prior to analysis. This precipitation can result in a lesser amount of strontium in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample.

Source: U.S. EPA, EMSL. 1980. "Method 905.0: Radioactive Strontium in Drinking Water." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*. Cincinnati, OH: U.S. EPA. EPA/600/4/80/032. http://www.epa.gov/sites/production/files/2015-06/documents/epa-905.0.pdf

6.2.6 EPA Method 906.0: Tritium in Drinking Water

Analyte(s)	CAS RN
Tritium (Hydrogen-3)	10028-17-8

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Tritium (as T_2O or HTO) in drinking water
Method Selected for: This method has been selected for qualitative and confirmatory analysis of drinking water and aqueous/liquid phase samples.

Description of Method: An unpreserved 100-mL aliquot of a drinking water sample is distilled after adjusting pH with a small amount of sodium hydroxide and adding potassium permanganate. The alkaline treatment prevents other radionuclides, such as radioiodine and radiocarbon, from distilling with the tritium. The permanganate treatment oxidizes trace organics that may be present in the sample and prevents their appearance in the distillate. To determine the concentration of tritium, the middle fraction of the distillate is used, because the early and late fractions are more apt to contain materials interfering with the liquid scintillation counting process. A portion of this collected fraction is added to a liquid scintillator cocktail, and the solution is mixed, dark adapted and counted for beta particle activity. The

efficiency of the system can be determined by the use of prepared tritiated water (HTO) standards having the same density and color as the sample.

Special Considerations: Some compounds present in decontamination agents, such as organic compounds containing oxygen, reductants, halogenated compounds or elevated levels of nitrates or nitromethane, can cause chemical quenching. Color quenching compounds, such as dyes and pigments also contained in some decontamination agents, can have a significant impact when using liquid scintillation methods.

Source: U.S. EPA, EMSL. 1980. "Method 906.0: Tritium in Drinking Water." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*. Cincinnati, OH: U.S. EPA. EPA/600/4/80/032. http://www.epa.gov/sites/production/files/2015-06/documents/epa-906.0.pdf

6.2.7 EPA Method 907.0: Actinide Elements in Drinking Water - Thorium, Uranium, Neptunium, Plutonium, Americium and Curium

Analyte(s)	CAS RN
Neptunium-237	13994-20-2
Thorium-227	15623-47-9
Thorium-228	14274-82-9
Thorium-230	14269-63-7
Thorium-232	7440-29-1

Analysis Purpose: Qualitative and confirmatory determination
Technique: Alpha spectrometry

Method Developed for: Alpha emitting actinide elements in drinking water
Method Selected for: This method has been selected for qualitative and confirmatory analysis of drinking water samples.

Description of Method: Actinide elements are concentrated by coprecipitation using ferric hydroxide. The ferric hydroxide is dissolved and thorium, neptunium, plutonium, americium and curium are coprecipitated with bismuth phosphate. The bismuth phosphate is dissolved in 8M hydrochloric acid and plutonium and neptunium are extracted in tri-isooctylamine (TIOA). The thorium is separated from americium and curium by extraction with trioctylphosphine oxide (TOPO). All separated and purified elements are coprecipitated on lanthanum fluoride and alpha counted.

Special Considerations: The technical contacts in Section 4.0 should be consulted regarding use of alpha spectrometry for analysis of samples prepared using this method to detect and measure specific isotopes. Ammonium ions interfere in the precipitation of neptunium with ferric hydroxide. If ammonium ions are present, adding sodium hydroxide to raise the pH should result in complete recovery of neptunium. Chelating agents, which are present in some decontamination agents, will interfere to varying extents by totally or partially comp1exing actinide elements. Dispersants and corrosion inhibitors, also present in decontaminating agents, can have chelating ability as well. When chelating agents are present, alternate methods, such as coprecipitation from acid solutions (Section 6.2.27), should be considered. Clays that are present in some decontamination agents can contain iron, magnesium and calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause interferences.

Source: U.S. EPA, EMSL. 1980. "Method 907.0: Actinide Elements in Drinking Water - Thorium, Uranium, Neptunium, Plutonium, Americium and Curium." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*. Cincinnati, OH: U.S. EPA. EPA/600/4/80/032. https://nepis.epa.gov/Exe/ZyPDF.cgi/30000QHM.PDF?Dockey=30000QHM.PDF

6.2.8 EPA Method EMSL-33: Isotopic Determination of Plutonium, Uranium, and Thorium in Water, Soil, Air, and Biological Tissue

Analyte(s)	CAS RN
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Isotopic plutonium, uranium and thorium, together or individually, in soil, water, air filters, urine or ashed residues of vegetation, animal tissues and bone
Method Selected for: This method has been selected for confirmatory analysis of plutonium-238 and -239 in drinking water samples.

Description of Method: This method is appropriate for the analysis of isotopic plutonium, uranium and thorium, together or individually, by alpha spectrometry. Plutonium-236, uranium-232 and thorium-234 tracer standards are added for the determination of chemical yields. Samples are decomposed by nitric-hydrofluoric acid digestion or ignition to assure that all of the plutonium is dissolved and chemically separated from the sample by coprecipitation with sodium and ammonium hydroxide, anion exchange and electrodeposition. The residues are dissolved in dilute nitric acid and successive sodium and ammonium hydroxide precipitations are performed in the presence of boric acid to remove fluoride and soluble salts. The hydroxide precipitate is dissolved, the solution is pH-adjusted with hydrochloric acid, and plutonium and uranium are adsorbed on an anion exchange column, separating them from thorium. Plutonium is eluted with hydrobromic acid. The actinides are electrodeposited on stainless steel discs from an ammonium sulfate solution and subsequently counted by alpha spectrometry. This method is designed to detect environmental levels of activity as low as 0.02 pCi per sample. To avoid possible cross-contamination, sample aliquot activities should be limited to 25 pCi or less.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.47). The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately form the entire sample. Such compounds include:

- Chelating compounds can compromise the collection of radionuclides prior to analysis by preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Some chelators also can tightly complex barium that may be present in the sample causing interferences when analyzing for plutonium-238. Dispersants and corrosion inhibitors can have chelating ability as well.
- Compounds containing carbonate, fluoride, hydroxide or phosphate can precipitate radionuclides out of solution prior to analysis.
- The presence of higher valence anions can lead to lower yields when using the evaporation option, due to competition with active sites on the resin used to collect the radionuclides.

Source: U.S. EPA, EMSL. 1979. "EMSL-33: Isotopic Determination of Plutonium, Uranium, and Thorium in Water, Soil, Air, and Biological Tissue." *Radiochemical Analytical Procedures for Analysis of Environmental Samples*. Cincinnati, OH: U.S. EPA. http://www.epa.gov/sites/production/files/2015-06/documents/epa-emsl-33.pdf

6.2.9 EPA Method Rapid Radiochemical Method for Phosphorus-32 in Water for Environmental Restoration Following Homeland Security Events

Analyte(s)	CAS RN
Phosphorus-32	14596-37-3

Analysis Purpose: Qualitative analysis
Technique: Liquid scintillation

Method Developed for: Phosphorus-32 in water
Method Selected for: This method has been selected for qualitative analysis of drinking water samples.

Description of Method: A 100-mL water sample is filtered and phosphate carrier is added to the filtered sample. The solution is then passed through a cation exchange resin, followed by a Diphonix® resin, to remove interferences from cation radionuclides. The eluent is treated with a mixture of 10 mL of 30 % hydrogen peroxide and 10 mL of concentrated nitric acid, reduced to approximately 2–5 mL by heating, and quantitatively transferred to a liquid scintillation vial for counting. The Čerenkov photons from the P-32 beta (1710 keV, E_{max}) decay are detected using a calibrated liquid scintillation counter (LSC). Following counting of the sample, an aliquot of the final solution is used for yield determination by the inductively coupled plasma-atomic emission spectrometry (ICP-AES) method.

Special Considerations: This method has been selected for rapid qualitative screening of drinking water samples. The method is not intended for use in compliance monitoring of drinking water. Organic compounds containing oxygen, reductants, halogenated compounds or elevated levels of nitrates or nitromethane, such as those contained in some decontamination agents, can cause chemical quenching. Chemical and color quenching compounds, such as dyes and pigments, also contained in some decontamination agents, can have a significant impact when using liquid scintillation methods. This method also can be impacted by high levels of phosphates or phosphorus compounds.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2011. "Rapid Radiochemical Method for Phosphorus-32 in Water for Environmental Remediation Following Homeland Security Events." Montgomery, AL: U.S. EPA. EPA/600/R-11/181. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.10 EPA Method R4-73-014: Radioactive Phosphorus

Analyte(s)	CAS RN
Phosphorus-32	14596-37-3

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Low background alpha/beta counter

Method Developed for: Phosphorus-32 in nuclear reactor solutions
Method Selected for: This method has been selected for qualitative and confirmatory analysis of aqueous and liquid phase samples, and for confirmatory analysis of drinking water samples.

Description of Method: 200 mL or less of a water sample is acidified with nitric acid and carriers of phosphorus (standardized), cobalt, zirconium, silver and manganese are added. Hydroxides are precipitated by the addition of hydrogen peroxide and potassium hydroxide, and the hot solution is filtered through filter paper. Carriers of cobalt and zirconium are added to the filtrate, and the hydroxides are precipitated by the addition of hydrogen peroxide and potassium hydroxide. The solution is filtered and the hydroxides are discarded. The filtrate is acidified with hydrochloric acid, and phosphorous is

precipitated as magnesium ammonium phosphate by the addition of a magnesium mixture and ammonium hydroxide. The magnesium ammonium phosphate is collected on a tared filter, dried, and weighed to determine the chemical yield. The precipitate is mounted and beta counted with a gas-flow proportional counter.

Special Considerations: Chelating compounds, such as those present in some decontamination agents, can compromise the collection of scavenging carriers that are added to the sample solution prior to analysis by preventing them from being precipitated out of solution, affecting the chemical yield of phosphorus-32. This method also can be impacted by the presence of high levels of phosphates or phosphorus compounds.

Source: U.S. EPA. EMSL. 1980. "Method R4-73-14: Radioactive Phosphorus." *Prescribed Procedures for Radiochemical Analysis of Nuclear Reactor Solutions*. Cincinnati, OH: U.S. EPA. http://www.epa.gov/sites/production/files/2015-06/documents/epa-r4-73-014.pdf

6.2.11 EPA Method: Determination of Radiostrontium in Food and Bioenvironmental Samples

Analyte(s)	CAS RN
Strontium-89	14158-27-1

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Low background alpha/beta counter

Method Developed for: Strontium-89 and strontium-90 in food, vegetation and tissue samples
Method Selected for: This method has been selected for qualitative analysis of strontium-89 in wipes and air filters and confirmatory analysis strontium-89 in wipes, air filters, soil and sediment, and vegetation.

Description of Method: This method is use for the determination of strontium-89 and strontium-90 in various bio-environmental samples. A sample of 10 g or less is placed in a nickel crucible. Barium and strontium (standardized) carriers are added to the sample. Sodium hydroxide pellets and anhydrous sodium carbonate are added and mixed, and the sample is fused as a carbonate. The strontium-calcium carbonates are dissolved in hydrochloric acid, complexed with di-sodium EDTA and passed through a cation column where the strontium is absorbed, and the complexed calcium passes through. The strontium is eluted from the column and precipitated as a carbonate. The strontium carbonate is weighed and mounted on a planchet for beta counting with a low background gas-flow alpha beta counter. The chemical yield is determined gravimetrically, using calculations provided in the method.

Special Considerations: This method was developed for analysis of food, vegetation and tissue. Additional laboratory development and testing is necessary for application to soil, sediment, air filters and wipes. At this time, there are no known interferences posed by decontamination agents that might be present in a sample.

Source: U.S. EPA, National Environmental Research Center. 1975. "Determination of Radiostrontium in Food and Bioenvironmental Samples." *Handbook of Radiochemical Methods*. Washington, DC: U.S. EPA. EPA-680/4-75-001. http://www.epa.gov/sites/production/files/2015-06/documents/radiostrontium_in_food.pdf

6.2.12 EPA Method: Rapid Radiochemical Method for Americium-241 in Water for Environmental Restoration Following Homeland Security Events

Analyte(s)	CAS RN
Americium-241	14596-10-2

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Americium-241 in water
Method Selected for: This method has been selected for qualitative analysis of drinking water samples.

Description of Method: The method is based on a sequence of two chromatographic extraction resins. Americium is concentrated, isolated and purified by removing interfering radionuclides as well as other components of the sample in order to prepare the americium fraction for counting by alpha spectrometry. The method utilizes vacuum-assisted flow to improve the speed of the separations. Prior to use of the extraction resins, the water sample is filtered as necessary to remove any insoluble fractions, equilibrated with americium-243 tracer, and concentrated by evaporation or calcium phosphate precipitation. The sample test source is prepared by microprecipitation with neodymium fluoride. Standard laboratory protocol for the use of an alpha spectrometer is used when the sample is ready for counting.

Special Considerations: This method has been selected for rapid qualitative screening of drinking water samples. It is not intended for use in compliance monitoring of drinking water. The presence of higher valence anions such as phosphates can lead to lower yields when using the evaporation option in this method, due to competition with active sites on the resin. High levels of iron, manganese, calcium or magnesium can also have an impact on exchange site availability and or poison the extraction resin used in this method.

The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately form the entire sample. Such compounds include:

- Reducing or oxidizing compounds can result in lower measured concentrations when using this method, which requires specific valence states for radionuclides.
- Compounds, such as clays that are also present in some decontamination agents, contain iron, magnesium and calcium, which can be released as ions via ion exchange in the presence of radionuclides, and cause interference when analyzing water samples.
- Compounds containing carbonate, fluoride, hydroxide or phosphate can precipitate radionuclides out of solution prior to analysis.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2010. "Rapid Radiochemical Method for Americium-241 in Water for Environmental Restoration Following Homeland Security Events." *Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events.* Montgomery, AL: U.S. EPA. EPA 402-R-10-001. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.13 EPA Method: Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Water for Environmental Restoration Following Homeland Security Events

Analyte(s)	CAS RN
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Plutonium-238 and -239 in water
Method Selected for: This method has been selected for qualitative analysis of drinking water samples.

Description of Method: This method is based on the sequential use of two chromatographic extraction resins to isolate and purify plutonium by removing interfering radionuclides as well as other components of the matrix in order to prepare the plutonium fraction for counting by alpha spectrometry. The method utilizes vacuum-assisted flow to improve the speed of the separations. Prior to using the extraction resins, a water sample is filtered as necessary to remove any insoluble fractions, equilibrated with plutonium-242 tracer, and concentrated by either evaporation or coprecipitation with calcium phosphate. The sample test source is prepared by microprecipitation with neodymium fluoride. Standard laboratory protocol for the use of an alpha spectrometer is used when the sample is ready for counting.

Special Considerations: This method has been selected for rapid qualitative screening of drinking water samples. It is not intended for use in compliance monitoring of drinking water. The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well.
- Compounds such as clays can contain iron, magnesium or calcium, which can be released as ions via ion exchange, and cause interference when analyzing water samples.
- High levels of iron, manganese, calcium or magnesium can also have an impact on exchange site availability and/or poison the extraction resins used in this method.
- Higher valence anions may lead to lower yields when using the evaporation option due to competition with active sites on the resin used to collect the radionuclides.
- The presence of fluoride can precipitate out plutonium prior to measurement.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2010. "Rapid Radiochemical Method for Plutonium-238 and -239 in Water for Environmental Restoration Following Homeland Security Events." *Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events.* Montgomery, AL: U.S. EPA. EPA 402-R-10-001. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.14 EPA Method: Rapid Radiochemical Method for Radium-226 in Water for Environmental Restoration Following Homeland Security Events

Analyte(s)	CAS RN
Radium-223	15623-45-7
Radium-226	13982-63-3

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Radium-226 in water
Method Selected for: This method has been selected for qualitative analysis of radium-226 in drinking water samples and for the qualitative and confirmatory analysis of radium-223 in drinking water, aqueous and liquid phase, soil and sediment, surface wipes, air filters and vegetation samples.

Description of Method: A known quantity of radium-225 is used as the yield determinant in this analysis. The sample is initially digested using concentrated nitric acid, followed by volume reduction and conversion to the chloride salt using concentrated hydrochloric acid. The solution is adjusted to a neutral pH and batch equilibrated with manganese resin to separate radium from any radioactive and/or non-radioactive matrix constituents. Further selectivity is achieved using a column containing Diphonix® resin. The radium (including radium-226 and -223) eluted from the column is prepared for counting by microprecipitation with barium sulfate. Low-level measurements are performed by alpha spectrometry. The activity measured in the radium-226 and -223 region of interest is corrected for chemical yield based on the observed activity of the alpha peak at 7.07 mega-electron volts [MeV].

Special Considerations: This method has been selected for rapid qualitative screening of drinking water samples. It is not intended for use in compliance monitoring of drinking water. Although the method has not been validated in sample types other than water, it is likely be applicable to the additional sample types for qualitative and confirmatory analyses of radium-223. The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, by preventing them from being trapped on an ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well.
- Permanganate and permanganic acid can be reduced to insoluble manganese (IV) oxide, which could remove radium.
- Clays can contain iron, magnesium or calcium, which can be released as ions via ion exchange in the presence of certain radionuclides, and cause interference in the analysis of the water.
- High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison the extraction resin used in this method.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2010. "Rapid Radiochemical Method for Radium-226 in Water for Environmental Restoration Following Homeland Security Events." *Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events*. Montgomery, AL: U.S. EPA. EPA 402-R-10-001. http://www.epa.gov/sites/production/files/2015-07/documents/epa-402-r-10-001.pdf

6.2.15 EPA Method: Rapid Radiochemical Method for Radiostrontium in Water for Environmental Restoration Following Homeland Security Events

Analyte(s)	CAS RN
Strontium-90	10098-97-2

Analysis Purpose: Qualitative analysis
Technique: Beta counting

Method Developed for: Strontium-90 in water
Method Selected for: This method has been selected for qualitative analysis of drinking water samples.

Description of Method: Strontium is isolated from the sample matrix and purified from potentially interfering radionuclides and matrix constituents using a strontium-specific, rapid chemical separation procedure. The sample is equilibrated with strontium carrier, and concentrated by coprecipitation with strontium/barium carbonate. If insoluble residues are noted during acid dissolution steps, the residue and precipitate mixture is digested in 8M nitric acid to solubilize strontium. The solution is passed through a chromatography column that selectively retains strontium while allowing most interfering radionuclides and matrix constituents to pass through to waste. If present in the sample, residual plutonium and several interfering tetravalent radionuclides are stripped from the column using an oxalic acid/ nitric acid rinse. Strontium is eluted from the column with 0.05M nitric acid and taken to dryness in a tared, stainless steel planchet. The planchet containing the strontium nitrate precipitate is weighed to determine the strontium yield. The sample test source is promptly counted on a gas flow proportional counter to determine the beta emission rate, which is used to calculate the total radiostrontium activity.

Special Considerations: This method has been selected for rapid qualitative screening of drinking water samples. It is not intended for use in compliance monitoring of drinking water. High levels of radioactive cesium or cobalt (>1000 times the activity of strontium being measured) may not be completely removed during ion exchange and can cause interferences. Chelating compounds, such as those present in some decontamination agents, can compromise the collection of strontium prior to analysis, by preventing it from being trapped on an ion exchange column or from being precipitated out of solution. Compounds containing carbonate, fluoride, phosphate or sulfate, also present in some decontamination agents, can precipitate radionuclides out of solution prior to analysis. This precipitation can result in a lesser amount of strontium in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2010. "Rapid Radiochemical Method for Radiostrontium in Water for Environmental Restoration Following Homeland Security Events." *Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events.* Montgomery, AL: U.S. EPA. EPA 402-R-10-001. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.16 EPA Method: Rapid Radiochemical Method for Isotopic Uranium in Water for Environmental Restoration Following Homeland Security Events

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Uranium-234, -235 and -238 in water
Method Selected for: This method has been selected for qualitative analysis of drinking water samples.

Description of Method: This method is based on the sequential elution of interfering radionuclides as well as other components of the sample matrix by extraction chromatography to isolate and purify uranium for counting by alpha spectrometry. The method utilizes vacuum assisted flow to improve the speed of the separations. Prior to the use of the extraction resins, a water sample is filtered as necessary to remove any insoluble fractions, equilibrated with uranium-232 tracer, and concentrated by either evaporation or coprecipitation with calcium phosphate. The sample test source is prepared by microprecipitation with neodymium fluoride. Standard laboratory protocol for the use of an alpha spectrometer is used when the sample is ready for counting.

Special Considerations: This method has been selected for rapid qualitative screening of drinking water samples. The method is not intended for use in compliance monitoring of drinking water. Higher valence anions may lead to lower yields when using the evaporation option due to competition with active sites on the resin. The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately form the entire sample. Such compounds include:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, by preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well.
- Clays can contain iron, magnesium or calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause interference in the analysis of water. High levels of iron, manganese, calcium or magnesium can also impact exchange site availability and/or poison the extraction resins used in this method.
- Compounds containing carbonate, fluoride, hydroxide or phosphate can precipitate uranium out of solution.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2010. "Rapid Radiochemical Method for Isotopic Uranium in Water for Environmental Restoration Following Homeland Security Events." *Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events*. Montgomery, AL: U.S. EPA. EPA 402-R-10-001. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.17 EPA Method: Rapid Method for Acid Digestion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Radium-226	13982-63-3
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Americium-241, plutonium-238 and -239, radium-226, strontium-90, uranium-234, -235 and -238 in surface wipes and air filters
Method Selected for: This method has been selected for qualitative analysis of surface wipe and air filter samples.

Description of Method: The method is based on the complete dissolution of both the filter material and deposited particulates. Glass-fiber filters (the siliceous filter as well as deposited silicates) are dissolved with direct application of hydrofluoric acid. The addition of nitric and hydrochloric acids facilitates dissolution of remaining solids. The sample digestate is taken to dryness and re-dissolved in nitric acid. Filters composed of organic materials, such as cellulose or polypropylene, are dry ashed in a 450°C

muffle furnace to destroy the organic filter material, then processed through the acid dissolution steps referenced above for non-organic filter material. Once sample dissolution is complete, it is re-dissolved in nitric acid solution. The sample is then processed for specific analyte determination using one of the following rapid methods contained in *Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events*
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

- Rapid Radiochemical Method for Americium-241 in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.12)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.13)
- Rapid Radiochemical Method for Radium-226 in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.14)
- Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.15)
- Rapid Radiochemical Method for Isotopic Uranium in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.16)

Special Considerations: This method is a gross pre-treatment technique, to be used prior to use of the appropriate rapid separation methods cited above. Filters that contain large amounts of particulate material may result in persistent undissolved particulates in the digestion process. These samples may require repeated application of the digestion procedure to cause a complete dissolution of the particulates. If refractory constituents are suspected in the sampled particulates or the acidic digestion procedure is otherwise deemed to be ineffective because of refractory residuals or constituents, the alternate *Rapid Method for Sodium Carbonate Fusion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events* (Section 6.2.18) should be considered for sample preparation.

Some concrete or brick materials can have native concentrations of uranium, radium, thorium, strontium or barium, all of which can have an effect on the chemical separations used following sample fusion. In some cases (e.g., radium or strontium analysis), elemental analysis of the digest prior to chemical separation may be necessary to determine native concentrations of carrier elements. Lanthanum is used in this method to pre-concentrate actinides, along with lanthanum (III) fluoride, to eliminate matrix interferences including silica, which can cause column flow problems. Compounds contained in decontamination agents are not expected to cause interferences during sample preparation; see the sections corresponding to the analytical methods listed in the description of this method for potential interferences caused by constituents of decontamination agents.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2012. "Rapid Method for Acid Digestion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events." Montgomery, AL: U.S. EPA. EPA 402-R-12-009.
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.18 EPA Method: Rapid Method for Sodium Carbonate Fusion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Radium-226	13982-63-3
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Americium-241, plutonium-238 and -239, radium-226, strontium-90, uranium-234, -235 and -238 in surface wipes and air filters
Method Selected for: This method has been selected for qualitative analysis of surface wipe and air filter samples.

Description of Method: The method is based on the complete dissolution of both the filter or swipe material and the deposited particulates. Glass-fiber media and deposited particulates are destroyed by fusion with molten sodium carbonate in a nickel or platinum crucible. The resulting fusion cake is dissolved in hydrochloric acid. Filters composed of organic materials, such as cellulose or polypropylene are charred in a crucible to destroy the organic filter material. The resulting charred media and deposited particulates are destroyed by fusion with molten sodium carbonate in a nickel or platinum crucible. The resulting fusion cake is dissolved in hydrochloric acid. Once sample fusion is complete and the fusion cake is dissolved in hydrochloric acid, the sample is processed for specific analyte determination using one of the following rapid methods:

- Rapid Radiochemical Method for Americium-241 in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.12)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.13)
- Rapid Radiochemical Method for Radium-226 in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.14)
- Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.15)
- Rapid Radiochemical Method for Isotopic Uranium in Water for Environmental Remediation Following Homeland Security Events (Section 6.2.16)

Special Considerations: This method is a gross pre-treatment technique, to be used prior to use of the appropriate rapid separation methods cited. Filters that contain large amounts of particulate material may result in persistent undissolved particulates in the digestion process. These samples may require repeated application of the digestion procedure to cause a complete dissolution of the particulates.

Some surface materials (e.g., concrete or brick) can contain native concentrations of uranium, radium, thorium, strontium or barium, all of which may have an effect on the chemical separations used following the fusion of the sample. In some cases (e.g., radium or strontium analysis), elemental analysis of the

digest prior to chemical separation may be necessary to determine concentrations of carrier elements. Trace levels of radium-226 may be present in sodium carbonate used in the pre-concentration step used in this method. Compounds contained in decontamination agents are not expected to cause interferences during sample preparation; see the sections corresponding to the analytical methods listed in the description of this method for potential interferences caused by constituents of decontamination agents.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2012. "Rapid Method for Sodium Carbonate Fusion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R-12-008.
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.19 Rapid Method for Radium in Soil Incorporating the Fusion of Soil and Soil-Related Matrices with the Radioanalytical Counting Method for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Radium-226 in soil samples
Method Selected for: This method has been selected for qualitative analysis of soil/sediment samples.

Description of Method: This method is based on the complete fusion of a representative, finely ground 1-g aliquot of dried sample with no insoluble residue remaining after dissolution of the fused melt in acid. For organic soils, the sample is dry ashed at 600°C in an appropriate vessel prior to fusion, then dissolved in a crucible with hydrofluoric acid and evaporated to dryness. Dry flux mix (equal weight of dried sodium carbonate, potassium carbonate and boric acid) is added, and the crucible is warmed under a flame until a reaction initiates. The crucible is then heated under full flame until the reaction subsides and the melt is completely liquid and homogeneous. After cooling, the solidified melt is dissolved in hydrochloric acid, and transferred to a digestion container while rinsing the crucible with 6M hydrochloric acid. The barium content is determined for a small aliquot of the dissolved flux by adding sufficient amount of barium so that the final mass (native plus added) is not more than 90 µg and then analyzing by ICP-AES. A manganese (IV) solution and phenolphthalein indicator are added to this mixture, and the pH is adjusted with sodium hydroxide until the solution turns pink. Hydrogen peroxide is slowly added forming insoluble manganese (II) oxide. The manganese (IV) oxide precipitate is centrifuged, rinsed with water and dissolved in a manganese (IV) oxide stripping agent. Ascorbic acid is added and the solution is passed through a resin column, rinsing with hydrochloric acid. The rinse solution is collected, ammonium sulfate and isopropanol are added, and the solution is placed in a cold water ultrasonic bath for 20 minutes, after which it is filtered, rinsing with a solution of ammonium sulfate in isopropanol. The filter is then placed in a Petri dish, dried, and stored for at least 24 hours. The sample is then counted by alpha spectrometry.

Special Considerations: The presence of discrete radioactive particles or particles larger than 150 µm can require additional sample preparation, as described in Sections A4 and A5.2.3 of the method (Interferences and Hot Particles, respectively). Soils with high silica content may require either additional fusing reagent and boric acid or a longer fusion melt. Platinum crucibles must be used when digesting samples with hydrofluoric acid. If platinum crucibles are not available, effective alternate methods are

available that use zirconium crucibles (see *Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick Matrices Prior to Am, Pu, Sr, Ra, and U Analyses* [Section 6.3.3] and *Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Building Materials for Environmental Remediation Following Radiological Incidents* [Section 6.3.1]). At this time, there are no known interferences posed by decontamination agents that might be present in a sample.

In some cases, elemental analysis of the digest prior to chemical separations may be necessary to determine native concentrations of carrier elements present in the sample. Trace levels of radium-226 might be present in the sodium carbonate used in the pre-concentration step.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2012. "Rapid Method for Radium in Soil Incorporating the Fusion of Soil and Soil-Related Matrices with the Radioanalytical Counting Method for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA-600-R-12-635.
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.20 Rapid Method for Fusion of Soil and Soil-Related Matrices Prior to Americium, Plutonium, and Uranium Analyses for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Americium-241, plutonium-238, plutonium-239, uranium-234, uranium-235 and uranium-238 in soil samples
Method Selected for: This method has been selected for qualitative analysis of soil/sediment samples

Description of Method: The method is based on the complete fusion of a representative finely ground 1-g aliquot of dried sample with no insoluble residue remaining after dissolution of the fused melt in acid. For organic soils, the sample is dry ashed at 600°C in an appropriate vessel prior to fusion. The sample is dissolved in a crucible with hydrofluoric acid, and evaporated to dryness on a hotplate at medium to high heat (~300°C). Dry flux mix (equal weight of dried sodium carbonate, potassium carbonate and boric acid) is added, and the crucible is warmed under a flame until a reaction initiates. The crucible is then heated under full flame until the reaction subsides and the melt is completely liquid and homogeneous. After cooling, the solidified melt is dissolved in nitric acid. The dissolved sample is transferred to an appropriately sized beaker, and the crucible is rinsed with nitric acid to ensure a quantitative transfer of material. The sample is then processed using one of the following methods:

- Rapid Radiochemical Method for Americium-241 in Water (Section 6.2.12)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Water (Section 6.2.13)
- Rapid Radiochemical Method for Isotopic Uranium in Water (Section 6.2.16)

Special Considerations: The presence of discrete radioactive particles or particles larger than 150 µm can require additional sample preparation as described in Sections A4 and A5.2.3 of the method (Interferences and Hot Particles, respectively). Soils with high silica content may require either additional fusing reagent and boric acid or a longer fusion melt. Platinum crucibles must be used when digesting samples with hydrofluoric acid. If platinum crucibles are not available, effective alternate methods are available that use zirconium crucibles (see *Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick Matrices Prior to Am, Pu, Sr, Ra, and U Analyses* [Section 6.3.3] and *Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Building Materials for Environmental Remediation Following Radiological Incidents* [Section 6.3.1]). Compounds contained in decontamination agents are not expected to cause interferences during sample preparation; see the sections corresponding to the analytical methods listed in the description of this method for potential interferences caused by constituents of decontamination agents.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2012. "Rapid Method for Fusion of Soil and Soil-Related Matrices Prior to Americium, Plutonium, and Uranium Analyses for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA-600-R-12-636, EPA-600-R-12-637 and EPA-600-R-12-638.
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.21 Rapid Method for Sodium Carbonate Fusion of Soil and Soil-Related Matrices Prior to Strontium-90 Analyses for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Strontium-90	10098-97-2

Analysis Purpose: Qualitative analysis
Technique: Beta counting

Method Developed for: Strontium-90 in soil samples
Method Selected for: This method has been selected for qualitative analysis of soil/sediment samples

Description of Method: The method is based on the complete fusion of a representative, finely ground 1-g aliquot of dried sample with no insoluble residue remaining after dissolution of the fused melt in acid. For organic soils, the sample is dry ashed at 600°C prior to fusion. The sample is dissolved in a crucible with hydrofluoric acid and evaporated to dryness on a hotplate at medium to high heat (~300°C). Dry flux mix (equal weight of dried sodium carbonate, potassium carbonate and boric acid) is added, and the crucible is heated under a flame until a reaction initiates. The crucible is then heated under full flame until the reaction subsides and the melt is completely liquid and homogeneous. After cooling, the solidified melt is dissolved in nitric acid. Calcium solution and phenolphthalein indicator are added to this mixture, and the pH is adjusted to 8.3 with sodium hydroxide. The sample will become pinkish-orange due to the indicator color change and the formation of hydroxide precipitate. Sodium carbonate and heat are added to complete precipitation. After cooling and allowing the precipitate to settle, the supernatant is decanted and the precipitate is transferred to a centrifuge tube and dissolved in nitric acid. The sample is then processed for strontium-90 determination using *Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Water for Environmental Restoration Following Homeland Security Events* (Section 6.2.15).

Special Considerations: The presence of discrete radioactive particles or particles larger than 150 µm can require additional sample preparation as described in Sections A4 and A5.2.3 of the method (Interferences and Hot Particles, respectively). Soils with high silica content may require either additional fusing reagent and boric acid or a longer fusion melt. Platinum crucibles must be used in this method

when digesting samples with hydrofluoric acid. If platinum crucibles are not available, an effective, alternate method is available that uses zirconium crucibles (see *Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick Matrices Prior to Am, Pu, Sr, Ra, and U Analyses* [Section 6.3.3] and *Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Building Materials for Environmental Remediation Following Radiological Incidents* [Section 6.3.1]). Compounds contained in decontamination agents are not expected to cause interferences during sample preparation; see the sections corresponding to the analytical methods listed in the description of this method for potential interferences caused by constituents of decontamination agents.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2012. "Rapid Method for Sodium Carbonate Fusion of Soil and Soil-Related Matrices Prior to Strontium-90 Analyses for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA-600-R-12-640.
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.22 Rapid Method for Sodium Hydroxide/Sodium Peroxide Fusion of Radioisotope Thermoelectric Generator Materials in Water and Air Filter Matrices Prior to Plutonium Analyses for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Plutonium-238 and -239 in water and air filters
Method Selected for: This method has been selected as a pre-treatment technique supporting analysis of refractory radioisotopic forms of plutonium in drinking water and air filters using the following qualitative techniques:

- Rapid methods for acid or fusion digestion (Sections 6.2.17 and 6.2.18)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium 239/240 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.5)

Description of Method: This method is a pre-treatment technique for qualitative analysis of water and air filters, and has been validated together with the chemical separation and analysis process described in EPA's *Rapid Radiochemical Method for Plutonium-238 and Plutonium 239/240 in Building Materials for Environmental Remediation Following Radiological Incidents* (Section 6.3.5). The method is based on total dissolution of radioisotope thermoelectric generator (RTG). Air filters are fused using rapid sodium hydroxide/sodium peroxide at 700°C. For water samples, refractory RTG particles are collected on a 0.45µm filter using a vacuum, and RTG activity in the filtrate is preconcentrated using calcium phosphate precipitation. Solid fractions and filtrate fractions are processed separately by fusing with sodium hydroxide/sodium peroxide prior to subsequent chemical separation and alpha spectrometric analysis. Pre-concentration steps are needed to eliminate the alkaline fusion matrix and collect the radionuclides. Plutonium is separated from the fusion matrix using a lanthanum/calcium fluoride matrix removal step in preparation for separation and analysis using the rapid separation method cited above. Assuming a 68 m^3 air volume, the method is capable of meeting a required minimum detectable concentration (MDC) of 0.003 pCi/m^3 or below for air filters (240-minute count time) and an uncertainty of 1.9 pCi/filter at and below the analytical action level of 15.0 pCi/filter (360-minute count time). Assuming a 1-L volume and

360-minute count time, the method is capable of satisfying an uncertainty of 2.1 pCi/L at and below an analytical action level of 16.3 pCi/L, and meeting a required MDC of 0.23 pCi/L for water samples (filtered solids, filtrate, or combined result) with a 240-minute count time.

Special Considerations: Organic-based materials, such as cellulose nitrate or cellulose acetate filters, may react vigorously upon addition of peroxide or during charring steps. Wet ashing with nitric acid and hydrogen peroxide is needed to destroy organic constituents prior to fusion. Samples with elevated activity or samples that require multiple analyses may need to be split after dissolution. Reducing or oxidizing compounds, such as those present in some decontamination agents, can impact this method, which requires specific valence states for radionuclides. All plutonium must be reduced to plutonium (+3 or +4) before isotopic exchange with the tracer can be achieved with reasonable certainty. Additionally, only plutonium (+3 or +4) will precipitate in the lanthanum fluoride/calcium fluoride pre-concentration step. Although peroxide may reduce plutonium +6 to +4, the valence must be controlled with certainty. Valence controls also ensure that plutonium will be present in the plutonium +4 form prior to separation. Although this method was validated using plutonium-242 tracer, plutonium-236 tracer can be used assuming it can be obtained with sufficient purity.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Method for Sodium Hydroxide/Sodium Peroxide Fusion of Radioisotope Thermoelectric Generator Materials in Water and Air Filter Matrices Prior to Plutonium Analyses for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R14-003. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.23 EPA Method: Rapid Radiochemical Method for Californium-252 in Water, Air Particulate Filters, Swipes and Soil for Environmental Restoration Following Homeland Security Events

Analyte(s)	CAS RN
Californium-252	13981-17-4

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Californium-252 in water, air particulate filters, swipes and soil samples

Method Selected for: This method has been selected for confirmatory analysis of californium-252 in drinking water, aqueous liquid, air filter, surface wipes and soil matrices.

Description of Method: This method is based on the use of extraction chromatography resins to isolate and purify californium by removing interfering radionuclides as well as other matrix components. The method utilizes vacuum-assisted flow to improve the speed of the separations. Americium-243 tracer equilibrated with the sample is used as a yield monitor.

- Water samples are concentrated using a calcium phosphate coprecipitation. The calcium phosphate precipitate is dissolved in a load solution containing ~3M nitric acid/1M aluminum nitrate before continuing with chemical separations.
- Glass-fiber or cellulose-based air particulate filter samples are wet ashed with repeated additions of nitric and hydrofluoric acids and hydrogen peroxide. The residues are treated with nitric-boric acid, and dissolved in a load solution containing 3M nitric acid/1M aluminum nitrate before continuing with chemical separations.
- Cotton-twill swipe and organic-polymer-based air particulate filter samples are dry ashed in a

beaker for 30−60 minutes using a ramped program to minimize the risk of flash-ignition. The residue is transferred to a polytetrafluoroethylene (PTFE) beaker with nitric acid and hydrogen peroxide, digested with hydrofluoric acid, and taken to dryness. The residues are wet ashed with nitric acid and hydrogen peroxide and taken to dryness before being treated with nitric-boric acid and dissolved in a load solution containing 3M nitric acid/1M aluminum nitrate for chemical separations.

- Soils are finely ground before being fused with sodium hydroxide in zirconium crucibles. The fusion cake is dissolved in water and californium preconcentrated from the alkaline matrix using an iron/titanium hydroxide precipitation (enhanced with calcium phosphate precipitation) followed by a lanthanum fluoride matrix removal step. The fluoride precipitate is dissolved with nitric-boric acid and diluted in nitric acid and aluminum nitrate to yield a load solution containing ~3M nitric acid/1M aluminum nitrate.

Extraction chromatography resins (TEVA® + DGA® resins [Eichrom Technologies, Lisle, IL, or equivalent]) are then used to isolate and purify californium and americium by removing interfering radionuclides and other matrix components. Following chemical separation of curium and americium, the sample test source is prepared by microprecipitation with cerium (III) fluoride.

Water: This method is capable of achieving a required method uncertainty for californium-252 of 2.0 pCi/L at an analytical action level of 15.3 pCi/L and a required MDC of 1.5 pCi/L, using a sample volume of 0.2 L and count time of at least 4 hours.

Air Particulate Filter: This method is capable of achieving a required method uncertainty for californium-252 of 0.57 pCi/filter at an analytical action level of 4.37 pCi/filter and a required MDC of 0.44 pCi/filter, using a sample aliquant of one filter and count time of at least 4 hours.

Swipe or Organic-Polymer-Based Air Particulate Filter: This method is capable of achieving a required method uncertainty for californium-252 of 0.12 pCi/swipe or filter at an analytical action level of 0.12 pCi/ swipe or filter and a required MDC of 0.15 pCi/ swipe or filter, using a sample aliquant of one swipe or filter and count time of at least 4 hours.

Soil: This method is capable of achieving a required method uncertainty for californium-252 of 0.18 pCi/g at an analytical action level of 1.38 pCi/g and a required MDC of 0.14 pCi/g, using a sample weight of 1 g and count time of at least 4 hours.

Special Considerations: Alpha emissions from californium-250 fall in the same region as californium-252, and cannot be differentiated from those of californium-252 using alpha spectrometry. Measurements should be reported in terms of the activity of californium-250/252. Since alpha spectrometry does not differentiate between californium-250 and californium-252, decay corrections based on the half-life of californium-252 will impart a positive bias to results as mixtures age. The effect can be minimized by keeping the time between the activity reference date (i.e., collection or standard reference date) short, or by reporting the activity at the time of the measurement.

Other radionuclides (or their short-lived progeny) that emit alpha particles that are isoenergetic with californium-252 (e.g., bismuth-212 at 6.1 MeV supported by thorium-228 and/or radium-224) must be chemically separated to prevent positive interference with the measurement. This method effectively separates these radionuclides.

The use of the americium-243 tracer assumes that both californium and americium are removed from the column at the time of elution. The separation scheme is designed to ensure that nitrates and lanthanum will not interfere with this elution. High levels of calcium in soil samples can have an adverse impact on the retention of californium and americium on the DGA resin. The method is designed to minimize calcium interference and enhance californium and americium affinity by increasing the nitrate

concentration in the load and initial rinse solutions. Non-radiological anions, including fluoride and phosphate, can complex californium and americium and lead to depressed yields. Boric acid added to the load solution will complex residual fluoride ions, while aluminum in the load solution will complex phosphate ions.

Chelating compounds, such as those present in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well. Clays, which are also present in some decontamination agents can contain iron, magnesium and calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause analytical interferences. High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison extraction resins used in this method.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. 2017. "[Validation of] Rapid Radiochemical Method for Cf-252 in Water, Air Particulate Filters, Swipes and Soils for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-S17-003. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides.

6.2.24 EPA Method: Rapid Radiochemical Method for Curium-244 in Water Samples for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Curium-244	13981-15-2

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Curium-244 in water samples
Method Selected for: This method has been selected for confirmatory analysis of curium-244 in water samples.

Description of Method: This method is based on the use of extraction chromatography resins to isolate and purify curium by removing interfering radionuclides and other matrix components and preparing the curium fraction for counting by alpha spectrometry. The method utilizes vacuum-assisted flow to improve the speed of the separations. An americium-243 tracer is equilibrated with the water sample and used as a yield monitor. Following chemical separation of curium and americium, the sample test source is prepared by microprecipitation with cerium (III) fluoride. Alpha emissions from the source are measured using an alpha spectrometer and used to calculate the activity of curium-244 in the sample.

Using a 0.2 L sample and a count time of four hours, this method is capable of achieving an uncertainty of 2.0 pCi/L californium-252 at an analytical action level of 15 pCi/L and a required MDC of 1.515 pCi/L.

Special Considerations: The alpha emissions from curium-243 fall in the same region as curium-244 and cannot be differentiated using alpha spectrometry. Although curium-243 and curium-244 alpha emissions overlap, monitoring the region of the spectrum between 5.8 and 6.0 MeV for less intense emissions of curium-243 can qualitatively indicate the presence of curium-243 in a sample. Alpha spectrometry measurements that show activity in the region of interest for curium-244 should be reported as curium-243/244. Americium and californium are chemical analogs of curium in the separations scheme used for this analysis. Several isotopes of californium or americium emit alpha particles within the region of interest for curium-244. These include californium-249 and californium-251. If high levels of californium could be present in samples, alpha spectrometry results should be monitored for other isotopes of californium. Radionuclides of other elements (or their short-lived progeny) that emit alpha

particles which are isoenergetic with curium-244 (e.g., thorium-227 or actinium-225 5.8 MeV) must be chemically separated using the method procedures to prevent positive interference.

Non-radiological anions that can complex curium, including fluoride and phosphate, can lead to depressed yields. Aluminum in the load solution will complex both fluoride and residual phosphate. High levels of calcium can have an adverse impact on curium and americium retention on DGA resin. Calcium retention is minimized, and curium and americium affinity is enhanced, by increasing nitrate concentrations in the load and initial rinse solutions. A dilute nitric acid rinse is performed on DGA resin to remove calcium that could otherwise end up in the sample test source as the fluoride. For samples containing elevated concentrations of calcium, it may be advisable to increase the volume of this rinse step slightly to better remove calcium ions and possibly improve alpha peak resolution.

Chelating compounds, such as those present in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well. Clays, which are also present in some decontamination agents, can contain iron, magnesium or calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause analytical interferences. High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison extraction resins used in this method.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. May 2017. "Rapid Radiochemical Method for Curium-244 in Water Samples for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-S17-001. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides.

6.2.25 EPA Method: Rapid Radiochemical Method for Curium-244 in Air Particulate Filters, Swipes and Soil

Analyte(s)	CAS RN
Curium-244	13981-15-2

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Curium-244 in air particulate filters, swipes and soil samples
Method Selected for: This method has been selected for confirmatory analysis of curium-244 in air particulate filters, swipes and soil matrices.

Description of Method: This method is based on the use of extraction chromatography resins to isolate and purify curium by removing interfering radionuclides and matrix components in order to prepare the curium fraction for counting by alpha spectrometry. The method utilizes vacuum-assisted flow to improve the speed of the separations. An americium-243 tracer is equilibrated with the sample as a yield monitor.

- Glass-fiber or cellulose-based air particulate filter samples are wet ashed with repeated additions of nitric acid and hydrofluoric acid, and hydrogen peroxide. The residues are treated with nitric-boric acid and dissolved in a load solution containing ~3M nitric acid/1M aluminum nitrate before continuing with chemical separations.
- Cotton-twill swipe and organic-polymer-based air particulate filter samples are dry ashed in a beaker for 30-60 minutes using a ramped program to minimize the risk of flash-ignition. The residue is transferred to a PTFE beaker with nitric acid and hydrogen peroxide, digested with hydrofluoric acid, and taken to dryness. The residues are then wet ashed with nitric acid and hydrogen peroxide and taken to dryness before being treated with nitric-boric acid and dissolved

in a load solution containing ~3M nitric acid/1M aluminum nitrate before continuing with chemical separations.

- Soils are finely ground before being fused with sodium hydroxide in zirconium crucibles. The fusion cake is dissolved in water and curium preconcentrated from the alkaline matrix using an iron/titanium hydroxide precipitation (enhanced with calcium phosphate precipitation) followed by a lanthanum fluoride matrix removal step. The fluoride precipitate is dissolved with nitric-boric acid and diluted in nitric acid and aluminum nitrate to yield a load solution containing ~3M nitric acid/1M aluminum nitrate before continuing with chemical separations.

Extraction chromatography resins are then used to isolate and purify curium by removing interfering radionuclides and other matrix components. The method utilizes vacuum-assisted flow to improve the speed of the separations. Following chemical separation of curium and americium, the sample test source (STS) is prepared by microprecipitation with cerium (III) fluoride.

Air Particulate Filters: This method is capable of achieving a required method uncertainty for curium-244 of 1.4 pCi/filter at an analytical action level of 10.5 pCi/filter and a required MDC of 0.25 pCi/filter, using a sample aliquant of one filter and a count time of at least four hours.

Swipes (or Organic-Polymer-Based Air Particulate Filters): This method is capable of achieving a required method uncertainty for curium-244 of 0.051 pCi/swipe at an analytical action level of 0.39 pCi/swipe and a required MDC for of 0.065 pCi/swipe, using one swipe and a count time of at least four hours.

Soil: This method is capable of achieving a required method uncertainty for curium-244 of 0.66 pCi/g at an analytical action level of 5.09 pCi/g and a required MDC of 0.66 pCi/g, using a sample weight of 1 gram and a count time of at least four hours.

Special Considerations: The alpha emissions from curium-243 fall in the same region as curium-244 and cannot be differentiated using alpha spectrometry. Although curium-243 and curium-244 alpha emissions overlap, monitoring the region of the spectrum between 5.8 and 6.0 MeV for less intense emissions of curium-243 can qualitatively indicate the presence of curium-243 in a sample. Alpha spectrometry measurements that show activity in the region of interest for curium-244 should be reported as curium-243/244. Americium and californium are chemical analogs of curium in the separations scheme used for this analysis. Several isotopes of californium or americium emit alpha particles within the region of interest for curium-244. These include californium-249 and californium-251. If high levels of californium could be present in samples, alpha spectrometry results should be monitored for other isotopes of californium. Radionuclides of other elements (or their short-lived progeny) that emit alpha particles which are isoenergetic with curium-244 (e.g., thorium-227 or actinium-225 5.8 MeV) must be chemically separated using the method procedures to prevent positive interference.

Non-radiological anions that can complex curium, including fluoride and phosphate, can lead to depressed yields. Aluminum in the load solution will complex both fluoride and residual phosphate. High levels of calcium can have an adverse impact on curium and americium retention on DGA resin. Calcium retention is minimized, and curium and americium affinity is enhanced, by increasing nitrate concentrations in the load and initial rinse solutions. A dilute nitric acid rinse is performed on DGA resin to remove calcium that could otherwise end up in the sample test source as the fluoride. For samples containing elevated concentrations of calcium, it may be advisable to increase the volume of this rinse step slightly to better remove calcium ions and possibly improve alpha peak resolution.

Chelating compounds, such as those present in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well. Clays, which are also present in some decontamination agents, can contain iron,

magnesium or calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause analytical interferences. High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison extraction resins used in this method.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. May 2017. "Rapid Radiochemical Method for Curium-244 in Air Particulate Filters, Swipes and Soil for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-S17-004. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides.

6.2.26 Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Radium-226 in building materials
Method Selected for: This method is selected for confirmatory analysis of radium-226 in surface wipes and air filters (also see Section 6.3.2 for application of this method to building materials).

Description of Method: A known quantity of radium-225 is used as the yield tracer in this analysis. Samples are fused using procedures in *Rapid methods for acid or fusion digestion* (Sections 6.2.17 and 6.2.18), and the radium isotopes are removed from the fusion matrix using a carbonate precipitation step. The sample is acidified and loaded onto a cation exchange resin to remove interferences such as calcium, and radium is eluted from the cation resin with 8M nitric acid. After evaporation of the eluate, the sample is dissolved in a minimal amount of 3M nitric acid and passed through Sr Resin to remove any barium. This solution is then evaporated to dryness, redissolved in 0.02M hydrochloric acid, and passed through Ln Resin to remove interferences such as calcium, and to remove the initial actinium-225. The radium (including radium-226) is prepared for counting by micro-precipitation with barium sulfate. Activity measured in the radium-226 region of interest is corrected for chemical yield based on observed activity of the alpha peak at 7.07 MeV (astatine-217, the third progeny of radium-225).

This method is suited for low-level measurements for radium-226 using alpha spectrometry and is capable of satisfying a method uncertainty of 0.83 pCi/g at an analytical action level of 6.41 pCi/g, using a sample aliquant of ~1g and count time of at least eight hours.

Special Considerations: Depending on actual spectral resolution, method performance may be compromised if samples contain high levels of other radium isotopes (e.g., ~3 times the radium-226 activity concentration) due to ingrowth of interfering decay progeny. Calcium, iron (+3 oxidation state), and radionuclides with overlapping alpha energies, such as thorium-229, uranium-234, and neptunium-237, will interfere if they are not removed effectively. Delaying the count significantly longer than one day may introduce positive bias in results near the detection threshold due to the decay progeny from the radium 225 tracer. If radium-226 measurements close to detection levels are required and sample counting cannot be performed within ~36 hours of tracer addition, the impact of tracer progeny tailing into the radium-226 may be minimized by reducing the amount of the tracer that is added to the sample. This will aid in improving the signal-to-noise ratio for the radium-226 peak by minimizing the amount of tailing from higher energy alphas of the radium-225 progeny. If actinium-225 is present prior to the final separation time and the flow rate through the column is too fast (>1.5 drops/second), then actinium-225 will break through the resin, resulting in a high bias in the tracer yield. Additional information regarding

procedures to remove or minimize interferences is provided in Section 4.0 of the method.
Clays that are present in some decontamination agents can contain iron, magnesium or calcium, which can be released as ions via ion exchange in the presence of certain radionuclides, and cause interferences. High levels of iron, manganese, calcium or magnesium might have an impact on exchange site availability and or poisoning of the extraction resins used. Chelators, also present in some decontamination agents, can tightly complex barium, calcium, iron and magnesium that may be present in the sample causing interferences when analyzing for radium-226.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R14-002.
https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.2.27 EPA Method: NAREL Standard Operating Procedure for Actinides in Environmental Matrices by Extraction Chromatography

Analyte(s)	CAS RN
Neptunium-237	13994-20-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Thorium-227	15623-47-9
Thorium-228	14274-82-9
Thorium-230	14269-63-7
Thorium-232	7440-29-1
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Alpha spectrometry / Beta counting (analysis of tracer)
Method Developed for: Americium-241; neptunium-237; plutonium-238 and -239; thorium-227, -228, -230 and -232; and uranium-234, -235 and -238 in water, soil, vegetation, air filters, swipes and tissue
Method Selected for: This method has been selected for qualitative and confirmatory analysis of aqueous and liquid phase, soil and sediment, surface wipes, air filters and vegetation samples.

- Standard Methods 7500-U B (Section 6.2.57) and 7500-U C (Section 6.2.58) should be used for qualitative (7500-U B) and confirmatory (7500-U C) analysis of uranium-234, -235 and -238 in aqueous and liquid phase samples.
- ASTM Method D3084-05 (Section 6.2.49) should be used for qualitative analysis of plutonium-238 and -239 in aqueous and liquid phase samples.
- EPA NAREL Rapid Method for Fusion of Soil and Soil-Related Matrices (Section 6.2.20) should be used for qualitative analysis of plutonium-238 and -239 and uranium-234, -235 and -238, in soil and sediment samples.
- EPA NAREL Rapid Methods for Acid or Fusion Digestion (Sections 6.2.17 and 6.2.18) should be used for qualitative analysis of plutonium-238 and -239 and uranium-234, -235 and -238, in surface wipes and air filters.
- DOE SRS Actinides and Sr-89/90 in Vegetation (Section 6.2.42) should be used for qualitative analysis of plutonium-238 and -239 and uranium-234, -235 and -238 in vegetation.
- EML HASL-300 Methods Am-06-RC (Section 6.2.30) and U-02-RC (Section 6.2.38) should be used for confirmatory analysis of plutonium -238 and -239 and uranium-234, -235 and -238 in

vegetation, respectively.

Description of Method: This method involves the use a tandem arrangement of cartridges containing extraction chromatographic resins connected in series, which effectively separate and isolate americium, plutonium, thorium, uranium and neptunium from a variety of environmental matrices. The oxidation states of the elements of interest in the load solution are as follows: americium (III), neptunium (IV), plutonium (III), thorium (IV) and uranium (VI). The sample is first loaded onto a TEVA®-resin (Eichrom Technologies, Lisle, IL, or equivalent) cartridge. Any thorium or neptunium present in the sample will be retained on the cartridge. The effluent passes through this cartridge and onto a transuranic (TRU)-resin cartridge, which will retain any americium, plutonium or uranium. The tandem cartridge arrangement is then separated and the elements of interest are selectively eluted, then co-precipitated as a fluoride complex and radio-assayed by alpha-particle spectrometry.

Special Considerations: Prior to adding americium-243 or neptunium-239 tracers, the sample should be analyzed for native neptunium-239 by the appropriate method (EPA Method 901.1 for drinking water; Standard Method 7120 for aqueous and liquid phase; and HASL-300 Method Ga-01-R for surface wipes, air filters and vegetation).

If present, iron (III) can interfere with retention of the actinides on the TRU resin, and must be reduced with ascorbic acid to iron (II) so that it will not interfere with desired chemical reactions. Phosphates, sulfates, and oxalates can cause interferences by forming insoluble complexes with actinides. These anions can be complexed to aluminum (III) so that they do not interfere with the analysis. In fact, the presence of aluminum (III) actually increases the retention factor of americium to the TRU resin. There may be instances when increasing the aluminum (III) concentration in the nitric acid/aluminum nitrate load solution can improve radiochemical separation and recovery.

Neptunium and americium cannot be determined sequentially and each must be analyzed using a separate sample aliquot. The tracer used for americium analysis is americium-243, while the one used for neptunium analysis is neptunium-239, which is in equilibrium with americium-243. The neptunium-239 yield is determined by beta counting using approximately the amount of neptunium-239 equivalent to ~50 dpm of americium-243. The calcium phosphate preparation option described may give low plutonium recoveries in unpreserved or weakly preserved samples. Until the calcium phosphate procedure is revised, analysts must use the evaporation/digestion option when preparing water samples for plutonium analysis.

High levels of iron, manganese, calcium or magnesium, which are present in some decontamination agents, can impact exchange site availability and/or poison the extraction resins. The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately form the entire sample. Such compounds include:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well.
- Clays can contain iron, magnesium and calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause analytical interferences.
- Compounds containing carbonate, fluoride, hydroxide, or phosphate, present in some decontamination agents can precipitate radionuclides out of solution prior to analysis of water samples, resulting in a lesser amount of polonium in cases where an aliquot of water sample is transferred prior to the precipitation step.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. "NAREL Standard

Operating Procedure for Actinides in Environmental Matrices by Extraction Chromatography." AM/SOP-1. Revision 7. Montgomery, AL: U.S. EPA. For access to this procedure, consult the appropriate contact in Section 4.0.

6.2.28 EML HASL-300 Method Am-01-RC: Americium in Soil

Analyte(s)	CAS RN
Americium-241	14596-10-2

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Americium in soil
Method Selected for: This method has been selected for confirmatory analysis of soil/sediment samples.

Description of Method: This method uses alpha spectrometry for determination of americium-241 in soil. Americium is leached from soil with nitric acid and hydrochloric acid. Americium-243 is added as a tracer to determine chemical yield. The soil is processed through the plutonium separation steps using ion exchange resin according to Method Pu-11-RC. Americium is collected with a calcium oxalate precipitation and finally isolated and purified by ion exchange. After source preparation by microprecipitation, americium-241 is determined by alpha spectrometry analysis. The counting period chosen depends on the sensitivity required of the measurement and the degree of uncertainty in the result that is acceptable. The lower limit of detection (LLD) for americium-241 is 0.5 milli Becquerel (mBq) when counted for 1000 minutes. In cases where less than 100 g of sample is available, use of EML HASL-300 Method Pu-12-RC is recommended.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.47). High levels of ammonium compounds or polyacrylamides (which can degrade into ammonium) present in some decontamination agents can potentially cause interference and should be removed during sample preparation by heating the extracts in nitric acid for 1 to 1.5 hours.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Am-01-RC: Americium in Soil." *EML Procedures Manual*, HASL-300, 28th Edition.
http://www.epa.gov/sites/production/files/2015-06/documents/eml-am-01-rc.pdf

6.2.29 EML HASL-300 Method Am-04-RC: Americium in QAP Water and Air Filters - Eichrom's TRU Resin

Analyte(s)	CAS RN
Americium-241	14596-10-2

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Americium (but not lanthanides) in water and air filters
Method Selected for: This method has been selected for confirmatory analysis of drinking water, aqueous/liquid samples, surface wipes and air filters.

Description of Method: This method is specific to measurement of americium isotopes in samples that do not contain lanthanides, but also can be used for measurement of californium and curium. The method

uses microprecipitation and determination by alpha spectrometry. Americium-243 is added to the sample to determine chemical yield. The sample is processed through separation steps using ion exchange resins. The eluate from the ion exchange column containing americium (and all other ions, except plutonium) is evaporated, redissolved, and loaded onto a TRU resin extraction column. Americium (and curium and californium, if present) is separated and purified on the column and finally stripped with dilute nitric acid stripping solution. Microprecipitation is used to prepare for alpha spectrometry. The LLD for total americium is 0.3 mBq when counted for 1000 minutes.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.47). Lanthanides, if present, will not be removed by this method and will significantly reduce the resolution of the alpha spectrograph. At this time, there are no known interferences posed by decontamination agents that might be present in a sample.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Am-04-RC: Americium in QAP Water and Air Filters - Eichrom's TRU Resin." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-06/documents/eml-am-04-rc.pdf

6.2.30 EML HASL-300 Method Am-06-RC: Americium and/or Plutonium in Vegetation

Analyte(s)	CAS RN
Americium-241	14596-10-2
Californium-252	13981-17-4
Curium-244	13981-15-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Americium and/or plutonium in vegetation
Method Selected for: This method has been selected for confirmatory analysis of vegetation.

Description of Method: Vegetation is either dry ashed in a ceramic crucible using a muffle furnace or wet ashed with nitric acid. Plutonium-236 and americium-243 tracers are added after dry ashing or before wet ashing. Wet ashing requires considerably more time and must be carefully monitored due to the highly reactive nature of vegetation. The sample is further digested with hydrofluoric acid to dissolve silicate compounds. Plutonium is separated by ion exchange and determined by alpha spectrometry using the plutonium-236 tracer to determine recovery. Americium (and californium-252 and curium-244, if present) is collected with calcium oxalate precipitation and finally isolated and purified by ion exchange. After source preparation by microprecipitation, americium -241 (and californium-252 and curium-244, if present) is determined by alpha spectrometry using americium-243 tracer to provide recovery data.

Special Consideration: PTFE beakers must be used when digesting samples with hydrofluoric acid. Clays that are present in some decontamination agents can contain iron, magnesium or calcium, which can be released via ion exchange in the presence of certain radionuclides, and cause analytical interferences. High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison the extraction resins used in this method.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Am-06-RC: Americium and/or Plutonium in Vegetation." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-06/documents/eml-am-06-rc.pdf

6.2.31 EML HASL-300 Method Ga-01-R: Gamma Radioassay

Analyte(s)	CAS RN
Americium-241	14596-10-2
Cesium-137	10045-97-3
Cobalt-60	10198-40-0
Europium-154	15585-10-1
Gamma	NA
Indium-111	15750-15-9
Iodine-131	10043-66-0
Iridium-192	14694-69-0
Molybdenum-99	14119-15-4
Neptunium-239	13968-59-7
Rhenium-188	14378-26-8
Rubidium-82	14391-63-0
Ruthenium-103	13968-53-1
Ruthenium-106	13967-48-1
Selenium-75	14265-71-5
Select Mixed Fission Products	NA
Technetium-99m	378784-45-3

Analysis Purpose: Qualitative and confirmatory analysis or gross gamma determination
Technique: Gamma spectrometry

Method Developed for: Gamma-ray emitting radionuclides in a variety of environmental matrices
Method Selected for: This method has been selected for qualitative and/or confirmatory analysis of select gamma emitters in aqueous/liquid, soil/sediment, surface wipe, air filters and/or vegetation.

Description of Method: This method uses gamma spectrometry for measurement of gamma photons emitted from radionuclides without separating them from the sample matrix. Samples are placed into a standard geometry for counting, typically using a high purity Germanium [HP(Ge)] detector. Ge(Li) or NaI(Tl) detectors also can be used. The sample is placed into a standard geometry for gamma counting. Soil samples and sludge are placed into an appropriately sized Marinelli beaker after drying and grinding for homogenization. Air filters and surface wipes can be counted directly or pressed into a planchet and counted. Samples are counted long enough to meet the required sensitivity. For typical counting systems and sample types, activity levels of approximately 40 Bq are measured, and sensitivities as low as 0.002 Bq can be achieved. Because of electronic limitations, count rates higher than 2000 counts per second (cps) should be avoided. High activity samples can be diluted, reduced in size, or moved away from the detector (a limited distance) to reduce the count rate. The method is applicable for analysis of samples that contain radionuclides emitting gamma photons with energies above approximately 20 keV for germanium (Ge) (both HP(Ge) and GeLi) detectors and above 50 keV for NaI(Tl) detectors.
Special Considerations: Clays and hydrated alumina, which are present in some decontamination agents, can sequester cesium-137 and iodine-131, respectively. Each would be released only upon complete dissolution and, therefore, not measured when using this method for analysis of water samples. Compounds containing carbonate, fluoride, hydroxide or phosphate, also present in some decontamination agents, can precipitate radionuclides out of solution prior to analysis, resulting in a lesser amount of gamma emitting radionuclides in cases where a water sample aliquot is transferred and analyzed separately from the entire sample.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Ga-01-R: Gamma Radioassay." *EML Procedures Manual*, HASL-300, 28th Edition.
http://www.epa.gov/sites/production/files/2015-06/documents/eml-ga-01-r.pdf

6.2.32 EML HASL-300 Method Po-02-RC: Polonium in Water, Vegetation, Soil, and Air Filters

Analyte(s)	CAS RN
Polonium-210	13981-52-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Polonium in water, vegetation, soil and air filters
Method Selected for: This method has been selected for qualitative and confirmatory analysis of drinking water, aqueous/liquid, soil/sediment and/or vegetation samples.

Description of Method: This method uses alpha spectrometry for determination of polonium in water, vegetation, soil and air filter samples. Polonium equilibrated with polonium-208 or polonium-209 tracer is isolated from most other elements by coprecipitation with lead sulfide. The sulfide precipitate is dissolved in weak hydrochloric acid solution. Polonium is quantitatively deposited on a nickel disc, and the plated disc is counted on an alpha spectrometer to measure chemical yield and activity of the sample. The solution from the deposition may be retained and analyzed for polonium-210. When counted for 1000 minutes, the LLD for polonium is 1.0 mBq for water and 1.3 mBq for vegetation, soil and filters.

Special Considerations: This method requires specific valence states for radionuclides; oxidizing and reducing agents, which are present in decontamination agents, can impact the analysis. Oxidizers can oxidize nickel or lead to form soluble metal ions that can cause interferences. Chelating compounds, such as those present in some decontamination agents, can complex tightly to nickel and lead that may be present in a sample, preventing their precipitation during sample preparation. These metal ions can potentially cause interferences when analyzing for polonium-210.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Po-02-RC: Polonium in Water, Vegetation, Soil, and Air Filters." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-07/documents/eml-po-02-rc.pdf

6.2.33 EML HASL-300 Method Pu-12-RC: Plutonium and/or Americium in Soil or Sediments

Analyte(s)	CAS RN
Americium-241	14596-10-2

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Plutonium and americium in soil
Method Selected for: This method has been selected for use when small soil and sediment sample sizes (≤100 g) will be analyzed.

Description of Method: A sample of soil of up to 100 g in size is equilibrated with americium-243 tracer. Contaminant isotopes are leached with nitric and hydrochloric acid. Plutonium is removed by ion exchange. The eluent from the plutonium separation is saved for determination of americium, curium and californium. Americium, curium and californium are collected with a calcium oxalate coprecipitation, isolated and purified by extraction chromatography. Microprecipitation is used to prepare the sample for analysis by alpha spectrometry of americium, curium and californium. The LLD for americium is 0.5 mBq when counted for 1000 minutes.

Special Considerations: In cases where only small sample sizes (≤100 g) will be analyzed, this method is recommended for confirmatory analysis. If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.47). High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison the extraction resins used in this method.

The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately form the entire sample. Such compounds include:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well.
- Clays can contain iron, magnesium or calcium that can be released via ion exchange in the presence of certain radionuclides, and cause analytical interferences.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Pu-12-RC: Plutonium and/or Americium in Soil or Sediments." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-07/documents/eml-pu-12-rc.pdf

6.2.34 EML HASL-300 Method Ra-03-RC: Radium-226 in Soil, Vegetable Ash, and Ion Exchange Resin

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Radon emanation / Gamma spectroscopy (analysis of tracer)

Method Developed for: Radium-226 in soil, vegetation ash and ion exchange resin
Method Selected for: This method has been selected for qualitative and confirmatory analysis of vegetation.

Description of Method: Soil, vegetation ash or ion exchange resin are prepared for radon-222 emanation measurement. The sample is pretreated with nitric acid-hydrogen fluoride, fused with potassium fluoride and transposed to pyrosulfate. The cake is dissolved in dilute hydrochloric acid. Radium-barium sulfate is precipitated, filtered, and dissolved in alkaline EDTA. The chemical yield is determined with the γ-emitting tracer barium-133. The solution is transferred to a radon bubbler. Radon is de-emanated into an ionization chamber or scintillation cell, and counted using a counter with a photomultiplier.

Special Consideration: Use of platinum crucibles is required in this method. Certain chelating compounds, which are present in some decontamination agents, can compromise the collection of radionuclides prior to analysis, by preventing them from being precipitated out of solution during precipitation procedures. Other chelators can tightly complex barium or strontium that may be present in the sample, causing interference when analyzing for radium-226. Dispersants and corrosion inhibitors, also present in decontaminating agents, can have chelating ability as well.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Ra-03-RC: Radium 226 in Soil, Vegetable Ash, and Ion Exchange resin." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-07/documents/eml-ra-03-rc.pdf

6.2.35 EML HASL-300 Method Sr-03-RC: Strontium-90 in Environmental Samples

Analyte(s)	CAS RN
Strontium-90	10098-97-2

Analysis Purpose: Confirmatory analysis
Technique: Beta counting / Gamma spectroscopy (analysis of tracer)

Method Developed for: Strontium-90 in vegetation, water, air filters and soil
Method Selected for: This method has been selected for confirmatory analysis of soil and sediment samples, vegetation, surface wipes and air filters.

Description of Method: Strontium is separated from calcium, other fission products and natural radioactive elements. Fuming nitric acid separations remove the calcium and most other interfering ions. Radium, lead and barium are removed with barium chromate. Traces of other fission products are scavenged with iron hydroxide. After strontium-90 and yttrium-90 equilibrium has been attained, yttrium-90 is precipitated as the hydroxide and converted to oxalate for counting on a low-background gas proportional beta counter. Chemical yield is determined with strontium-85 tracer by counting in a gamma well detector.

Special Consideration: If analyzing highly calcareous soils, or if carbonate compounds found in some decontamination agents are present in the sample, an additional quantity of hydrochloric acid should be added to replace the acid required to decompose the carbonates. At this time, there are no known interferences posed by decontamination agents that might be present in a sample.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Sr-03-RC: Strontium-90 in Environmental Samples." *EML Procedures Manual*, HASL-300, 28th Edition.
http://www.epa.gov/sites/production/files/2015-07/documents/eml-sr-03-rc.pdf

6.2.36 EML HASL-300 Method Tc-01-RC: Technetium-99 in Water and Vegetation

Analyte(s)	CAS RN
Technetium-99	14133-76-7

Analysis Purpose: Confirmatory analysis
Technique: Beta counting / Gamma spectrometry

Method Developed for: Technetium-99 in water and vegetation
Method Selected for: This method has been selected for confirmatory analysis of vegetation.

Description of Method: Samples are wet ashed with nitric acid. After wet ashing is complete, samples are evaporated to the smallest volume possible with no salting out. The resulting solution is cooled, transferred to a 1-L beaker, and diluted to 800 mL with reagent water. The sample solution is then stirred and filtered with suction through a 15-cm glass fiber filter, and the filter is washed with water. The filter containing the silica and insoluble material is discarded. Technetium-99 is equilibrated with technetium-95m tracer in the wet ashing step. Technetium is separated from other elements by anion exchange and electro-deposition, and technetium-99 is beta counted. Gamma spectrometry measurement of technetium-95m tracer provides the chemical yield.

Special Consideration: Technetium-95m tracer is no longer readily available from the source cited in the method. If technetium-95m cannot be obtained, technetium-99m tracer may be substituted.

Compounds containing carbonate, hydroxide, phosphate or sulfate, which are present in some decontamination agents, can precipitate radionuclides out of solution prior to analysis. This precipitation can result in a lesser amount of technetium in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Tc-01-RC: Technetium-99 in Water and Vegetation." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-07/documents/eml-tc-01-rc.pdf

6.2.37 EML HASL-300 Method Tc-02-RC: Technetium-99 in Water – TEVA® Resin

Analyte(s)	CAS RN
Technetium-99	14133-76-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Technetium-99 in water
Method Selected for: This method has been selected for qualitative and confirmatory analysis of drinking water samples.

Description of Method: The sample containing technetium-99 is mixed with technetium-95m added as a gamma-emitting tracer. The two isotopes of technetium are brought to an isotopic equilibrium and separated from other elements by ferrous and ferric hydroxide coprecipitation. The precipitate is dissolved with dilute nitric acid and passed through a TEVA-resin column,which is highly specific for technetium in the pertechnetate form. The resin is washed with dilute nitric acid to remove possible interferences and then it is eluted directly into a suitable liquid scintillation cocktail. The sample is typically counted for 1 hour to simultaneously determine technetium-99 activity and the technetium-95m radiochemical yield. Quench/efficiency calibration curves need to be established for the liquid scintillation spectrometer for both technetium-95m and technetium-99.

Special Considerations: Chemical and color quenching can have a significant impact when using liquid scintillation methods. Several compounds contained in decontamination agents can cause this quenching, such as organic compounds containing oxygen; halogenated compounds; elevated levels of nitrates or nitromethane; and dyes, pigments or other colored compounds. Chelators can compromise the collection of radionuclides prior to analysis, by causing them to avoid being precipitated out of solution during precipitation procedures.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Tc-02-RC: Technetium-99 in Water – TEVA® Resin." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-07/documents/eml-tc-02-rc.pdf

6.2.38 EML HASL-300 Method U-02-RC: Isotopic Uranium in Biological and Environmental Materials

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Confirmatory analysis

Technique: Alpha spectrometry
Method Developed for: Isotopic uranium in biological and environmental materials
Method Selected for: This method has been selected for confirmatory analysis of vegetation.

Description of Method: Uranium from acid leached, dry-ashed and wet-ashed materials is equilibrated with uranium-232 tracer, and isolated by anion exchange chromatography. The separated uranium isotopes are microprecipitated for alpha spectrometry.

Special Considerations: For microprecipitation procedures, refer to HASL-300 Method G-03. Chelating or complexing compounds, such as those present in some decontamination agents, can compromise the collection of radionuclides prior to analysis, by preventing them from being trapped on the ion exchange column or from being precipitated out of solution during precipitation procedures. Dispersants and corrosion inhibitors can have chelating ability as well.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method U-02-RC: Isotopic Uranium in Biological and Environmental Materials." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sites/production/files/2015-07/documents/eml-u-02-rc.pdf

6.2.39 DOE FRMAC Method Volume 2, Page 33: Gross Alpha and Beta in Air

Analysis Purpose: Gross alpha and gross beta determination
Technique: Alpha/Beta counting

Method Developed for: Gross alpha and beta in air
Method Selected for: This method has been selected for gross alpha and gross beta determination in air filters, and for direct counting of surface wipes.

Description of Method: A gas-flow proportional counter is used for counting gross alpha and beta radioactivity. The method supplies an approximation of the alpha and beta activity present in the air or the removable surface activity dependent on the sample type. The method provides an indication of the presence of alpha and beta emitters, including the following analytes:

- Americium-241 (CAS RN 14596-10-2) Alpha emitter
- Californium-252 (CAS RN 13981-17-4) Alpha emitter
- Cesium-137 (CAS RN 10045-97-3) Beta emitter
- Cobalt-60 (CAS RN 10198-40-0) Beta emitter
- Curium-244 (CAS RN 13981-15-2) Alpha emitter
- Europium-154 (CAS RN 15585-10-1) Beta emitter
- Iridium-192 (CAS RN 14694-69-0) Beta emitter
- Plutonium-238 (CAS RN 13981-16-3) Alpha emitter
- Plutonium-239 (CAS RN 15117-48-3) Alpha emitter
- Polonium-210 (CAS RN 13981-52-7) Alpha emitter
- Radium-226 (CAS RN 13982-63-3) Alpha emitter
- Ruthenium-103 (CAS RN 13968-53-1) Beta emitter
- Ruthenium-106 (CAS RN 13967-48-1) Beta emitter
- Strontium-90 (CAS RN 10098-97-2) Beta emitter
- Uranium-234 (CAS RN 13966-29-5) Alpha emitter
- Uranium-235 (CAS RN 15117-96-1) Alpha emitter
- Uranium-238 (CAS RN 7440-16-1) Alpha emitter

For this application, the procedure requires the use of thorium-230 for alpha counting efficiency and cesium-137 for beta counting efficiency in the calibration of the detector. An air filter or swipe sample is placed onto a planchet then counted for alpha and beta radioactivity. Activity is reported in activity units

per volume of air sampled, as units of activity per surface area sampled, or as total units of activity in cases where sample collection information is not available.

Special Considerations: High levels of particulate loading on the air filter or swipe will affect the alpha efficiency. Accurate results for radionuclides, other than cesium-137 and thorium-230, may be difficult because of the difference in efficiencies for the uncalibrated radionuclides. At this time, there are no known interferences posed by decontamination agents that might be present in a sample.

Source: FRMAC. 1998. "Gross Alpha and Beta in Air." *FRMAC Monitoring and Analysis Manual – Sample Preparation and Analysis* - Volume 2, DOE/NV/11718-181 Vol. 2, UC-707, p. 33. Las Vegas, NV: U.S. DOE. http://www.epa.gov/sites/production/files/2015-06/documents/frmac-vol2-pg33.pdf

6.2.40 DOE RESL Method P-2: P-32 Fish, Vegetation, Dry Ash, Ion Exchange

Analyte(s)	CAS RN
Phosphorus-32	14596-37-3

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Čerenkov counting with Liquid Scintillation

Method Developed for: Phosphorus-32 in fish and vegetation
Method Selected for: This method has been selected for qualitative and confirmatory analysis of soil, sediment, wipes, air filters and vegetation.

Description of Method: Samples up to 500 g are dry ashed at 550°C and dissolved in two portions of nitric acid. The sample is evaporated to half volume and transferred to a perchloric acid hood. Concentrated nitric acid and concentrated perchloric acid are added, and the sample is evaporated to dryness. The residue is dissolved in hydrochloric acid and filtered through a glass fiber filter. Iron-55 is removed by precipitation with cupferron. The solution containing phosphate is purified by passing it through anion and cation columns to remove possible contaminants. The purified phosphate is precipitated as magnesium ammonium phosphate, filtered onto a glass fiber filter, and dried. The magnesium ammonium phosphate is dissolved in nitric acid and transferred to a counting vial. Phopsphorus-32 is assayed by counting the Čerenkov radiation with a liquid scintillation counter.

Special Considerations: Laboratories using this method must have a designated perchloric acid fume hood. This method was developed for analysis of fish and vegetation. Additional development and testing is necessary for application to soil, sediment, wipes and air filters. Phosphorus and iron carrier must be added to matrices that do not contain milligram quantities of both elements.

Chemical and color quenching can have a significant impact when using liquid scintillation methods. Several compounds contained in decontamination agents can cause this quenching, such as organic compounds containing oxygen; halogenated compounds; elevated levels of nitrates or nitromethane; and dyes, pigments or other colored compounds. Chelators also can tightly complex calcium that may be present in the sample causing interference when analyzing for phosphorus-32.

Source: RESL, DOE. 1977. "Method P-2: P-32 Fish, Vegetation, Dry Ash, Ion Exchange." *RESL Analytical Chemistry Branch Procedures Manual*, IDO-12096.
http://www.epa.gov/sites/production/files/2015-07/documents/resl-p-2.pdf

6.2.41 DOE SRS Actinides and Sr-89/90 in Soil Samples

Analyte(s)	CAS RN
Strontium-89	14158-27-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry and beta counting

Method Developed for: Actinides and strontium-89 and -90 in soil samples
Method Selected for: This method has been selected for qualitative analysis of strontium-89 in soil and sediment samples.

Description of Method: Radioactive tracers are added to samples prior to sample fusion at 600°C using sodium hydroxide in zirconium crucibles. An iron hydroxide precipitation is performed. After dissolution by acidification of the precipitate, a lanthanum fluoride precipitation is used to further eliminate the sample matrix. The lanthanum fluoride precipitate is redissolved in nitric acid, boric acid, and aluminum nitrate. A column separation using TEVA, TRU and DGA resins is applied to separate the actinides into four fractions: thorium, plutonium-neptunium, uranium and americium/curium. Plutonium-242 (or plutonium-236 if neptunium-237 is measured), thorium-229, americium-243 and uranium-232 are used as tracers to determine yield. Actinide tracers are not needed when analyzing samples only for Sr-89. The various fractions of actinides are eluted from the resin columns and precipitated with cerium fluoride, dried, and counted by alpha spectrometry. Strontium resin is used to separate strontium-89/90 for measurement by beta counting.

Special Considerations: The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well.
- Clays can contain iron, magnesium or calcium, which can be released as ions via ion exchange in the presence of certain radionuclides, and cause interference in the analysis of the water.
- High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison extraction resins used in this method.
- Reducing agents also can impact this method, which requires specific valence states for radionuclides.

Source: SRS, DOE. 2011. "Actinides and Sr-89/90 in Soil." *SRS Manual L3.23*, Procedure L3.23-10054. http://www.epa.gov/sites/production/files/2015-07/documents/l3.23-10054.pdf

6.2.42 DOE SRS Actinides and Sr-89/90 in Vegetation: Fusion Method

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15517-48-3
Strontium-89	14158-27-1
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry / Beta counting

Method Developed for: Actinides and strontium-89 and -90 in vegetation
Method Selected for: This method has been selected for qualitative analysis of vegetation.

Description of Method: Radioactive tracers are added to samples prior to sample fusion at 600°C using sodium hydroxide in zirconium crucibles. An iron hydroxide precipitation is performed. After dissolution by acidification of the precipitate, a lanthanum fluoride precipitation is used to further eliminate the sample matrix. The lanthanum fluoride precipitate is redissolved in nitric acid, boric acid and aluminum nitrate. A column separation using commercially available resins (TEVA, TRU and DGA) is applied to separate the actinides into three fractions: plutonium/neptunium, uranium and americium/curium. Plutonium-242 (or plutonium-236 if neptunium-237 is measured), thorium-229, americium-243 and uranium-232 are used as tracers to determine yield. The various fractions of actinides are eluted from the resin columns and precipitated with cerium fluoride, dried, and counted by alpha spectrometry. Strontium resin is used to separate strontium-89/90 for measurement by beta counting.

Special Considerations: Thorium-228, if present as a daughter of uranium-232 tracer, will interfere with thorium-228 analysis. Self-cleaning uranium-232 tracer, with thorium-228 removed, is required if thorium isotopes are separated and measured with uranium. If uranium-232 is present in a sample, the procedure of standard addition can be used to determine the amount of uranium-232 contamination. The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well.
- Clays can contain iron, magnesium or calcium, which can be released as ions via ion exchange in the presence of certain radionuclides, and cause interference in the analysis of the water.
- High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison extraction resins used in this method.
- Reducing agents also can impact this method, which requires specific valence states for radionuclides.

Source: SRS, DOE. 2011. "Actinides and Sr-89/90 in Vegetation: Fusion Method." *SRS Manual L3.23*, Procedure L3.23-10055. http://www.epa.gov/sites/production/files/2015-07/documents/l3.23-10055.pdf

6.2.43 ORISE Method AP1: Gross Alpha and Beta for Various Matrices

Analysis Purpose: Gross alpha and gross beta determination
Technique: Alpha/Beta counting

Method Developed for: Gross alpha and beta in water, soil, vegetation and other solids
Method Selected for: This method has been selected for gross alpha and gross beta determination in soil/sediment and vegetation.

Description of Method: This method provides an indication of the presence of alpha and beta emitters, including the following analytes:

- Americium-241 (CAS RN 14596-10-2) Alpha emitter
- Californium-252 (CAS RN 13981-17-4) Alpha emitter
- Cesium-137 (CAS RN 10045-97-3) Beta emitter

- Cobalt-60 (CAS RN 10198-40-0) Beta emitter
- Curium-244 (CAS RN 13981-15-2) Alpha emitter
- Europium-154 (CAS RN 15585-10-1) Beta emitter
- Iridium-192 (CAS RN 14694-69-0) Beta emitter
- Plutonium-238 (CAS RN 13981-16-3) Alpha emitter
- Plutonium-239 (CAS RN 15117-48-3) Alpha emitter
- Polonium-210 (CAS RN 13981-52-7) Alpha emitter
- Radium-226 (CAS RN 13982-63-3) Alpha emitter
- Ruthenium-103 (CAS RN 13968-53-1) Beta emitter
- Ruthenium-106 (CAS RN 13967-48-1) Beta emitter
- Strontium-90 (CAS RN 10098-97-2) Beta emitter
- Uranium-234 (CAS RN 13966-29-5) Alpha emitter
- Uranium-235 (CAS RN 15117-96-1) Alpha emitter
- Uranium-238 (CAS RN 7440-16-1) Alpha emitter

This procedure provides screening measurements to indicate whether specific analyses are required for water, soil, vegetation and other solids. Liquid samples are acidified, concentrated, dried in a planchet, and counted in a low-background proportional counter. Solid samples are dried and homogenized, and a known quantity is transferred to a planchet and counted in a low-background proportional counter.

Special Considerations: Volatile radionuclides will not be accurately determined using this procedure. At this time, there are no known interferences posed by decontamination agents that might be present in a sample.

Source: ORISE, Oak Ridge Associated Universities (ORAU). 2001. "Method AP1: Gross Alpha and Beta for Various Matrices." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program*. http://www.epa.gov/sites/production/files/2015-06/documents/orise-ap1.pdf

6.2.44 ORISE Method AP2: Determination of Tritium

Analyte(s)	CAS RN
Tritium (Hydrogen-3)	10028-17-8

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Tritium in soil, sediment, animal tissue, vegetation, smears and water samples
Method Selected for: This method has been selected for qualitative and confirmatory analysis of soil/sediment, surface wipes and vegetation.

Description of Method: The tritium in aqueous and solid samples is distilled using an Allihn condenser. For solid samples, an appropriate volume of laboratory reagent water is added to facilitate distillation. Certain solid samples may be refluxed to ensure distribution of any tritium that may be in the sample. The sample may be spiked with a standard tritium solution to evaluate quenching and counting efficiency. After the sample has been distilled, an aliquot of the distillate is added to a scintillation cocktail and the sample is counted using a liquid scintillation analyzer.

Special Considerations: Other volatile radionuclides such as iodine and carbon isotopes may interfere and may require that the sample be made alkaline using solid sodium hydroxide before distillation. Organic impurities may interfere and may require the addition of an oxidizing agent to the sample as well as spiking the samples with a standard tritium solution. The addition of a standard tritium solution to each sample allows for counting efficiencies to be calculated for each individual sample.

Chemical and color quenching can have a significant impact when using liquid scintillation methods. Some decontamination agents include organic compounds that contain oxygen, halogenated compounds, elevated levels of nitrates or nitromethane, or dyes, pigments or other colored compounds that can cause chemical or color quenching.

Source: ORISE, ORAU. 2001. "Method AP2: Determination of Tritium." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program*.
http://www.epa.gov/sites/production/files/2015-06/documents/orise-ap2.pdf

6.2.45 ORISE Method AP5: Determination of Technetium-99

Analyte(s)	CAS RN
Technetium-99	14133-76-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Technetium-99 in sediment, soil, smears and water at environmental levels
Method Selected for: This method has been selected for qualitative and confirmatory analysis of soil/sediment, surface wipe and air filter samples; and qualitative analysis of vegetation.

Description of Method: Solid samples are leached with dilute nitric acid. The leachates are passed through a TEVA-resin column, which is highly specific for technetium in the pertechnetate form. The technetium is absorbed onto the extraction resin. The resin is added to a scintillation vial containing an appropriate cocktail and counted using a liquid scintillation analyzer. Most interfering beta emitting radionuclides (including carbon-14, phosphorus-32, sulfur-35, strontium-90, yttrium-90 and thorium-234) are effectively removed using TEVA resin under the conditions in this procedure.

Special Considerations: Tritium may follow technetium due to the absorption of some tritium-labeled compounds by the resin. Possible tritium interferences are eliminated by setting the technetium counting window above the maximum energy of tritium beta particles. Chelating compounds, such as those contained in some decontamination agents, can compromise the collection of radionuclides prior to analysis, by preventing them from being precipitated out of solution during precipitation procedures. Some decontamination agents include organic compounds that contain oxygen, halogenated compounds, elevated levels of nitrates or nitromethane, or dyes, pigments or other colored compounds can cause chemical or color quenching.

Source: ORISE, ORAU. 2001. "Method AP5: Determination of Technetium-99." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program*.
http://www.epa.gov/sites/production/files/2015-06/documents/orise-ap5.pdf

6.2.46 ORISE Method AP7: Determination of Radium-226 in Water and Soil Samples Using Alpha Spectroscopy

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry / Gamma spectroscopy (analysis of tracer)
Method Developed for: Radium-226 in water and soil

Method Selected for: This method has been selected for confirmatory analysis of radium-226 in soil/sediment samples.

Description of Method: The tracer (barium-133) and potassium hydrogen fluoride are added to a soil sample aliquot in a platinum crucible. The sample is heated until the potassium hydrogen fluoride has completely dried. Heating is continued at 900°C until total dissolution of the sample. After allowing the sample to cool slightly, sulfuric acid is added and the mixture is heated to dissolve the fluoride cake. Sodium sulfate is added to the slurry and the temperature is slowly raised until the slurry melts completely. Water and 12M hydrochloric acid are added to dissolve the pyrosulfate cake. Sulfuric acid (9M), potassium sulfate and sodium sulfate are added and the solution is evaporated to 35–40 mL. Three portions of lead (II) in solution are added while stirring, waiting 5 minutes between each addition. After centrifuging, the supernatant is discarded and the precipitate is dissolved in diethylenetriaminepentaacetic acid (DTPA). Barium (II) in solution is added to form barium sulfate, which acts to separate radium-226 from possible interfering radionuclides. The barium precipitate is filtered, and radium-226 is counted by alpha spectroscopy. Barium-133 is used to quantify the yield by gamma spectroscopy.

Special Considerations: High levels of barium will add mass to the final sample causing self-attenuation and degradation of the alpha spectrum. If the amount of barium in the sample can be predetermined, it may be possible to adjust sample size and not add the barium (II) in step 4.2.11 of the method. Contamination with barium-133 will interfere with the yield determination. This may be corrected by gamma counting before analysis and adjusting the barium yield accordingly.

Chelating compounds, such as those contained in some decontamination agents, can compromise the collection of radionuclides prior to analysis, by preventing them from being precipitated out of solution during precipitation procedures. Other chelators can tightly complex barium or strontium that may be present in the sample causing interference when analyzing for radium-226. Dispersants and corrosion inhibitors, also present in decontaminating agents, can have chelating ability as well.

Source: ORISE, ORAU. 2001. "Method AP7: Determination of Radium-226 in Water and Soil Samples Using Alpha Spectroscopy." Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program.
https://www.epa.gov/sites/production/files/2017-10/documents/ap7_ra-226_water_soil_alpha_spec.pdf

6.2.47 ORISE Method AP11: Sequential Determination of the Actinides in Environmental Samples Using Total Sample Dissolution and Extraction Chromatography

Analyte(s)	CAS RN
Americium-241	14596-10-2
Californium-252	13981-17-4
Curium-244	13981-15-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Americium, curium, plutonium, neptunium, thorium and/or uranium in water and solid samples

Method Selected for: This method has been selected for confirmatory analysis when a sample exists in a refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem. In the event of refractory radioactive material, this method is recommended for both qualitative determination and confirmatory analysis of drinking water, aqueous/liquid, soil/sediment, surface wipes and air filter samples.

Description of Method: Solid and unfiltered aqueous samples (after evaporation to dryness) are dissolved completely by a combination of potassium hydrogen fluoride and pyrosulfate fusions. Filtered aqueous samples are evaporated to dryness followed by a pyrosulfate fusion. The fusion cake is dissolved and, for analyses requiring uranium only, two barium sulfate precipitations are performed, and the uranium is separated using EDTA. For all other analyses, one barium sulfate precipitation is performed and all alpha emitters are coprecipitated on barium sulfate. The barium sulfate is dissolved and the actinides are separated by extraction chromatography. An optional section is presented for the separation of americium from the lanthanides. All actinides are coprecipitated on cerium fluoride and counted with an alpha spectrometer system.

Special Considerations: Chelating compounds, such as those present in some decontamination agents, can compromise the collection of radionuclides prior to analysis by preventing them from being trapped on the ion exchange column or from being precipitated out of solution. Dispersants and corrosion inhibitors can have chelating ability as well. Clays, which are also present in some decontamination agents, can contain iron, magnesium or calcium that can be released via ion exchange in the presence of certain radionuclides, and cause analytical interferences. High levels of iron, manganese, calcium or magnesium can impact exchange site availability and/or poison extraction resins used in this method.

Source: ORISE, ORAU. 2001. "Method AP11: Sequential Determination of the Actinides in Environmental Samples Using Total Sample Dissolution and Extraction Chromatography." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program*. http://www.epa.gov/sites/production/files/2015-06/documents/orise-ap11.pdf

6.2.48 ORISE Method Procedure #9: Determination of I-125 in Environmental Samples

Analyte(s)	CAS RN
Iodine-125	14158-31-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Gamma spectrometry

Method Developed for: Iodine-125 in environmental samples, such as soil, sediment, vegetation, water, milk, filters (air or water), etc.
Method Selected for: This method has been selected for qualitative and confirmatory analysis of drinking water, aqueous/liquid, soil/sediment, surface wipe, air filter and vegetation samples.

Description of Method: In this method a direct comparison is made between the sample and a source prepared from a National Institute of Standards and Technology (NIST) traceable standard. If it is known, either by the sample preparation procedure or by a qualitative analysis on some device (high resolution intrinsic planar detector) that iodine-125 is the only radionuclide contributing to the observed peak, then this method can be used as a rapid quantitative method.

The sample is prepared by matrix specific techniques and the final sample is placed in a 16-mL culture tube and counted in a 3" x 3" sodium iodide (NaI(Tl)) well detector attached to a pulse height analyzer. Iodine-125 gamma counting rate is determined in the 25 to 35 keV energy range by pulse height analysis. NIST traceable liquid standards are also counted in the same geometric configuration as the samples to

determine iodine-125 counting efficiency.

Special Considerations: Due to the low photon energy of iodine-125, the Compton scattering and x-ray photons from other radionuclides may cause significant interferences in this procedure. Chelating compounds, such as those contained in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column. Hydrated alumina, also present in certain decontamination agents, can sequester iodine-125 and iodine-131, which would only be released upon complete dissolution and therefore not be measured when using this method.

Source: ORISE, ORAU. 1995. "Procedure #9: Determination of I-125 in Environmental Samples." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program*. http://www.epa.gov/sites/production/files/2015-06/documents/orise-procedure9-1995.pdf

6.2.49 ASTM Method D3084-05 (2012): Standard Practice for Alpha Spectrometry in Water

Analyte(s)	CAS RN
Americium-241	14596-10-2
Californium-252	13981-17-4
Curium-244	13981-15-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3

Analysis Purpose: Qualitative determination
Technique: Alpha spectrometry

Method Developed for: Alpha particle spectra in water
Method Selected for: This method has been selected for qualitative determination of californium-252 and curium-244 in drinking water, surface wipes, air filters and vegetation; americium-241, californium-252, curium-244, and plutonium-238 and -239 in aqueous and liquid phase samples; and californium-252 and curium-244 in soil and sediment.

Description of Method: This standard practice covers the process that is required to obtain well-resolved alpha spectra from water samples and discusses the associated problems. This practice is typically preceded with specific chemical separations and mounting techniques that are included in referenced methods. A chemical procedure is required to isolate and purify the radionuclides (see Section 10.1 of the method), and a radioactive tracer is added to determine yield. A source is prepared by employing electrodeposition, microprecipitation or evaporation (depositing the solution onto a stainless steel or platinum disc). Electrodeposition and microprecipitation are preferred. The source's radioactivity is then measured in an alpha spectrometer according to manufacturer's operating instructions. The counting period chosen depends on the sensitivity required of the measurement and the degree of uncertainty in the result that is acceptable.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.47) for sample preparation instead of the methods referenced in ASTM Method D3084. At this time, there are no known interferences posed by decontamination agents that might be present in a sample.

Source: ASTM. 2012. "Method D3084-05 (2012): Standard Practice for Alpha Spectrometry in Water." *Annual Book of ASTM Standards*, Vol. 11.02. West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D3084.htm

6.2.50 ASTM Method D3972-09 (2015): Standard Test Method for Isotopic Uranium in Water by Radiochemistry

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Alpha-particle-emitting isotopes of uranium in water
Method Selected for: This method has been selected for confirmatory analysis of drinking water samples.

Description of Method: Uranium is chemically separated from a water sample by coprecipitation with ferrous hydroxide followed by anion exchange, and electrodeposition. When suspended matter is present, an acid dissolution step is added to ensure that all of the uranium dissolves. The sample is acidified, and uranium-232 is added as an isotopic tracer to determine chemical yield. Uranium is coprecipitated from the sample with ferrous hydroxide. This precipitate is dissolved in concentrated hydrochloric acid, or is subjected to acid dissolution with concentrated nitric and hydrofluoric acids, if the hydrochloric acid fails to dissolve the precipitate. Uranium is separated from other radionuclides by adsorption on anion exchange resin, followed by elution with hydrochloric acid. The uranium is finally electrodeposited onto a stainless steel disc and counted using alpha spectrometry.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.47). The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column or from being precipitated out of solution during precipitation procedures. Dispersants and corrosion inhibitors can have chelating ability as well.
- Compounds containing carbonate, hydroxide, or phosphate can precipitate uranium out of solution prior to analysis.

Source: ASTM. 2015. "Method D3972-09 (2015): Standard Test Method for Isotopic Uranium in Water by Radiochemistry." *Annual Book of ASTM Standards*, Vol. 11.02. West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D3972.htm

6.2.51 ASTM Method D5811-08 (2013): Standard Test Method for Strontium-90 in Water

Analyte(s)	CAS RN
Strontium-90	10098-97-2

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Beta counting

Method Developed for: Strontium-90 in water samples
Method Selected for: This method has been selected for qualitative and confirmatory analysis of aqueous and liquid phase samples.

Description of Method: An aliquot of the sample is measured into a beaker, and strontium carrier is added. The sample is digested with nitric acid, sorbed on an ion exchange column, eluted, and evaporated to dryness. The residue is redissolved in nitric acid and then is selectively sorbed on a solid phase extraction column. Strontium is eluted with dilute nitric acid, dried on a planchet, weighed, and counted for beta radiation.

Special Considerations: Significant amounts of stable strontium, if present in the sample, will interfere with the yield determination. The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Chelating compounds can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column or from being precipitated out of solution during precipitation procedures. Dispersants and corrosion inhibitors can have chelating ability as well.
- Compounds containing carbonate, fluoride, phosphate or sulfate can precipitate radionuclides out of solution prior to analysis, resulting in a lesser amount of strontium in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample.

Source: ASTM. 2013. "Method D5811-08 (2013): Standard Test Method for Strontium-90 in Water." *Annual Book of ASTM Standards*, Vol. 11.02. West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D5811.htm

6.2.52 ASTM Method D7168-16: Standard Test Method for Technetium-99 in Water by Solid Phase Extraction Disk

Analyte(s)	CAS RN
Technetium-99	14133-76-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Technetium-99 in water samples
Method Selected for: This method has been selected for qualitative and confirmatory analysis of aqueous and liquid phase samples.

Description of Method: A measured aliquot of sample is transferred to a beaker and hydrogen peroxide is added to facilitate the formation of the extractable pertechnetate ion. The sample may be heated to oxidize organics, if suspected to be present. The entire sample is passed through a technetium-selective solid-phase extraction (SPE) disk onto which the pertechnetate is adsorbed. The disk is transferred to a liquid scintillation vial, scintillation cocktail is added, and the contents are well mixed. The beta-emission rate of the sample is determined by liquid scintillation spectrometry. Chemical yield corrections are determined by the method of standard additions.

Special Considerations: Suspended materials must be removed by filtration or centrifuging prior to processing the sample. High levels of iodate, iron (III) and antimony can interfere with the measurement of technetium-99 and lead to a positive bias in sample results.

Chelating compounds, such as those contained in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being precipitated out of solution during precipitation procedures. Chemical and color quenching can have a significant impact when using liquid scintillation methods. Some decontamination agents include organic compounds that contain oxygen, halogenated compounds, elevated levels of nitrates or nitromethane, or dyes, pigments or other colored compounds that can cause chemical or color quenching, which can significantly impact liquid scintillation methods.

Source: ASTM. 2016. "Method D7168-16: Standard Test Method for Technetium-99 in Water by Solid Phase Extraction Disk." *Annual Book of ASTM Standards*, Vol. 11.02. West Conshohocken, PA: ASTM International. http://www.astm.org/Standards/D7168.htm

6.2.53 Standard Method 7110 B: Gross Alpha and Gross Beta Radioactivity (Total, Suspended, and Dissolved)

Analysis Purpose: Gross alpha and gross beta determination
Technique: Alpha/Beta counting

Method Developed for: Gross alpha and gross beta activity in water
Method Selected for: This method has been selected for gross alpha and gross beta determination in aqueous/liquid samples.

Description of Method: This method allows for measurement of gross alpha and gross beta radiation in water samples. The method provides an indication of the presence of alpha and beta emitters, including the following analytes:

- Americium-241 (CAS RN 14596-10-2) Alpha emitter
- Californium-252 (CAS RN 13981-17-4) Alpha emitter
- Cesium-137 (CAS RN 10045-97-3) Beta emitter
- Cobalt-60 (CAS RN 10198-40-0) Beta emitter
- Curium-244 (CAS RN 13981-15-2) Alpha emitter
- Europium-154 (CAS RN 15585-10-1) Beta emitter
- Iridium-192 (CAS RN 14694-69-0) Beta emitter
- Plutonium-238 (CAS RN 13981-16-3) Alpha emitter
- Plutonium-239 (CAS RN 15117-48-3) Alpha emitter
- Polonium-210 (CAS RN 13981-52-7) Alpha emitter
- Radium-226 (CAS RN 13982-63-3) Alpha emitter
- Ruthenium-103 (CAS RN 13968-53-1) Beta emitter
- Ruthenium-106 (CAS RN 13967-48-1) Beta emitter
- Strontium-90 (CAS RN 10098-97-2) Beta emitter
- Uranium-234 (CAS RN 13966-29-5) Alpha emitter
- Uranium-235 (CAS RN 15117-96-1) Alpha emitter
- Uranium-238 (CAS RN 7440-16-1) Alpha emitter

This method recommends using a thin-window gas-flow proportional counter for counting gross alpha and beta radioactivity. An internal proportional or Geiger counter may also be used. An aliquot of sample is evaporated to a small volume and transferred to a tared counting pan. The sample residue is dried to constant weight, cooled, and reweighed to determine dry residue weight, then counted for alpha and beta radioactivity.

Special Considerations: Ground water samples containing elevated levels of dissolved solids will require use of smaller sample volumes. Compounds containing carbonate, fluoride, hydroxide, or

phosphate, which are present in some decontamination agents, can precipitate radionuclides out of solution prior to analysis. This precipitation can result in a lesser amount of radionuclides in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. The presence of hydroxide, in particular, can have a significant impact. At a pH above 5, it will precipitate out iron, carrying actinides (e.g., uranium and plutonium) with it.

Source: APHA, AWWA and WEF. 2005. "Method 7110 B: Gross Alpha and Gross Beta Radioactivity (Total, Suspended, and Dissolved)." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: APHA. http://www.standardmethods.org/

6.2.54 Standard Method 7120: Gamma-Emitting Radionuclides

Analyte(s)	CAS RN
Americium-241	14596-10-2
Cesium-137	10045-97-3
Cobalt-60	10198-40-0
Europium-154	15585-10-1
Iridium-192	14694-69-0
Neptunium-239	13968-59-7
Ruthenium-103	13968-53-1
Ruthenium-106	13967-48-1
Selenium-75	14265-71-5

Analysis Purpose: Qualitative and confirmatory determination
Technique: Gamma spectrometry

Method Developed for: Gamma emitting radionuclides in water
Method Selected for: This method has been selected for qualitative and confirmatory analysis of select gamma emitters in aqueous/liquid samples.

Description of Method: The method uses gamma spectroscopy using either Ge detectors or NaI(Tl) crystals for the measurement of gamma photons emitted from radionuclides present in water. The method can be used for qualitative and confirmatory determinations with Ge detectors or semi-qualitative and semi-quantitative determinations (using NaI(Tl) detectors). Exact confirmation using NaI is possible for single nuclides or when the gamma emissions are limited to a few well-separated energies. A homogeneous water sample is placed into a standard geometry (normally a Marinelli beaker) for gamma counting. Sample portions are counted long enough to meet the required sensitivity of measurement. A radioactive standard, in the same geometry as the samples, containing a mixture of gamma energies from approximately 50 to 2000 keV is used for energy and efficiency calibration.

Special Considerations: The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Reducing agents can potentially convert radionuclides into an insoluble zero-valent state that can precipitate out of solution. The addition of nitric acid during sample collection can prevent this precipitation from occurring. Iridium and ruthenium would likely still precipitate in the presence of reducing agents.
- Clays can sequester cesium-137, which would only be released upon complete dissolution when using this method.
- Compounds containing carbonate, fluoride, hydroxide, or phosphate can precipitate radionuclides

out of solution prior to analysis.

Source: APHA, AWWA and WEF. 2005. "Method 7120: Gamma-Emitting Radionuclides." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: APHA. http://www.standardmethods.org/

6.2.55 Standard Method 7500-Ra B: Radium: Precipitation Method

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Qualitative determination
Technique: Alpha counting

Method Developed for: Alpha-emitting isotopes of radium in water
Method Selected for: This method has been selected for qualitative determination in aqueous/liquid samples.

Description of Method: This method is for determination of all alpha-emitting radium isotopes by alpha decay analysis. Lead and barium carriers are added to the sample containing alkaline citrate, then sulfuric acid is added to precipitate radium, barium and lead as sulfates. The precipitate is purified by washing with nitric acid, dissolving in alkaline EDTA, and re-precipitating as radium-barium sulfate after pH adjustment to 4.5. This slightly acidic EDTA keeps other naturally occurring alpha-emitters and the lead carrier in solution. Radium-223, -224 and -226 are identified by the rate of ingrowth of their daughter products in barium sulfate precipitate. The results are corrected by the rate of ingrowth of daughter products to determine radium activity. This method involves alpha counting by a gas-flow internal proportional counter, scintillation counter or thin end-window gas-flow proportional counter.

Special Considerations: The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation. Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Chelating compounds, such as those contained in some decontamination agents, can complex tightly to calcium, nickel, lead, magnesium or strontium that may be present in a sample, preventing their precipitation during sample preparation. These metal ions can potentially cause interferences when analyzing for radium-226.
- Compounds containing sulfate, carbonate, oxalate or phosphate, which are also present in some decontamination agents, can precipitate radium out of solution prior to analysis.

Source: APHA, AWWA and WEF. 2005. "Method 7500-Ra B: Radium: Precipitation Method." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: APHA. http://www.standardmethods.org/

6.2.56 Standard Method 7500-Ra C: Radium: Emanation Method

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Confirmatory determination
Technique: Alpha counting

Method Developed for: Soluble, suspended and total radium-226 in water
Method Selected for: This method has been selected for confirmatory analysis of aqueous/liquid samples.
Description of Method: Radium in water is concentrated and separated from sample solids by coprecipitation with a relatively large amount of barium as the sulfate. The precipitate is treated to remove silicates, if present, and to decompose insoluble radium compounds, fumed with phosphoric acid to remove sulfite, and dissolved in hydrochloric acid. The completely dissolved radium is placed in a bubbler, which is then closed and stored for a period of several days to 4 weeks for ingrowth of radon. The bubbler is connected to an evacuation system and the radon gas is removed from the liquid by aeration and helium, dried with a desiccant, and collected in a counting cell. Four hours after radon collection, the cell is counted. The activity of the radon is equal to the radium concentration. The MDC depends on counter characteristics, background-counting rate of scintillation cell, cell efficiency, length of the counting period, and contamination of the apparatus and environment by radium-226. Without reagent purification, the overall reagent blank (excluding background) should be between 0.03 and 0.05 pCi radium-226, which may be considered the minimum detectable amount under routine conditions.

Special Considerations: The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation, Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Chelating compounds can complex tightly to calcium, nickel, lead, magnesium or strontium that may be present in a sample, preventing their precipitation during sample preparation. These metal ions can potentially cause interferences when analyzing for radium-226.
- Compounds containing sulfate, carbonate, oxalate or phosphate can precipitate radium out of solution prior to analysis. This precipitation can result in a lesser amount of radium in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample.
- Oxidizers present in these agents can oxidize nickel or lead to form soluble metal ions that can also cause interferences.

Source: APHA, AWWA and WEF. 2005. "Method 7500-Ra C: Radium: Emanation Method." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: APHA. http://www.standardmethods.org/

6.2.57 Standard Method 7500-U B: Uranium: Radiochemical Method

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative determination
Technique: Alpha counting

Method Developed for: Total uranium alpha activity in water
Method Selected for: This method has been selected for qualitative determination in aqueous/liquid samples.

Description of Method: The sample is acidified with hydrochloric or nitric acid and boiled to eliminate carbonate and bicarbonate ions. Uranium is coprecipitated with ferric hydroxide and subsequently separated. The ferric hydroxide is dissolved, passed through an anion-exchange column, washed with

acid, and the uranium is eluted with dilute hydrochloric acid. The acid eluate is evaporated to near dryness, the residual salt is converted to nitrate, and the alpha activity is counted by a gas-flow proportional counter or alpha scintillation counter.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.47). The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure due to precipitation, Precipitation can result in a lesser amount of radionuclide in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample. Such compounds include:

- Certain chelating agents, such as those contained in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column or from being precipitated out of solution during precipitation procedures.
- Compounds containing carbonate, hydroxide, or phosphate, which are present in some decontamination agents can precipitate uranium out of solution.

Source: APHA, AWWA and WEF. 2005. "Method 7500-U B: Uranium: Radiochemical Method." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: APHA. http://www.standardmethods.org/

6.2.58 Standard Method 7500-U C: Uranium: Isotopic Method

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Confirmatory determination
Technique: Alpha spectrometry

Method Developed for: Isotopic content of the uranium alpha activity; determining the differences among naturally occurring, depleted and enriched uranium in water
Method Selected for: This method has been selected for confirmatory analysis of aqueous/liquid samples.

Description of Method: This method is a radiochemical procedure for determination of the isotopic content of uranium alpha activity. The sample is acidified with hydrochloric or nitric acid and uranium-232 is added as an isotopic tracer. Uranium is coprecipitated with ferric hydroxide and subsequently separated from the sample. The ferric hydroxide precipitate is dissolved and the solution passed through an anion-exchange column. The uranium is eluted with dilute hydrochloric acid. The acid eluate is evaporated to near dryness, and the residual salt is converted to nitrate and electrodeposited onto a stainless steel disc and counted by alpha spectrometry.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 (Section 6.2.47). Chelating compounds, such as those present in some decontamination agents, can compromise the collection of radionuclides prior to analysis, preventing them from being trapped on an ion exchange column or from being precipitated out of solution during precipitation procedures. Dispersants and corrosion inhibitors can have chelating ability as well. Compounds containing carbonate, hydroxide or phosphate, which are also present in some

decontamination agents, can precipitate uranium out of solution prior to analysis, resulting in a lesser amount of uranium in cases where an aliquot of water sample is transferred and analyzed separately from the entire sample.

Source: APHA, AWWA and WEF. 2005. "Method 7500-U C: Uranium: Isotopic Method." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: APHA. http://www.standardmethods.org/

6.2.59 Y-12 (DOE) Preparation of Samples for Total Activity Screening

Analyte(s)	CAS RN
Total Activity Screening	NA

Analysis Purpose: Total activity screening
Technique: Liquid scintillation
Method Developed for: Total activity screening
Method Selected for: This method has been selected for gross total activity screening of drinking water, liquid and aqueous phase, soil and sediment, wipe, air filter and vegetation samples.

Description of Method: Aqueous sample aliquots that require no preparation are added directly to the scintillation cocktail. Solid and semi-solid sample aliquots are digested in nitric acid on a hot plate, cooled, filtered, and diluted to a specified volume. Oil sample aliquots are weighed directly into a tared counting vial. A specified volume of liquid scintillation cocktail is added to each vial and mixed with the sample aliquot. The samples are then counted for total activity.

Special Considerations: The method assumes 100% counting efficiencies for both beta and alpha emitters. Low energy beta emitters will not be counted at 100% efficiency, which can introduce a negative bias in the measurement.

Chemical and color quenching can have a significant impact when using liquid scintillation methods. Organic compounds containing oxygen; halogenated compounds; elevated levels of nitrates or nitromethane; and dyes, pigments or other colored compounds present in certain decontamination agents, can cause quenching. Chelators, also present in decontamination agents, can tightly complex calcium that may be present in a sample, causing analytical interference (e.g., excessive levels of calcium can interfere with detection and measurement of phosphorus-32).

Source: Y-12 (DOE). 2005. "Preparation of Samples for Total Activity Screening." Procedure Y50-AC-65-7230. http://www.epa.gov/sites/production/files/2015-07/documents/y50-ac-65-7230.pdf

6.3 Method Summaries (Outdoor Infrastructure and Building Material Samples)

Summaries corresponding to the methods selected for analysis of outdoor infrastructure and building material samples listed in Appendix B2 are provided in Sections 6.3.1 through 6.3.8. These summaries contain information that has been extracted from the selected methods. Each method summary contains a table identifying the contaminants listed in Appendix B2 to which the method applies, a brief description of the analytical method, and a link to the full version of the method or a source for obtaining a full version of the method. Summaries are provided for informational use. The full version of the method should be consulted prior to sample analysis. For information regarding sample collection considerations for samples to be analyzed by these methods, see the companion *Sample Collection Information Document for Chemicals, Radiochemicals and Biotoxins – Companion to SAM 2017.*

6.3.1 Rapid Radiochemical Method for Total Radiostrontium (Sr-90) In Building Materials for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Strontium-90	10098-97-2

Analysis Purpose: Confirmatory analysis
Technique: Beta counting

Method Developed for: Strontium-89 and -90 in building materials
Method Selected for: This method has been selected for confirmatory analysis of strontium-90 in asphalt singles, asphalt paving materials, concrete and brick.

Description of Method: Strontium is solubilized and purified by sodium hydroxide fusion using procedures described in Section 6.3.3 for concrete and brick matrix samples, Section 6.3.7 for asphalt matrix samples and Section 6.3.8 for asphalt shingles, and purified from potentially interfering radionuclides and matrix constituents using a strontium-specific, rapid chemical separation method. The sample is equilibrated with strontium carrier, and preconcentrated by strontium/calcium carbonate coprecipitation from the alkaline fusion matrix. The carbonate precipitate is dissolved in hydrochloric acid and strontium is precipitated with calcium fluoride to remove silicates. The strontium fluoride precipitate is dissolved in strong nitric acid and the solution is passed through an Sr Resin extraction chromatography column. The sample test source is promptly counted on a gas flow proportional counter to determine the beta emission rate, which is used to calculate the total radiostrontium activity. The method is capable of satisfying a method uncertainty for total strontium-90 of 0.31 pCi/g at an analytical action level of 2.4 pCi/g, using a sample weight of 1.5 g and a count time of approximately 1.5 hours.

If differentiating between strontium-89 and strontium-90 is needed, then the same prepared sample can be recounted after ~10 days. If the initial and second counts agree (based on the expected ingrowth of yttrium-90) then strontium-89 is not present in significant amounts relative to strontium-90. Computational methods are available for resolving the concentration of strontium-89 and strontium-90 from two sequential counts of the sample (see Appendix B of the method). If significant amounts of strontium-89 are suspected, it can be determined more rapidly using Čerenkov counting; however, the minimum detectable activity levels will be higher than that of determination with gas proportional counting and may or may not meet measurement quality objectives.

Special Considerations: Count results should be monitored for detectable alpha activity and appropriate corrective actions should be taken when this is observed. Failure to address the presence of alpha emitters in the sample test source may lead to high bias in the results, due to alpha-to-beta crosstalk.

Elevated levels of tetravalent plutonium, neptunium, cerium or ruthenium in the sample may hold up on the column and co-elute with strontium. The method uses an oxalic acid rinse that should address low to moderate levels of these interferences. Significant levels of strontium-90 also will interfere with the total radiostrontium analysis (see Appendix B of the method for an alternative approach should this situation arise). High levels of lead-210 can interfere with low level strontium analysis due to ingrowth of short-lived bismuth-210 during chemical separations, where lead is retained by the resin, but is not eluted. If lead-210 is known to be present in samples, minimizing the time between the final rinse and the elution of strontium to less than 15 minutes will minimize the levels of interfering bismuth-210.

The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure:

- Clays and other compounds containing iron, magnesium and calcium, which can be released as ions via ion exchange in the presence of certain radionuclides, can cause interferences.
- High levels of iron or magnesium can impact exchange site availability and/or poison the extraction resins.
- Chelating compounds can tightly complex barium, iron, lead, magnesium and potassium causing interference when analyzing for strontium-89 or -90.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Radiochemical Method for Total Radiostrontium (Sr-90) In Building Materials for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R14-001. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.2 Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Radium-226 in building materials
Method Selected for: This method has been selected for confirmatory analysis of radium-226 in asphalt shingle, asphalt paving materials, concrete and brick.

Description of Method: A known quantity of radium-225 is used as the yield tracer in this analysis. The sample is fused using the procedures described in Section 6.3.3 for concrete and brick matrix samples, Section 6.3.7 for asphalt matrix samples and Section 6.3.8 for asphalt roofing matrix samples. Radium isotopes are removed from the fusion matrix using a carbonate precipitation step. The sample is acidified and loaded onto a cation exchange resin to remove interferences, such as calcium. Radium is eluted from the cation resin with 8M nitric acid. After evaporation of the eluate, the sample is dissolved in a minimal amount of 3M nitric acid and passed through Sr Resin to remove any barium present. This solution is then evaporated to dryness, redissolved in 0.02M hydrochloric acid, and passed through Ln Resin to remove interferences such as residual calcium, and to remove the initial actinium-225 present. The radium (including radium-226) is prepared for counting by microprecipitation with barium sulfate. The activity measured in the radium-226 region of interest is corrected for chemical yield based on the observed activity of the alpha peak at 7.07 MeV (astatine-217, the third progeny of radium-225).

This method is suited for low-level measurements for radium-226 using alpha spectrometry and is capable of satisfying a method uncertainty of 0.83 pCi/g at an analytical action level of 6.41 pCi/g, using a sample aliquant of approximately 1 g and count time of 8 hours (or longer).

Special Considerations: Depending on actual spectral resolution, method performance may be compromised if samples contain high levels of other radium isotopes (e.g., ~3 times the radium-226 activity concentration) due to ingrowth of interfering decay progeny. Calcium, iron (+3 oxidation state), and radionuclides with overlapping alpha energies, such as thorium-229, uranium-234, and neptunium-237, will interfere if they are not removed effectively. Delaying the count significantly longer than one day may introduce positive bias in results near the detection threshold due to the decay progeny from the radium 225 tracer. If radium-226 measurements close to detection levels are required and sample counting cannot be performed within ~36 hours of tracer addition, the impact of tracer progeny tailing into the radium-226 may be minimized by reducing the amount of the tracer that is added to the sample. This will aid in improving the signal-to-noise ratio for the radium-226 peak by minimizing the amount of tailing from higher energy alphas of the radium-225 progeny. If actinium-225 is present prior to the final separation time and the flow rate through the column is too fast (>1.5 drops/second), then actinium-225 will break through the resin, resulting in a high bias in the tracer yield. Additional information regarding procedures to remove or minimize interferences is provided in Section 4.0 of the method.

The presence of compounds contained in various decontamination agents can impact the results of analysis using this procedure:

- Clays can contain iron, magnesium or calcium, which can be released as ions via ion exchange in the presence of certain radionuclides, and cause interferences.
- High levels of iron, manganese, calcium or magnesium might have an impact on exchange site availability and or poisoning of the extraction resins used.
- Chelators can tightly complex barium, calcium, iron and magnesium that may be present in the sample causing interferences when analyzing for radium-226.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R14-002. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.3 Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Radium-226	13982-63-3
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Sample Preparation
Sample Preparation Technique: Fusion
Determinative Technique: Alpha spectrometry/beta counting

Method Developed for: Americium-241, plutonium-238, plutonium-239, radium-226, strontium-90, uranium-234, uranium-235 and uranium-238 in concrete and brick samples.

Method Selected for: This method has been selected for preparation of concrete and brick samples to be analyzed for americium-241, plutonium-238, plutonium-239, radium-226, strontium-90, uranium-234, uranium-235 and uranium-238.

Description of Method: Concrete and brick samples may be received as core samples, pieces of various sizes, dust or particles (wet or dry) from scabbling, or powder. The method is based on the rapid fusion, in zirconium crucibles, of a representative, finely ground (5–100 mesh sized) 1–1.5 gram aliquant using rapid sodium hydroxide fusion at 600°C. Plutonium, uranium and americium are separated from the alkaline matrix using an iron/titanium hydroxide precipitation (enhanced with calcium phosphate precipitation) followed by a lanthanum fluoride matrix removal step. Strontium is separated from the alkaline matrix using a carbonate precipitation, followed by calcium fluoride precipitation to remove silicates. Radium is separated from the alkaline matrix using a carbonate precipitation. These sample preparation procedures are performed prior to the chemical separation procedures described in the following:

- Rapid Radiochemical Method for Total Radiostrontium (Strontium-90) in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.1)
- Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.2)
- Rapid Radiochemical Method for Isotopic Uranium in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.4)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.5)
- Rapid Radiochemical Method for Americium-241 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.6)

Special Considerations: In samples where native constituents may be present that could interfere with determination of the chemical yield (e.g., strontium for strontium-90 analysis) or with the creation of a sample test source (e.g., barium for radium-226 analysis by alpha spectrometry), it may be necessary to determine the concentration of the native constituents in advance of chemical separation (using a separate aliquant of fused material) and make appropriate adjustments to the yield calculations or amount of carrier added. Concrete and brick can contain native barium or radium, which can cause interferences with the analysis of radium-226. Compounds contained in decontamination agents are not expected to cause interferences during sample preparation; see the sections corresponding to the analytical methods listed in the description of this method for potential interferences caused by constituents of decontamination agents.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R-14-004. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.4 Rapid Radiochemical Method for Isotopic Uranium in Building Materials for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Uranium-234, -235 and -238 in concrete and brick samples
Method Selected for: This method has been selected for confirmatory analysis of uranium-234, uranium-235 and uranium-238 in asphalt shingles, asphalt building materials, concrete and brick.

Description of Method: This method is based on the use of extraction chromatography resins to isolate and purify uranium isotopes by removing interfering radionuclides as well as other components of the sample matrix in order to prepare the uranium fraction for counting by alpha spectrometry. The method utilizes vacuum-assisted flow to improve the speed of the separations. Uranium-232 tracer, added to the building materials sample, is used as a yield monitor. A 1.0- to 1.5-gram sample is fused using the procedure described in Section 6.3.3 for concrete and brick samples, Section 6.3.7 for asphalt samples and Section 6.3.8 for asphalt roofing materials. The uranium isotopes are then removed from the fusion matrix using iron hydroxide and lanthanum fluoride precipitation steps. The sample test source is prepared by microprecipitation with cerium (III) fluoride. The method is capable of achieving a method uncertainty for uranium-234, uranium-235, and uranium-238 of 1.9 pCi/g at an analytical level of 14.7 pCi/g, using a sample weight of approximately 1 g and count time of at least 3 to 4 hours.

Special Considerations: Alpha-emitting radionuclides with peaks at energies that cannot be adequately resolved from the tracer or analyte (e.g., polonium-210 [5.304 MeV], thorium-228 [5.423 MeV, 5.340 MeV] and americium-243 [5.275 MeV, 5.233 MeV]) must be chemically separated to enable radionuclide-specific measurements (see Section 4.0 of the method for procedures to remove specific interferences). Nonradiological anions such as fluoride and phosphate that complex uranium ions may cause lower chemical yields. Aluminum that is added in the column load solution complexes fluoride, as well as any residual phosphate that may be present. Lanthanum, added to preconcentrate uranium from the sample matrix as lanthanum fluoride, can have a slight adverse impact on uranium retention on TRU resin, but this impact is minimal at the level added. Iron (3+) can also have an adverse impact on uranium retention on TRU resin, but the residual iron levels after preconcentration steps are acceptable.

Clays, which are present in some decontamination agents, can contain iron, magnesium or calcium that can be released as ions via ion exchange, in the presence of certain radionuclides, and cause analytical interferences. High levels of iron, manganese, calcium or magnesium can also have an impact on exchange site availability and/or poison extraction resins used in alpha spectrometry. Higher valence anions such as phosphates may lead to lower yields when using the evaporation option due to competition with active sites on the resin. Concrete and brick can contain native uranium isotopes, which can cause interferences with the analysis of uranium isotopes.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Radiochemical Method for Isotopic Uranium in Building Materials for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R14-005. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.5 Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Building Materials for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Plutonium-238 and -239 in building materials
Method Selected for: This method has been selected for confirmatory analysis of plutonium-238 and -239 in asphalt shingles, asphalt paving materials, concrete and brick.

Description of Method: This method is based on the use of TEVAresin to isolate and purify plutonium by removing interfering radionuclides as well as other components of the sample matrix in order to prepare the plutonium fraction for counting by alpha spectrometry. The method utilizes vacuum-assisted flow to improve the speed of the separations. The sample may be fused using the procedure described in Section 6.3.3 for concrete and brick matrix samples, Section 6.3.7 for asphalt matrix samples and Section 6.3.8 for asphalt roofing matrix samples. The plutonium isotopes are then removed from the fusion matrix using iron hydroxide and lanthanum fluoride precipitation steps. Plutonium-242 or plutonium-236 tracer, added to the sample, is used as a yield monitor. The sample test source is prepared by microprecipitation with cerium (III) fluoride. The method is capable of achieving a required method uncertainty of 0.25 pCi/g for plutonium-238, -239/240, at an analytical action level of 1.89 pCi/g, using a sample weight of approximately 1 g and a count time of at least 3 to 4 hours.

Special Considerations: Alpha-emitting radionuclides with irresolvable alpha energies, such as plutonium-238 (5.50 MeV), americium-241 (5.48 MeV) and thorium-228 (5.42 MeV) must be chemically separated to enable measurement. This method separates these radionuclides effectively. The significance of peak overlap is determined by the detector's alpha energy resolution characteristics and the quality of the final precipitate that is counted.

Non-radiological interferences include very high levels of anions such as phosphates, which may lead to lower yields due to competition with active sites on the resin and/or complexation with plutonium ions. The presence of fluoride (e.g., from hydrofluoric or fluoroboric acids) can precipitate out plutonium prior to sample measurement. Aluminum is added in the column load solution to complex interfering anions such as fluoride and phosphate. Compounds such as clays containing iron, magnesium or calcium, which are present in some decontamination agents, can release these elements as ions via ion exchange in the presence of certain radionuclides, and cause interference. High levels of iron, manganese, calcium or magnesium can also have an impact on exchange site availability and/or poison extraction resins used in alpha spectrometry.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Building Materials for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R14-006. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.6 Rapid Radiochemical Method for Americium-241 in Building Materials for Environmental Remediation Following Radiological Incidents

Analyte(s)	CAS RN
Americium-241	14596-10-2

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Americium-241 in building materials
Method Selected for: This method has been selected for confirmatory analysis of americium-241 in asphalt shingles, asphalt paving materials, concrete and brick.

Description of Method: This method is based on the use of extraction chromatography resins (TEVA and DGA resins) to isolate and purify americium by removing interfering radionuclides as well as other matrix components to prepare the americium fraction for counting by alpha spectrometry. The method uses vacuum-assisted flow to improve the speed of separations. The sample is fused using procedures described in Section 6.3.3 for concrete and brick, Section 6.3.7 for asphalt matrix samples and Section 6.3.8 for asphalt roofing matrix samples. The americium isotopes removed from the fusion matrix using iron hydroxide and lanthanum fluoride precipitation steps. Americium-243 tracer, added to the sample, is used as a yield monitor. The STS is prepared by microprecipitation with cerium (III) fluoride.

The method is capable of achieving a required method uncertainty for Am-241 of 0.20 pCi/g at an analytical action level of 1.5 pCi/g, using a sample weight of approximately 1 g and a count time of at least 4 hours.

Special Considerations: Alpha-emitting radionuclides with irresolvable alpha energies, such as plutonium-238 (5.50 MeV) and thorium-228 (5.42 MeV) can interfere with measurement of americium-241, and must be chemically separated to enable measurement. This method separates these radionuclides effectively. The significance of peak overlap is determined by the detector's alpha energy resolution characteristics and the quality of the final precipitate that is counted. A thorium removal rinse is performed on DGA resin in the event that any thorium ions pass through TEVA resin onto DGA resin. A dilute nitric acid rinse is performed to remove calcium and lanthanum ions that could end up on the final alpha source filter as fluoride solids.

Non-radiological interferences include anions that can complex americium, such as fluoride and phosphate, and lead to lower yields. Higher valence anions (e.g., phosphate) may lead to lower yields when using the evaporation option due to competition with active sites on the resin. Boric acid added in the load solution complexes fluoride ions, while aluminum complexes both fluoride as well as any residual phosphate that may be present. Clays that are present in some decontamination agents can contain iron, magnesium and calcium, which can be released as ions, via ion exchange, in the presence of certain radionuclides and cause interferences. High levels of calcium can have an adverse impact on americium retention on DGA resin. This interference is minimized by increasing the nitrate concentration to lower calcium retention and increase americium affinity. High levels of iron, manganese or magnesium can also have an impact on exchange site availability and/or poisoning of the Eichrom extraction resins used in this method.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. April 2014. "Rapid Radiochemical Method for Americium-241 in Building Materials for Environmental Remediation Following Radiological Incidents," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R14-007. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.7 Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Radium-226	13982-63-3
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Sample Preparation
Sample Preparation Technique: Fusion
Determinative Technique: Alpha spectrometry/beta counting

Method Developed for: Americium-241, plutonium-238, plutonium-239, radium-226, strontium-90, uranium-234, uranium-235 and uranium-238 in asphalt samples
Method Selected for: This method has been selected for preparation of americium-241, plutonium-238, plutonium-239, radium-226, strontium-90, uranium-234, uranium-235 and uranium-238 in asphalt matrices.

Description of Method: The method is based on heating a representative, finely milled 1- to 1.5-g aliquant asphalt sample to remove organic components, followed by rapid fusion using sodium hydroxide fusion at 600°C. Plutonium, uranium and americium are separated from the alkaline matrix using an iron/titanium hydroxide precipitation (enhanced with calcium phosphate precipitation), followed by a lanthanum fluoride matrix removal step. Strontium is separated from the alkaline matrix using a phosphate precipitation, followed by a calcium fluoride precipitation to remove silicates. Radium is separated from the alkaline matrix using a carbonate precipitation. The method is applicable to the sodium hydroxide fusion of asphalt samples, prior to the chemical separation procedures described in the following procedures:

- Rapid Radiochemical Method for Total Radiostrontium (Strontium-90) in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.1)
- Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.2)
- Rapid Radiochemical Method for Isotopic Uranium in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.4)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.5)
- Rapid Radiochemical Method for Americium-241 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.6)

Special Considerations: Asphalt samples with larger particle size may require a longer fusion time. Information regarding the elemental composition of the sample may be helpful to determine any native concentrations of uranium, radium, thorium, strontium or barium, all of which may have an effect on the chemical separations used following the fusion of the sample. In those samples where native constituents are present that could interfere with the determination of the chemical yield (e.g., strontium for strontium-90 analysis) or with the creation of a sample test source (e.g., barium for radium-226 analysis by alpha spectrometry), it may be necessary to determine the concentration of these constituents in advance of chemical separation (using a separate aliquant of fused material) and to make appropriate adjustments to

the yield calculations or amount of carrier added. Aluminum nitrate reagent typically contains trace levels of uranium contamination. To achieve the lowest possible blanks for isotopic uranium measurements, the aluminum nitrate reagent can be passed through ~7 mL TRU resin or UTEVA resin. Compounds contained in decontamination agents are not expected to cause interferences during sample preparation; see the sections corresponding to the analytical methods listed in the description of this method for potential interferences caused by constituents of decontamination agents.

Source: U.S. EPA, National Analytical and Radiation Environmental Laboratory. May 2017. "Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R16-001. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

6.3.8 Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Material Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Radium-226	13982-63-3
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Sample Preparation
Sample Preparation Technique: Fusion
Determinative Technique: Alpha spectrometry/beta counting

Method Developed for: Americium-241, plutonium-238, plutonium-239, radium-226, strontium-90, uranium-234, uranium-235 and uranium-238 in asphalt roofing material samples
Method Selected for: This method has been selected for preparation of americium-241, plutonium-238, plutonium-239, radium-226, strontium-90, uranium-234, uranium-235 and uranium-238 in asphalt shingles.

Description of Method: Asphalt roofing material samples should be cut into very small pieces prior to taking a representative aliquant for furnace heating and fusion. The method is based on ashing a 25-g subsample of asphalt roofing material sample in a furnace to remove organic components, followed by taking a representative aliquant from the ashed sample. A 1- to 1.5-g aliquant is fused using sodium hydroxide fusion at 600°C. Plutonium, uranium and americium are separated from the alkaline matrix using an iron/titanium hydroxide precipitation (enhanced with calcium phosphate precipitation) followed by a lanthanum fluoride matrix removal step. Strontium is separated from the alkaline matrix using a phosphate precipitation, followed by a calcium fluoride precipitation to remove silicates. Radium is separated from the alkaline matrix using a carbonate precipitation. The method is applicable to the sodium hydroxide fusion of asphalt shingle samples, prior to the chemical separation procedures described in the following procedures:

- Rapid Radiochemical Method for Total Radiostrontium (Strontium-90) in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.1)
- Rapid Radiochemical Method for Radium-226 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.2)

- Rapid Radiochemical Method for Isotopic Uranium in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.4)
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.5)
- Rapid Radiochemical Method for Americium-241 in Building Materials for Environmental Remediation Following Radiological Incidents (Section 6.3.6)

Special Considerations: The term "asphalt roofing materials" is used in this procedure to mean asphalt organic shingles or asphalt fiberglass shingles typically used for residential or commercial roofs. This roofing material procedure was validated with asphalt fiberglass shingles. Roofing material samples should be cut into very small pieces prior to taking a representative aliquant for furnace heating and fusion.

Bitumen components, which may have affinity for the radionuclides, are destroyed in this method. Radionuclides deposited on the surface of the asphalt roofing material are effectively digested, including refractory radionuclide particles. A small amount of mineralized granules may remain after the fusion. Information regarding the elemental composition of the sample may be helpful. For example, asphalt roofing materials may have native concentrations of uranium, radium, thorium, stable strontium or stable barium, all of which may have an effect on the chemical separations used following the fusion of the sample. In those samples where constituents are present that could interfere with the determination of the chemical yield (e.g., strontium for strontium-90 analysis) or with the creation of a sample test source (e.g., barium for radium-226 analysis by alpha spectrometry), it may be necessary to determine the concentration of these constituents in advance of chemical separation (using a separate aliquant of fused material) and make appropriate adjustments to the yield calculations or amount of carrier added. Compounds contained in decontamination agents are not expected to cause interferences during sample preparation; see the sections corresponding to the analytical methods listed in the description of this method for potential interferences caused by constituents of decontamination agents.

Source: U.S. EPA, National Air and Radiation Environmental Laboratory. August 2016. "Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Material Matrices Prior to Americium, Plutonium, Strontium, Radium, and Uranium Analyses," Revision 0. Montgomery, AL: U.S. EPA. EPA 402-R16-003. https://www.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides

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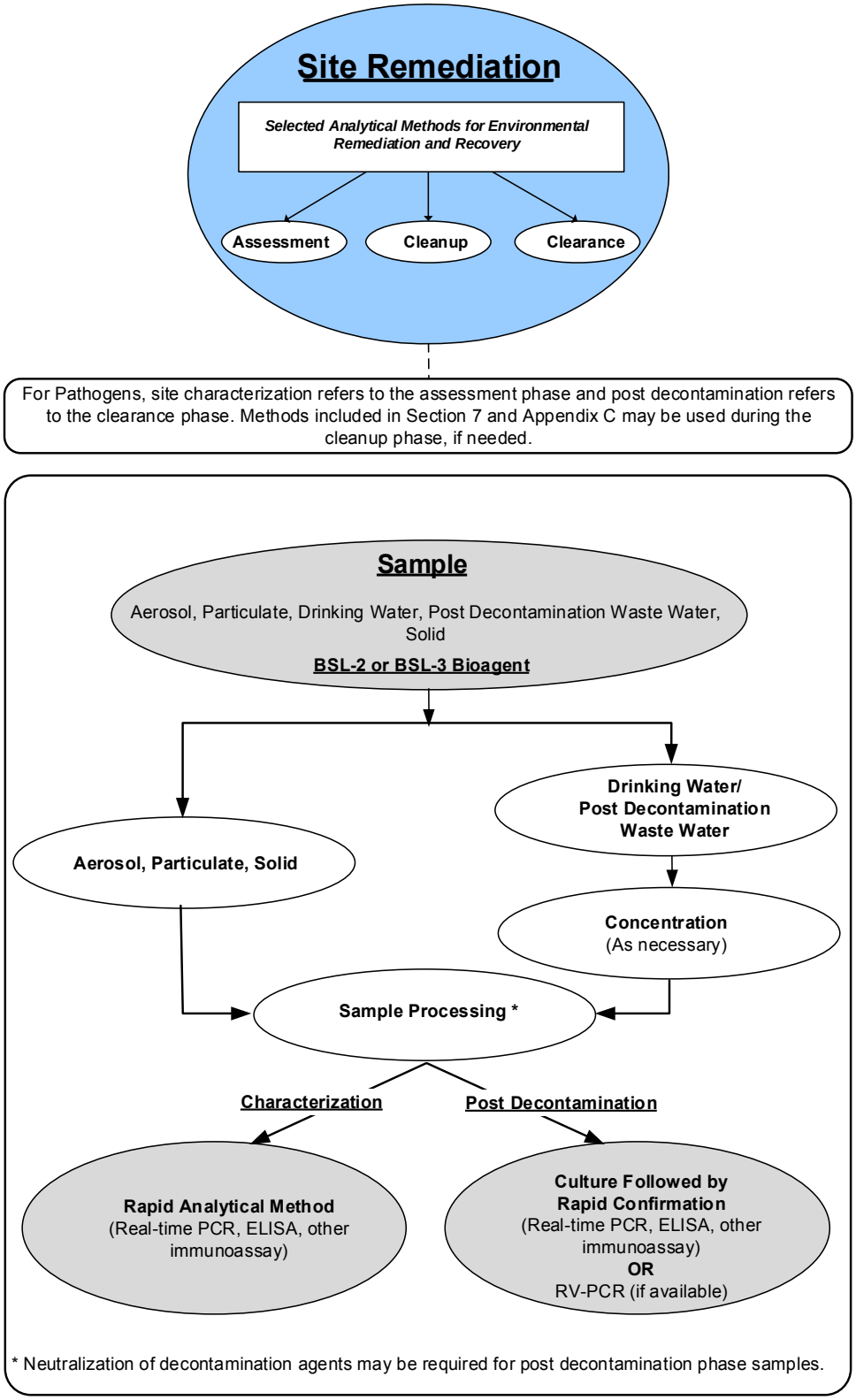
Section 7.0: Selected Pathogen Methods

Following a microbial contamination incident, it is assumed that the identification, confirmation and strain-level characterization of the pathogen have been completed before the U.S. Environmental Protection Agency's (EPA) remediation actions begin. The first phase of EPA's actions includes site characterization, to determine the extent and magnitude of contamination and to guide remediation planning. Based on the results of sample analyses for site characterization, EPA will determine the approach for site decontamination. During the post decontamination (clearance) phase of remediation, samples are collected and analyzed to determine the efficacy of the decontamination treatment.

The purpose of this section is to provide guidance to stakeholders in determining the appropriate methods for each remedial phase (site characterization and/or post decontamination) of a response to a contamination incident. Emphasis is given to the following environmental sample types: aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water.

Selection of methods from Appendix C should be based on specific data and information needs, including consideration of the remediation phase and whether there is a need to determine either the presence of a pathogen, the viability of a pathogen or both. The flow chart in Figure 7-1 presents a summary of the sample types, overall steps in sample analysis, and analytical techniques that should be used to address pathogens during EPA site remediation activities following a contamination incident. As depicted in Figure 7-1, for Pathogens, site characterization refers to the assessment phase, decontamination refers to the cleanup phase and post decontamination refers to the clearance phase.

Figure 7-1. Sample Analysis During Site Characterization and Post Decontamination Phases Following a Biological Contamination Event



Methods for Site Characterization Phase: Since decontamination of the affected site has to quickly follow the site characterization phase, rapid analytical methods should be selected to determine the extent and magnitude of contamination. It is assumed here that, prior to site characterization, the identity and viability of the pathogen have been determined. Therefore, in most cases, the analytical methods selected for the site characterization phase may not have to determine the viability of the pathogen. The methods chosen should also provide a high throughput analytical capability, so that a large number of samples can be rapidly analyzed to determine the presence or absence of the pathogen and allow for site decontamination planning in a time-efficient manner. For most pathogens, such methods routinely include polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA) or other immunoassay-based methods. Depending on the pathogen, type of incident and response, culture methods could be appropriate for use during site characterization. In certain cases, the determination of the extent of pathogen contamination within this phase may drive decontamination planning.

Methods for Post Decontamination Phase: It is extremely critical that the analytical methods used for sample analysis during the post decontamination phase be highly sensitive, specific, rapid and able to determine pathogen viability. For post decontamination phase samples, neutralization or removal of the decontamination agent may be required prior to analysis to minimize false negative results. Traditional microbiological culture methods typically include plating on selective medium to determine the viability of the pathogen and to minimize or eliminate non-target growth. The absence of growth on the medium generally indicates the absence of live pathogen in the sample (with the exception of some pathogens which may become viable but non-culturable [VBNC]). To minimize the analytical time needed to obtain results, typical colonies should be quickly analyzed to confirm the presence of the pathogen using reliable and rapid methods such as PCR, ELISA or other immunoassay-based methods, as opposed to time and labor intensive traditional biochemical and serological procedures. For *Bacillus anthracis* (Létant *et al.* 2011[10], U.S. EPA 2011[11], U.S. EPA 2012[12]) and *Yersinia pestis* (U.S. EPA 2016[13]) the Rapid Viability-PCR (RV-PCR) method may be used because it provides rapid and high throughput results in addition to viability determination.

A list of methods that have been selected by the Pathogen Methods Work Group for use in analyzing environmental samples for pathogens is provided in Appendix C. These methods should be used during remediation activities following a contamination incident. Appendix C is sorted alphabetically within pathogen categories (i.e., bacteria, viruses, protozoa and helminths). Protocols from peer-reviewed journal articles are listed where verified and/or validated methods for pathogens are not currently available. The literature references will be replaced as fully developed and validated protocols become available.

***Please note*:** This section provides guidance for selecting pathogen methods that have a high likelihood of assuring analytical consistency when laboratories analyze a large number of samples during remediation. Not all methods have been verified for the pathogen/sample type combination listed in Appendix C. Please refer to the specified method to identify analyte/sample type combinations for which the method has been verified. Any questions regarding information discussed in this section should be addressed to the appropriate contact(s) listed in Section 4.0.

[10] Létant, S. E., Murphy, G.A., Alfaro, T. M., Avila, J. R., Kane, S. R., Raber, E., Bunt, T. M. and Shah, S. R. 2011. "Rapid-Viability PCR Method for Detection of Live, Virulent *Bacillus anthracis* in Environmental Samples." Applied Environmental Microbiology. 77(18): 6570–6578.
[11] U.S. EPA. 2011. "Development and Verification of Rapid Viability Polymerase Chain Reaction (RV-PCR) Protocols for *Bacillus anthracis* – For Application to Air Filters, Water and Surface Samples." EPA/600/R-10/156.
[12] U.S. EPA. 2012. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577.
[13] U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109.

Pathogens that require biosafety level (BSL)-4 containment and practices, such as hemorrhagic fever viruses and Variola major (smallpox) will be handled only by reference laboratories with BSL-4 capability and are not included in this document. All other pathogens should be handled using BSL-2 or BSL-3 containment and practices, as appropriate. If known, the BSL classification for each pathogen is provided in the method summaries in Sections 7.2 through 7.5. Pathogens that are considered to be solely of agricultural concern (i.e., animal and plant pathogens) are not currently included. However, such pathogens may be considered for possible inclusion in future revisions.

Culture-based methods have been selected for many of the pathogens; however, due to technical difficulty and time constraints, molecular techniques such as PCR will likely be used for viruses.

Some of the methods in Appendix C include multiple analytical techniques by inference. The analytical technique listed in Appendix C for each pathogen is intended to be a description of the predominant technique that is required to provide the data quality parameter (viability or detection and identification). This description does not preclude the use of other techniques that are within or referenced by the method. For example, a viability method or procedure listed as "culture" might include immunochemical or PCR-based assays for the identification and/or confirmation of isolates. Several of the methods listed in Appendix C also include options such as the use of multiple cell culture media for primary isolation and a selection of a defined subset of biochemical tests for confirmation. To expedite time-to-results, however, isolates should be confirmed using rapid techniques (e.g., PCR, ELISA).

Appendix C includes the following information:

- **Pathogen(s).** A specific causative agent (e.g., viruses, bacteria) of disease.
- **Analytical technique.** An analytical procedure used to determine the identity, quantity and/or viability of a pathogen.
- **Method type.** Two method types (sample preparation and analytical) are used to complete sample analysis. In some cases, a single method contains information for both sample preparation and the analytical procedure. In most instances, however, two separate methods may need to be used.
- **Analytical method.** A series of techniques which together isolate, concentrate and detect a microorganism or group of microorganisms. In some cases, a unique identifier or number is assigned to an analytical method by the method publisher. Analytical methods can be developed for various sample types, including:
    - **Aerosol (growth media, filters, liquids).** The recommended method/procedure for the pathogen of interest in air sample collectors such as growth media, filters or liquids.
    - **Particulate (swabs, wipes, Sponge-Sticks, vacuum socks, filters).** The recommended method/procedure for the pathogen of interest in particulate sample collection tools such as swabs, wipes, Sponge-Sticks, and high-efficiency particulate air (HEPA) collecting socks and filters used for vacuum collection.
    - **Soil.** The recommended method/procedure for the pathogen of interest in soils.
    - **Drinking water.** The recommended method/procedure for the pathogen of interest in potable water (concentrated and small volume grab samples).
    - **Post decontamination waste water.** The recommended method/procedure for the pathogen of interest in post decontamination waste water (concentrated and small volume grab samples).

Sample Processing: It is widely recognized in the scientific community that the processing of biologically contaminated environmental samples is one of the most challenging issues prior to sample analysis. Although details regarding sample processing are not included, it is critical that end users and stakeholders select the most appropriate sample processing procedure for a given sample type and analytical method. It is highly unlikely that a single procedure will be applicable to all sample types and analytical methods. Inadequate sample processing may not only decrease recovery efficiency of biological targets (e.g., pathogen, deoxyribonucleic acid/ribonucleic acid [DNA/RNA], antigen/protein)

from the samples, but also prevent accurate quantitation and high throughput. Samples should not be stored indefinitely, and should be processed and analyzed as soon as possible upon receipt. ***Note***: For post decontamination samples it may be necessary to neutralize the decontamination agent.

The methods listed attempt to address multiple environmental sample types, each with different physical, chemical and biological properties (e.g., pH, inhibitory substances and background microorganisms). In this edition, emphasis is given to the environmental sample types that are predominately used to fulfill EPA's responsibilities following a contamination incident (e.g., aerosols, particulates [wipes or swabs], soils, drinking water, post decontamination waste water). Other sample types may have to be analyzed, and for those sample types, specific requests should be sent to the Pathogen Methods Lead and Alternate Lead (see Section 4.0).

7.1 General Guidelines

This section provides a general overview of how to identify the appropriate method(s) for a given pathogen as well as recommendations for quality control (QC) procedures.

For additional information on the properties of the pathogens listed in Appendix C, Toxicology Data Network (TOXNET) (http://toxnet.nlm.nih.gov/index.html), a cluster of databases on toxicology, hazardous chemicals and related areas maintained by the National Library of Medicine, is an excellent resource. Also informative are the Centers for Disease Control and Prevention's (CDC's) Emergency Preparedness and Response website (https://emergency.cdc.gov/bioterrorism/index.asp) and the U.S. Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition (CFSAN) 2012, "Bad Bug Book" (https://www.fda.gov/food/foodborneillnesscontaminants/causesofillnessbadbugbook/). Further research on pathogens is ongoing within EPA. Databases to manage this information are currently under development.

7.1.1 Standard Operating Procedures for Identifying Pathogen Methods

To determine the appropriate analytical method that is to be used for an environmental sample, locate the pathogen in Appendix C: Selected Pathogen Methods, under the "Pathogen(s)" column. After locating the pathogen, continue across the table and select an analytical technique. After an analytical technique has been chosen (e.g., culture, PCR, immunoassay), select the analytical method applicable to the sample type of interest (aerosol, particulate [wipes or swabs], soil, drinking water or post decontamination waste water).

The fitness of a method for an intended use is related to site-specific data quality objectives (DQOs) for a particular environmental remediation activity. These selected pathogen methods have been assigned tiers (below) to indicate a level of method usability for the specific analyte and sample type. The assigned tiers pertain only to technical aspects of method usability, and do not pertain to aspects such as cost, equipment availability and sample throughput.

Tier I: The method was developed for the pathogen and sample type. The method has been evaluated by multiple laboratories, a detailed protocol has been developed, and suitable QC measures and checks are provided. (Examples: EPA Method 1623.1 [*Cryptosporidium* in drinking water]; Standard Methods 9260 E [*Shigella* culture method].)

Tier II: The pathogen is the target of the method, and the method has been evaluated by one or more laboratories. The available data and/or information indicate that additional testing and/or modifications will likely be needed. (Example: Cunningham *et al.* 2010 [*Shigella* molecular method].)

Tier III: The pathogen is not the target of the method but the method is for the specific sample type and the pathogen is similar to the target of the method (i.e., vegetative bacteria, spore-forming bacteria, virus or protozoan). Data and expert opinion suggest, however, that the method(s) may be applicable with modifications. (Example: EPA *Yersinia pestis* protocol for *Salmonella enterica* serovar Typhi (*S.* Typhi) in post decontamination waste water.)

Once a method has been identified in Appendix C, the corresponding method summary can be found in Sections 7.2 through 7.5. Method summaries are listed in alphabetical order within each pathogen subcategory (i.e., bacteria, virus, protozoa, helminthes) and then by order of method selection hierarchy (see Figure 2-1), starting with EPA methods, followed by methods from other federal agencies, voluntary consensus standard bodies (VCSBs), and literature references. Where available, a direct link to the full text of the method is provided with the method summary. For additional information regarding sample preparation and analysis procedures available through consensus standards organizations, other federal agencies and journals, please use the source information provided in **Table 7-1**.

Table 7-1. Sources of Pathogen Methods

Name	Publisher	Reference
Antimicrobial Agents and Chemotherapy*	American Society for Microbiology (ASM)	http://aac.asm.org/
Applied and Environmental Microbiology*	ASM	http://aem.asm.org/
Applied Biosafety*	SAGE Journals	http://apb.sagepub.com/
Archives of Virology*	Springer	http://link.springer.com/journal/705
Bacteriological Analytical Manual (BAM)	FDA CFSAN	http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm
BMC Microbiology	Springer	http://www.springer.com/life+sciences/microbiology/journal/12866
Canadian Journal of Microbiology*	NRC Research Press	http://www.nrcresearchpress.com/loi/cjm
Clinical Chemistry*	American Association for Clinical Chemistry	http://www.clinchem.org/content/by/year
Clinical Infectious Diseases*	Oxford	https://cid.oxfordjournals.org/
Current Protocols in Microbiology*	Wiley	http://onlinelibrary.wiley.com/book/10.1002/9780471729259
Emerging Infectious Diseases	CDC	http://wwwnc.cdc.gov/eid/
Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage and Sludge	EPA, National Risk Management Research Laboratory (NRMRL)	https://www.epa.gov/biosolids/control-pathogens-and-vector-attraction-sewage-sludge
Environmental Science and Technology*	American Chemical Society (ACS)	http://pubs.acs.org/journal/esthag
EPA Analytical Protocols	EPA, National Homeland Security Research Center (NHSRC)	https://www.epa.gov/homeland-security-research
EPA Microbiology Home Page	EPA	https://www.epa.gov/cwa-methods
FEMS Immunology & Medical Microbiology*	Oxford	http://femsim.oxfordjournals.org/
International Organization for Standardization (ISO) Methods*	ISO	http://www.iso.org/iso/home.html
Journal of Clinical Microbiology*	ASM	http://jcm.asm.org/

Name	Publisher	Reference
Journal of Environmental Monitoring*	Royal Society of Chemistry	http://pubs.rsc.org/en/journals/journalissues/em?issueid=em014011&issnprint=1464-0325#!issueid=em014011&type=archive&issnprint=1464-0325
Journal of Food Protection*	International Association for Food Protection	https://www.foodprotection.org/publications/journal-of-food-protection/
Journal of Medical Virology*	Wiley	http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1096-9071
Journal of Microbiological Methods*	Elsevier	http://www.sciencedirect.com/science/journal/01677012
Journal of Parasitology*	American Society of Parasitologists	http://www.journalofparasitology.org/
Journal of Parasitology Research*	Hindawi Publishing Corporation	https://www.hindawi.com/journals/jpr/contents/
Journal of Virological Methods*	Elsevier	http://www.sciencedirect.com/science/journal/01660934
Legionella: Methods and Protocols, Methods in Molecular Biology	Springer	http://link.springer.com/book/10.1007%2F978-1-62703-161-5
Methods in Molecular Biology	Springer	http://link.springer.com/search?facet-series=7651&facet-content-type=Book
Molecular and Cellular Probes*	Elsevier	http://www.sciencedirect.com/science/journal/08908508
National Institute for Occupational Safety and Health (NIOSH) Methods	CDC NIOSH	http://www.cdc.gov/niosh/nmam/
Neglected Tropical Diseases	PLOS	http://journals.plos.org/plosntds/
Occupational Safety and Health Administration (OSHA) Methods	OSHA	http://www.osha.gov
Parasitology Research*	Springer	http://www.springer.com/biomed/medical+microbiology/journal/436
Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases	ASM	http://www.asm.org/index.php/guidelines/sentinel-guidelines
Standard Methods for the Examination of Water and Wastewater, 22nd Edition, 2012*	American Public Health Association (APHA)	http://www.standardmethods.org
Transactions of the Royal Society of Tropical Medicine and Hygiene*	Oxford	http://trstmh.oxfordjournals.org/
U.S. Department of Agriculture (USDA) *Food Safety and Inspection Service (FSIS) Microbiology Laboratory Guidebook*	USDA FSIS	http://www.fsis.usda.gov/wps/portal/fsis/topics/science/laboratories-and-procedures/guidebooks-and-methods/microbiology-laboratory-guidebook/microbiology-laboratory-guidebook
U.S. Department of Health and Human Services *Procedures for the Recovery of Legionella from the Environment*	CDC	https://www.cdc.gov/legionella/health-depts/inv-tools-cluster/lab-inv-tools/procedures-manual.pdf

* Subscription and/or purchase required. ***Note***: ASM does not require a subscription or purchase 6 months after the publication date.

7.1.2 General QC Guidelines for Pathogen Methods

Generation of analytical data of known and documented quality is a critical factor in the accurate assessment of and appropriate response to emergency situations. The generation of data of sufficient quality requires that analytical laboratories: (1) have appropriately trained and proficient personnel; (2) acquire and maintain required supplies, equipment and reagents; (3) conduct the appropriate QC procedures to ensure that all measurement systems are in control and operating properly; (4) properly document all analytical results; (5) properly document analytical QC procedures and corrective actions; (6) conduct training and proficiency testing; and (7) maintain personnel training and proficiency testing records.[14]

The level or amount of QC needed depends on the intended purpose of the data generated. Specific data needs should be identified and QC requirements, based on those needs, applied consistently across laboratories when multiple laboratories are used. The individual methods listed, sampling and analytical protocols or contractual statements of work should be consulted to determine if additional QC procedures are required.

Method-specific QC requirements are described in many of the methods cited in this manual and will be included in protocols developed to address specific pathogen/sample type combinations of concern. In general, analytical QC requirements for pathogen methods include an initial demonstration of measurement system capability, as well as the capability of the laboratory and the analyst to perform the method with the required precision and accuracy. In addition, for molecular techniques (e.g., PCR) general guidelines are provided in EPA's 2004 *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (Cincinnati,OH: U.S. EPA. EPA 815-B-04-001) at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf.

Ongoing analysis of control samples to ensure the continued reliability of the analytical results should also be performed. At a minimum, the following QC analyses should be conducted on an ongoing basis:

- Media and reagent sterility checks
- Positive and negative controls
- Method blanks
- Reference matrix spikes to evaluate initial and ongoing method/analyst performance, if available
- Matrix spikes (where possible) to evaluate method performance in the sample type of interest
- Matrix spike duplicates (MSD) and/or sample replicates to assess method precision
- Sample processing controls to evaluate processing procedures (e.g., extraction, concentration) in the sample type of interest
- Instrument calibration checks and temperature controls

QC procedures and proper calibration and maintenance of ancillary laboratory equipment (e.g., thermometers, autoclaves, pipettors) should be performed as frequently as necessary to ensure the reliability of analytical results.

***Please note*:** The appropriate points of contact identified in Section 4.0 should be consulted regarding appropriate quality assurance (QA)/QC procedures prior to sample analysis. These contacts should be consulted regarding any method deviations or modifications, sample problems or interferences, QC requirements, the use of alternative methods, or the need to address analytes or sample types other than those listed. As previously indicated, any deviations from the recommended method(s) should be reported immediately to ensure data comparability is maintained. Method deviations or modifications must be approved by the Environmental Response Laboratory Network (ERLN) or Water Laboratory Alliance (WLA) prior to use.

[14] Information regarding EPA's DQO process, considerations, and planning is available at: http://www.epa.gov/QUALITY/dqos.html

7.1.3 Safety and Waste Management

Laboratories should have a documented health and safety plan for handling samples that might contain target chemical, biological and/or radiological (CBR) contaminants. Laboratory staff should be trained in the safety and waste handling procedures included in the plan and implement those procedures. Pathogens in samples taken from areas contaminated as the result of a homeland security event may be more hazardous than naturally occurring pathogens of the same genus and species. The pathogens may have been manufactured, engineered or treated to enhance dispersion or virulence characteristics. Laboratories should carefully consider implementing additional safety measures before agreeing to accept these samples. Sample disposal should follow federal and local regulations.

In addition, many of the methods listed in Appendix C and summarized or cited in Sections 7.2 through 7.5 contain specific requirements, guidelines or information regarding safety precautions that should be followed when handling or processing environmental samples and reagents. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. BSL-3 is applicable when performing manipulations of indigenous or exotic agents that can cause serious or potentially lethal disease and also have the potential for aerosol transmission. Whenever available, BSLs are provided in the method summaries in Sections 7.2 through 7.5. However, some pathogens that are normally handled at BSL-2 may require BSL-3 procedures and facilities if large volumes, high concentrations or potential aerosols are expected as a part of the analytical process. For more information on BSL practices and procedures, the following references should be consulted:

- CDC. 2009. "Biosafety in Microbiological and Biomedical Laboratories" (BMBL), 5th Edition. Available at: http://www.cdc.gov/biosafety/publications/bmbl5/
- CDC. 2002. "Laboratory Security and Emergency Response Guidance for Laboratories Working with Select Agents." *Morbidity and Mortality Weekly Report*, Vol. 51, No. RR-19, 1-6, December 6, 2002. Available at: http://www.cdc.gov/mmwr/pdf/rr/rr5119.pdf
- Select Agent Rules and Regulations found at the National Select Agent Registry. Available at: http://www.selectagents.gov/ and http://www.selectagents.gov/regulations.html

The following sources provide information regarding waste management:

- U.S. EPA – Hazardous Waste Management (40 CFR part 260) and U.S. EPA Administered Permit Programs (40 CFR part 270). Available at: http://www.ecfr.gov/
- U.S. EPA. 2010. Laboratory Environmental Sample Disposal Information Document Companion to Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events (SAM) Revision 5. EPA/600/R-10/092. Available at: http://www.epa.gov/sites/production/files/2015-06/documents/lesdid.pdf

Other resources that can be consulted for additional information include the following:

- OSHA – Hazardous Waste Operations and Emergency Response (29 CFR part 1910.120). Available at: http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9765
- OSHA – Occupational Exposure to Hazardous Chemicals in Laboratories (29 CFR part 1910.1450). Available at: http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10106
- OSHA – Respiratory Protection (29 CFR part 1910.134). Available at: http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=12716&p_table=STANDARDS
- DOT Hazardous Materials Shipment and Packaging (49 CFR parts 171–180). Available at: http://www.ecfr.gov/cgi-bin/text-idx?gp=&SID=994b04d45ee6d584ce676138929280b3&mc=true&tpl=/ecfrbrowse/Title49/49tab_02.tpl

7.1.4 Laboratory Response Network (LRN)

The LRN is a national network of local, state, federal, military, food, agricultural, veterinary and environmental laboratories that was created in accordance with Presidential Decision Directive 39, which established terrorism preparedness responsibilities for federal agencies. The CDC provides technical and scientific support to member laboratories as well as secure access to standardized procedures (e.g., immunoassay, PCR) and reagents for rapid (4–6 hours) presumptive detection of select agents. The algorithm for a confirmed result is often a combination of one or more presumptive positive results from a rapid assay in combination with a positive result from one of the "gold standard" methods, such as culture. The standardized procedures, reagents and agent-specific algorithms are considered to be sensitive and are available only to LRN member laboratories. Thus, these procedures are not available to the general public and are not discussed in this document. However, EPA has published methods for the analysis of *Bacillus anthracis* and *Yersinia pestis* in environmental matrices that are included in this document.

It is important to note that, in some cases, the procedures may not be fully developed or validated for each environmental sample type/pathogen combination listed in Appendix C. Except for *Coxiella burnetii*, culture methods are available for all of these pathogens as American Society for Microbiology's (ASM) Sentinel Laboratory Guidelines. Available at: https://www.asm.org/index.php/guidelines/sentinel-guidelines

The pathogens identified below and listed in Appendix C are included in the U.S. Health and Human Services (HHS)/U.S. Department of Agriculture (USDA) select agent list and should be analyzed in accordance with appropriate regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121, available at http://www.ecfr.gov/cgi-bin/ECFR?page=browse) and safety and BSL requirements (see CDC's BMBL, 5th Edition, available at: http://www.cdc.gov/biosafety/publications/bmbl5/)

Select Agents Listed in Appendix C

Pathogen(s) [Disease]	Agent Category
Bacillus anthracis [Anthrax]	Bacteria
Brucella spp. [Brucellosis]	Bacteria
Burkholderia mallei [Glanders]	Bacteria
Burkholderia pseudomallei [Melioidosis]	Bacteria
Coxiella burnetii [Q-fever]	Bacteria
Francisella tularensis [Tularemia]	Bacteria
Yersinia pestis [Plague]	Bacteria

For additional information on the LRN, including selection of a laboratory capable of receiving and processing the specified sample type/pathogen, please use the contact information provided below or visit https://emergency.cdc.gov/lrn/.

Centers for Disease Control and Prevention (CDC)
Laboratory Response Branch
Division of Bioterrorism Preparedness and Response (DBPR)
National Center for the Prevention, Detection, and Control of Infectious Diseases (NCPDCID)
Coordinating Center for Infectious Diseases (CCID)
CDC
1600 Clifton Road NE, Mailstop C-18
Atlanta, GA 30333
Telephone: (866) 576-5227

E-mail: lrn@cdc.gov
Website: https://emergency.cdc.gov/lrn/contact.asp

Local public health laboratories, private laboratories and commercial laboratories with questions about the LRN should contact their state public health laboratory director or the Association of Public Health Laboratories (APHL) (contact information provided below).

Association of Public Health Laboratories
8515 Georgia Avenue, Suite 700
Silver Spring, MD 20910
Telephone: (240) 485-2745
Fax: (240) 485-2700
Website: http://www.aphl.org
E-mail: info@aphl.org

The following references and information sources provide additional information regarding Select Agents Culture Methods – LRN Sentinel Labs (website references for individual pathogens are included in their respective summaries):

- Avian Influenzae: http://www.asm.org/images/PSAB/Novel_Influenza_July2013.pdf
- *Brucella*: http://www.asm.org/images/PSAB/LRN/Brucella316.pdf
- *Burkholderia mallei and B. pseudomallei*: http://www.asm.org/images/PSAB/LRN/Burkholderia316.pdf
- *Coxiella burnetii*: http://www.asm.org/images/PSAB/LRN/Coxiella316.pdf
- *Fransicella tularensis*: http://www.asm.org/images/PSAB/LRN/Tularemia316.pdf
- *Yersinia pestis*: http://www.asm.org/images/PSAB/LRN/Ypestis316.pdf

Sources:

ASM. 2010. Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases.
https://www.asm.org/index.php/guidelines/sentinel-guidelines

CDC. 2009. "Biosafety in Microbiological and Biomedical Laboratories" (BMBL), 5th Edition.
http://www.cdc.gov/biosafety/publications/bmbl5/

7.2 Method Summaries for Bacteria

Summaries for the analytical methods listed in Appendix C are provided in Sections 7.2.1 through 7.2.17. Each summary contains a brief description of the analytical methods selected for each bacterial pathogen, and links to, or sources for, obtaining full versions of the methods. Summaries are provided for informational use. Tiers that have been assigned to each method/analyte pair (see Section 7.1.1) can be found in Appendix C. The full version of the method should be consulted prior to sample analysis. For information regarding sample collection considerations for samples to be analyzed by these methods, see the companion *Sample Collection Information Document for Pathogens – Companion to SAM 2017*.[15]

7.2.1 *Bacillus anthracis* [Anthrax] – BSL-3

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.1.1
Post Decontamination	Rapid Viability-PCR (RV-PCR)	7.2.1.2
	Culture and Real-Time PCR	7.2.1.3

[1] See Appendix C for corresponding method usability tiers.

7.2.1.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols (filters), particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to EPA's "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (U.S. EPA 2012), referred to as the "EPA BA Protocol."

Nucleic acid extraction and purification for all sample types should be performed according to procedures within the EPA BA Protocol, Section 9.2 (U.S. EPA 2012).

Analytical Technique: Use real-time PCR (U.S. EPA 2012).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2 [U.S. EPA 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *B. anthracis* spores.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at:

[15] EPA's *Sample Collection Information Document for Pathogens – Companion to SAM 2017* can be found at: https://www.epa.gov/homeland-security-research/sam-companion-documents-and-sample-collection-procedures

https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Bacillus anthracis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed (see CDC's BMBL, 5th Edition [CDC 2009]). http://www.cdc.gov/biosafety/publications/bmbl5/.

Sources:

Silvestri, E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2012. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

7.2.1.2 Post Decontamination Sample Analyses (RV-PCR)

Note: Laboratories without RV-PCR capability should analyze samples according to the culture procedure provided in Section 7.2.1.3.

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols (filters), particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA BA Protocol (U.S. EPA 2012).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within the EPA BA Protocol, Section 10.4 (U.S. EPA 2012).

Analytical Technique: Use RV-PCR (U.S. EPA 2012).

Description of Method: The RV-PCR procedure is a combination of a broth culture and real-time PCR. Culturing the sample allows the germination of *Bacillus anthracis* spores recovered from a processed sample. The real-time PCR provides rapid detection of *Bacillus anthracis.* By combining both culture and PCR the protocol allows for the detection of viable *Bacillus anthracis* spores. Prior to analysis, samples (e.g., aerosols, particulates, soils, water) are processed using multiple extraction and wash steps. After brain heart infusion broth is added to the spores, an aliquot (Time 0 [T_0]) is removed and stored at 4°C. The remaining broth is then incubated for 9 to 15 hours at 37°C. After the incubation, an aliquot is removed (Time Final [T_f]). Both T_0 and T_f aliquots then go through DNA extraction and purification followed by real-time PCR analysis. The cycle threshold (C_T) values for the T_0 and T_f aliquots are then compared. The difference in C_T values between the T_0 and T_9 is used to detect viable *Bacillus anthracis* spores. A change (decrease) in the PCR $C_T \geq 6$ represents increased DNA amplification in the T_f aliquot relative to the T_0 aliquot, which in turn, represents an increase in DNA as a result of the germination and growth of viable spores in the sample during the incubation period.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Bacillus anthracis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed (see BMBL, 5th Edition [CDC 2009]).
http://www.cdc.gov/biosafety/publications/bmbl5/

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:
Silvestri, E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13.
http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2012. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577.
https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

7.2.1.3 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols (filters), particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA BA Protocol (U.S. EPA 2012).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within the EPA BA Protocol, Section 11.6 (U.S. EPA 2012).

Analytical Technique: Use culture and real-time PCR (U.S. EPA 2012).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the sample is streaked for isolation onto tryptic soy agar with 5% sheep's blood. Plates are incubated at 35°C to 37°C for 18–24 hours. Isolated typical colonies are resuspended in sterile distilled water. The bacterial suspensions are then heated at 95°C to 98°C to release the DNA from the cells (EPA BA Protocol, Section 11 [U.S. EPA 2012]). DNA extracts are then used in real-time PCR to confirm the presence of *Bacillus anthracis*. Combining the culture component with confirmation using real-time PCR analyses allows for detection and

viability results within 24–30 hours as compared to traditional culture procedures that require a minimum of 48 hours.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Bacillus anthracis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed (see BMBL, 5th Edition [CDC 2009]). http://www.cdc.gov/biosafety/publications/bmbl5

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:

Silvestri, E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2012. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

7.2.2 *Brucella* spp. [Brucellosis] – BSL-3

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.2.1
Post Decontamination	Real-Time PCR/Immunoassay	7.1.4 [2]
	Culture and Real-Time PCR	7.2.2.2

[1] See Appendix C for corresponding method usability tiers.

[2] Standardized procedures, reagents and agent-specific algorithms are available to LRN member laboratories (see Section 7.1.4).

7.2.2.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to EPA's "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (U.S. EPA 2016), referred to as the EPA YP Protocol.

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Hinić *et al.* 2008 or the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use real-time PCR (Hinić *et al.* 2008).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (Hinić *et al.* 2008 or EPA YP Protocol, Section 9.6 [U.S. EPA. 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Hinić *et al.* 2008). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Brucella* spp.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Brucella* spp. are select agents requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed see (BMBL, 5th Edition [CDC 2009]). http://www.cdc.gov/biosafety/publications/bmbl5/

Sources:

Silvestri, E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Hinić, V., Brodard, I., Thomann, A., Cvetnić, Ž., Makaya, P.V., Frey, J. and Abril, C. 2008. "Novel Identification and Differentiation of *Brucella melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* Suitable for Both Conventional and Real-time PCR Systems." Journal of Microbiological Methods. 75(2): 375-378. http://www.sciencedirect.com/science/article/pii/S0167701208002522

7.2.2.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or

swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Hinić *et al.* 2008 or the EPA YP Protocol, Section 10.5 (U.S. EPA. 2016).

Analytical Technique: Use culture (ASM 2004) and real-time PCR (Hinić *et al.* 2008).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are plated directly on selective and non-selective agars and incubated at 35°C (5–10% carbon dioxide) for up to 7 days. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Hinić *et al.* 2008 or EPA YP Protocol, Section 10.5 [U.S. EPA. 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Hinić *et al.* 2008). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Brucella* spp.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Brucella* spp. are select agents requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed (BMBL, 5th Edition [CDC 2009]). http://www.cdc.gov/biosafety/publications/bmbl5/

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:

Silvestri, E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). EPA/600/R-16/109. Cincinnati, OH: U.S. EPA. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Hinić, V., Brodard, I., Thomann, A., Cvetnić, Ž., Makaya, P.V., Frey, J. and Abril, C. 2008. "Novel Identification and Differentiation of *Brucella melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* Suitable for Both Conventional and Real-Time PCR Systems." Journal of Microbiological Methods. 75(2): 375-378. http://www.sciencedirect.com/science/article/pii/S0167701208002522

ASM. 2004. "Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species." https://www.asm.org/images/PSAB/LRN/Brucella316.pdf

7.2.3 *Burkholderia mallei* [Glanders] – BSL-3 and *Burkholderia pseudomallei* [Melioidosis] – BSL-3

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.3.1
Post Decontamination	Real-Time PCR/Immunoassay	7.1.4 [2]
	Culture and Real-Time PCR	7.2.3.2

[1] See Appendix C for corresponding method usability tiers.

[2] Standardized procedures, reagents and agent-specific algorithms are available to LRN member laboratories (see Section 7.1.4).

7.2.3.1 Site Characterization Sample Analysis (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Limmathurotsakul *et al.* 2013.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within the analytical technique references cited below or the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use real-time PCR (Tomaso *et al.* 2006 and Novak *et al.* 2006).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (analytical technique references cited above or the EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Tomaso *et al.* 2006 and Novak *et al.* 2006). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Burkholderia mallei* and *Burkholderia pseudomallei*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Burkholderia mallei* and *Burkholderia pseudomallei* are select agents requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (see BMBL, 5th Edition [CDC 2009]). http://www.cdc.gov/biosafety/publications/bmbl5/

Sources:

Limmathurotsakul, D., Dance, D. A.B., Wuthiekanun, V., Kaestli, M., Mayo, M., Warner, J., Wagner, D.M., Tuanyok, A., Wertheim, H., Cheng, T.Y., Mukhopadhyay, C., Puthucheary, S., Day, N.P.J., Steinmetz, I., Currie, B.J. and Peacock, S. 2013. "Systematic Review and Consensus Guidelines for Environmental Sampling of *Burkholderia pseudomallei*." PLoS Neglected Tropical Diseases. 7(3): e2105. http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002105

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Tomaso, H., Scholz, H.C., Al Dahouk, S., Eickhoff, M., Treu, T.M., Wernery, R., Wernery, U. and Neubauer, H. 2006. "Development of a 5'-Nuclease Real-Time PCR Assay Targeting fliP for the Rapid Identification of *Burkholderia mallei* in Clinical Samples." Clinical Chemistry. 52(2): 307-310. http://www.clinchem.org/content/52/2/307.full.pdf+html

Novak, R.T., Glass, M.B., Gee, J.E., Gal, D., Mayo, M.J., Currie, B.J. and Wilkins, P.P. 2006. "Development and Evaluation of a Real-Time PCR Assay Targeting the Type III Secretion System of *Burkholderia pseudomallei*." Journal of Clinical Microbiology. 44(1): 85-90. http://jcm.asm.org/content/44/1/85.full.pdf+html

7.2.3.2 Post Decontamination (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Limmathurotsakul *et al.* 2013.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within the real-time PCR analytical techniques cited below or the EPA YP Protocol, Section 10.5 (U.S. EPA 2016).

Analytical Technique: Use culture (ASM 2008) and real-time PCR (Tomaso *et al.* 2006 and Novak *et al.* 2006).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are plated directly on sheep blood agar and incubated at 35°C–37°C for 48 hours. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (real-time PCR analytical techniques cited above or the EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR

primers, probes and assay parameters (Tomaso *et al.* 2006 and Novak *et al.* 2006). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Burkholderia mallei* and *Burkholderia pseudomallei*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Burkholderia mallei* and *Burkholderia pseudomallei* are select agents requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (see BMBL, 5th Edition [CDC 2009]). http://www.cdc.gov/biosafety/publications/bmbl5/

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:

Limmathurotsakul, D., Dance, D. A. B., Wuthiekanun, V., Kaestli, M., Mayo, M., Warner, J., Wagner, D. M., Tuanyok, A., Wertheim, H., Cheng, T.Y., Mukhopadhyay, C., Puthucheary, S., Day, N. P. J., Steinmetz, I., Currie, B.J. and Peacock, S. 2013. "Systematic Review and Consensus Guidelines for Environmental Sampling of *Burkholderia pseudomallei*." PLoS Neglected Tropical Diseases. 7(3): e2105. http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002105

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ASM. 2008. "Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*." http://www.asm.org/images/pdf/Clinical/Protocols/bpseudomallei2008.pdf

Tomaso, H., Scholz, H.C., Al Dahouk, S., Eickhoff, M., Treu, T.M., Wernery, R., Wernery, U. and Neubauer, H. 2006. "Development of a 5'-Nuclease Real-Time PCR Assay Targeting fliP for the Rapid Identification of *Burkholderia mallei* in Clinical Samples." Clinical Chemistry. 52(2): 307-310. http://www.clinchem.org/content/52/2/307.full.pdf+html

Novak, R.T., Glass, M.B., Gee, J.E., Gal, D., Mayo, M.J., Currie, B.J. and Wilkins, P.P. 2006. "Development and Evaluation of a Real-Time PCR Assay Targeting the Type III Secretion System of *Burkholderia pseudomallei*." Journal of Clinical Microbiology. 44(1): 85-90. http://jcm.asm.org/content/44/1/85.full.pdf+html

7.2.4 *Campylobacter jejuni* [Campylobacteriosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.4.1
Post Decontamination	Culture and Real-Time PCR	7.2.4.2

[1] See Appendix C for corresponding method usability tiers.

7.2.4.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Cunningham *et al.* 2010 or the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use real-time PCR (Cunningham *et al.* 2010).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (Cunningham *et al.* 2010 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Cunningham *et al.* 2010). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Campylobacter jejuni*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Cunningham, S.A., Sloan, L.M., Nyre, L.M., Vetter, E.A., Mandrekar, J. and Patel, R. 2010. "Three-Hour Molecular Detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* Species in Feces With Accuracy as High as That of Culture." Journal of Clinical Microbiology.48(8): 2929-2933. http://jcm.asm.org/content/48/8/2929.full.pdf+html

7.2.4.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Cunningham *et al.* 2010 or the EPA YP Protocol, Section 10.5 (U.S. EPA 2016).

Analytical Technique: Use culture (ISO 2005) and real-time PCR (Cunningham *et al.* 2010).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are inoculated into broth media and incubated, and then plated onto selective agar. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Cunningham *et al.* 2010 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Cunningham *et al.* 2010). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Campylobacter jejuni*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Cunningham, S.A., Sloan, L.M., Nyre, L.M., Vetter, E.A., Mandrekar, J. and Patel, R. 2010. "Three-Hour Molecular Detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* Species in Feces With Accuracy as High as That of Culture." Journal of Clinical Microbiology. 48(8): 2929-2933. http://jcm.asm.org/content/48/8/2929.full.pdf+html

ISO. 2005. ISO 17995: Water quality – Detection and Enumeration of Thermotolerant *Campylobacter* Species.
http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=42082

7.2.5 *Chlamydophila psittaci* [Psittacosis] (formerly known as *Chlamydia psittaci*) – BSL-2; BSL-3 for Aerosols and Large Volumes

Remediation Phase	Analytical Technique [1]	Section
Site Characterization	PCR	7.2.5.1
Post Decontamination	Tissue Culture and PCR	7.2.5.2

[1] See Appendix C for corresponding method usability tiers.

7.2.5.1 Site Characterization Sample Analyses (PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Madico *et al.* 2000 or the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use PCR (Madico *et al.* 2000).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (Madico *et al.* 2000 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Madico *et al.* 2000). The use of PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Chlamydophila psittaci*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13.
http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Madico, G., Quinn, T.C., Boman, J. and Gaydos, C.A. 2000. "Touchdown Enzyme Time Release-PCR for Detection and Identification of *Chlamydia trachomatis, C. pneumoniae, and C. psittaci* Using the 16S and 16S-23S Spacer rRNA Genes." Journal of Clinical Microbiology. 38(3): 1085-1093. http://jcm.asm.org/content/38/3/1085.full.pdf+html

7.2.5.2 Post Decontamination Sample Analyses (Tissue Culture and PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Madico *et al.* 2000 or the EPA YP Protocol, Section 10.5 (U.S. EPA 2016).

Analytical Technique: Use tissue culture and PCR (Madico *et al.* 2000).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are inoculated onto buffalo green monkey kidney (BGMK) cells to increase sensitivity. Target nucleic acid should be extracted, purified (Madico *et al.* 2000 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Madico *et al.* 2000). The use of PCR analyses directly on isolates allows for rapid confirmation of *Chlamydophila psittaci*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA.

EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Madico, G., Quinn, T.C., Boman, J. and Gaydos, C.A. 2000. "Touchdown Enzyme Time Release-PCR for Detection and Identification of *Chlamydia trachomatis, C. pneumoniae, and C. psittaci* Using the 16S and 16S-23S Spacer rRNA Genes." Journal of Clinical Microbiology. 38(3): 1085-1093. http://jcm.asm.org/content/38/3/1085.full.pdf+html

7.2.6 *Coxiella burnetii* [Q-fever] – BSL-3

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.6.1
Post Decontamination	Real-Time PCR/Immunoassay	7.1.4 [2]
	Tissue Culture and Real-Time PCR	7.2.6.2

[1] See Appendix C for corresponding method usability tiers.

[2] Standardized procedures, reagents and agent-specific algorithms are available to LRN member laboratories (see Section 7.1.4).

7.2.6.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012).
- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011 or the EPA BA Protocol (U.S. EPA 2012).
- Soil samples should be prepared according to Silvestri *et al.* 2016.
- Water samples should be prepared according to the EPA and CDC Ultrafiltration (UF) Report (U.S. EPA and CDC 2011), referred to as the EPA and CDC UF Report.

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Panning *et al.* 2008 or the EPA BA Protocol, Section 9.2 (U.S. EPA 2012)

Analytical Technique: Use real-time PCR (Panning *et al.* 2008).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (Panning *et al.* 2008 or EPA BA Protocol, Section 9.2 [U.S. EPA 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Panning *et al.* 2008). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Coxiella burnetii*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality*

Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Coxiella burnetii* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed (BMBL, 5th Edition [CDC 2009]). http://www.cdc.gov/biosafety/publications/bmbl5/

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA. 2012. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Panning, M., Kilwinski, J., Greiner-Fischer, S., Peters, M., Kramme, S., Frangoulidis, D., Meyer, H., Henning, K. and Drosten, C. 2008. "High Throughput Detection of *Coxiella burnetii* by Real-Time PCR With Internal Control System and Automated DNA Preparation." BMC Microbiology. 8:77. http://www.biomedcentral.com/1471-2180/8/77

7.2.6.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012).

- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, or Rose *et al.* 2011 or the EPA BA Protocol (U.S. EPA 2012).
- Soil samples should be prepared according to Silvestri *et al.* 2016.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Panning *et al.* 2008 or the EPA BA Protocol, Section 11.6 (U.S. EPA 2012).

Analytical Technique: Use tissue culture (Raoult *et al.* 1991) and real-time PCR (Panning *et al.* 2008).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are inoculated onto human erythroleukemia cells and incubated for 6 days at 37°C. Target nucleic acid should be extracted, purified (Panning *et al.* 2008 or EPA BA Protocol, Section 11.6 [U.S. EPA 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Panning *et al.* 2008). The use of real-time PCR analyses directly on isolates allows for rapid confirmation of *Coxiella burnetii*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Coxiella burnetii* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (see BMBL, 5th Edition [CDC 2009]).
http://www.cdc.gov/biosafety/publications/bmbl5/

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods. 81(2): 141-146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA. 2012 "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

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Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Panning, M., Kilwinski, J., Greiner-Fischer, S., Peters, M., Kramme, S., Frangoulidis, D., Meyer, H., Henning, K. and Drosten, C. 2008. "High Throughput Detection of *Coxiella burnetii* by Real-Time PCR With Internal Control System and Automated DNA Preparation." BMC Microbiology. 8:77. http://www.biomedcentral.com/1471-2180/8/77

Raoult, D. Torres, H. and Drancourt, M. 1991. "Shell-Vial Assay: Evaluation of a New Technique for Determining Antibiotic Susceptibility, Tested in 13 Isolates of *Coxiella burnetii*." Antimicrobial Agents and Chemotherapy. 35(10): 2070-2077. http://aac.asm.org/content/35/10/2070.long

7.2.7 *Escherichia coli* O157:H7 – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.7.1
Post Decontamination	Culture and Real-Time PCR	7.2.7.2

[1] See Appendix C for corresponding method usability tiers.

7.2.7.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- Drinking water samples should be prepared according to the EPA *Escherichia coli* O157:H7 Protocol (referred to as the EPA EC Protocol [U.S. EPA 2010]) or the EPA YP Protocol (U.S. EPA 2016).
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Sen *et al.* 2011 or the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use real-time PCR (Sen *et al.* 2011).
Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (Sen *et al.* 2011 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Sen *et al.* 2011). The use of real-time

PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *E. coli* O157:H7.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. September 2010. "Standard Analytical Protocol for *Escherichia coli* O157:H7 in Water" (EPA EC Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-10/056. http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=498725

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Sen, K., Sinclair, J.L., Boczek, L. and Rice, E.W. 2011. "Development of a Sensitive Detection Method for Stressed *E. coli* O157:H7 in Source and Finished Drinking Water by Culture-qPCR." Environmental Science and Technology. 45(6): 2250-2256. http://pubs.acs.org/doi/abs/10.1021/es103365b

7.2.7.2 Post Decontamination (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- Drinking water samples should be prepared according to the EPA EC Protocol (U.S. EPA 2010) or the EPA YP Protocol (U.S. EPA 2016).
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Sen *et al.* 2011 or the EPA YP Protocol, Section 10.5 (U.S. EPA 2016).
Analytical Technique: Use culture (EPA EC Protocol [U.S. EPA 2010]) and real-time PCR (Sen *et al.* 2011).

Description of Method: Following the appropriate sample preparation procedure (see Sample

Preparation procedures above), samples are cultured using multiple media and immunomagnetic separation (IMS) (EPA EC Protocol [U.S. EPA 2010]). Typical isolates are then confirmed using biochemical and serological tests. To expedite time to results, isolates should be confirmed using real-time PCR analyses. Target nucleic acid should be extracted, purified (Sen *et al.* 2011 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Sen *et al.* 2011). The use of real-time PCR analyses allows for rapid confirmation of *E. coli* O157:H7.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. September 2010. "Standard Analytical Protocol for *Escherichia coli* O157:H7 in Water" (EPA EC Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-10/056. http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=498725

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Sen, K., Sinclair, J.L., Boczek, L. and Rice, E.W. 2011. "Development of a Sensitive Detection Method for Stressed *E. coli* O157:H7 in Source and Finished Drinking Water by Culture-qPCR." Environmental Science and Technology. 45(7): 2250-2256. http://pubs.acs.org/doi/abs/10.1021/es103365b

7.2.8 *Francisella tularensis* [Tularemia] – BSL-3

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.8.1
Post Decontamination	Real-Time PCR /Immunoassay	7.1.4 [2]
	Culture and Real-Time PCR	7.2.8.2

[1] See Appendix C for corresponding method usability tiers.

[2] Standardized procedures, reagents and agent-specific algorithms are available to LRN member laboratories (see Section 7.1.4).

7.2.8.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is

needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Versage *et al.* 2003 or the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use real-time PCR (Versage *et al.* 2003).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (Versage *et al.* 2003 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Versage *et al.* 2003). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Francisella tularensis*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Francisella tularensis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (see BMBL, 5th Edition [CDC 2009]). http://www.cdc.gov/biosafety/publications/bmbl5/

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Versage, J., Severin, D.D.M., Chu, M.C. and Petersen, J.M. 2003. "Development of a Multitarget Real-Time TaqMan PCR Assay for Enhanced Detection of *Francisella tularensis* in Complex Specimens." Journal of Clinical Microbiology. 41(12): 5492-5499. http://jcm.asm.org/content/41/12/5492.full.pdf+html

7.2.8.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Versage *et al.* 2003 or the EPA YP Protocol, Section 10.5 (U.S. EPA 2016).

Analytical Technique: Use culture (CDC *et al.* 2001) and real-time PCR (Versage *et al.* 2003).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are plated directly onto selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Versage *et al.* 2003 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Versage *et al.* 2003). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Francisella tularensis*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Francisella tularensis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (see BMBL, 5th Edition [CDC 2009]). http://www.cdc.gov/biosafety/publications/bmbl5/

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:
Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA.

EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Versage, J.L., Severin, D.D, Chu, M.C. and Petersen, J.M. 2003. "Development of a Multitarget Real-Time TaqMan PCR Assay for Enhanced Detection of *Francisella tularensis* in Complex Specimens." Journal of Clinical Microbiology. 41(12): 5492-5499. http://jcm.asm.org/content/41/12/5492.full.pdf+html

CDC, ASM and APHL. 2001. "Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Francisella tularensis*." Washington, DC: ASM. http://www.asm.org/images/pdf/Clinical/Protocols/tularemia.pdf

Humrighouse, B.W., Adcock, N.J. and Rice, E.W., 2011. "Use of Acid Treatment and a Selective Medium to Enhance the Recovery of *Francisella tularensis* from Water." Applied and Environmental Microbiology. 77(18): 6729-6732. http://aem.asm.org/content/77/18/6729.full.pdf+html

7.2.9 *Legionella pneumophila* [Legionellosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.9.1
Post Decontamination	Culture and Real-Time PCR	7.2.9.2

[1] See Appendix C for corresponding method usability tiers.

7.2.9.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to U.S. DHHS 2005.
- All other environmental sample types should be prepared according to Kozak *et. al*. 2013.

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Benitez and Winchell 2013 or the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use real-time PCR (Benitez and Winchell 2013).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (Benitez and Winchell 2013 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Benitez and Winchell 2013). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Legionella pneumophila*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing

analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. Department of Health and Human Services. 2005. "Procedures for the Recovery of *Legionella* from the Environment." Atlanta, GA: CDC. http://www.cdc.gov/legionella/health-depts/inv-tools-cluster/lab-inv-tools/procedures-manual.html

Kozak, N.A., Lucas, C.E. and Winchell, J.M. 2013. "Identification of *Legionella* in the Environment." *Legionella*: Methods and Protocols, Methods in Molecular Biology. 954: 3-25. https://www.ncbi.nlm.nih.gov/pubmed/23150387

Benitez, A.J. and Winchell, J.M. 2013. "Clinical Application of a Multiplex Real-Time PCR Assay for Simultaneous Detection of *Legionella* Species, *Legionella pneumophila*, and *Legionella pneumophila* Serogroup 1." Journal of Clinical Microbiology. 51(1): 348-351. https://www.ncbi.nlm.nih.gov/pubmed/23135949

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

7.2.9.2 Post Decontamination (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to U.S. DHHS 2005.
- All other environmental sample types should be prepared according to Kozak *et al*. 2013.

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Benitez and Winchell 2013 or the EPA YP Protocol, Section 10.5 (U.S. EPA 2016).

Analytical Technique: Use culture (Kozak *et al*. 2013) and real-time PCR (Benitez and Winchell 2013).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are cultured using multiple media (buffered charcoal yeast extract [BCYE] with polymyxin B, cycloheimide and vancomycin [BCYE PCV]; or BCYE with glycine, polymyxin B, cycloheximide and vancomycin [BCYE GPCV]). Typical isolates are then confirmed using serological tests. To expedite time to results, isolates should be confirmed using real-time PCR analyses. Target nucleic acid should be extracted, purified (Benitez and Winchell 2013 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]) and analyzed using the referenced

target-specific PCR primers, probes and assay parameters (Benitez and Winchell 2013). The use of real-time PCR analyses allows for rapid confirmation of *Legionella pneumophila*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. Department of Health and Human Services. 2005. "Procedures for the Recovery of Legionella from the Environment." Atlanta, GA: CDC. http://www.cdc.gov/legionella/health-depts/inv-tools-cluster/lab-inv-tools/procedures-manual.html

Kozak, N.A., Lucas, C.E. and Winchell, J.M. 2013. "Identification of *Legionella* in the Environment." *Legionella*: Methods and Protocols, Methods in Molecular Biology. 954: 3-25. https://www.ncbi.nlm.nih.gov/pubmed/23150387

Benitez, A.J. and Winchell, J.M. 2013. "Clinical Application of a Multiplex Real-Time PCR Assay for Simultaneous Detection of *Legionella* Species, *Legionella pneumophila*, and *Legionella pneumophila* Serogroup 1." Journal of Clinical Microbiology. 51(1): 348-351. https://www.ncbi.nlm.nih.gov/pubmed/23135949

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

7.2.10 *Leptospira interrogans* [Leptospirosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.10.1
Post Decontamination	Culture and Real-Time PCR	7.2.10.2

[1] See Appendix C for corresponding method usability tiers.

7.2.10.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Palaniappan *et al.* 2005 or the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use real-time PCR (Palaniappan *et al.* 2005).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (Palaniappan *et al.* 2005 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Palaniappan *et al.* 2005). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Leptospira interrogans*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Palaniappan, R.U.M., Chang, Y.F., Chang, C., Pan, M.J., Yang, C.W., Harpending, P., McDonough, S.P., Dubovi, E., Divers, T., Qu, J. and Roe, B. 2005. "Evaluation of Lig-based Conventional and Real Time PCR for the Detection of Pathogenic Leptospires." Molecular and Cellular Probes. 19(2): 111-117. http://www.sciencedirect.com/science/article/pii/S0890850804000970

7.2.10.2 Post Decontamination Sample Analyses (Culture and Real-time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures in Palaniappan *et al.* 2005 or the EPA YP Protocol, Section 10.5 (U.S. EPA 2016).

Analytical Technique: Use culture (Standard Method 9260 I [APHA *et al.* 2012]) and real-time PCR (Palaniappan *et al.* 2005).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are inoculated into selective broth media and incubated for up to six weeks at 30°C. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Palaniappan *et al.* 2005 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Palaniappan *et al.* 2005). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Leptospira interrogans*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Palaniappan, R.U.M., Chang, Y.F., Chang, C., Pan, M.J., Yang, C.W., Harpending, P., McDonough, S.P., Dubovi, E., Divers, T., Qu, J. and Roe, B. 2005. "Evaluation of Lig-based Conventional and Real Time PCR for the Detection of Pathogenic Leptospires." Molecular and Cellular Probes. 19(2): 111-117. http://www.sciencedirect.com/science/article/pii/S0890850804000970

APHA, AWWA and WEF. 2012. "Method 9260 I: *Leptospira*." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: American Public Health Association. http://www.standardmethods.org/

7.2.11 *Listeria monocytogenes* [Listeriosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR[1]	7.2.11.1
Post Decontamination	Culture and Real-Time PCR	7.2.11.2

[1] See Appendix C for corresponding method usability tiers.

7.2.11.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within USDA FSIS 2009 or the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use real-time PCR (USDA FSIS 2009).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (USDA FSIS 2009 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (USDA FSIS 2009). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Listeria monocytogenes*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

USDA, FSIS. 2009. "FSIS Procedure for the Use of a *Listeria monocytogenes* Polymerase Chain Reaction (PCR) Screening Test." Chapter MLG 8A.04 in *Microbiology Laboratory Guidebook*. Athens, GA: USDA. http://www.epa.gov/sites/production/files/2015-07/documents/usda-mlg-8a.03.pdf

7.2.11.2 Post Decontamination Sample Analyses (Culture and Real-time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is

needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within the USDA FSIS 2009 or the EPA YP Protocol, Section 10.5 (U.S. EPA 2016).

Analytical Technique: Use culture (Hitchins *et al.* 2003) and real-time PCR (USDA FSIS 2009).

Description of Method: Following appropriate sample preparation (see Sample Preparation procedures above), samples are inoculated into broth media, incubated for 48 hours, and then plated onto selective agar. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (USDA FSIS 2009 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (USDA FSIS 2009). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Listeria monocytogenes*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

USDA, FSIS. 2009. "FSIS Procedure for the Use of a *Listeria monocytogenes* Polymerase Chain Reaction (PCR) Screening Test." Chapter MLG 8A.04 in *Microbiology Laboratory Guidebook*. Athens, GA: USDA. http://www.epa.gov/sites/production/files/2015-07/documents/usda-mlg-8a.03.pdf

Hitchins, A.D., Jinneman, K. and Chen, Y., FDA, CFSAN. 2003. "Chapter 10 – Detection and Enumeration of *Listeria monocytogenes* in Foods." *Bacteriological Analytical Manual Online.* http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm

7.2.12 Non-typhoidal *Salmonella* (Not applicable to *S.* Typhi) [Salmonellosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.12.1
Post Decontamination	Culture and Real-Time PCR	7.2.12.2

[1] See Appendix C for corresponding method usability tiers.

7.2.12.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to EPA Method 1682 (U.S. EPA 2006).
- Drinking water samples should be prepared according to EPA Method 1200 (U.S. EPA 2012), Standard Method 9260 B (APHA *et al.* 2012) or the EPA YP Protocol (U.S. EPA 2016).
- Post decontamination waste water samples should be prepared according to EPA Method 1200 (U.S. EPA 2012), EPA Method 1682 (U.S. EPA 2006), Standard Method 9260 B (APHA *et al.* 2012) or the EPA YP Protocol (U.S. EPA 2016).
- All other environmental sample types should be prepared according to procedures within the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Jyoti *et al.* 2011 or the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use real-time PCR (Jyoti *et al.* 2011).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (Jyoti *et al.* 2011 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Jyoti *et al.* 2011). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of non-typhoidal *Salmonella*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. http://www.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2012. ”Method 1200: Analytical Protocol for Non-Typhoidal *Salmonella* in Drinking Water and Surface Water.” Washington, DC: U.S. EPA. EPA 817-R-12-004. https://www.epa.gov/sites/production/files/2015-08/documents/epa817r12004.pdf

APHA, AWWA and WEF. 2012. “Method 9260 B: *Salmonella*.” *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: American Public Health Association. http://www.standardmethods.org/

U.S. EPA. 2016. “Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident” (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Jyoti, A., Vajpayee, P., Singh, G., Patel, C.B., Gupta, K.C. and Shanker, R. 2011. “Identification of Environmental Reservoirs of Nontyphoidal Salmonellosis: Aptamer-Assisted Bioconcentration and Subsequent Detection of *Salmonella Typhimurium* by Quantitative Polymerase Chain Reaction.” Environmental Science and Technology. 45(20): 8996-9002. http://pubs.acs.org/doi/abs/10.1021/es2018994

7.2.12.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to EPA Method 1682 (U.S. EPA 2006).
- Drinking water samples should be prepared according to EPA Method 1200 (U.S. EPA 2012), Standard Method 9260 B (APHA *et al.* 2012) or the EPA YP Protocol (U.S. EPA 2016).
- Post decontamination waste water samples should be prepared according to EPA Method 1200 (U.S. EPA 2012), EPA Method 1682 (U.S. EPA 2006), Standard Method 9260 B (APHA *et al.* 2012) or the EPA YP Protocol (U.S. EPA 2016).
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Jyoti *et al.* 2011 or the EPA YP Protocol, Section 10.5 (U.S. EPA 2016).

Analytical Technique: Use culture (Method 1682 [U.S. EPA 2006] or EPA Method 1200 [U.S. EPA 2012]) and real-time PCR (Jyoti *et al.* 2011).

Description of Method: Following appropriate sample preparation (see Sample Preparation procedures above), samples are inoculated into broth media, incubated for 24 hours, and then plated onto multiple selective agars. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Jyoti *et al.* 2011 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Jyoti *et al.* 2011). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of non-typhoidal *Salmonella*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." Washington, DC: U.S. EPA. EPA-821-R-06-14. http://www.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2012. "Method 1200: Analytical Protocol for Non-Typhoidal *Salmonella* in Drinking Water and Surface Water." Washington, DC: U.S. EPA. EPA 817-R-12-004. https://www.epa.gov/sites/production/files/2015-08/documents/epa817r12004.pdf

APHA, AWWA and WEF. 2012. "Method 9260 B: *Salmonella*." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: American Public Health Association. http://www.standardmethods.org/

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Jyoti, A., Vajpayee, P., Singh, G., Patel, C.B., Gupta, K.C. and Shanker, R. 2011. "Identification of Environmental Reservoirs of Nontyphoidal Salmonellosis: Aptamer-Assisted Bioconcentration and Subsequent Detection of *Salmonella Typhimurium* by Quantitative Polymerase Chain Reaction." Environmental Science and Technology. 45(20): 8996-9002. http://pubs.acs.org/doi/abs/10.1021/es2018994

7.2.13 *Salmonella enterica* serovar Typhi (*S.* Typhi) [Typhoid fever] – BSL-2; BSL-3 for Aerosol Release

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.13.1
Post Decontamination	Culture and Real-Time PCR	7.2.13.2

[1] See Appendix C for corresponding method usability tiers.

7.2.13.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.

- Drinking water samples should be prepared according to the *Salmonella* Typhi Protocol (referred to as the EPA ST Protocol [U.S. EPA 2010]), Standard Method 9260 B (APHA *et al.* 2012) or the EPA YP Protocol (U.S. EPA 2016).
- Post decontamination waste water samples should be prepared according to Standard Method 9260 B (APHA *et al.* 2012) or the EPA YP Protocol (U.S. EPA 2016).
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within the CDC Laboratory Assay or the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use real-time PCR (CDC Laboratory Assay).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (CDC Laboratory Assay or the EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (CDC Laboratory Assay). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Salmonella* Typhi.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2010. "Standard Analytical Protocol for *Salmonella* Typhi in Drinking Water" (EPA ST Protocol). Washington, DC: U.S. EPA. EPA 600/R-10/133. https://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=230138

APHA, AWWA and WEF. 2012. "Method 9260 B: *Salmonella*." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: American Public Health Association. http://www.standardmethods.org/

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

CDC Laboratory Assay. "Triplex PCR for Detection of *S.* Typhi Using SmartCycler®." Contact: Dr. Eija Trees, Foodborne and Diarreheal Diseases Branch, CDC, Atlanta, GA. https://www.nemi.gov/methods/method_summary/10303/

7.2.13.2 Post Decontamination (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- Drinking water samples should be prepared according to the EPA ST Protocol (U.S. EPA 2010), Standard Method 9260 B (APHA *et al.* 2012) or the EPA YP Protocol (U.S. EPA 2016).
- Post decontamination waste water samples should be prepared according to Standard Method 9260 B (APHA *et al.* 2012) or the EPA YP Protocol (U.S. EPA 2016).
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within the CDC Laboratory Assay or the EPA YP Protocol, Section 10.5 (U.S. EPA 2016).

Analytical Technique: Use culture (EPA ST Protocol [U.S. EPA 2010]) and real-time PCR (CDC Laboratory Assay).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are inoculated into broth media, incubated for 24 hours, and then inoculated and plated onto multiple selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (CDC Laboratory Assay or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (CDC Laboratory Assay). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Salmonella* Typhi.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2010. "Standard Analytical Protocol for *Salmonella* Typhi in Drinking Water" (EPA ST Protocol). Washington, DC: U.S. EPA. EPA 600/R-10/133. https://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=230138

APHA, AWWA and WEF. 2012. "Method 9260 B: *Salmonella*." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: American Public Health Association. http://www.standardmethods.org/

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

CDC Laboratory Assay. "Triplex PCR for Detection of *S.* Typhi Using SmartCycler®." Contact: Dr. Eija Trees, Foodborne and Diarreheal Diseases Branch, CDC, Atlanta, GA. https://www.nemi.gov/methods/method_summary/10303/

7.2.14 *Shigella* spp. [Shigellosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.14.1
Post Decontamination	Culture and Real-Time PCR	7.2.14.2

[1] See Appendix C for corresponding method usability tiers.

7.2.14.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Cunningham *et al.* 2010 or the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use real-time PCR (Cunningham *et al.* 2010).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (Cunningham *et al.* 2010 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Cunningham *et al.* 2010). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Shigella* spp.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control*

Guidance for Laboratories Performing PCR Analyses on Environmental Samples (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Cunningham, S.A., Sloan, L.M., Nyre, L.M., Vetter, E.A., Mandrekar, J. and Patel, R. 2010. "Three-Hour Molecular Detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* Species in Feces with Accuracy as High as That of Culture." Journal of Clinical Microbiology. 48(8): 2929-2933. http://jcm.asm.org/content/48/8/2929.full.pdf+html

7.2.14.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes and swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Cunningham *et al.* 2010 or the EPA YP Protocol, Section 10.5 (U.S. EPA 2016).

Analytical Technique: Use culture (Standard Method 9260 E [APHA *et al.* 2012]) and real-time PCR (Cunningham *et al.* 2010).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are inoculated into broth media, incubated for 24 hours, and then plated onto multiple selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Cunningham *et al.* 2010 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Cunningham *et al.* 2010). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Shigella* spp.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

PCR QC checks should be performed according EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Cunningham, S.A., Sloan, L.M., Nyre, L.M., Vetter, E.A., Mandrekar, J. and Patel, R. 2010. "Three-Hour Molecular Detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* Species in Feces With Accuracy as High as That of Culture." Journal of Clinical Microbiology. 48(8): 2929-2933. http://jcm.asm.org/content/48/8/2929.full.pdf+html

APHA, AWWA and WEF. 2012. "Method 9260 Detection of Pathogenic Bacteria E: *Shigella*." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: American Public Health Association. http://www.standardmethods.org/

7.2.15 *Staphylococcus aureus* – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.15.1
Post Decontamination	Culture and Real-Time PCR	7.2.15.2

[1] See Appendix C for corresponding method usability tiers.

7.2.15.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Chiang *et al.* 2007 or the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use real-time PCR (Chiang *et al.* 2007).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (Chiang *et al.* 2007 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Chiang *et al.* 2007). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Staphylococcus aureus*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Chiang, Y.C, Fan, C.M., Liao, W.W., Lin, C.K. and Tsen, H.Y. 2007. "Real-Time PCR Detection of *Staphylococcus aureus* in Milk and Meat Using New Primers Designed From the Heat Shock Protein Gene htrA Sequence." Journal of Food Protection. 70(12): 2855-2859. http://jfoodprotection.org/doi/abs/10.4315/0362-028X-70.12.2855

7.2.15.2 Post Decontamination Sample Analyses (Culture and Real-time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Chiang *et al.* 2007 or the EPA YP Protocol, Section 10.5 (U.S. EPA 2016).

Analytical Technique: Use culture (Standard Method 9213 B: *Staphylococcus aureus* [APHA *et al.* 2012]) and real-time PCR (Chiang *et al.* 2007).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are inoculated into broth media, incubated for 24 hours,

and then plated onto selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Chiang *et al.* 2007 or EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Chiang *et al.* 2007). Use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Staphylococcus aureus*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Chiang, Y.C, Fan, C.M., Liao, W.W., Lin, C.K. and Tsen, H.Y. 2007. "Real-Time PCR Detection of *Staphylococcus aureus* in Milk and Meat Using New Primers Designed From the Heat Shock Protein Gene htrA Sequence." Journal of Food Protection. 70(12): 2855-2859. http://jfoodprotection.org/doi/abs/10.4315/0362-028X-70.12.2855

APHA, AWWA and WEF. 2012. "Method 9213 B: *Staphylococcus aureus*." *Standard Methods for the Examination of Water and Wastewater*. 22nd Edition. Washington, DC: American Public Health Association. http://www.standardmethods.org/

7.2.16 *Vibrio cholerae* [Cholera] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.16.1
Post Decontamination	Culture and Real-Time PCR	7.2.16.2

[1] See Appendix C for corresponding method usability tiers.

7.2.16.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.

- Water samples should be prepared according to the EPA *Vibrio cholerae* Protocol (referred to as the EPA VC Protocol [U.S. EPA 2010]) or the EPA YP Protocol (U.S. EPA 2016).
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Blackstone *et al.* 2007 or the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use real-time PCR (Blackstone *et al.* 2007).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (Blackstone *et al.* 2007 or EPA YP Protocol, Section 9.6 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Blackstone *et al.* 2007). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Vibrio cholerae*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2010. "Standard Analytical Protocol for *Vibrio cholerae* O1 and O139 in Drinking Water and Surface Water" (EPA VC Protocol). Washington, DC: U.S. EPA. EPA 600/R-10/139. http://nepis.epa.gov/Adobe/PDF/P100978K.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Blackstone, G.M., Nordstrom, J.L., Bowen, M.D., Meyer, R.F., Imbro, P. and DePaola, A. 2007. "Use of a Real Time PCR Assay for Detection of the ctxA Gene of *Vibrio cholerae* in an Environmental Survey of Mobile Bay." Journal of Microbiological Methods. 68(2): 254-259. http://www.sciencedirect.com/science/article/pii/S016770120600248X

7.2.16.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- Water samples should be prepared according to the EPA VC Protocol (U.S. EPA 2010) or the EPA YP Protocol (U.S. EPA 2016).
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Blackstone *et al.* 2007 or the EPA YP Protocol, Section 10.5 (U.S. EPA 2010).

Analytical Technique: Use culture (EPA VC Protocol [U.S. EPA 2010]) and real-time PCR (Blackstone *et al.* 2007).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are inoculated into enrichment broth, incubated for 8 hours, and then plated onto selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Blackstone *et al.* 2007 or EPA YP Protocol, Section 10.5 [U.S. EPA 2010]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Blackstone *et al.* 2007). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Vibrio cholerae*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. October 2010. "Standard Analytical Protocol for *Vibrio cholerae* O1 and O139 in Drinking Water and Surface Water" (EPA VC Protocol). Cincinnati, OH: U.S. EPA. EPA 600/R-10/139. http://nepis.epa.gov/Adobe/PDF/P100978K.pdf

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

Blackstone, G.M., Nordstrom, J.L., Bowen, M.D., Meyer, R.F., Imbro, P. and DePaola, A. 2007. "Use of a Real Time PCR Assay for Detection of the ctxA Gene of *Vibrio cholerae* in an Environmental Survey of Mobile Bay." Journal of Microbiological Methods. 68(2): 254-259. http://www.sciencedirect.com/science/article/pii/S016770120600248X

7.2.17 *Yersinia pestis* [Plague] – BSL-3

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.2.17.1
Post Decontamination	RV-PCR (Water Samples)	7.2.17.2
	Culture and Real-Time PCR	7.2.17.3

[1] See Appendix C for corresponding method usability tiers.

7.2.17.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al*. 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within the EPA YP Protocol, Section 9.6 (U.S. EPA 2016).

Analytical Technique: Use real-time PCR (EPA YP Protocol [U.S. EPA 2016]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (EPA YP Protocol, Section 9.6), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Yersinia pestis*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Yersinia pestis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed (BMBL, 5th Edition [CDC 2009]). http://www.cdc.gov/biosafety/publications/bmbl5/

Sources:
Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

7.2.17.2 Post Decontamination Sample Analyses (RV-PCR) – Water Samples

Note: Laboratories without RV-PCR capability should analyze samples according to the culture procedure provided in Section 7.2.17.3.

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation: Water samples should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within the EPA YP Protocol, Section 11.3 (U.S. EPA 2016).

Analytical Technique: Use RV-PCR (EPA YP Protocol [U.S. EPA 2016]).

Description of Method: The RV-PCR procedure serves as an alternative to the traditional culture-based methods for detection of viable pathogens. The RV-PCR procedure integrates high-throughput sample processing, short-incubation broth culture, and highly sensitive and specific real-time PCR assays to detect low concentrations of viable *Y. pestis*. Specifically, the procedure uses the change in real-time PCR response, referred to as the change in cycle threshold, or ΔCT, between the initial cycle threshold (C_T) at time 0 (T_0) (just before sample incubation) and the final C_T after incubation (C_T T_f).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Yersinia pestis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (see BMBL, 5th Edition [CDC 2009]).
http://www.cdc.gov/biosafety/publications/bmbl5/

Sources:
U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

7.2.17.3 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils and drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Soil samples should be prepared according to Silvestri *et al.* 2016.
- All other environmental sample types should be prepared according to the EPA YP Protocol (U.S. EPA 2016).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within the EPA YP Protocol, Section 10.5 (U.S. EPA 2016).

Analytical Technique: Use culture and real-time PCR (EPA YP Protocol [U.S. EPA 2016]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples can be inoculated into enrichment broth prior to plating or plated directly on non-selective media and incubated for a minimum of three days. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA YP Protocol, Section 10.5 [U.S. EPA 2016]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters. The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Yersinia pestis*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: *Yersinia pestis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed (see BMBL, 5th Edition [CDC 2009]).
http://www.cdc.gov/biosafety/publications/bmbl5/

Some laboratories may not have access to a positive control for this agent for culture analyses. For laboratories that may not have access to a virulent strain for the positive control, an avirulent strain may be used to meet the laboratory's BSL.

Sources:

Silvestri E.E., Feldhake, D., Griffin, D., Lisle, J., Nichols, T.L., Shah, S.R., Pemberton, A. and Schaefer, F.W. III. 2016. "Optimization of a Sample Processing Protocol for Recovery of *Bacillus anthracis* Spores from Soil." Journal of Microbiological Methods. 130: 6-13. http://www.sciencedirect.com/science/article/pii/S0167701216302238

U.S. EPA. 2016. "Protocol for Detection of *Yersinia pestis* in Environmental Samples During the Remediation Phase of a Plague Incident" (EPA YP Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-16/109. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=329170

7.3 Method Summaries for Viruses

Summaries for the analytical methods listed in Appendix C for analysis of viral pathogens are provided in Sections 7.3.1 through 7.3.10. Each summary contains a brief description of the analytical methods selected for each viral pathogen, and links to, or sources for, obtaining full versions of the methods. Summaries are provided for informational use. Tiers that have been assigned to each method/analyte pair (see Section 7.1.1) can be found in Appendix C. The full version of the method should be consulted prior to sample analysis. For information regarding sample collection considerations for samples to be analyzed by these methods, see the companion *Sample Collection Information Document for Pathogens – Companion to SAM 2017*.

7.3.1 Adenoviruses: Enteric and Non-enteric (A-F) – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.3.1.1
Post Decontamination	Tissue Culture and Real-Time PCR	7.3.1.2

[1] See Appendix C for corresponding method usability tiers.

7.3.1.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or EPA Method 1615 (Fout *et al.* 2012). ***Note***: Since the concentration of adenovirus using the Nanoceram® filter (Argonide Corporation, Sanford, FL) has not been optimized, use of a 1MDS filter is recommended.

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Jothikumar *et al.* 2005 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use real-time PCR (Jothikumar *et al.* 2005).

Description of Method: Following appropriate sample preparation (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (Jothikumar *et al.* 2005 or EPA Method 1615 [Fout *et al.* 2012]), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters (Jothikumar *et al.* 2005). Use of real-time PCR directly on samples (e.g., no tissue culture component) allows for rapid detection of adenoviruses.

At a minimum, the following QC checks should be performed and evaluated when using this

protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

Cao, G., Noti, J.D., Blachere, F.M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M.,and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

Jothikumar, N., Cromeans, T.L., Hill, V.R., Lu, X., Sobsey, M.D. and Erdman, D.D. 2005. "Quantitative Real-Time PCR Assays for Detection of Human Adenoviruses and Identification of Serotypes 40 and 41." Applied and Environmental Microbiology. 71(6): 3131-3136. http://aem.asm.org/content/71/6/3131.full.pdf+html

7.3.1.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA

and CDC 2011) or EPA Method 1615 (Fout *et al.* 2012). ***Note***: Since the concentration of adenovirus using the Nanoceram filter has not been optimized, use of the 1MDS filter is recommended.

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Jothikumar *et al.* 2005 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use tissue culture (Boczek *et al.* 2016 or Green and Loewenstein 2005) and real-time PCR (Jothikumar *et al.* 2005).
Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples should be cultured to assess viability (Boczek *et al.* 2016 or Green and Loewenstein 2005). For confirmation, target nucleic acid should be extracted, purified (Jothikumar *et al.* 2005 or EPA Method 1615 [Fout *et al.* 2012]), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters (Jothikumar *et al.* 2005).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
Cao, G., Noti, J.D., Blachere, F.M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

Jothikumar, N., Cromeans, T.L., Hill, V.R., Lu, X., Sobsey, M.D. and Erdman, D.D. 2005. "Quantitative Real-Time PCR Assays for Detection of Human Adenoviruses and Identification of Serotypes 40 and 41." Applied and Environmental Microbiology. 71(6): 3131-3136. http://aem.asm.org/content/71/6/3131.full.pdf+html

Boczek, L.A., Rhodes, E.R., Cashdollar, J.L., Ryu, J., Popovici, J., Hoelle, J.M., Sivaganesan, M., Hayes, S.L., Rodgers, M.R. and Ryu, H. 2016. "Applicability of UV Resistant *Bacillus pumilus* Endospores as a Human Adenovirus Surrogate for Evaluating the Effectiveness of Virus Inactivation in Low-pressure UV Treatment Systems." Journal of Microbiological Methods. 122: 43-49. http://www.sciencedirect.com/science/article/pii/S0167701216300124

Green, M., and Loewenstein, P.M. 2005. "UNIT 14C.1 Human Adenoviruses: Propagation, Purification, Quantification, and Storage." Current Protocols in Microbiology. 00:C:14C.1.1-14C.1.19. http://onlinelibrary.wiley.com/doi/10.1002/9780471729259.mc14c01s00/abstract

7.3.2 Astroviruses – BSL not specified

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.2.1
Post Decontamination	Integrated Cell Culture and Real-Time Reverse Transcription-PCR	7.3.2.2

[1] See Appendix C for corresponding method usability tiers.

7.3.2.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription- PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or EPA Method 1615 (Fout *et al.* 2012). ***Note***: Since the concentration of astrovirus using the Nanoceram filter has not been optimized, use of the 1MDS filter is recommended.

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Grimm *et al.* 2004 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use real-time reverse transcription-PCR (Grimm *et al.* 2004).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (Grimm *et al.* 2004 or EPA Method 1615 [(Fout *et al.* 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Grimm *et al.* 2004). The use of real-time reverse transcription-PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of astroviruses.

At a minimum, the following QC checks should be performed and evaluated when using this

protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate ribonuclease (RNAse) inhibitors should be included during sample processing and analysis.

Sources:

Cao, G., Noti, J.D., Blachere, F.M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

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Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

Grimm, A.C., Cashdollar, J.L., Williams, F.P. and Fout, G.S. 2004. "Development of an Astrovirus RT-PCR Detection Assay for Use With Conventional, Real-Time, and Integrated Cell Culture/RT-PCR." Canadian Journal of Microbiology. 50(4): 269-278. http://www.nrcresearchpress.com/doi/abs/10.1139/w04-012

7.3.2.2 Post Decontamination Sample Analyses (Integrated Cell Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.

- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or EPA Method 1615 (Fout *et al.* 2012). ***Note***: Since the concentration of astrovirus using the Nanoceram filter has not been optimized, use of the 1MDS filter is recommended.

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Grimm *et al.* 2004 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use integrated cell culture and real-time reverse transcription-PCR (Grimm *et al.* 2004).

Description of Method: The method is a real-time reverse transcription-PCR procedure that can be integrated with cell culture (CaCo-2 cells) to enhance sensitivity. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), concentrated samples are analyzed directly or indirectly, after cell culture, by a two-step real-time reverse transcription-PCR (i.e., reverse transcription followed by real-time PCR) assay using astrovirus-specific primers, probes and assay parameters (Grimm *et al.* 2004). For confirmation, target nucleic acid should be extracted, purified (Grimm *et al.* 2004 or EPA Method 1615 [Fout *et al.* 2012]), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters (Grimm *et al.* 2004).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Cao, G., Noti, J.D., Blachere, F.M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

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Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

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7.3.3 Caliciviruses: Noroviruses – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.3.1
Post Decontamination	No method available to determine viable virus after decontamination	7.3.3.2

[1] See Appendix C for corresponding method usability tiers.

7.3.3.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or EPA Method 1615 Fout *et al.* 2012).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use real-time reverse transcription-PCR (EPA Method 1615 [Fout *et al.* 2012]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (U.S. EPA 2012), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (EPA Method 1615 [Fout *et al.* 2012]).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Cao, G., Noti, J.D., Blachere, F. M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

7.3.3.2 Post Decontamination Sample Analyses

No method available to determine viable virus after decontamination.

7.3.4 Caliciviruses: Sapovirus – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.4.1
Post Decontamination	Tissue Culture and Real-Time Reverse Transcription-PCR	7.3.4.2

[1] See Appendix C for corresponding method usability tiers.

7.3.4.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or EPA Method 1615 (Fout *et al.* 2012).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Oka *et al.* 2006 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use real-time reverse transcription-PCR (Oka *et al.* 2006).

Description of Method: The method is a TaqMan® (Thermo Fisher Scientific, Waltham, MA, or equivalent)-based real-time reverse transcriptase PCR assay that can detect four of the five distinct sapovirus genogroups (GI–GV) using a multiplex assay. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (Oka *et al.* 2006 or EPA Method 1615 [Fout *et al.* 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Oka *et al.* 2006).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Cao, G., Noti, J.D., Blachere, F. M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method

1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181.
https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

Oka, T., Katayama, K., Hansman, G.S., Kageyama, T., Ogawa, S., Wu, F.T., White, P.A. and Takeda, N. 2006. "Detection of Human Sapovirus by Real-Time Reverse Transcription-Polymerase Chain Reaction." Journal of Medical Virology. 78(10): 1347-1353.
http://onlinelibrary.wiley.com/doi/10.1002/jmv.20699/abstract

7.3.4.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or EPA Method 1615 (Fout *et al.* 2012).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Oka *et al.* 2006 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use tissue culture (Parwani *et al*. 1991) and real-time reverse transcription-PCR (Oka *et al.* 2006).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples should be cultured using LL-PK cells supplemented with intestinal contents preparation (ICP) to assess viability (Parwani *et al.* 1991). For confirmation, target nucleic acid should be extracted, purified (Oka *et al.* 2006 or EPA Method 1615 [Fout *et al.* 2012]), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters (Oka *et al.* 2006).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis. Culture procedure is for porcine sapovirus and may not be appropriate for all strains of sapoviruses.

Sources:
Cao, G., Noti, J.D., Blachere, F.M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of

an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

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Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

Oka, T., Katayama, K., Hansman, G.S., Kageyama, T., Ogawa, S., Wu, F.T., White, P.A. and Takeda, N. 2006. "Detection of Human Sapovirus by Real-Time Reverse Transcription-Polymerase Chain Reaction." Journal of Medical Virology. 78(10): 1347-1353. http://onlinelibrary.wiley.com/doi/10.1002/jmv.20699/abstract

Parwani, A.V., Flynn, W.T., Gadfield, K.L and Saif L.J. 1991. "Serial Propagation of Porcine Enteric Calicivirus in a Continuous Cell Line. Effect of Medium Supplementation With Intestinal Contents or Enzymes." Archives of Virology. 120(1-2): 115-122. http://www.springerlink.com/content/u3v0041507k032h1/

7.3.5 Coronaviruses: Severe Acute Respiratory Syndrome (SARS) -associated Human Coronavirus (HCoV) – BSL-2; BSL-3 for Propagation

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Reverse Transcription-PCR	7.3.5.1
Post Decontamination	Tissue Culture and Reverse Transcription-PCR	7.3.5.2

[1] See Appendix C for corresponding method usability tiers.

7.3.5.1 Site Characterization Sample Analyses (Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Adachi *et al.* 2004 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use reverse transcription-PCR (Adachi *et al.* 2004).

Description of Method: This method uses a conventional single-tube reverse transcription-PCR procedure using the Stratagene Robocycler® (Stratagene Corporation, La Jolla, California, or equivalent). End-point amplicon analysis is by electrophoresis and subsequent visualization. The assay can detect the SARS-HCoV as well as several other human respiratory coronaviruses (HCoV-OC43 and HCoV-229E). Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (Adachi *et al.* 2004 or EPA Method 1615 [Fout *et al.* 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Adachi *et al.* 2004).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Cao, G., Noti, J.D., Blachere, F M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

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Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

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Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

7.3.5.2 Post Decontamination Sample Analyses (Tissue Culture and Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Adachi *et al.* 2004 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use tissue culture (Pagat *et al.* 2007) and reverse transcription-PCR (Adachi *et al.* 2004).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are inoculated onto Vero cell monolayers; the cells are examined for cytopathic effects (CPE) to assess viability (Pagat *et al.* 2007). For confirmation, target nucleic acid should be extracted, purified (Adachi *et al.* 2004 or EPA Method 1615 [Fout *et al.* 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Adachi *et al.* 2004).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Cao, G., Noti, J.D., Blachere, F.M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

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Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

Pagat, A., Seux-Goepfert, R., Lutsch, C., Lecouturier, V., Saluzzo, J. and Kusters, I.C. 2007. "Evaluation of SARS-Coronavirus Decontamination Procedures." Applied Biosafety 12(2): 100-108. http://journals.sagepub.com/doi/pdf/10.1177/153567600701200206

7.3.6 Hepatitis E Virus (HEV) – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.6.1
Post Decontamination	Tissue Culture and Real-Time Reverse Transcription-PCR	7.3.6.2

[1] See Appendix C for corresponding method usability tiers.

7.3.6.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or EPA Method 1615 (Fout *et al.* 2012). ***Note***: Since the concentration of hepatitis E virus using the Nanoceram filter has not been evaluated, use of the 1MDS filter is recommended.

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Jothikumar *et al.* 2006 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use real-time reverse transcription-PCR (Jothikumar *et al.* 2006).

Description of Method: The method uses a TaqMan® real-time reverse transcription-PCR assay using the R.A.P.I.D.® PCR systems to detect and quantitate all four major HEV genotypes. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (Jothikumar *et al.* 2006 or EPA Method 1615 [Fout *et al.* 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Jothikumar *et al.* 2006).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Cao, G., Noti, J.D., Blachere, F.M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R.,

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https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

Jothikumar, N., Cromeans, T.L., Robertson, B.H., Meng, X.J. and Hill, V.R. 2006. "A Broadly Reactive One-Step Real-Time RT-PCR Assay for Rapid and Sensitive Detection of Hepatitis E Virus." Journal of Virological Methods. 131(1): 65-71.
http://www.sciencedirect.com/science/article/pii/S0166093405002417?via%3Dihub

7.3.6.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or EPA Method 1615 (Fout *et al.* 2012). ***Note***: Since the concentration of hepatitis E virus using the Nanoceram filter has not been evaluated, use of the 1MDS filter is recommended.

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Jothikumar *et al.* 2006 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use tissue culture (Zaki *et al.* 2009) and real-time reverse transcription-PCR (Jothikumar *et al.* 2006).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are inoculated onto HPG11 cells; the cells are examined for CPEs to assess viability (Zaki *et al.* 2009). For confirmation, target nucleic acid should be extracted, purified (Jothikumar *et al.* 2006 or EPA Method 1615 [Fout *et al.* 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Jothikumar *et al.* 2006).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Cao, G., Noti, J.D., Blachere, F.M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

Jothikumar, N., Cromeans, T.L., Robertson, B.H., Meng, X.J. and Hill, V.R. 2006. "A Broadly Reactive One-Step Real-Time RT-PCR Assay for Rapid and Sensitive Detection of Hepatitis E Virus." Journal of Virological Methods. 131(1): 65-71. http://www.sciencedirect.com/science/article/pii/S0166093405002417?via%3Dihub

Zaki, M., Foud, M.F. and Mohamed, A. F. 2009. "Value of Hepatitis E Virus Detection by Cell Culture Compared With Nested PCR and Serological Studies by IgM and IgG." FEMS Immunology Medical Microbiology. 56(1): 73-79. http://onlinelibrary.wiley.com/doi/10.1111/j.1574-695X.2009.00552.x/pdf

7.3.7 Influenza H5N1 virus – BSL-3

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.7.1
Post Decontamination	Tissue Culture and Real-Time Reverse Transcription-PCR	7.3.7.2

[1] See Appendix C for corresponding method usability tiers.

7.3.7.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is

needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Hermann *et al.* 2006.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Ng *et al.* 2005 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use real-time reverse transcription-PCR (Ng *et al.* 2005).

Description of Method: This is a two-step, real-time reverse transcriptase-PCR multiplex assay. The assay is specific for the H5 subtype. ***Note***: Influenza H5N1 virus samples are to be handled with BSL-3 containment and practices. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (Ng *et al.* 2005 or EPA Method 1615 [Fout *et al.* 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Ng *et al.* 2005).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hermann, J.R., Hoff, S.J., Yoon, K.J., Burkhardt, A.C., Evans, R.B. and Zimmerman, J.J. 2006. "Optimization of a Sampling System for Recovery and Detection of Airborne Porcine Reproductive and Respiratory Syndrome Virus and Swine Influenza Virus." Applied and Environmental Microbiology. 72(7): 4811-4818. http://aem.asm.org/content/72/7/4811

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Ng, E.K.O., Cheng, P.K.C., Ng, A.Y.Y., Hoang, T.L. and Lim, W.W.L. 2005. "Influenza A H5N1 Detection." Emerging Infectious Diseases. 11(8): 1303-1305. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3320469/

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

7.3.7.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Hermann *et al.* 2006.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Ng *et al.* 2005 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use tissue culture (Krauss *et al.* 2012) and real-time reverse transcription-PCR (Ng *et al.* 2005).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are inoculated onto Madin-Darby Canine Kidney Cells (MDCK); the cells are examined for CPEs to assess viability (Krauss *et al.* 2012). For confirmation, target nucleic acid should be extracted, purified (Ng *et al.* 2005 or EPA Method 1615 [Fout *et al.* 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Ng *et al.* 2005).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hermann, J.R., Hoff, S.J., Yoon, K.J., Burkhardt, A.C., Evans, R.B. and Zimmerman, J.J. 2006. "Optimization of a Sampling System for Recovery and Detection of Airborne Porcine Reproductive and Respiratory Syndrome Virus and Swine Influenza Virus." Applied and Environmental Microbiology. 72(7): 4811-4818. http://aem.asm.org/content/72/7/4811

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Ng, E.K.O., Cheng, P.K.C., Ng, A.Y.Y., Hoang, T.L. and Lim, W.W.L. 2005. "Influenza A H5N1 Detection." Emerging Infectious Diseases. 11(8): 1303-1305. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3320469/

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

Krauss, S., Walker, D. and Webster, R.G. 2012. "Influenza Virus Isolation." Methods in Molecular Biology. 865: 11-24. https://www.ncbi.nlm.nih.gov/pubmed/22528151

7.3.8 Picornaviruses: Enteroviruses – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.8.1
Post Decontamination	Tissue Culture	7.3.8.2

[1] See Appendix C for corresponding method usability tiers.

7.3.8.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.

- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or EPA Method 1615 (Fout *et al.* 2012).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use real-time reverse transcription-PCR (EPA Method 1615 [Fout *et al.* 2012]).

Description of Method: The method uses a TaqMan® real-time reverse transcriptase-PCR assay to detect and quantify enteroviruses. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified, and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Method 1615 [Fout *et al.* 2012]).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Cao, G., Noti, J.D., Blachere, F.M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G. W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K. E., Radosevich, M. and Wommack, K. E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

7.3.8.2 Post Decontamination Sample Analyses (Tissue Culture)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use tissue culture (EPA Method 1615 [Fout *et al.* 2012]).

Description of Method: This method describes procedures for determining infectivity and quantifying enteroviruses using BGMK cells. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), aliquots of the sample are used to inoculate BGMK cells. Cell culture flasks are examined for evidence of CPE for a total of 14 days (EPA Method 1615 [Fout *et al.* 2012]).

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:
Cao, G., Noti, J.D., Blachere, F.M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

7.3.9 Picornaviruses: Hepatitis A Virus (HAV) – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.9.1
Post Decontamination	Integrated Cell Culture and Real-Time Reverse Transcription-PCR	7.3.9.2

[1] See Appendix C for corresponding method usability tiers.

7.3.9.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription- PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or EPA Method 1615 (Fout *et al.* 2012).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Hyeon *et al.* 2011 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use real-time reverse transcription-PCR (Hyeon *et al.* 2011).

Description of Method: The method is a multiplex real-time reverse transcription-PCR procedure optimized for the simultaneous detection of enteroviruses, HAV, reoviruses and rotaviruses. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (Hyeon *et al.* 2011 or EPA Method 1615 [Fout *et al.* 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Hyeon *et al.* 2011).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:
Cao, G., Noti, J.D., Blachere, F.M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

Hyeon, J. Y, Chon, J.Y, Park, C., Lee, J.B., Choi, I.S., Kim, M.S. and Seo, K.H. 2011. "Rapid Detection Method for Hepatitis A Virus from Lettuce by a Combination of Filtration and Integrated Cell Culture-Real-Time Reverse Transcription PCR." Journal of Food Protection. 74(10): 1756-1761. http://www.ncbi.nlm.nih.gov/pubmed/22004827

7.3.9.2 Post Decontamination Sample Analyses (Integrated Cell Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or EPA Method 1615 (Fout *et al.* 2012).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Hyeon *et al.* 2011 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use integrated cell culture and real-time reverse transcription-PCR (Hyeon *et al.* 2011).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are inoculated onto fetal rhesus monkey kidney (FRhK-4) cells, and the cells are examined for CPE to assess viability. For confirmation, target nucleic acid should be extracted, purified (Hyeon *et al.* 2011 or EPA Method 1615 [Fout *et al.* 2012]),

and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Hyeon *et al.* 2011).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Cao, G., Noti, J.D., Blachere, F. M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

Hyeon, J.Y, Chon, J.Y, Park, C., Lee, J.B., Choi, I.S., Kim, M.S. and Seo, K.H. 2011. "Rapid Detection Method for Hepatitis A Virus from Lettuce by a Combination of Filtration and Integrated Cell Culture-Real-Time Reverse Transcription PCR." Journal of Food Protection. 74(10): 1756-1761. http://www.ncbi.nlm.nih.gov/pubmed/22004827

7.3.10 Reoviruses: Rotavirus (Group A) – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.10.1
Post Decontamination	Tissue Culture and Real-Time Reverse Transcription-PCR	7.3.10.2

[1] See Appendix C for corresponding method usability tiers.

7.3.10.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015).
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or EPA Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (Fout *et al.* 2012). ***Note***: Since the concentration of reoviruses using the Nanoceram filter has not been optimized, use of the 1MDS filter is recommended.

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Jothikumar *et al.* 2009 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use real-time reverse transcription-PCR (Jothikumar *et al.* 2009).

Description of Method: The method is used to detect rotavirus using a one-step real-time reverse-transcription PCR. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (Jothikumar *et al.* 2009 or EPA Method 1615 [Fout *et al.* 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Jothikumar *et al.* 2009).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:
Cao, G., Noti, J.D., Blachere, F.M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K.E., Radosevich, M. and Wommack, K.E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

Jothikumar, N., Kang, G. and V.R. Hill. 2009. "Broadly Reactive TaqMan® Assay for Real-Time RT-PCR Detection of Rotavirus in Clinical and Environmental Samples." Journal of Virological Methods. 155(2): 126-131. http://www.sciencedirect.com/science/article/pii/S0166093408003571

7.3.10.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Preparation:

- Aerosol samples should be prepared according to Cao *et al.* 2011.
- Particulate samples (swabs and wipes) should be prepared according to Park *et al.* 2015.
- Soil samples should be prepared according to Williamson *et al.* 2005.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or EPA Method 1615 (Fout *et al.* 2012). ***Note***: Since the concentration of reoviruses using the Nanoceram filter has not been optimized, use of the 1MDS filter is recommended.

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Jothikumar *et al.* 2009 or EPA Method 1615 (Fout *et al.* 2012).

Analytical Technique: Use tissue culture (EPA Method 1615 [Fout *et al.* 2012]) and real-time reverse transcription-PCR (Jothikumar *et al.* 2009).

Description of Method: This method describes procedures for determining infectivity and quantifying enteroviruses using BGMK cells. Following appropriate sample preparation (see Sample Preparation Procedures above), aliquots of the sample are used to inoculate BGMK cells. Cell culture flasks are examined for evidence of CPE for a total of 14 days (EPA Method 1615 [Fout *et al.* 2012]). For confirmation, target nucleic acid should be extracted, purified (Jothikumar *et al.* 2009 or EPA Method 1615 [Fout *et al.* 2012]), and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Jothikumar *et al.* 2009).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Cao, G., Noti, J.D., Blachere, F.M., Lindsley, W.G. and Beezhold, D.H. 2011. "Development of an Improved Methodology to Detect Infectious Airborne Influenza Virus Using the NIOSH Bioaerosol Sampler." Journal of Environmental Monitoring. 13: 3321-3328. http://pubs.rsc.org/en/content/articlelanding/2011/em/c1em10607d#!divAbstract

Park, G.W., Lee, D., Treffiletti, A., Hrsak, M., Shugart, J. and Vinjé, J. 2015. "Evaluation of a New Environmental Sampling Protocol for Detection of Human Norovirus on Inanimate Surfaces." Applied and Environmental Microbiology. 81(17): 5987-5992. http://aem.asm.org/content/81/17/5987.full.pdf+html

Williamson, K. E., Radosevich, M. and Wommack, K. E. 2005. "Abundance and Diversity of Viruses in Six Delaware Soils." Applied and Environmental Microbiology. 71(6): 3119-3125. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1151856/

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Fout, G.S., Brinkman, N.E., Cashdollar, J.L., Griffin, S.M., McMinn, B.R., Rhodes, E.R., Varughese, E.A., Karim, M.R., Grimm, A.C., Spencer, S.K. and Borchardt, M.A. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR." Cincinnati, OH: U.S. EPA. EPA/600/R-10/181. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100LX19.PDF?Dockey=P100LX19.PDF

Jothikumar, N., Kang, G. and V.R. Hill. 2009. "Broadly Reactive TaqMan® Assay for Real-Time RT-PCR Detection of Rotavirus in Clinical and Environmental Samples." Journal of Virological Methods. 155(2): 126-131. http://www.sciencedirect.com/science/article/pii/S0166093408003571

7.4 Method Summaries for Protozoa

Summaries for the analytical methods listed in Appendix C for analysis of protozoa are provided in Sections 7.4.1 through 7.4.5. Each summary contains a brief description of the analytical methods selected for each protozoan, and links to, or sources for, obtaining full versions of the methods. Tiers that have been assigned to each method/analyte pair (see Section 7.1.1) can be found in Appendix C. The full version of the method should be consulted prior to sample analysis. For information regarding sample collection considerations for samples to be analyzed by these methods, see the companion *Sample Collection Information Document for Pathogens – Companion to SAM 2017*.

7.4.1 *Cryptosporidium* spp. [Cryptosporidiosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.4.1.1
	IMS/immunofluorescence assay (FA)	7.4.1.2 [2]
	IMS/FA	7.4.1.3 [2]
Post Decontamination	Cell Culture Immunofluorescence Procedure	7.4.1.4

[1] See Appendix C for corresponding method usability tiers.

[2] Methods 1622 and 1623.1 include the same sample processing and analytical procedures for *Cryptosporidium*; either method could be used.

7.4.1.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012a).
- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011, or the EPA BA Protocol (U.S. EPA 2012a).
- Soil samples should be prepared according to Liang and Keeley 2011.
- Water samples should be prepared according to EPA Method 1622 (U.S. EPA 2005), EPA Method 1623.1 (U.S. EPA 2012b), or the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Guy *et al.* 2003, Jiang *et al.* 2005 or the EPA BA Protocol, Section 9.2 (U.S. EPA 2012a).

Analytical Technique: Use real-time PCR (Guy *et al.* 2003 and Jiang *et al.* 2005).

Description of Method: Following appropriate sample preparation (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (Guy *et al.* 2003, Jiang *et al.* 2005 or EPA BA Protocol, Section 9.2 [U.S. EPA 2012a]), and analyzed using the referenced

target-specific real-time PCR primers, probes and assay parameters (Guy *et al.* 2003). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Cryptosporidium* spp.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2012a. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores from Surfaces." Journal of Microbiological Methods. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use after Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." Applied and Environmental Microbiology. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

U.S. EPA. 2005. "Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 815-R-05-001. https://www.epa.gov/sites/production/files/2015-07/documents/epa-1622.pdf

U.S. EPA. 2012b. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Guy, R.A., Payment, P., Krull, U.J. and Horgen, P.A. 2003. "Real-Time PCR for Quantification of *Giardia* and *Cryptosporidium* in Environmental Water Samples and Sewage." Applied and Environmental Microbiology. 69(9): 5178-5185. http://aem.asm.org/content/69/9/5178.full.pdf+html

Jiang, J., Alderisio, K.A., Singh, A. and Xiao, L. 2005. "Development of Procedures for Direct Extraction of *Cryptosporidium* DNA from Water Concentrates and for Relief of PCR Inhibitors." Applied and Environmental Microbiology. 71(3): 1135-1141. http://aem.asm.org/content/71/3/1135.full.pdf+html

7.4.1.2 Site Characterization Sample Analyses (Immunomagnetic Separation/Immunofluorescence Assay [IMS/FA])

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012a).
- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011, or the EPA BA Protocol (U.S. EPA 2012a).
- Soil samples should be prepared according to Liang and Keeley 2011.
- Water samples should be prepared according to EPA Method 1622 (U.S. EPA 2005), Method 1623.1 (U.S. EPA 2012b) or the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Analytical Technique: Use IMS and FA microscopy (EPA Method 1622 [U.S. EPA 2005]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are centrifuged to pellet the oocysts, and the supernatant fluid is aspirated. A solution containing anti-*Cryptosporidium* antibodies conjugated to magnetic beads is added to the pellet and mixed. The oocyst magnetic bead complex is separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts. The oocysts are stained on well slides with fluorescently labeled monoclonal antibodies (mAbs) and 4',6-diamidino-2-phenylindole (DAPI). The stained sample is examined using fluorescence and differential interference contrast (DIC) microscopy. Qualitative analysis is performed by scanning each slide well for objects that meet the size, shape, and fluorescence characteristics of *Cryptosporidium* oocysts. Quantitative analysis is performed by counting the total number of objects on the slide confirmed as oocysts. This method is not intended to determine viability, species, or infectivity of the oocysts.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control, matrix spike/matrix spike duplicate (MS/MSD) and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed as stipulated in the method.

Sources:
U.S. EPA. 2012a. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples during the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores from Surfaces." Journal of Microbiological Methods. 81(2): 141-146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use after Sampling *Bacillus anthracis* Spores from Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." Applied and Environmental Microbiology. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

U.S. EPA. 2005. "Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 815-R-05-001. https://www.epa.gov/sites/production/files/2015-07/documents/epa-1622.pdf

U.S. EPA. 2012b. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

7.4.1.3 Site Characterization Sample Analyses (IMS/FA)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012a).
- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011 or the EPA BA Protocol (U.S. EPA 2012a).
- Soil samples should be prepared according to Liang and Keeley 2011.
- Water samples should be prepared according to EPA Method 1622 (U.S. EPA 2005), EPA Method 1623.1 (U.S. EPA 2012b) or the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Analytical Technique: Use IMS and FA microscopy (EPA Method 1623.1 [U.S. EPA 2012b]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are centrifuged to pellet the oocysts and cysts, and the supernatant fluid is aspirated. A solution containing anti-*Cryptosporidium* and anti-*Giardia* antibodies conjugated to magnetic beads is added to the pellet and mixed. The oocyst and cyst magnetic bead complex is separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts and cysts. The oocysts and cysts are stained on well slides with fluorescently labeled mAbs and DAPI. The stained sample is examined using fluorescence and DIC microscopy. Qualitative analysis is performed by scanning each slide well for objects that meet the size, shape, and fluorescence characteristics of *Cryptosporidium* oocysts and *Giardia* cysts. Quantitative analysis is performed by counting the total number of objects on the slide confirmed as oocysts or cysts.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control, MS/MSD and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

U.S. EPA. 2012a. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores from Surfaces." Journal of Microbiological Methods. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use after Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." Applied and Environmental Microbiology. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

U.S. EPA. 2005. "Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 815-R-05-001. https://www.epa.gov/sites/production/files/2015-07/documents/epa-1622.pdf

U.S. EPA. 2012b. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

7.4.1.4 Post Decontamination Sample Analyses (Cell Culture Immunofluorescence Procedure)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012a).
- Particulate samples (surface swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011 or the EPA BA Protocol (U.S. EPA 2012a).

- Soil samples should be prepared according to Liang and Keeley 2011.
- Water samples should be prepared according to EPA Method 1622 (U.S. EPA 2005), EPA Method 1623.1 (U.S. EPA 2012b) or the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Analytical Technique: Use cell culture immunofluorescence procedure (Bukhari *et al.* 2007).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are used to inoculate HCT-8 monolayers and incubated. Following incubation, the monolayers are examined using immunofluorescence to determine the number of viable oocysts present in the sample. The use of cell culture immunofluorescence analyses is a cost effective and expedient alternative to mouse infectivity assays to determine in vitro infectivity of *Cryptosporidium* oocysts.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

U.S. EPA. 2012a. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." Applied and Environmental Microbiology. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

U.S. EPA. 2005. "Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 815-R-05-001. https://www.epa.gov/sites/production/files/2015-07/documents/epa-1622.pdf

U.S. EPA. 2012b. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Bukhari, Z., Holt, D.M., Ware, M.W. and Schaefer III, F.W. 2007. "Blind Trials Evaluating In Vitro Infectivity of *Cryptosporidium* Oocysts Using Cell Culture Immunofluorescence."

Canadian Journal of Microbiology. 53(5): 656–663.
http://www.nrcresearchpress.com/doi/abs/10.1139/W07-032?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed

7.4.2 *Entamoeba histolytica* – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.4.2.1
Post Decontamination	Cell Culture	7.4.2.2

[1] See Appendix C for corresponding method usability tiers.

7.4.2.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012).
- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011 or the EPA BA Protocol (U.S. EPA 2012).
- Soil samples should be prepared according to Liang and Keeley 2011.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Roy *et al.* 2005 or the EPA BA Protocol, Section 9.2 (U.S. EPA 2012).

Analytical Technique: Use real-time PCR (Roy *et al.* 2005).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (Roy *et al.* 2005 or EPA BA Protocol, Section 9.2 [U.S. EPA 2012]), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters (Roy *et al.* 2005). The use of real-time PCR analyses directly on samples allows for rapid detection of *Entamoeba histolytica*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:
U.S. EPA. 2012. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA.

EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." Applied and Environmental Microbiology. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Roy, S., Kabir, M., Mondal, D., Ali, I.K.M., Petri Jr., W.A. and Haque, R. 2005. "Real-Time-PCR Assay for Diagnosis of *Entamoeba histolytica* Infection." Journal of Clinical Microbiology. 43(5): 2168-2172. http://jcm.asm.org/content/43/5/2168.full.pdf+html

7.4.2.2 Post Decontamination Sample Analyses (Cell Culture)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012).
- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011 or the EPA BA Protol (U.S. EPA 2012).
- Soil samples should be prepared according to Liang and Keeley 2011.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Analytical Technique: Use cell culture (Stringert 1972).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), *Entamoeba histolytica* cysts are placed in a modified trypticase-panmede liver digest-serum medium and incubated for 10 hours. Live amoebae excyst through a rupture in the cyst wall, whereas non-viable amoebae remain encysted. Microscopic examination of an aliquot of the incubated excystation culture allows calculation of the percent of empty (live) cysts and full (dead) cysts in a population.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

U.S. EPA. 2012. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." Applied and Environmental Microbiology. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Stringert, R.P. 1972. "New Bioassay System for Evaluating Percent Survival of *Entamoeba histolytica* Cysts." The Journal of Parasitology. 58(2): 306-310. http://www.jstor.org/discover/10.2307/3278094?uid=3739704&uid=2129&uid=2&uid=70&uid=4&uid=3739256&sid=47698759181407

7.4.3 *Giardia* spp. [Giardiasis] – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.4.3.1
	IMS/FA	7.4.3.2
Post Decontamination	Cell Culture	7.4.3.3

7.4.3.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012a).
- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011 or the EPA BA Protocol (U.S. EPA 2012a).
- Soil samples should be prepared according to Liang and Keeley 2011.
- Water samples should be prepared according to EPA Method 1623.1 (U.S. EPA 2012b) or the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Guy *et al*. 2003 or the EPA BA Protocol, Section 9.2 (U.S. EPA 2012a).

Analytical Technique: Use real-time PCR (Guy *et al.* 2003).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (Guy *et al.* 2003 or the EPA BA Protocol [U.S. EPA 2012a]), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters (Guy *et al.* 2003). The use of real-time PCR analyses directly on samples allows for rapid detection of *Giardia*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2012a. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." Applied and Environmental Microbiology. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

U.S. EPA. 2012b. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Guy, R.A., Payment, P., Krull, U.J. and Horgen, P.A. 2003. "Real-Time PCR for Quantification of *Giardia* and *Cryptosporidium* in Environmental Water Samples and Sewage." Applied and Environmental Microbiology. 69(9): 5178-5185. http://aem.asm.org/content/69/9/5178.full.pdf+html

7.4.3.2 Site Characterization Sample Analyses (IMS/FA)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012a).
- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011, or EPA BA Protocol (U.S. EPA 2012a).
- Soil samples should be prepared according to Liang and Keeley 2011.
- Water samples should be prepared according to EPA Method 1623.1 (U.S. EPA 2012b) or the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Analytical Technique: Use IMS and FA microscopy (Method 1623.1 [U.S. EPA 2012b]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are centrifuged to pellet the oocysts and cysts, and the supernatant fluid is aspirated. A solution containing anti-*Cryptosporidium* and anti-*Giardia* antibodies conjugated to magnetic beads is added to the pellet and mixed. The oocyst and cyst magnetic bead complex is separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts and cysts. The oocysts and cysts are stained on well slides with fluorescently labeled mAbs and DAPI. The stained sample is examined using fluorescence and DIC microscopy. Qualitative analysis is performed by scanning each slide well for objects that meet the size, shape, and fluorescence characteristics of *Cryptosporidium* oocysts and *Giardia* cysts. Quantitative analysis is performed by counting the total number of objects on the slide confirmed as oocysts or cysts.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control, MS/MSD and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:
U.S. EPA. 2012a. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." Applied and Environmental Microbiology. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

U.S. EPA. 2012b. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

7.4.3.3 Post Decontamination Sample Analyses (Cell Culture)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012a).
- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011 or the EPA BA Protocol (U.S. EPA 2012a).
- Soil samples should be prepared according to Liang and Keeley 2011.
- Water samples should be prepared according to EPA Method 1623.1 (U.S. EPA 2012b) or the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Analytical Technique: Use cell culture (Keister 1983).

Description of Method: Procedures are described for analysis of cell culture samples and may be adapted for assessment of aerosols, wipes or swabs, and water samples (see Sample Preparation Procedures above). Trypticase-yeast-iron-serum medium supplemented with bovine bile and additional cysteine is used to isolate and culture *Giardia lamblia*. *G. lamblia* is incubated for intervals of 72 and 96 hours at 36°C in borosilicate glass tubes. The cells form a dense, adherent monolayer on the surface of the glass or are observed swimming through the liquid medium.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical

results should also be performed.

Sources:

U.S. EPA. 2012a. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." Applied and Environmental Microbiology. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

U.S. EPA. 2012b. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Keister, D. 1983. "Axenic Culture of *Giardia lamblia* in TYI-S-33 Medium Supplemented With Bile." Transactions of the Royal Society of Tropical Medicine and Hygiene. 77(4): 487-488. http://www.sciencedirect.com/science/article/pii/0035920383901207

7.4.4 *Naegleria fowleri* [Naegleriasis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.4.4.1
Post Decontamination	Culture and Real-Time PCR	7.4.4.2

[1] See Appendix C for corresponding method usability tiers.

7.4.4.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012).
- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011 or the EPA BA Protocol (U.S. EPA 2012).
- Soil samples should be prepared according to Mull *et al*. 2013.
- Water samples should be prepared according to Cope *et al.* 2015 or the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Nucleic acid extraction should be performed according to procedures within Mull *et al.* 2013 or the EPA BA Protocol, Section 9.2 (U.S. EPA 2012).

Analytical Technique: Use real-time PCR (Mull *et al.* 2013).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are concentrated by centrifugation. The pellet is then resuspended and further concentrated using IMS. Target nucleic acid should be extracted, purified (Mull *et al.* 2013 or EPA BA Protocol, Section 9.2 [U.S. EPA 2012]) and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Mull *et al.* 2013). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Naegleria fowleri*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2012. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Mull, B.J., Jothikumar, N. and Hill, V.R. 2013. "Improved Method for the Detection and Quantification of *Naegleria fowleri* in Water and Sediment Using Immunomagnetic Separation and Real-Time PCR." Journal of Parasitology Research. Article ID 608367: 8 pages. https://www.hindawi.com/journals/jpr/2013/608367/

Cope, J.R., Ratard, R.C., Hill, V.R., Sokol, T., Causey, J.J., Yoder, J.S., Mirani, G., Mull, B. Mukerjee, K.A., Narayanan, J., Doucet, M., Qvarnstrom, Y., Poole, C.N., Akingbola, O.A., Ritter, J.M., Xiong, Z., da Silva, A.J., Roellig, D., Van Dyke, R.B., Stern, H., Xiao, L. and Beach, M.J. 2015. "The First Association of a Primary Amebic Meningoencephalitis Death With Culturable *Naegleria fowleri* in Tap Water From a US Treated Public Drinking Water System." Clinical Infectious Diseases, 60(8): e36-42. https://www.ncbi.nlm.nih.gov/pubmed/25595746

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

7.4.4.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to procedures within the EPA BA Protocol (U.S. EPA 2012).
- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011 or the EPA BA Protocol (U.S. EPA 2012).
- Soil samples should be prepared according to Mull *et al*. 2013.
- Water samples should be prepared according to Cope *et al.* 2015 or the EPA and CDC UF Report (U.S. EPA and CDC 2011).

Nucleic acid extraction should be performed according to Mull *et al.* 2013 or the EPA BA Protocol, Section 11.6 (U.S. EPA 2012).

Analytical Technique: Use culture and real-time PCR (Mull *et al.* 2013).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are centrifuged to pellet the trophozoites and cysts and a portion of the pellet is plated with *E. coli*. The remaining pellet is further concentrated using IMS and then plated with *E. coli*. Plates are incubated for seven days. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (Mull *et al.* 2013 or EPA BA Protocol, Section 11.6 [U.S. EPA 2012]) and analyzed using the referenced target-specific PCR primers, probes and assay parameters (Mull *et al.* 2013). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Naegleria fowleri*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: https://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2012. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Mull, B.J., Jothikumar, N. and Hill, V.R. 2013. "Improved Method for the Detection and Quantification of *Naegleria fowleri* in Water and Sediment Using Immunomagnetic Separation and Real-Time PCR." Journal of Parasitology Research. Article ID 608367: 8 pages. https://www.hindawi.com/journals/jpr/2013/608367/

Cope, J.R., Ratard, R.C., Hill, V.R., Sokol, T., Causey, J.J., Yoder, J.S., Mirani, G., Mull, B. Mukerjee, K.A., Narayanan, J., Doucet, M., Qvarnstrom, Y., Poole, C.N., Akingbola, O.A., Ritter, J.M., Xiong, Z., da Silva, A. J., Roellig, D., Van Dyke, R.B., Stern, H., Xiao, L. and Beach, M.J. 2015. "The First Association of a Primary Amebic Meningoencephalitis Death With Culturable *Naegleria fowleri* in Tap Water From a US Treated Public Drinking Water System." Clinical Infectious Diseases. 60(8): e36-42. https://www.ncbi.nlm.nih.gov/pubmed/25595746

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

7.4.5 *Toxoplasma gondii* [Toxoplasmosis] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.4.5.1
Post Decontamination	Cell Culture	7.4.5.2

[1] See Appendix C for corresponding method usability tiers.

7.4.5.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S.

EPA 2012a).

- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011, or the EPA BA Protocol (U.S. EPA 2012a).
- Soil samples should be prepared according to Liang and Keeley 2011.
- Water samples should be prepared according to Villegas *et al.* 2010 or EPA Method 1623.1 (U.S. EPA 2012b).

Nucleic acid extraction should be performed according to procedures within Yang *et al.* 2009 or the EPA BA Protocol, Section 9.2 (U.S. EPA 2012a).

Analytical Technique: Use real-time PCR (Yang *et al.* 2009).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (Yang *et al.* 2009 or EPA BA Protocol, Section 9.2 [U.S. 2012a]), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters (Yang *et al.* 2009). The use of real-time PCR analyses directly on samples allows for rapid detection of *Toxoplasma gondii*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2012a. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." Applied and Environmental Microbiology. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

Villegas, E.N., Augustine, S.A., Villegas, L.F., Ware, M.W., See, M.J., Lindquist, H.D.A., Schaefer, III, F.W. and Dubey, J.P. 2010. "Using Quantitative Reverse Transcriptase PCR and Cell Culture Plaque Assays to Determine Resistance of *Toxoplasma gondii* Oocysts to Chemical Sanitizers." Journal of Microbiological Methods. 81(3): 219-225. http://www.sciencedirect.com/science/article/pii/S0167701210001107

U.S. EPA. 2012b. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

Yang, W., Lindquist, H.D. A., Cama, V., Schaefer III, F.W., Villegas, E., Fayer, R., Lewis, E.J., Feng, Y. and Xiao, L. 2009. "Detection of *Toxoplasma gondii* Oocysts in Water Sample Concentrates by Real-Time PCR." Applied and Environmental Microbiology. 75(11): 3477-3483. http://aem.asm.org/content/75/11/3477.full.pdf+html

7.4.5.2 Post Decontamination Sample Analyses (Cell Culture)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012a).
- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011) or the EPA BA Protocol (U.S. EPA 2012a).
- Soil samples should be prepared according to Liang and Keeley 2011.
- Water samples should be prepared according to Villegas *et al.* 2010 or EPA Method 1623.1 (U.S. EPA 2012b).

Analytical Technique: Use cell culture (Villegas *et al.* 2010).

Description of Method: Samples are subjected to a series of mechanical and chemical digestion steps to release sporozoites from the *Toxoplasma gondii* oocysts and then inoculated onto confluent fibroblast monolayers. Inoculated monolayers are then incubated undisturbed for ten days to allow for plaque formation. After ten days, the monolayers are fixed, stained with crystal violet, and examined for plaque formation. The literature reference also includes a quantitative polymerase chain reaction (qPCR) procedure to determine viability of *Toxoplasma gondii* oocysts, however, it may not be appropriate depending on the type of disinfection used during remediation.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:
U.S. EPA. 2012a. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." Applied and Environmental Microbiology. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

Villegas, E.N., Augustine, S.A., Villegas, L.F., Ware, M.W., See, M. J., Lindquist, H.D.A., Schaefer, III, F.W. and Dubey, J.P. 2010. "Using Quantitative Reverse Transcriptase PCR and Cell Culture Plaque Assays to Determine Resistance of *Toxoplasma gondii* Oocysts to Chemical Sanitizers." Journal of Microbiological Methods. 81(3): 219-225. http://www.sciencedirect.com/science/article/pii/S0167701210001107

U.S. EPA. 2012b. "Method 1623.1: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." Washington, DC: U.S. EPA. EPA 816-R-12-001. https://nepis.epa.gov/Exe/ZyPDF.cgi/P100J7G4.PDF?Dockey=P100J7G4.PDF

7.5 Method Summaries for Helminths

Summaries for the analytical methods listed in Appendix C for analysis of helminths are provided in Section 7.5.1. The section contains a brief description of the analytical methods selected, and links to, or sources for, obtaining full versions of the methods. Tiers that have been assigned to each method/analyte pair (see Section 7.1.1) can be found in Appendix C. The full version of the method should be consulted prior to sample analysis. For information regarding sample collection considerations for samples to be analyzed by these methods, see the companion *Sample Collection Information Document for Pathogens – Companion to SAM 2017*.

7.5.1 *Baylisascaris procyonis* [Raccoon roundworm fever] – BSL-2

Remediation Phase	Analytical Technique[1]	Section
Site Characterization	Real-Time PCR	7.5.1.1
Post Decontamination	Embryonation of Eggs and Microscopy	7.5.1.2

[1] See Appendix C for corresponding method usability tiers.

7.5.1.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below

Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012).
- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*.

2010, Rose *et al.* 2011 or the EPA BA Protocol (U.S. EPA 2012).

- Soil samples should be prepared according to Liang and Keeley 2011.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or Gatcombe *et al.* 2010.

Nucleic acid extraction and purification for all sample types should be performed according to procedures within Gatcombe *et al.* 2010 or the EPA BA Protocol, Section 9.2 (U.S. EPA 2012).

Analytical Technique: Use real-time PCR (Gatcombe *et al.* 2010).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (Gatcombe *et al.* 2010 or EPA BA Protocol, Section 9.2 [U.S. EPA 2012]), and analyzed using the referenced target-specific real-time PCR primers, probes and assay parameters (Gatcombe *et al.* 2010). The use of real-time PCR analyses directly on samples (e.g., no embryonation or microscopic examination) allows for rapid detection of *Baylisascaris procyonis*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.0.

Sources:

U.S. EPA. 2012. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods. 81(2): 141-146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." Applied and Environmental Microbiology. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Gatcombe, R.R., Jothikumar, N., Dangoudoubiyam, S., Kazacos, K.R. and Hill, V.R. 2010. "Evaluation of a Molecular Beacon Real-time PCR Assay for Detection of *Baylisascaris procyonis* in Different Soil Types and Water Samples." Parasitology Research. 106: 499-504. http://www.springerlink.com/content/k8t3581t07n82562

7.5.1.2 Post Decontamination Sample Analyses (Embryonation of Eggs and Microscopy)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: This method is listed for analysis of aerosols, particulates (wipes or swabs), soils, drinking water and post decontamination waste water samples. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types.

Sample Preparation:

- Aerosol samples (filters) should be prepared according to the EPA BA Protocol (U.S. EPA 2012).
- Particulate samples (swabs and wipes) should be prepared according to Hodges *et al*. 2010, Rose *et al.* 2011 or the EPA BA Protocol (U.S. EPA 2012).
- Soil samples should be prepared according to Liang and Keeley 2011.
- Water samples should be prepared according to the EPA and CDC UF Report (U.S. EPA and CDC 2011) or Gatcombe *et al.* 2010.

Analytical Technique: Use microscopy and embryonation of eggs (U.S. EPA. 2003).

Description of Method: The protocol describes procedures for analysis of soil and wastewater samples. Samples are processed by blending with buffered water containing a surfactant. The blend is screened to remove large particles, the soils in the screened portion are allowed to settle out, and the supernatant is decanted. The sediment is subjected to density gradient centrifugation using magnesium sulfate. This flotation procedure yields a layer likely to contain *Ascaris* and other parasite eggs, if present in the sample. Small particulates are removed by a second screening on a small mesh size screen. The resulting concentrate is incubated until control helminth eggs are fully embryonated. The concentrate is then microscopically examined for the categories of helminth ova on a counting chamber.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:
U.S. EPA. 2012. "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol). Cincinnati, OH: U.S. EPA. EPA/600/R-12/577. https://www.epa.gov/homeland-security-research/protocol-detection-bacillus-anthracis-environmental-samples

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods. 81(2): 141-146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology. 77(23): 8355-8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

Liang, Z. and Keeley, A. 2011. "Detection of Viable *Cryptosporidium parvum* in Soil by Reverse Transcription–Real-Time PCR Targeting *hsp70* mRNA." Applied and Environmental Microbiology. 77(18): 6476-6485. http://aem.asm.org/content/77/18/6476.abstract

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (EPA and CDC UF Report). Cincinnati, OH: U.S. EPA. EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

Gatcombe, R.R., Jothikumar, N., Dangoudoubiyam, S., Kazacos, K.R. and Hill, V.R. 2010. "Evaluation of a Molecular Beacon Real-time PCR Assay for Detection of *Baylisascaris procyonis* in Different Soil Types and Water Samples." Parasitology Research. 106:499-504. http://www.springerlink.com/content/k8t3581t07n82562

U.S. EPA. 2003. "Appendix I: Test Method for Detecting, Enumerating, and Determining the Viability of Ascaris Ova in Sludge." *U.S. EPA Environmental Regulations and Technology: Control of Pathogens and Vector Attractions in Sewage Sludge*. Cincinnati, OH: U.S. EPA EPA/625/R-92/013. https://www.epa.gov/sites/production/files/2015-07/documents/epa-625-r-92-013.pdf

Section 8.0: Selected Biotoxin Methods

Appendix D provides a list of methods to be used in analyzing environmental samples for biotoxin contaminants during remediation activities following a contamination incident. For the purposes of this document, biotoxins are defined as poisonous chemicals or group of related chemicals that are derived from plants or animals, and include those that can be artificially produced in sufficient quantities as to represent a substantial hazard. Procedures and methods have been selected for each biotoxin that may need to be identified and/or quantified during remediation. Analytical procedures are not currently available for all the biotoxin/sample type combinations included in this document, and on-going research efforts include identification of additional methods, as well as development and testing of some of the procedures listed. Appendix D is sorted alphabetically by biotoxin.

Please note: This section provides guidance for selecting biotoxin methods that have a high likelihood of assuring analytical consistency when laboratories are faced with a large-scale environmental remediation crisis. Not all methods have been verified for the biotoxin/sample type combination listed in Appendix D, and method usability tiers have been assigned to indicate the fitness of each method for its intended use. Users should refer to the specified methods and reference citations provided throughout Sections 8.2 and 8.3 to identify biotoxin/sample type combinations that have been verified, and should also consider the possibility of analytical interferences inherent to environmental sample types (e.g., sample composition, the presence and concentrations of additional or competing biotoxins or biotoxin variants. Any questions regarding this information should be addressed to the appropriate contact(s) listed in Section 4.0.

Appendix D provides the following information:

- **Analyte(s).** The biotoxin of interest.
- **Chemical Abstracts Service Registry Number (CAS RN) / Description.** A unique identifier for substances that provides an unambiguous way to identify a biotoxin or biotoxin isoform when there are many possible systematic, generic or trivial names, and/or a brief statement describing the biotoxin.
- **Analysis type.** Tests are either for presumptive identification, confirmatory identification or biological activity determination; test types are described below.
- **Analytical technique.** Type of analytical instrumentation or assay used to determine the quantity and identity of compounds or components in a sample.
- **Analytical method.** The recommended method or procedure, and the corresponding publisher.
- **Aerosol (filter/cassette or liquid impinger).** The recommended method/procedure to measure the analyte of interest in air sample collection media.
- **Solid (soil, powder).** The recommended method/procedure to measure the analyte of interest in solid samples.
- **Particulate (swabs, wipes, filters).** The recommended method/procedure to measure the analyte of interest in particulate sample collection media.
- **Non-drinking water.** The recommended method/procedure to measure the analyte of interest in water samples other than drinking water.
- **Drinking water.** The recommended method/procedure to measure the analyte of interest in drinking water samples.

Depending on site- and incident-specific goals, a determination of whether contaminant concentrations are above pre-existing levels may be necessary. Such determinations could involve investigations of background levels at potentially uncontaminated locations in close proximity to the site, using methods listed in Appendix D. Other means might include examination of historical information regarding

contaminant occurrence. For example, periodic episodes of some of the biotoxins (such as microcystins [MC] and other algal biotoxins) have been detected and measured in surface waters throughout the United States (U.S.) by methods similar to those in Appendix D (see, for example, http://toxics.usgs.gov/highlights/algal_toxins/). When using historical data, knowledge of the analytical methods and techniques used would be necessary, particularly in terms of their similarity in performance and quality control (QC) to the methods listed in Appendix D.

The "analysis types" identified in Appendix D are intended to address: (1) the level of certainty of results needed and (2) the remediation phase during which analytical support is needed (e.g., site assessment, clearance). Many of the presumptive methods that have been selected are immunoassays, which can be adapted for large-scale sample analysis while maintaining an appropriate level of analytical certainty. Confirmatory methods are generally more time consuming and expensive, and are intended to provide results with a higher level of certainty than those provided by presumptive methods. Methods that address biological activity tend to be even more time consuming and expensive, and are intended to provide a high level of certainty in corroborating other assay results. Note that the use of the terms "presumptive" and "confirmatory" in this document is not intended to redefine or supersede the Laboratory Response Network's (LRN) or any other organization's use of these terms.

A tiered approach, or algorithm, can be used when implementing the analysis types, particularly when needed to address a large number of samples. For example, methods identified as presumptive are generally more rapid than confirmatory methods, and might be used during the initial stages of remediation to evaluate the extent of contamination. Presumptive methods also might be used to identify samples that should be analyzed using the more extensive confirmatory methods. Confirmatory methods should be considered for use when: (1) presumptive analysis indicates the presence of the biotoxin, (2) a smaller subset of samples requires analysis, or (3) as required for a tiered approach to remediation. Depending on the goals of the remediation phase, biological activity methods may be needed because biotoxins are sometimes detectable but inactive; therefore, these assays may also provide information about potential impact on human safety.

For small molecule biotoxins in Appendix D, the presence of intact compound structure is an indication of biological activity; therefore, the confirmatory method listed for these biotoxins also serves as a measure of biological activity. For protein biotoxins, biological activity may be determined directly using *in vivo* (e.g., mouse bioassay) or *in vitro* (e.g., enzymatic activity) methods. However, both biological availability (i.e., biotoxin accessibility to site of action) and activity are required to elicit toxicity, and some *in vitro* methods may not address both parameters.

Numerous analytical techniques using a variety of instrumentation have been selected and are cited in Appendix D. It is recognized that advances in procedures for analysis of biotoxins are frequently reported in the literature, and commercially available equipment for these analyses is evolving rapidly. Accordingly, the individual techniques and methods listed in Appendix D are to be regarded as a starting point – the user is encouraged to consult the SAM website (https://www.epa.gov/homeland-security-research/sam) for updates. The availability of critical reagents (e.g., antibodies) and reference standards required for the selected analytical methods might be limited; in cases where specific information regarding their availability is known, it is provided in the method summaries throughout Section 8.2. The biotoxin methods points of contact listed in Section 4.0 should be contacted for additional information.

Additional research on biotoxin contaminants is ongoing within the U.S. Environmental Protection Agency (EPA) and includes the impact of disinfectants and preservatives. The presence of disinfectants (e.g., chlorine) and/or preservatives added during water sample collection (e.g., pH adjustors, de-chlorinating agents) may affect analytical results. When present, the impact of these agents on method performance should be evaluated, if not previously determined. EPA's National Homeland Security Research Center (NHSRC) continues to maintain sample collection information documents that are intended as companions to SAM. These sample collection documents provide information regarding sampling container/media, preservation, holding time, sample sizes and shipping, and are available at:

https://www.epa.gov/homeland-security-research/sam-companion-documents-and-sample-collection-procedures.

8.1 General Guidelines

This section provides a general overview of how to identify the appropriate method(s) for a given biotoxin, as well as recommendations for QC procedures.

For additional information on the properties of the biotoxins listed in Appendix D, users are referred to the Toxicology Data Network (TOXNET) (http://toxnet.nlm.nih.gov/index.html), a cluster of databases on toxicology, hazardous chemicals, and related areas maintained by the National Library of Medicine. There are other biotoxins that may be of interest in environmental samples, in addition to those listed in Appendix D. It is beyond the scope of this section and Appendix D to discuss every possible biotoxin. Some examples are included in the resources listed below; these resources also contain additional general information that may be of use to laboratories performing biotoxin analysis.

Additional resources:

- *Defense Against Toxin Weapons*, published by the U.S. Army Medical Research Institute of Infectious Diseases (http://www.usamriid.army.mil/education/defensetox/toxdefbook.pdf) contains information regarding sample collection, biotoxin analysis and identification, as well as decontamination and water treatment.
- The Centers for Disease Control and Prevention (CDC) has additional information regarding select agent biotoxins (http://www.cdc.gov/od/sap/sap/toxinamt.htm).
- CDC's "Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition" (https://www.cdc.gov/biosafety/publications/bmbl5/) includes some biotoxins.
- INCHEM contains both chemical and toxicity information (http://www.inchem.org/).
- The Registry of Toxic Effects of Chemical Substances (RTECS) database, accessed via the National Institute for Occupational Safety and Health (NIOSH) website at http://www.cdc.gov/niosh/rtecs/default.html for toxicity information.
- The *Forensic Science and Communications Journal* published by the Laboratory Division of the Federal Bureau of Investigation (FBI), accessed via http://www.fbi.gov/about-us/lab/forensic-science-communications.
- The U.S. National Response Team publishes Quick Reference Guides on a number of biotoxins (https://www.nrt.org/Main/Resources.aspx?ResourceType=Hazards&ResourceSection=2).

8.1.1 Standard Operating Procedures for Identifying Biotoxin Methods

To determine the appropriate method for analysis of an environmental sample, locate the biotoxin of concern in Appendix D: Selected Biotoxin Methods under the "Analyte(s)" column. After locating the biotoxin, continue across the table row and identify the appropriate analysis type. After an analysis type has been chosen, find the analytical technique (e.g., immunoassay) and analytical method applicable to the sample type of interest (aerosol, solid, particulate, drinking water or non-drinking water).

The fitness of a method for its intended use is related to data quality objectives (DQOs) for a particular remediation activity. The tiers below have been assigned to the methods selected for each biotoxin/sample type pair to indicate a level of method usability for the specific biotoxin and sample type for which it has been selected. The assigned tiers reflect the conservative view for DQOs involving timely implementation of methods for analysis of a high number of samples (such that multiple laboratories are necessary), and appropriate QC. The sample types reflect representative examples and are not necessarily inclusive of all sample types that might be encountered by laboratories following a contamination incident.

Tier I: The biotoxin and sample type are both targets of the method(s). Data are available for all aspects of method performance and QC measures supporting its use without modifications.

Tier II: The biotoxin is a target of the method, and the method has been evaluated by one or more laboratories. The sample type may or may not be a target of the method, and available data and/or information regarding sample preparation indicate that analyses of similar sample types were successful. However, additional testing and/or modifications may be needed.

Tier III: The sample type is not a target of the method, and no reliable data supporting the method's fitness for its intended use are available. Data suggest, however, that the method(s) may be applicable with significant modification.

Once a procedure has been identified in Appendix D, the corresponding procedure summary can be found in Section 8.2. Section 8.2 follows the organization of Appendix D, with biotoxins listed in alphabetical order and method summaries provided for each analysis type. Where available, a direct link to the references cited or to a source to obtain the reference cited is provided with the method summary. For additional information on sample preparation procedures and methods available through consensus standards organizations, please use the reference contact information provided in **Table 8-1**.

Table 8-1. Sources of Biotoxin Methods

Name	Publisher	Reference
EPA Analytical Methods	EPA Office of Water (OW) EPA NHSRC	https://www.epa.gov/homeland-security-research/sam
Food and Drug Administration (FDA), Bacteriological Analytical Manual (BAM) Online	FDA	http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm
Official Methods of Analysis of AOAC International*	AOAC International	http://www.aoac.org
American Public Health Association (APHA) Press Compendium	American Public Health Association	http://www.apha.org
Analytical Biochemistry*	Science Direct	http://www.sciencedirect.com/
Analytical Chemistry*	American Chemical Society (ACS)	http://www.acs.org/
Analytical and Bioanalytical Chemistry*	Springer	http://www.springer.com/chemistry/analytical+chemistry/journal/216
Analytical Methods*	Royal Society of Chemistry	http://www.rsc.org/journals-books-databases/about-journals/analytical-methods/
Applied and Environmental Microbiology*	American Society for Microbiology (ASM)	http://aem.asm.org/
Austin Immunology*	Austin Publishing Group	http://austinpublishinggroup.com/austin-immunology/
Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science*	Health Security	http://online.liebertpub.com/bsp
Canadian Journal of Microbiology*	NRC Research Press	http://www.nrcresearchpress.com/loi/cjm

Name	Publisher	Reference
Environmental Pollution*	Elsevier Science Publishers	https://www.journals.elsevier.com/environmental-pollution/
Environmental Science and Technology*	ACS Publications	http://pubs.acs.org/page/esthag/about.html
Food Chemistry*	Elsevier Science Publishers	https://www.journals.elsevier.com/food-chemistry
Food Research International*	Canadian Institute of Food Science and Technology	http://www.sciencedirect.com/science/journal/09639969
Forensic Science International*	Elsevier Science Publishers	https://www.journals.elsevier.com/Forensic-Science-International
Harmful Algae*	Elsevier Science Publishers	https://www.journals.elsevier.com/harmful-algae
International Journal of Food Microbiology*	Science Direct	http://www.sciencedirect.com/science/journal/01681605
Journal of Agricultural and Food Chemistry*	ACS Publications	http://pubs.acs.org/journal/jafcau
Journal of AOAC International*	AOAC International	http://aoac.org/aoac_prod_imis/AOAC/Publications/Journal_Of_AOAC/AOAC_Member/Publications/Journal_of_AOAC/The_Journal_of_AOAC.aspx
Journal of Chromatography A*	Elsevier Science Publishers	https://www.journals.elsevier.com/journal-of-chromatography-a/
Journal of Clinical Immunology	Austin Publishing Group	https://www.journals.elsevier.com/clinical-immunology
Journal of Food Protection*	International Association for Food Protection	http://www.foodprotection.org/
Journal of the Mexican Chemical Society*	Mexican Chemical Society	http://www.jmcs.org.mx/index.php/jmcs
Journal of Pharmaceutical and Biomedical Analysis*	Elsevier Science Publishers	https://www.journals.elsevier.com/journal-of-pharmaceutical-and-biomedical-analysis
Journal of the Science of Food and Agriculture*	Society of Chemical Industry	http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1097-0010/issues?year=2014
Journal of Shellfish Research*	National Shellfisheries Association	http://www.bioone.org/toc/shre/current
Letters in Applied Microbiology	Wiley Online Library	http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1472-765X
Rapid Communications in Mass Spectrometry*	John Wiley And Sons Ltd.	http://www.wiley.com/WileyCDA/WileyTitle/productCd-RCM.html
Toxicon*	Elsevier Science Publishers	http://www.journals.elsevier.com/toxicon/
Toxins*	Molecular Diversity Preservation International (MDPI)	http://www.mdpi.com/journal/toxins

* Subscription and/or purchase required.

8.1.2 General QC Guidelines for Biotoxin Methods

Public officials must have data of known and documented quality to accurately assess the activities that may be needed in remediating a site during and following emergency situations. Having such data requires that laboratories: (1) conduct the necessary QC to ensure that measurement systems are in control and operating properly; (2) properly document results of the analyses; and (3) properly document measurement system evaluation of the analysis-specific QC.[16] Ensuring data quality also requires that laboratory results are properly evaluated and the results of the data quality evaluation are included within the data report when transmitted to decision makers.

The level or amount of QC needed often depends on the intended purpose of the data that are generated, and on the need to support timely decisions. Various levels of QC may be required if the data are generated during presence/absence determinations versus confirmatory analyses. The specific needs for data generation should be identified. QC requirements and DQOs should be derived based on those needs and applied consistently across laboratories when multiple laboratories are used. For example, during rapid sample screening, minimal QC samples (e.g., blanks, replicates) and documentation might be required to ensure data quality. Sample analyses for environmental evaluation during site assessment through site clearance, such as those identified in this document, might require additional QC (e.g., demonstrations of method sensitivity, precision and accuracy). It is also important to consider that, during the course of remediation, the concentration of biotoxins and these interferences are expected to change, potentially affecting certain analytical systems. For example, some immunologically based approaches and mass spectrometer designs – due to the underlying chemistry and physics – can yield false negatives or inaccurately low results if the amount of biotoxins and/or interferences exceeds the test's design criteria. Procedural and QC steps (e.g., dilution, matrix spikes) should be applied to ensure appropriate method performance for concentratinos in sample taken throughout the remediation, including higher initial concentrations than might normally be expected in environmental samples.

The following describes a minimum set of QC samples and procedures that should be conducted for all analyses. Method-specific QC requirements may be included in some of the procedures cited in this document, and will be referenced in any EPA methods that are developed to address specific analytes and sample types of concern. Individual methods, sampling and analysis protocols, or contractual statements of work should be consulted to determine any additional QC that may be needed. QC tests should be run as frequently as necessary to ensure the reliability of analytical results. In general, sufficient QC includes an initial demonstration of measurement system capability as well as ongoing assessments to ensure the continued reliability of the analytical results.

Examples of sufficient QC for the **presumptive** tests listed in Appendix D include:

- Blanks (e.g., method blanks, matrix blanks, solvent blanks, calibration blanks, reagent blanks)
- Positive test samples / negative test samples
- Calibration check samples
- Use of test kits and reagents prior to expiration date
- Accurate temperature controls (sample and reagent storage)

Examples of sufficient QC for the **confirmatory** tests listed in Appendix D include:

- Demonstration that the measurement system is operating properly
  - Initial calibration
  - Method blanks

- Demonstration of measurement system suitability for intended use
  - Precision and recovery (verify measurement system has adequate accuracy)
  - Analyte/sample type/level of concern-specific QC samples (verify that measurement system has adequate sensitivity at levels of concern)

[16] Information regarding EPA's DQO process, considerations, and planning is available at: https://www.epa.gov/quality.

- Demonstration of continued measurement system reliability
  - Matrix spike/matrix spike duplicates (MS/MSDs) recovery and precision
  - QC samples (system accuracy and sensitivity at levels of concern)
  - Continuing calibration verification
  - Method blanks

Please note: The appropriate point of contact identified in Section 4.0 should be consulted regarding appropriate quality assurance (QA)/QC procedures prior to sample analysis. These contacts will consult with the EPA Environmental Response Laboratory Network (ERLN) and Water Laboratory Alliance (WLA) coordinators responsible for laboratory activities during the specific event to ensure QA/QC procedures are performed consistently across laboratories. EPA program offices will be responsible for ensuring that the QA/QC practices are implemented.

8.1.3 Safety and Waste Management

All appropriate safety precautions should be used during collection, processing, and analysis of environmental samples. Laboratories should have a documented health and safety plan for handling samples that may contain target chemical, biological and/or radiological (CBR) contaminants, and laboratory staff should be trained in and implement the safety procedures included in the plan. Many of the methods summarized or cited in Section 8.2 contain some specific requirements, guidelines or information regarding safety precautions that should be followed when handling or processing environmental samples and reagents. These methods also provide information regarding waste management. Additional information may be found in the following resources:

- American Biological Safety Association, Risk Group Classifications for Infectious Agents. Available at: https://my.absa.org/Riskgroups
- CDC. 2009. *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 5th Edition. Available at: http://www.cdc.gov/biosafety/publications/bmbl5/index.htm
- Fleming, D.O. and Hunt, D.L. (editors). 2006. *Biological Safety: Principles and Practices*, 4th Ed. American Society for Microbiology (ASM) Press: Herndon, VA
- CDC – Title 42 of the Code of Federal Regulations part 72 (42 CFR part 72). Interstate Shipment of Etiologic Agents
- CDC – 42 CFR part 73. Select Agents and Toxins
- Department of Transportation (DOT) – 49 CFR part 172. Hazardous Materials Table, Special Provisions, Hazardous Materials Communications, Emergency Response Information, and Training Requirements
- EPA – 40 CFR part 260. Hazardous Waste Management System: General. Available at: http://www.ecfr.gov/
- EPA – 40 CFR part 270. EPA Administered Permit Programs: The Hazardous Waste Permit Program. Available at: http://www.access.gpo.gov/nara/cfr/waisidx_07/40cfr270_07.html
- Occupational Safety and Health Administration (OSHA) – 29 CFR part 1910.1450. Occupational Exposure to Hazardous Chemicals in Laboratories. Available at: http://www.gpo.gov/fdsys/pkg/CFR-2014-title29-vol6/pdf/CFR-2014-title29-vol6-sec1910-1450.pdf
- OSHA – 29 CFR part 1910.120. Hazardous Waste Operations and Emergency Response
- U.S. Department of Agriculture (USDA) – 9 CFR part 121. Possession, Use, and Transfer of Select Agents and Toxins
- The Electronic Code of Federal Regulations (e-CFR). Available at: http://www.ecfr.gov/

8.2 Method Summaries

Summaries of the analytical methods for the biotoxins listed in Appendix D are provided in Sections 8.2.1 through 8.2.17. Each section contains a brief description of the analytical methods selected, and links to, or sources for, obtaining full versions of the methods. For information regarding sample collection considerations for samples to be analyzed by these methods, see the companion *Sample Collection Information Document for Chemicals, Radiochemicals and Biotoxins – Companion to SAM 2017*.

8.2.1 Abrin / Abrine

CAS RN (Abrin): 1393-62-0
CAS RN (Abrine): 526-31-8

Description (Abrin): Toxin found in the seeds of jequirity pea (or rosary pea) plants. Contains glycoproteins, and consists of deadenylase (25–32 kDa A chain) and lectin (35 kDa B chain); an agglutinin (A2B2) may be present in crude preparations.
Description (Abrine): Small molecule, indole alkaloid marker for abrin.

Selected Methods	Analysis Type	Analytical Technique	Section
Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science. 2014. 12(1): 49-62	Presumptive	Lateral Flow Immunoassay (LFA)	8.2.1.1
EPA/600-R-13/022. 2013. Version 1.0	Presumptive	Liquid Chromatography-Tandem Mass Spectrometry (LC-MS-MS)	8.2.1.2
Journal of Food Protection. 2008. 71(9): 1868-1874	Confirmatory	Immunoassays (Enzyme-Linked Immunosorbent Assay [ELISA] and Electrochemiluminescence [ECL])	8.2.1.3
Analytical Biochemistry. 2008. 378: 87-89	Biological Activity	Enzyme activity	8.2.1.4

8.2.1.1 Presumptive Analysis

Analytical Technique: LFA

Method Developed for: Abrin in buffer, aerosol filters, and food powders
Method Selected for: These procedures have been selected for presumptive analysis of abrin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This assay involves a lateral flow immunochromatographic device that uses two antibodies in combination to specifically detect target antigen in solution. One of the specific antibodies is labeled with a colloidal gold derivative. Samples applied to the test strips mix with the colloidal gold-labeled antibody and move along the strip membrane by capillary action. The second specific antibody captures the colloidal gold-labeled antibody and bound target. When a sufficient amount of target antigen is present, the colloidal gold label accumulates in the sample window on the test strip, forming a visible reddish-brown colored line. As an internal control, a second band in the control window indicates that the test strip functioned properly. Two bands or colored lines (in the sample and control windows) are required for a positive result determination. To complete a test on a liquid sample, the sample is mixed with the provided buffer, and five or six drops are added to the sample well of the test strip. A positive result is indicated by the appearance of a colored line in the test window of the test strip and can be read visually or with a reader.

The method source (below) describes a multicenter evaluation of the sensitivity, specificity, reproducibility, and limitations of the LFA for abrin that can be used as a rapid qualitative test to detect the presence of abrin in environmental samples. Samples analyzed in this study included various powders of food and non-food substances (prepared in buffer) and aerosol samples (filter extracts). Using the test strip and the manufacturer's recommended threshold, the estimated limit of detection (LOD) of the LFA is approximately 10ng/mL or 1.5ng/reaction, which is well below clinically relevant levels (median lethal dose [LD_{50}] 3.3mg/kg inhaled and 20mg/kg ingested). Because this assay does not discriminate between abrin and *Abrus precatorius* agglutinin-1 (APA-1), it can only be used as a qualitative screening assay when testing unknown samples.

Special Considerations: This LFA is listed as Tier I for presumptive analysis of abrin in solid and aerosol samples, and Tier II for presumptive analysis of abrin in other environmental sample types. Like some other types of immunoassays, this assay is subject to the "hook effect," which is an interference that occurs when analyte is present in amounts significantly higher than the amounts for which the assay was designed. The end result is a decreased response and, under extreme conditions, a false-negative. The incorporation of a serial dilution step in the sample protocol can eliminate such potential errors.

Source: Ramage, J.G., Prentice, K.W., Morse, S.A., Carter, A.J., Datta, S., Drumgoole, R., Gargis, S.R., Griffin-Thomas, L., Hastings, H.P., Masri, H.P., Reed, M.S., Sharma, S.K., Singh, A.K., Swaney, E., Swanson, T., Gauthier, C., Toney, D., Pohl, J., Shakamuri, P., Stuchlik, O., Elder, I.A., Estacio, P.L., Farber, E.A.E., Hojvat, S., Kellogg, R.B., Kovacs, G., Stanker, L., Weigel, L., Hodge, D.R. and Pillai, S.P. 2014. "Comprehensive Laboratory Evaluation of a Specific Lateral Flow Assay for the Presumptive Identification of Abrin in Suspicious White Powders and Environmental Samples." Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science. 12(1): 49-62.
http://online.liebertpub.com/bsp

8.2.1.2 Presumptive Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Abrine in drinking water
Method Selected for: This method has been selected for presumptive analysis of abrin by abrine detection in aerosol, solid, particulate and water samples. Abrine, an alkaloid present in equal concentrations with abrin in rosary peas (*Abrus precatorius* L.), is found in crude preparations of abrin and may be an indicator of abrin contamination. Further research is needed to adapt and verify the procedures for environmental sample types other than drinking water.

Description of Method: This method involves solid-phase extraction (SPE) of samples, followed by analysis of the extracts for abrine by liquid chromatography and LC-MS-MS. Samples are combined with isotopically-labeled internal standards and sample extracts are concentrated to dryness under nitrogen and heat, then adjusted to a 100-µL volume in high performance liquid chromatography (HPLC)-grade water. Accuracy and precision data are provided for application of the method to reagent water, as well as finished ground water and surface waters containing residual chlorine and/or chloramine. The method has a detection limit (DL) of 0.06 µg/mL and a minimum reporting level (MRL) of 0.50 µg/mL for abrine. The stability of 50 µg/mL abrine was tested in ground water and surface water samples stored at 4°C for up to 28 days. Percent recoveries of abrine were significantly reduced in chlorine-containing samples after five hours. Percent recoveries for chlorine-containing samples at five hours ranged from 9 to 21; percent recoveries at 28 days ranged from 8 (± 0) to 19% (± 1). Abrine was much more stable in monochloramine-containing samples. Percent recoveries for monochloramine-containing samples at 5 days ranged from 103 (± 3) to 107 (± 3); percent recoveries at 28 days ranged from 90 (± 4) to 93 (± 5).

Special Considerations: This method is listed as Tier I for presumptive analysis of abrin (as abrine) in drinking water and Tier II for presumptive analysis of abrin (as abrine) in all other environmental sample types. Performance data were generated using preserved water samples. If

concentrations of abrine are unexpectedly low (or absent), additional QC steps may be needed. The biotoxin methods points of contact listed in Section 4.0 should be consulted for additional information regarding analysis of sample types other than drinking water. While abrine can be used to indicate the presence of abrin, it can also be found alone, which can limit the usefulness of this determination.

Source: U.S. EPA and CDC. August 2013. "High Throughput Determination of Ricinine, Abrine, and Alpha-Amanitin in Drinking Water by Solid Phase Extraction and High Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC/MS/MS)," Version 1.0. Cincinnati, OH: EPA/Atlanta, GA: CDC. EPA 600/R-13/022.
https://nepis.epa.gov/Exe/ZyPDF.cgi/P100I5I0.PDF?Dockey=P100I5I0.PDF

Additional Resource: Knaack, J.S., Pittman, C.T., Wooten, J.V., Jacob, J.T, Magnuson, M., Silvestri, E. and Johnson, R.C. 2013. "Stability of ricinine, abrine, and alpha-amanitin in finished tap water." Analytical Methods. 20(5): 5804-5811.

8.2.1.3 Confirmatory Analysis

Analytical Technique: Immunoassay (ELISA and ECL-based immunoassay)

Method Developed for: Abrin in food
Method Selected for: These procedures have been selected for confirmatory analysis of abrin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: These commercially available immunoassays are used for detecting abrin in various food products. The procedures use mouse monoclonal antibodies (mAbs) and rabbit-derived polyclonal antibodies prepared against a mixture of abrin isozymes for three separate ELISA and ECL-based assays. The three assays vary by use of antibody combination (e.g., assay configuration): (1) polyclonal (capture)/polyclonal (detection) ELISA, (2) polyclonal/monoclonal ELISA and (3) polyclonal/monoclonal ECL assay. The LODs, with purified Abrin C and various abrin extracts in buffer, are between 0.1 and 0.5 ng/mL for all three assays. The LOD for abrin spiked into food products ranged from 0.1 to 0.5 ng/mL, using the ECL assay. The LOD for abrin spiked into food products for the ELISA assays ranged between 0.5 and 10 ng/mL depending on the antibody combination. In all cases, the LODs were less than the concentration at which abrin has been shown to pose a health concern.

Special Considerations: These procedures are listed as Tier II for confirmatory analysis of abrin in aerosol, solid, particulate and water samples. Sample preparation procedures used for foods suggest that similar aqueous extraction procedures may be applicable to environmental samples. Crude preparations of abrin may also contain agglutinins that are unique to rosary peas and that can cross-react in the immunoassays. Addition of non-fat milk powder to the sample buffer may eliminate false-positive results (Dayan-Kenigsberg, J., Bertocchi, A. and Garber, E.A.E. 2008. "Rapid Detection of Ricin in Cosmetics and Elimination of Artifacts Associated With Wheat Lectin." Journal of Immunological Methods. 336(2): 251-254.)
http://www.sciencedirect.com/science/journal/00221759

Source: Garber, E.A.E., Walker, J.L. and O'Brien, T.W. 2008. "Detection of Abrin in Food Using Enzyme-Linked Immunosorbent Assay and Electrochemiluminescence Technologies." Journal of Food Protection. 71(9): 1868-1874.
http://jfoodprotection.org/doi/abs/10.4315/0362-028X-71.9.1868

8.2.1.4 Analysis of Biological Activity

Analytical Technique: Enzyme activity

Method Developed for: Jequirity seed (abrin) and castor bean (ricin) extracts in buffer
Method Selected for: These procedures have been selected for biological activity analysis of abrin in aerosol, solid, particulate and water samples. Further research is needed to adapt and

verify the procedures for environmental sample types.

Description of Method: This *in vitro* assay is a ribonucleic acid (RNA) N-glycosidase enzyme activity assay that can be used for the detection of purified abrin or abrin in jequirity seed extracts. The method can be applied to both abrin and ricin, due to the similarity in enzyme activities of the two toxins. Synthetic biotinylated RNA substrates with varied loop sequences are cleaved by abrin toxin and the RNA products are hybridized to ruthenylated-oligodeoxynucleotides to generate an ECL signal. Assays require incubation for 2 hours at 48°C. Commercially available ECL-based reagents and ribonuclease (RNAse) inactivators are used. Control experiments for the jequirity seed experiments demonstrate lack of non-specific cleavage for the assay. The undiluted jequirity seed extract was assayed, with a resultant 21.6 ± 0.6 mg/mL total protein and 3.7 ± 0.3 µg/mL equivalents of toxin. Dilutions were performed to determine effective signal-to-background ratio and the linear range for calculation of toxin activity. Undiluted jequirity seed extract contained a calculated level of 740 ± 50 µg/mL ricin activity equivalents, which greatly exceeded the immunoassay-based value.

Special Considerations: These procedures are listed as Tier II for analysis of the biological activity of abrin in aerosol, solid, particulate and water samples. The enzyme activity assay does not test for cell binding; other cell binding assays are in development, but are not currently available. At this time, the mouse bioassay is the only readily available assay to test for both cell binding and enzymatic activity of the intact (whole) toxin.

Source: Keener, W.K., Rivera, V.R., Cho, C.R., Hale, M.L., Garber, E.A.E. and Poli, M.A. 2008. "Identification of the RNA N-glycosidase Activity of Ricin in Castor Bean Extracts by an Electrochemiluminescence-Based Assay." Analytical Biochemistry. 378(1): 87-89. http://www.sciencedirect.com/science/journal/00032697

8.2.2 Aflatoxin

CAS RN: 27261-02-5 (B1), 22040-96-6 (B2), (1385-95-1 (G1), 7241-98-7 (G2)
Considered Variants: Aflatoxins B1, B2, G1 and G2

Description: Toxin produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Composed of difuranocoumarin molecules; B-group aflatoxins (B1 and B2) have a cyclopentane ring, while the G-group aflatoxins (G1 and G2) contain a lactone ring

Selected Methods	Analysis Type	Analytical Technique	Section
AOAC Method 991.31	Presumptive and Confirmatory	Immunoaffinity (column) purification / LC-FL (detection)	8.2.2.1

8.2.2.1 Presumptive and Confirmatory Analysis

Analytical Technique: Immunoaffinity column purification and LC-fluorescence detector (FL)

Method Developed for: Aflatoxins in corn, raw peanuts and peanut butter
Method Selected for: These procedures have been selected for presumptive and confirmatory analyses of aflatoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This method involves extraction of samples with methanol/water, followed by sample filtration, dilution with water, and application to a commercially available affinity column containing mAbs specific for aflatoxins B_1, B_2, G_1 and G_2. Antibody-bound aflatoxins are eluted from the column with methanol. Reaction with bromine solution and subsequent fluorescence measurement is performed for detection and quantitation of total aflatoxins. Post-column iodine derivatization and LC-FL are performed for quantitation of individual aflatoxins. Method performance was characterized using various commodities (e.g., corn) for aflatoxin levels ranging from 10 to 30 ng/g.

Special Considerations: These procedures are listed as Tier II for presumptive and confirmatory analysis of aflatoxins in aerosol, solid, particulate and water samples. The method was originally designed for the analysis of aflatoxins (B_1, B_2, G_1 and G_2) in samples where cleanup was necessary to remove food components such as fats and proteins. Research results indicate that clean up and analyte concentration in water samples can be accomplished using an immunoaffinity column containing a mAbs specific for aflatoxin B_1, B_2, G_1 and G_2 serotypes (Paterson, 2007, see additional resource citation below). Additional research indicates that soil samples can be extracted overnight in water/ethyl acetate and the supernatant evaporated to dryness, reconstituted in methanol/water, then filtered and eluted through a mini-column packed with aluminum oxide (Accinelli, 2008, see additional resource citation below). The method notes that AOAC Official Method 994.08: Aflatoxin in Corn, Almonds, Brazil Nuts, Peanuts, and Pistachio Nuts, which uses derivatization and a multifunctional clean-up column, can be used as a complementary LC-FL procedure.

Source: AOAC International. 1994. "Method 991.31: Aflatoxins in Corn, Raw Peanuts, and Peanut Butter." *Official Methods of Analysis of AOAC International*. 16th Edition. 4th Revision; Vol. II. http://www.aoac.org/

Additional Resources:

- AOAC International. 1998. AOAC Official Method 994.08: Aflatoxin in Corn, Almonds, Brazil Nuts, Peanuts, and Pistachio Nuts. *Official Methods of Analysis of AOAC International*, 16th Edition. 4th Revision, Vol. II. http://www.aoac.org/.
- Paterson, R. R. M., Kelly, J. and Gallagher, M. 2007. "Natural occurrence of aflatoxins and *Aspergillus flavus* (Link) in water." Letters in Applied Microbiology. 25: 435-436.
- Accinelli, C., Abbas, H. K., Zablotowsicz, R. M. and Wilkinson, J. R. 2008. "*Asperigjullus flavus* aflatoxin biosynthesis genes in soil." Canadian Journal of Microbiology. 54: 371-379.

8.2.3 α-Amanitin

CAS RN: 23109-05-9
Considered Variants: NA

Description: Toxins produced by the poisonous death cap mushroom, *Amanita phalloides*. One of a group of thermostable bicyclic octapeptides

Selected Methods	Analysis Type	Analytical Technique	Section
Journal of Food Protection. 2005. 68(6): 1294–1301	Presumptive	Immunoassay (ELISA)	8.2.3.1
EPA 600/R-13/022	Confirmatory	LC-MS-MS	8.2.3.2

8.2.3.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: α-Amanitin, ricin and T-2 mycotoxin in food and beverages
Method Selected for: These procedures have been selected for presumptive analysis of α-amanitin and T-2 toxin (see Section 8.2.16.1) in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: These commercially available ELISAs detect α-amanitin in a variety of food and beverage samples at levels below those shown to be a health concern. Solid food samples are extracted with sodium phosphate buffer, then diluted with phosphate-buffered saline. Liquid beverage samples are diluted in sodium phosphate buffer. Prepared samples are analyzed according to the kit manufacturer's directions, except for the incorporation of an eight-point calibration curve and reading the plates at both 405 and 650 nm after 26 minutes of incubation at

37°C. α-Amanitin can be detected in food products at 1 µg/g. Background responses are noted for some samples, but at less than the equivalent of 0.5 µg/g for most samples.

Special Considerations: These procedures are listed as Tier II for presumptive analysis of α-amanitin in aerosol, solid, particulate and water samples. Sample preparation procedures used for foods and beverages suggest that similar procedures may be applicable to environmental samples. If appropriate concentrations are present, sample dilution can be used to minimize background interferences.

Source: Garber, E.A.E., Eppley, R.M., Stack, M.E., McLaughlin, M.A. and Park, D.L. 2005. "Feasibility of Immunodiagnostic Devices for the Detection of Ricin, Amanitin, and T-2 Toxin in Food." Journal of Food Protection. 68(6): 1294-1301.
http://jfoodprotection.org/doi/abs/10.4315/0362-028X-68.6.1294?=

8.2.3.2 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Determination of ricinine, abrine, and α-amanitin in drinking water
Method Selected for: This method has been selected for presumptive analysis of α-amanitin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for sample types other than drinking water.

Description of Method: An isotope dilution LC-MS-MS method is used for the determination of α-amanitin in drinking water. Sample aliquots are combined with mixture containing an internal quantification standard for α-amanitin, pipetted into a 96-well SPE plate, and extracted. Extracts are concentrated to dryness under nitrogen and heat, adjusted to 100 µL with HPLC-grade water, and injected onto an HPLC-MS-MS operated in MRM mode. α-Amanitin is identified by comparing the acquired mass spectra, including ion ratios and retention times, to reference spectra and retention times for calibration standards acquired under identical HPLC-MS-MS conditions. Quantitation is performed using the internal standard technique.

Special Considerations: These procedures are listed as Tier I for confirmatory analysis of α-amanitin in drinking water and Tier II for confirmatory analysis of α-amanitin in all other environmental sample types. Extraction of non-aqueous samples prior to SPE may be required (Kaya, 2013). These procedures might be modified for application to soil, air filter and particulate samples. Isotopically labeled α-amanitin internal standards were not available during method development, but efforts to synthesize these standards are in progress. The biotoxin contacts listed in Section 4.0 should be consulted for current status and availability of α-amanitin internal standards**.**

Source: U.S. EPA and CDC. August 2013. "High Throughput Determination of Ricinine, Abrine, and Alpha Amanitin in Drinking Water by Solid Phase Extraction and High Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC/MS/MS)," Version 1.0. Cincinnati, OH: EPA/Atlanta, GA: CDC. EPA 600/R-13/022.
https://nepis.epa.gov/Exe/ZyPDF.cgi/P100I5I0.PDF?Dockey=P100I5I0.PDF

Additional Resource: Kaya, E., Yilmaz, I., Sinirlioglu, Z.A., Karahan, S., Bayram, R., Yaykasli, K.O., Colakoglu, S., Saritas, A. and Severoglu, Z. 2013. "Amanitin and pallotoxin concentration in *Amanita phalloides var. alba* mushroom." Toxicon. 76: 225-233.
http://www.sciencedirect.com/science/article/pii/S0041010113003942

8.2.4 Anatoxin-a

CAS RN: 64285-06-9
Considered Variants: NA

Description: Tropane-related bicyclic alkaloid produced by a variety of cyanobacteria species

Selected Methods	Analysis Type	Analytical Technique	Section
See Source Citation	Presumptive	Immunoassay (ELISA)	8.2.4.1
EPA Method 545	Confirmatory	LC-MS-MS	8.2.4.2

8.2.4.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Anatoxin-a in drinking and recreational water
Method Selected for: This immunoassay procedure has been selected for presumptive analysis of anatoxin-a in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercially available immunoassay is a direct competitive ELISA based on the recognition of anatoxin-a by a mAbs. When present in a sample, anatoxin-a and an anatoxin-a-enzyme conjugate compete for the binding sites of mouse anti-anatoxin-a antibodies in solution. The anatoxin-a antibodies are then bound by a second antibody (anti-mouse) immobilized on the microtiter plate. After a wash step and addition of the substrate solution, a color signal is generated. The color reaction is stopped after a specified time and the color is evaluated using an ELISA reader. The intensity of the blue color is inversely proportional to the concentration of anatoxin-a present in the sample. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run. A DL of approximately 0.1 µg/L, based on anatoxin-a, is reported by the manufacturer of this kit.

Special Considerations: This commercial ELISA is currently listed as Tier III for presumptive analysis of anatoxin-a in aerosol, solid, particulate and water samples, pending results of ongoing assay verification in water samples. Please consult with the technical contacts provided in Section 4.0 for information regarding the status of verification efforts.

Source: A commercially available ELISA immunoassay kit is currently undergoing testing and verification. Updates will be provided once the results are available. Please consult technical contacts listed in Section 4.0 for information regarding the status of these results and availability of corresponding procedures.

8.2.4.2 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Anatoxin-a in potable water
Method Selected for: This method has been selected for confirmatory analysis of anatoxin-a in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than drinking water.

Description of Method: This method is used to detect anatoxin-a in water samples. Samples are frozen and thawed three times, then filtered. A 1-mL sample aliquot from the supernatant is combined with internal standards and analyzed using LC-MS-MS with ESI. Anatoxin-a is identified by comparing retention times and signals produced by unique mass to those of procedural calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined using the integrated peak area and the internal standard technique. The method reports a lowest concentration minimum reporting level

(LCMRL) of 0.018 µg/L for anatoxin-a in fortified reagent water. The working range reported in the method is 0.029–5.87 µg/L.

Special Considerations: This method is listed as Tier I for confirmatory analysis of anatoxin-a in drinking water and Tier II for confirmatory analysis of anatoxin-a in all other environmental sample types. Samples containing intact cyanobacteria must be treated to disrupt the cells in order to recover intracellular toxins. It may be possible to analyze relatively clean water samples by direct injection into the LC-MS-MS. Extraction of anatoxin-a from cyanobacterial biocrusts has been achieved (Chrapusta, 2015). These procedures might be modified for application to soil, air filter and particulate samples, for analysis using the LC-MS-MS conditions described in EPA Method 545. LC-MS-MS methods for anatoxin-a, cylindrospermopsin and microcystins also have been developed by EPA's Office of Research and Development, and are currently undergoing review. Once published, these methods will be provided on the SAM website https://www.epa.gov/homeland-security-research/sam.

Source: U.S. EPA. April 2015. Method 545: Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS). Cincinnati, OH: U.S. EPA. EPA 815-R-15-009**.** https://www.epa.gov/sites/production/files/2017-10/documents/epa_815-r-15-009_method_545.pdf

Additional Resource: Chrapusta, E., Wegrzyn, M., Zabaglo, K., Kaminski, A., Adamski, M., Wietrzyk, P. and Bialczyk, J. 2015. "Microcystins and anatoxin-a in Arctic biocrust cyanobacterial communities." Toxicon. 101: 35-40. http://www.sciencedirect.com/science/article/pii/S0041010115001130

8.2.5 Botulinum Neurotoxins (BoNTs)

CAS RN: 93384-43-1 (BoNT-A), 93384-44-2 (BoNT-B), 93384-45-3 (BoNT-C), 93384-46-4 (BoNT-D), 93384-47-5 (BoNT-E), 107231-15-2 (BoNT-F), 107231-16-3 (BoNT-G)
Considered Variants: A–G

Description: Protein neurotoxin produced by *Clostridium botulinum* and related species. Composed of ~100 kDa heavy chain and ~50 kDa light chain; can be complexed with hemagglutinin and non-hemagglutinin components for total molecular weight (MW) of ~900 kDa.

Selected Methods	Analysis Type	Analytical Technique	Section
EPA Environmental Technology Verification (ETV) Program Reports	Presumptive	Immunoassay (LFA)	8.2.5.1
Analytical Biochemistry. 2011. 411 (2): 200-209	Presumptive	Immunocapture-Forster Resonance Energy Transfer (FRET)-based activity assay	8.2.5.2
U.S. Department of Homeland Security (DHS) Report	Presumptive	Immunoassay (fluorescent bead-based)	8.2.5.3
Journal of the Science of Food and Agriculture. 2014. 94: 707–712	Presumptive	Immunoassay (ECL)	8.2.5.4
Journal of Agricultural and Food Chemistry. 2015. 63(4): 1133-1141	Confirmatory	LC-MS-MS Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF-MS)	8.2.5.5
APHA Press Compendium of Methods, Chapter 32	Biological Activity	Mouse bioassay	8.2.5.6

8.2.5.1 Presumptive Analysis

Analytical Technique: Immunoassay (LFA)

Method Developed for: BoNTs (Types A, B) in buffer or water samples
Method Selected for: These procedures have been selected for presumptive analysis of BoNTs in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water.

Description of Method: The commercial test strip is a lateral flow immunochromatographic device that uses two antibodies in combination to specifically detect target antigen in solution. One of the specific antibodies is labeled with a colloidal gold derivative. Samples applied to the test strips mix with the colloidal gold-labeled antibody and move along the strip membrane by capillary action. The second specific antibody captures the colloidal gold-labeled antibody and bound target. When a sufficient amount of target antigen is present, the colloidal gold label accumulates in the sample window on the test strip, forming a visible reddish-brown colored line. As an internal control, a second band in the control window indicates that the test strip functioned properly. Two bands or colored lines (in the sample and control windows) are required for a positive result determination. For a liquid sample, the sample is mixed with the provided buffer, and five or six drops are added to the sample well of the test strip. A positive result is indicated by the appearance of a colored line in the test window of the test strip and is read visually or with a reader.

The LFA kits have been evaluated by the EPA ETV Program for the detection of BoNTs Types A and B in concentrated (ultrafiltration [UF]) and unconcentrated drinking water. The reference source reports the lowest detectable concentration of BoNT Type A as 0.01 mg/L and Type B as 0.05 mg/L, with no false negatives detected when interferents are present. Reports and information associated with these evaluations are available at:
http://www.epa.gov/sites/production/files/2015-07/documents/etv-biothreat092104.pdf.

Special Considerations: This assay is listed as Tier I for presumptive analysis of BoNTs in drinking water samples and Tier II for presumptive analysis of BoNTs in other environmental sample types. Like some other types of immunoassays, this assay is subject to the "hook effect," which is an interference that occurs when analyte is present in amounts significantly higher than the amounts for which the assay was designed. The end result is a decreased response and, under extreme conditions, a false-negative.The incorporation of a serial dilution step can eliminate such potential errors.

Source: Environmental Technology Verification Report. 2004. Anthrax, Botulinum Toxin, and Ricin Immunoassay Test Strips; available at:
http://www.epa.gov/sites/production/files/2015-07/documents/etv-biothreat092104.pdf.

8.2.5.2 Presumptive Analysis

Analytical Technique: Immunocapture FRET-based activity assay

Method Developed for: BoNTs Serotypes A, B, D, E, F and G
Method Selected for: These procedures have been selected for presumptive analysis of BoNTs in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercial FRET-based assay detects BoNT serotypes A and E, and serotypes B, D, F and G, using separate kits for each serotype group. The assays measure the ability of BoNTs to proteolytically cleave synthetic substrates that mimic the natural BoNT substrates (SNAP25 or VAMP2) in a sensitive, FRET-based format using most standard fluorescent plate readers. The substrates used in the assay encompass both the exosite binding sites and cleavage site of BoNT, resulting in high BoNT affinity for the substrate with femtomolar to picomolar detection sensitivities within a few minutes to a few hours. The FRET-

based nature of the assays allows for real-time detection of BoNT proteolytic activity, enabling the determination of kinetic constants and enzymatic activity.

Special Considerations: This assay is listed as Tier II for presumptive analysis of BoNTs in aerosol, solid, particulate and water samples. Application of the assay to complex or dilute sample types may require a preliminary antibody capture/enrichment procedure using magnetic beads conjugated to serotype-specific antibodies. Non-liquid samples such as soils, powders and aerosol filters will require an aqueous extraction step prior to antibody capture. The FRET-based detection assay and antibody-coated beads are both commercially available.

Source: Ruge, D.R., Dunning, F.M., Piazza, T.M., Molles, B.E., Adler, M., Zeytin, F.N. and Tucker, W.C. 2011. "Detection of six serotypes of BoNT using fluorogenic reporters." Analytical Biochemistry. 411: 200-209.
https://www.ncbi.nlm.nih.gov/pubmed/21216216

8.2.5.3 Presumptive Analysis

Analytical Technique: Immunoassay (fluorescent bead-based)

Method Developed for: BoNT Serotypes A, B, C, D, E, F and G
Method Selected for: These procedures have been selected for presumptive analysis of BoNTs in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This multiplexed immunoassay is based on a commercially available technology that uses antibody-coated fluorescent polystyrene microspheres or beads as an immunoassay reaction surface. The beads are optically encoded internally with two spectrally distinct fluorescent dyes which identify each of the beads. Using a fluidics system, individual beads pass by a red laser that identifies each bead set based on their unique internal dye signatures and, hence, the antigen specificity assigned to each bead set. A green laser is used to detect a third spectrally distinct fluorescent dye that quantifies the extent of antibody-antigen reactions on the surface of each bead. Distinct bead sets, coupled with unique capture antibodies with specific reactivity to each of the seven BoNT Serotypes (A–G), are added as a mixture to an unknown sample. As specific beads contact specific antigenic components in the sample, the BoNTs are captured on the surface of the beads. The mixture is washed to remove unbound sample; then a biotin-labeled detection antibody is added and allowed to bind to the complex, followed by addition of fluorescent reporter streptavidin phycoerythrin (SA-PE). The fluorescent intensity of the reporter as read by the fluorescence reader is proportional to the amount of toxin bound to the bead. LODs ranged from 20 to 200 pg/mL for each serotype, which is similar to the definition of 1 mouse LD_{50} by the mouse bioassay.

Special Considerations: This method is listed as Tier II for presumptive analysis of BoNTs in aerosol, solid, particulate and water samples. Non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction and clarification (e.g., centrifugation) prior to assay.

Source: DHS. 2015. Multi-agency Report. Rapid Botulinum Toxin Assay Test, Evaluation and Validation Study Report. [***Note**: This document is available only to select government agencies. Please consult technical contacts listed in Section 4.0 for additional information regarding this report.*]

8.2.5.4 Presumptive Analysis

Analytical Technique: Immunoassay (ECL)

Method Developed for: BoNT A in milk products
Method Selected for: These procedures have been selected for presumptive analysis of BoNTs in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the

procedures for environmental sample types.

Description of Method: This commercial imaging-based ECL immunoassay detects BoNT-A. The imaging-based ECL detection method is a sandwich-format immunoassay that uses a target-specific immobilized capture antibody and a biotinylated detection antibody. The ECL assay uses 96-well multiarray assay plates with integrated screen-printed carbon electrodes. The electrodes act both as solid phase supports to capture reagents used in the solid phase binding assays, and as the source of electrical energy for inducing ECL. The basis of the ECL measurement is the ability of these labels to emit light when oxidized at an electrode surface in the presence of tertiary amine (i.e. tripropylamine). During the ECL measurement, the plate reader applies a voltage to electrodes in the wells of the multiarray plates and measures the resulting ECL from labeled detector antibodies incorporated in sandwich complexes on each spot in the well with a cooled charge-coupled device (CCD) sensor. The LOD of this ECL assay is 40 pg/mL for BoNT-A complex. The additional resource listed below (Cheng, L.W. and Stanker, L.H. 2013) describes the use of a similar ECL immunoassay for detection of both BoNT-A and BoNT-B in different liquids, liquified solid foods, and horse serum.

Special Considerations: This method is listed as Tier II for presumptive analysis of BoNTs in aerosol, solid, particulate and water samples. Sample preparation procedures described in both the source and additional resource citations, suggest that similar aqueous extraction may be applicable to environmental samples. Soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay.

Source: Sachdeva, A., Singh, A.K. and Sharma, S.K. 2014. "An electrochemiluminescence assay for the detection of bio threat agents in selected food matrices and in the screening of *Clostridium botulinum* outbreak strains associated with type A botulism." Journal of the Science of Food and Agriculture. 94: 707-712.
http://onlinelibrary.wiley.com/doi/10.1002/jsfa.6310/abstract

Additional Resource: Cheng, L.W. and Stanker, L.H. 2013. "Detection of Botulinum Neurotoxin Serotypes A and B Using a Chemiluminescent versus Electrochemiluminescent Immunoassay in Food and Serum." Journal of Agricultural and Food Chemistry. 61: 755-760.
https://www.ncbi.nlm.nih.gov/pubmed/23265581

8.2.5.5 Confirmatory Analysis

Analytical Technique: LC-MS-MS (BoNT Serotypes A, B, E and F) and MALDI-TOF-MS (BoNT Serotypes A–G)

Method Developed for: BoNT Serotypes A, B, C, D, E, F and G in serum, stool and food
Method Selected for: These procedures have been selected for confirmatory analysis of BoNTs in aerosol, solid, particulate and water samples. Further research is needed to validate the procedures for environmental sample types.

Description of Method: This Endopep-MS assay has been developed to detect specific activities of all seven BoNT (serotypes A–G). Peptide products are cleaved by the enzymatic action of the BoNTs on four target peptide substrates in a reaction buffer created to maximize the enzymatic activity of the BoNT toxins. An inert peptide substrate is added as the internal standard, and the reaction mixture is incubated at 37°C for a minimum of 2 hours. If present, BoNT A–G will react with the target peptides to form cleaved peptide products that can be measured by LC-MS-MS with ESI or MALDI-TOF-MS, although the source cited below evaluated application of LC-MS-MS to measurement of only A, B, E and F. For MALDI-TOF-MS analysis, the reaction is quenched by the addition of a solution of α-cyano-4-hydroxycinnamic acid (CHCA) matrix. For LC-MS-MS analysis, the reaction is quenched by adding 5 µL of 10% acetic acid to the sample. A sample aliquot is then injected into and analyzed by LC-MS-MS, or spotted onto a MALDI sample plate and analyzed by MALDI-TOF-MS.

Quantification of the toxins is performed by comparing the area ratios of the unknowns to those of calibration standards. LOD concentrations are given in units of U/mL, where 1U is defined as the enzyme catalysis of 1 micromole (µmole) of substrate per minute, and mL represents the sample volume. LODs varied for all serotypes and ranged from 1.25 to 6.25 U/mL (MALDI-TOF-MS) and 0.078 to 1.25 U/mL (LC-MS-MS) after four hours of incubation in the reaction buffer; and 0.313 to 6.25 U/mL (MALDI-TOF-MS) and 0.039 to 1.00 U/mL (LC-MS-MS) after incubation for 10 or 17 hours. The additional resource listed below (Kalb et al., 2015) describes a modified Endopep-MS assay, using antibody-based toxin capture followed by synthetic peptide cleavage and MALDI-TOF-MS detection. The MALDI-TOF-MS analysis required less than one minute to record the mass spectrum, and LODs in food matrices ranged from (depending on the serotype) 0.01 mouse lethal dose (mLD_{50}) to 0.75 mLD_{50}.

Special Considerations: This assay is listed as Tier II for confirmatory analysis of BoNTs in aerosol, solid, particulate and water samples. Only qualitative information (presence/absence) using MALDI-TOF-MS exists for BoNT-C, -D and -G, and LODs for these serotypes have not been reported using either LC-MS-MS or MALDI-TOF-MS. The sample preparation procedures used for food products also may be applicable to environmental sample types. Non-water samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay. Although procedures are not provided, the reference source notes that this method has also been used to test human stool and serum; and results indicate no false positives.

Source: Kalb, S.R., Krilich, J.C., Dykes, J.K., Luquez, C., Maslanka, S.E. and Barr, J.R. 2015. "Detection of Botulinum Toxins A, B, E, and F in Foods by Endopep-MS." Journal of Agricultural and Food Chemistry. 63(4): 1133-1141.
http://pubs.acs.org/doi/abs/10.1021/jf505482b

8.2.5.6 Analysis of Biological Activity

Analytical Technique: Mouse bioassay

Method Developed for: BoNTs in food products and clinical samples
Method Selected for: These procedures have been selected for confirmatory analysis of BoNTs in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: The mouse bioassay is the standard for government agencies testing for BoNT-containing food and clinical samples. Mice are injected with 0.5 mL sample, each sample dilution, or a heat-inactivated control sample. Mice are observed over a period of 1 to 7 days for symptoms of botulism and/or death. Results for the mouse bioassay are reported in median lethal dose (LD_{50}) units, where 1 LD_{50} is the amount of BoNT required to kill 50% of injected mice after a defined time interval. The mouse bioassay has a DL of 5 − 10 pg for BoNT serotype A. Some BoNTs are produced by non-proteolytic strains of *C. botulinum* (group II) and require trypsin activation prior to testing in the mouse bioassay. It is important to observe for symptoms of botulism in the test mice since death without clinical symptoms of botulism is not sufficient evidence that the material injected contained BoNT. BoNT typing can be conducted by injecting pairs of test mice with monovalent antitoxins prior to injecting with suspected toxin sample and noting a protective or neutralizing effect of the initial sample toxicity.

Special Considerations: This assay is listed as Tier I for analysis of the biological activity of BoNTs in aerosol, solid, particulate and water samples. It is a low-throughput, labor intensive assay and is not suitable for screening a large number of samples.

Source: Maslanka, S.E, Solomon, H.M., Sharma, S. and Johnson, E.A. 2015. "*Clostridium botulinum* and Its Toxins" APHA Press Compendium of Methods for the Microbiological Examination of Foods. Fifth Edition, Chapter 32: 397. Washington, DC: APHA Press.
https://secure.apha.org/imis/ItemDetail?iProductCode=978-087553-2738&CATEGORY=BK

8.2.6 Brevetoxins (BTX)

CAS RN: 98112-41-5 (A Form), 79580-28-2 (B Form)
Considered Variants: BTX-B1, BTX-B2, BTX-B5

Description: Suite of cyclic polyether neurotoxin compounds produced by a species of dinoflagellate, *Karenia brevis*.

Selected Methods	Analysis Type	Analytical Technique	Section
Toxicon. 2015. 96: 82-88	Presumptive	Immunoassay (ELISA)	8.2.6.1
Toxicon. 2015. 96: 82-88	Confirmatory	LC-MS	8.2.6.2

8.2.6.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Determination of brevetoxins (BTX) in shellfish and salt water
Method Selected for: These procedures have been selected for presumptive analysis of brevetoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercially available ELISA is used for determination of brevetoxin in shellfish and sea water. It is a direct competitive assay in which the antibody is directed against the brevetoxin B-type backbone, responding to B-type brevetoxin analogues with intact epitopic region. Brevetoxin in the sample and brevetoxin conjugated to peroxidase enzyme compete for the binding sites of sheep antibrevetoxin antibodies that have been immobilized in the wells of the microtiter plate. BTX-3 is used as the standard and composite toxins are expressed as mg BTX-3 equivalents. Results are determined by measuring absorbance at 450 nm using a microplate ELISA photometer. The LOD was calculated as 0.05 ng/mL in water. The concentration of residue necessary to cause 50% inhibition is approximately 0.16 ng/mL.

Special Considerations: This ELISA is listed as a Tier II procedure for presumptive analysis of brevetoxin in aerosol, solid, particulate and water samples. Sample preparation procedures used for shellfish and sea water suggest that similar extraction procedures may be applicable to environmental samples. Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, interferences caused by matrix effects cannot be completely excluded.

Source: Abraham, A., El Said, K.R., Wang, Y., Jester, E.L.E., Plakas, S.M., Flewelling, L.J., Henry, M.S., Pierce, R.H. 2015. "Biomarkers of brevetoxin exposure and composite toxin levels in hard clam (Mercenaira sp.) exposed to Karenia brevis blooms." Toxicon. 96: 82-88. https://www.ncbi.nlm.nih.gov/pubmed/25620222

8.2.6.2 Confirmatory Analysis

Analytical Technique: LC-MS

Method Developed for: Determination of brevetoxins in shellfish
Method Selected for: These procedures have been selected for confirmatory analysis of brevetoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This method involved extraction of clam tissue homogenates with either acetone or 80% methanol in water. Extracts are then defatted using 95% n-hexane, followed by SPE using a C_{18} column. The SPE eluent is evaporated to dryness under nitrogen,

redissolved in methanol at a concentration of 0.5 g tissue/mL of solution, filtered through a syringe filter and analyzed by LC-MS equipped with a C_8 column.

Special Considerations: This method is listed as a Tier II procedure for confirmatory analysis of brevetoxin in aerosol, solid, particulate and water samples. The procedures described in the source method are for extraction of clam homogenates (Abraham, 2015). Additional resource citation listed below (Abraham, 2006) includes extraction procedures for aerosol filters, shellfish, laboratory cultures and natural blooms of *Karenia brevis,* which may be applicable to water, soil, aerosol filter and particulate sample types.

Source: Abraham, A., El Said, K.R., Wang, Y., Jester, E.L.E., Plakas, S.M., Flewelling, L.J., Henry, M.S. and Pierce, R.H. 2015. "Biomarkers of brevetoxin exposure and composite toxin levels in hard clam (Mercenaira sp.) exposed to Karenia brevis blooms." Toxicon. 96: 82-88. https://www.ncbi.nlm.nih.gov/pubmed/25620222

Additional Resource: Abraham, A., Plakas, S.M., Wang, Z., Jester, E.L.E., El Said, K.R., Granade, H.R., Henry, M.S., Blum, P.C., Pierce, R.H. and Dickey, R.W. 2006. "Characterization of polar brevetoxin derivatives isolated from *Karenia brevis* cultures and natural blooms." Toxicon. 48: 104-115. http://www.sciencedirect.com/science/article/pii/S0041010106001632

8.2.7 Cylindrospermopsin

CAS RN: 143545-90-8
Considered Variants: NA

Description: Polycyclic uracil derivative containing guanidino and sulfate groups produced by a variety of freshwater cyanobacteria.

Selected Methods	Analysis Type	Analytical Technique	Section
Environ. Sci. Technol. 44: 7361-7368	Presumptive	Immunoassay (ELISA)	8.2.7.1
EPA Method 545	Confirmatory	LC-MS-MS	8.2.7.2

8.2.7.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Cylindrospermopsin in ground water, surface water and well water
Method Selected for: These procedures have been selected for presumptive analysis of Cylindrospermopsin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Cylindrospermopsin is detected using a commercially available colorimetric immunoassay (competitive ELISA) procedure. Cyanobacterial cells in the sample are lysed by three sequential freeze-thaw cycles to allow determination of total toxin. Sample (0.05 mL), enzyme conjugate (cylindrospermopsin-horseradish peroxidase [HRP]), and an antibody solution containing rabbit anti-cylindrospermopsin antibodies are added to plate wells containing immobilized sheep anti-rabbit antibodies. Both the cylindrospermopsin (if present) in the sample and cylindrospermopsin-HRP conjugate compete in solution to bind to the rabbit anti-cylindrospermopsin antibodies in proportion to their respective concentrations. After incubation, the unbound molecules are washed and decanted. A specific substrate is then added which is converted from a colorless to a blue solution by the HRP enzyme conjugate solution. The reaction is terminated with the addition of a dilute acid. The concentration of cylindrospermopsin in the sample is determined photometrically by comparing sample absorbance to the absorbance of

calibrators at a specific wavelength (450 nm). The applicable concentration range is 0.4–2.0 µg/L, with a minimum detection level of 0.4 µg/L.

Special Considerations: This method is listed as Tier II for presumptive analysis of cylindrospermopsin in aerosol, solid, particulate and water samples. The source citation listed below (Graham, 2010) details the use of this ELISA for assessing cylindrospermopsin in naturally occurring freshwater cyanobacterial blooms. Non-aqueous samples will require an aqueous extraction procedure prior to assay. Samples containing intact cyanobacteria must be treated to disrupt the cells in order to recover intracellular toxins.

Source: Graham, J.L., Loftin, K.A., Meyer, M.T. and Ziegler, A.C. 2010. "Cyanotoxin Mixtures and Taste-and-Odor Compounds in Cyanobacterial Blooms from the Midwestern United States." Environmental Science and Technology. 44: 7361-7368. (***Note***: Descriptive lake data, analytical details for LC/MS/MS, cyanobacterial community composition data, and dissolved toxin data are available at: http://pubs.acs.org/.)

8.2.7.2 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Cylindrospermopsin in potable water
Method Selected for: This method has been selected for confirmatory analysis of cylindrospermopsin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than drinking water.

Description of Method: This method is used for detection of cylindrospermopsin in water samples. Samples are frozen and thawed three times, then filtered. A 1-mL sample aliquot from the supernatant is combined with internal standards and analyzed using LC-MS-MS with ESI. Cylindrospermopsin is identified by comparing retention times and signals produced by unique mass transitions to those of procedural calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined using the integrated peak area and the internal standard technique. The method reports an LCMRL of 0.063 µg/L for anatoxin-a in fortified reagent water. The working range reported in the method is 0.050–10.0 µg/L.

Special Considerations: This method is listed as Tier I for confirmatory analysis of cylindrospermopsin in drinking water and Tier II for confirmatory analysis of cylindrospermopsin in all other environmental sample types. Non-aqueous samples will require additional extraction procedures. SPE also may be needed for additional cleanup and concentration of these sample types. Extraction of cylindrospermopsin from ground up cyanobacterial mat material has been achieved (Wood, 2008). These procedures might be modified for analysis of soil, air filter and particulate samples using the LC-MS-MS conditions described in this method. LC-MS-MS methods for anatoxin-a, cylindrospermopsin and microcystins also have been developed by EPA's Office of Research and Development, and are currently undergoing review. Once published, these methods will be provided on the SAM website https://www.epa.gov/homeland-security-research/sam.

Source: U.S. EPA. April 2015. Method 545: Determination of Cylindrospermopsin and Anatoxin-a in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS). Washington, DC: U.S. EPA. EPA 815-R-15-009**.** https://www.epa.gov/sites/production/files/2017-10/documents/epa_815-r-15-009_method_545.pdf **Additional Resource:** Wood, S., Mountfourt, D., Selwood, A., Holland, P., Puddick, J. and Cary, S. 2008. "Widespread Distribution and Identification of Eight Novel Microcystins in Antarctic Cyanobacterial Mats." Applied Environmental Microbiology. 74(23): 7243-7251. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2592942/

8.2.8 Diacetoxyscirpenol (DAS)

CAS RN: 2270-40-8
Considered Variants: NA

Description: Trichothecene mycotoxin produced by *Fusarium* spp.

Selected Methods	Analysis Type	Analytical Technique	Section
International Journal of Food Microbiology. 1988. 6(1): 9-17	Presumptive	Immunoassay (ELISA)	8.2.8.1
Food Research International. 2015. 72: 247-255	Confirmatory	LC-MS-MS	8.2.8.2

8.2.8.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: DAS in food
Method Selected for: These procedures have been selected for presumptive analysis of DAS in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This ELISA is used for the detection of DAS in food samples. Antibodies against DAS are obtained after immunization of rabbits with DAS-hemiglutarate-human serum albumin (DAS-HG-HSA), and a DAS-hemisuccinate-HRP conjugate (DAS-HS-HRP) is prepared by an ester method for use as enzyme-labeled toxin in the competitive assay. The DL for DAS using this assay is approximately 10 pg/mL. This assay will cross-react with related toxins.

Special Considerations: This immunoassay is listed as Tier III for presumptive analysis of DAS in aerosol, solid, particulate and water samples due to concerns regarding the availability of the rabbit polyclonal antibodies and DAS enzyme conjugate. Efforts to identify and verify a readily available immunoassay for analysis of DAS in environmental sample types are ongoing. Please consult the technical contacts listed in Section 4.0 for information regarding the status of these efforts.

Source: Klaffer, U., Martlbauer, E. and Terplan, G. 1988. "Development of a Sensitive Enzyme-Linked Immunosorbent Assay for the Detection of Diacetoxyscirpenol." International Journal of Food Microbiology. 6(1): 9-17.
http://www.sciencedirect.com/science/article/pii/0168160588900797

8.2.8.2 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Mycotoxins in brewed coffee
Method Selected for: These procedures have been selected for confirmatory analysis of DAS in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This liquid-liquid extraction procedure using either triple quadrupole or ion trap LC-MS/MS is used for determination of DAS in brewed coffee. Samples are first clarified with a diluted Carrez solution and filtration, extracted, and evaporated to dryness. The residue is reconstituted, filtered, and analyzed by LC-MS-MS. The limits of detection using an ion trap and triple quadrupole are 1.38 and 18.70 µg/kg, respectively. The limits of quantification using an ion trap and triple quadrupole are 5.99 and 39.64, respectively.

Special Considerations: This method is listed as Tier II for all confirmatory analysis of DAS in aerosol, solid, particulate and water samples. The procedures described will need to be modified

for water, soil, air filter and particulate samples. Non-aqueous samples will require additional extraction, cleanup and concentration procedures (e.g., SPE). Non-drinking water samples or water samples containing low concentrations of DAS may require additional concentration and clean up.

Source: García-Moraleja, A., Font, G., Mañes, J. and Ferrer, E. 2015. "Development of a new method for the simultaneous determination of 21 mycotoxins in coffee beverages by liquid chromatography tandem mass spectrometry." Food Research International. 72: 247-255. http://www.sciencedirect.com/science/journal/09639969/72

8.2.9 Domoic Acid (DA)

CAS RN: 14277-97-5
Considered Variants: NA

Description: Water-soluble, nonprotein amino acid neurotoxin produced by microscopic algae, specifically the diatom species *Pseudo-nitzschia.* Composed of a proline ring, one imino group and three carboxyl groups

Selected Methods	Analysis Type	Analytical Technique	Section
Journal of AOAC International. 2007. 90(4): 1011-1027	Presumptive	Immunoassay (ELISA)	8.2.9.1
Journal of Shellfish Research. 2008. 27(5): 1301-1310	Presumptive	Immunoassay (ELISA)	8.2.9.2
Journal of the Mexican Chemical Society. 2011. 55(2): 65-71	Confirmatory	Liquid chromatography-ultraviolet detection (LC-UV)	8.2.9.3

8.2.9.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Determination of DA in shellfish
Method Selected for: These procedures have been selected for presumptive analysis of DA in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercially available ELISA assay is used to determine DA in shellfish. The ELISA assay is a direct competitive format, where free DA in the sample competes with DA-conjugated protein coated on plastic wells for binding to anti-DA antibodies free in the solution. Resulting color intensity is measured spectrophotometrically on a plate reader at 450 nm, and is inversely proportional to the concentration of DA in the sample solution. The assay is calibrated using dilutions of a DA standard. The LOD for shellfish extracts is 0.01 mg/kg. Based on the results of a collaborative study (Kleivdal, 2007), the ELISA kit is under consideration as an official AOAC method.

Special Considerations: These procedures are listed as Tier II for presumptive analysis of DA in aerosol, solid, particulate and water samples. The methanol/water extraction method used for shellfish may be applicable to non-aqueous environmental sample types. Liquid samples may only require dilution prior to assay while those containing intact organisms (e.g., phytoplankton), may require cell disruption prior to dilution and assay. Results of a multi-laboratory study are described in the source reference (Kleivdal, 2007) and indicate that the ELISA method may slightly overestimate DA levels in shellfish when compared to LC-MS (see additional resource citation, Quilliam, 1995).

Source: Kleivdal, H., Kristiansen, S.I., Nilsen, M.V., Goksoyr, A., Briggs, L., Holland, P. and

McNabb, P. 2007. "Determination of Domoic Acid Toxins in Shellfish by Biosense ASP ELISA – A Direct Competitive Enzyme-Linked Immunosorbent Assay: Collaborative Study." Journal of AOAC International. 90(4): 1011-1027.
http://www.ingentaconnect.com/content/aoac/jaoac/2007/00000090/00000004/art00016

Additional Resource: Quilliam, M.A., Xie, M. and Hardstaff, W.R. 1995. "Rapid extraction and cleanup for liquid chromatographic determination of domoic acid in unsalted seafood." Journal of AOAC International. 78(2): 543-554.
http://www.aoac.org/aoac_ref_imis/AOAC_Member/PUBSCF/JAOACCF/JN_M.aspx?&Journal_Information=2

8.2.9.2 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: DA in shellfish
Method Selected for: These procedures have been selected for presumptive analysis of DA in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercially available ELISA test kit is used for detecting DA using a mAbs. The sequential competitive ELISA gives equivalent results to those obtained using standard HPLC, fluorenylmethoxycarbonyl HPLC, or liquid chromatography-mass spectrometry (LC-MS) methods. It has a linear range from 0.1 to 3 ppb and was used to measure DA in razor clams, mussels, scallops, and phytoplankton. The assay requires approximately 1.5 h to complete and has a standard 96-well format.

Special Considerations: These procedures are listed as Tier II for presumptive analysis of DA in aerosol, solid, particulate and water samples. The methanol/water extraction method used for shellfish may be applicable to non-aqueous environmental sample types. Liquid samples may only require dilution prior to assay while those containing intact organisms (e.g., phytoplankton), may require cell disruption prior to dilution and assay.

Source: Litaker, R.W., Stewart, T. N., Eberhart, B-T. L., Wekell, J.C., Trainer, V.L., Kudela, R.M., Miller, P.E., Roberts, A., Hertx, C., Johnson, T.A., Frankfurter, G., Smith, G.J., Schnetzer, A., Schumacker, J., Bastian, J.L., Odell, A., Gentien, P., Le Gal, D., Hardison, D.R. and Tester, P.A. 2008. "Rapid Enzyme-Linked Immunosorbent Assay for Detection of the Algal Toxin Domoic Acid." Journal of Shellfish Research. 27(5): 1301-1310.
http://www.bioone.org/doi/abs/10.2983/0730-8000-27.5.1301

8.2.9.3 Confirmatory Analysis

Analytical Technique: LC-Ultraviolet Detection (UV)

Method Developed for: DA in seawater
Method Selected for: These procedures have been selected for confirmatory analysis of DA in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This modified LC-UV detection procedure is based on a previous study (Mafra, 2009), and was used to detect DA in seawater. An on-line SPE step was added to the off-line SPE step in order to allow for the use of larger sample volumes to increase sensitivity. Off-line SPE was performed using a C_{18} column. On-line SPE is performed on the diluted sample extract using a polymeric reversed phase (PLRP-S) pre-column for sample concentration, followed by elution onto a reverse-phase chromatography column. Detection is performed using a variable wavelength UV detector set at 239 nm. The method detection limit (MDL) for DA in synthetic saline water is estimated to be 0.04 µg/L.

Special Considerations: This method is listed as Tier II for confirmatory analysis of DA in aerosol, solid, particulate and water samples. The procedures described in this method may need to be modified for water, soil, air filter and particulate samples. The use of LC-MS (Tor, 2003) and LC-MS-MS (Barbaro, 2013) might provide additional sensitivity without requiring an online SPE step. It may be possible to analyze relatively clean water samples by direct injection into the LC-MS/MS; however, dirty water samples or water samples with low concentrations of DA may require cleanup and concentration.

Source: Vera-Avila, L.E., Marin-Perez, D.Y. and Covarrubias-Herrera, R. 2011. "Trace Level Determination of Domoic Acid in Seawater by Off-line/on-line Solid-phase Extraction Coupled to HPLC-UV." Journal of the Mexican Chemical Society. 55(2): 65-71. http://www.scielo.org.mx/pdf/jmcs/v55n2/v55n2a1.pdf

Additional Resources:

- Mafra Jr., L.L. Léger, C., Bates, S.S. and Quilliama, M.A. 2009. "Analysis of trace levels of domoic acid in seawater and plankton by liquid chromatography without derivatization, using UV or mass spectrometry detection." Journal of Chromatography A. 1216: 6003-6011. http://www.sciencedirect.com/science/article/pii/S0021967309009303?np=y&npKey=bd9c944498548a090bb6e1724cdf4d78a00bc2512277155380318615837 31ea7
- Tor, E.R., Puschner, B. and Whitehead, W.E. 2003. "Rapid Determination of Domoic Acid in Serum and Urine by Liquid Chromatography-Electrospray Tandem Mass Spectrometry." Journal of Agriculture and Food Chemistry. 51: 1791-1796. http://pubs.acs.org/doi/abs/10.1021/jf020947f
- Barbaro, E., Zangrando, R, Rossi, S., Cairns, W.R.L., Piazza, R., Corami, F., Barbante, C. and Gambaro, A. 2013. "Domoic acid at trace levels in lagoon waters: assessment of a method using internal standard quantification." Analytical and Bioanalytical Chemistry. 405(28): 9113-9123. http://link.springer.com/article/10.1007/s00216-013-7348-5

8.2.10 Microcystins

CAS RNs: 96180-79-9 (LA), 154037-70-4 (LF), 101043-37-2 (LR), 123304-10-9 (LY), 111755-37-4 (RR), 101064-48-6 (YR)
Considered Variants: LA, LF, LR, LY, RR, YR

Description: Cyclic heptapeptide hepatotoxins produced by a variety of cyanobacteria

Selected Methods	Analysis Type	Analytical Technique	Section
EPA Method 546	Presumptive	Immunoassay (ELISA)	8.2.10.1
EPA Method 544	Confirmatory	LC-MS-MS	8.2.10.2

8.2.10.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Microcystins (MC) in water
Method Selected for: This method has been selected for presumptive analysis of microcystins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: EPA Method 546 determines total microcystins (MC) and nodularins (NOD) in finished drinking water and in ambient water using a commercially available indirect competitive ELISA. The term "total microcystins and nodularins" is defined as the sum of the congener-independent, intracellular and extracellular microcystins and nodularins that are measurable in a sample. Cyanobacterial cells in the sample are lysed by three sequential freeze-

thaw cycles to allow determination of total toxin, and the total concentration of toxin is measured based on detection of a characteristic feature common to microcystin and nodularin congeners (structural variants), specifically, the Adda amino acid side chain (see Section 1.1 of Method 546). To ensure comparability between laboratories, the ELISA is calibrated against one congener, MC-LR. The minimum reporting level (MRL) for MC-LR is 0.30 µg/L. The method describes required laboratory QC and demonstration of capability procedures.

Special Considerations: This method is listed as Tier I for presumptive analysis of microcystins in water samples and Tier II for presumptive analysis of microcystins in all other environmental sample types. Non-aqueous samples will require aqueous extraction prior to analysis. Samples containing intact cyanobacteria must be treated to disrupt the cells in order to recover intracellular MC.

Source: Zaffiro, A., Rosenblum, L. and Wendelken, S. August 2016. Method 546: Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay. Washington, DC: U.S. EPA. https://www.epa.gov/sites/production/files/2016-09/documents/method-546-determination-total-microcystins-nodularins-drinking-water-ambient-water-adda-enzyme-linked-immunosorbent-assay.pdf

8.2.10.2 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Determination of microcystins and nodularin in drinking water
Method Selected for: This method has been selected for confirmatory analysis of microcystins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than drinking water.

Description of Method: Method 544 determines six microsytins (including MC-LR) and nodularin in drinking water using SPE and LC-MS-MS. Samples are fortified with a surrogate, then filtered, and the filter is placed in a methanol solution to release toxin. The filtered sample and methanol solution are combined, passed through an SPE cartridge, eluted, evaporated to dryness, and redissolved in methanol solution. A 10-µL aliquot of extracted sample is analyzed by LC-MS-MS equipped with a C_8 column. Microcystins are identified by comparing the acquired mass spectra and retention times to those of calibration standards acquired under identical LC-MS-MS conditions. The concentration of each analyte is determined by external standard calibration. DLs for analytes in this method range from 1.2 to 4.6 ng/L.

Special Considerations: This method is listed as Tier I for confirmatory analysis of microcystins in drinking water, Tier II for confirmatory analysis of microcystins in non-drinking water, and Tier III for confirmatory analysis of microcystins in all other environmental sample types. Non-aqueous samples will require aqueous extraction prior to analysis. For example, a procedure resulting in 90% recovery of microcystins in soil and sediment has been reported by Chen, 2006 (see additional resource citation below). These procedures may need to be modified for application to air filters and particulates and for analysis using the LC-MS-MS conditions described in the EPA method selected for confirmatory analysis of these toxins. It may be possible to analyze relatively clean water samples for extracellular toxins by direct injection into an LC-MS-MS; however, dirty water samples or water samples with low concentrations of toxin may require cleanup and concentration using SPE. LC-MS-MS methods for anatoxin-a, cylindrospermopsin and microcystins have been developed by EPA's Office of Research and Development, and are currently undergoing review. Once published, these methods will be provided on the SAM website https://www.epa.gov/homeland-security-research/sam.

Source: Shoemaker, J., Tettenhorst, D. and Delacruz, A. 2015. Method 544: Determination of Microcystins and Nodularin in Drinking Water by Solid Phase Extraction and Liquid

Chromatography/Tandem Mass Spectrometry (CL/MS/MS), Version 1.0. Cincinnati, OH: EPA. EPA/600/R-14/474. https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=306953

Additional Resource: Chen, W., Li, L., Gan, N. and Song, L. 2006. "Optimization of an effective extraction procedure for the analysis of microcystins in soils and lake sediments." Environmental Pollution. 143: 241–246. http://www.sciencedirect.com/science/article/pii/S0269749105006172

8.2.11 Picrotoxin

CAS RN (Picrotoxin): 124-87-8
CAS RN (Picrotin): 21416-53-5
CAS RN (Picrotoxinin): 17617-45-7
Considered Variants: NA

Description: Alkaloid toxin produced by the climbing plant, *Anamirta cocculus*, and consisting of picrotin and picrotoxinin.

Selected Methods	Analysis Type	Analytical Technique	Section
To be determined (TBD)	Presumptive	TBD	NA
Journal of Pharmaceutical and Biomedical Analysis. 1989. 7(3): 369-375	Confirmatory	LC-UV	8.2.11.1

8.2.11.1 Confirmatory Analysis

Analytical Technique: LC-UV

Method Developed for: Picrotoxin in serum
Method Selected for: These procedures have been selected for confirmatory analysis of picrotoxin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Picrotoxin (picrotin and picrotoxinin) is quantified in serum by reversed phase HPLC. Serum samples are prepared by washing with *n*-hexane, followed by extraction with chloroform. The chloroform is evaporated and the sample is reconstituted in acetonitrile-1 mM ammonium acetate buffer (pH 6.4) 34:66 (v/v) for assay. The effluent is monitored at 200 nm, and quantification is based on peak-height ratio of analyte to the internal standard. A linear response is obtained for both analytes (picrotin and picrotoxinin) in the range of 0.2 to 20.0 µg/mL with mean recoveries > 94.2%. Increased sensitivity may be possible using LC-TOF-MS analyses (Ogawa, 2016).

Special Considerations: This method is listed as Tier II for confirmatory analysis of picrotoxin in aerosol, solid, particulate and water samples. The procedures described may need to be modified for water, soils, air filters and particulates, such as use of agitation (e.g., shaking, ultrasonication). The extraction solvent specified (chloroform) may be suitable for these sample types, but has not been evaluated. Extracts also may require cleanup and concentration by SPE. Although it may be possible to analyze relatively clean water samples by direct injection into a reversed-phase LC, dirty water samples or water samples with low levels of picrotoxin may require cleanup and concentration using SPE.

Source: Soto-Otero, R., Mendez-Alvarez, E., Sierra-Paredes, G., Galan-Valiente, J., Aguilar-Veiga, E. and Sierra-Marcuno, G. 1989. "Simultaneous Determination of the Two Components of Picrotoxin in Serum by Reversed-Phase High-Performance Liquid Chromatography With Application to a Pharmacokinetic Study in Rats." Journal of Pharmaceutical & Biomedical Analysis. 7(3): 369-375. http://www.sciencedirect.com/science/article/pii/0731708589801049

Additional Resource: Ogawa, T., Tada, M., Hattori, H., Shiraishi, Y., Suzuki, T., Iwai, M., Kusano, M., Zaitsu, K., Ishii, A. and Seno, H. May 2016. "Sensitive determination of picrotoxin by liquid chromatography-quadrupole time-of-flight mass spectrometry." Letter to the Editor. Legal Medicine. 20: 8-11. http://www.legalmedicinejournal.com/article/S1344-6223(16)30018-9/abstract

8.2.12 Ricin (Ricinine)

Ricin – CAS RN: 9009-86-3.
Description: Toxic lectin (carbohydrate-binding protein) found in the seeds of the castor oil plant, *Ricinus communis*. 60 kDa glycoprotein consisting of a deadenylase (~32 kDa A chain) and lectin (~34 kDa B chain); an agglutinin of MW 120 kDa may be present in crude castor bean preparations.

Ricinine – CAS RN: 5254-40-3.
Description: Small molecule, alkaloid marker for ricin.

Selected Methods	Analysis Type	Analytical Technique	Section
Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science. 2013. 11 (4): 237−250	Presumptive	LFA	8.2.12.1
Journal of Food Protection. 2005. 68(6): 1294–1301	Presumptive	Immunoassay (ELISA)	8.2.12.2
Journal of AOAC International. 2008. 91(2): 376−382	Presumptive	Immunoassay (ECL)	8.2.12.3
EPA 600/R-13/022 (EPA/CDC)	Presumptive	LC-MS-MS	8.2.12.4
Analytical Chemistry, 2011. 83: 2897−2905	Confirmatory	Immunocapture / LC-MS-MS	8.2.12.5
Analytical Chemistry, 2011. 83: 2897−2905	Biological Activity	Immunocapture / MALDI-TOF-MS	8.2.12.6

8.2.12.1 Presumptive Analysis

Analytical Technique: LFA

Method Developed for: Ricin in buffer, food products, powders and aerosol filter extracts
Method Selected for: These procedures have been selected for presumptive analysis of ricin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This lateral flow immunochromatographic device uses two antibodies in combination to specifically detect target antigen in solution. One of the specific antibodies is labeled with a colloidal gold derivative. Samples applied to the test strips mix with the colloidal gold-labeled antibody and move along the strip membrane by capillary action. The second specific antibody captures the colloidal gold-labeled antibody and bound target. When a sufficient amount of target antigen is present, the colloidal gold label accumulates in the sample window on the test strip, forming a visible reddish-brown colored line. As an internal control, a second line in the control window indicates that the test strip functioned properly. Two colored lines (in the sample and control windows) are required for a positive result determination. To perform a test on a liquid sample, the sample is mixed with the provided buffer, and five or six drops are added to the sample well of the test strip. A positive result is indicated by the appearance of a colored line in the test window of the test strip and can be read visually or with a reader.

The source reference (below) details a multicenter evaluation of the sensitivity, specificity, reproducibility, and limitations of a LFA for ricin that can be used in the field or in the laboratory to qualitatively screen for ricin in environmental samples. Using the recommended test strip

reader, the LFA could reproducibly detect >3.6 ng ricin/mL (0.54 ng/test) in various 'white powder' samples and aerosol filter extracts. Because this assay does not discriminate among *Ricinus communis* agglutinin (RCA) 60, RCA120, and ricin A chain, it can be used only as a qualitative screening assay when testing unknown samples. The test strips have also been evaluated by the EPA ETV Program for the detection of ricin in water samples. Reports and information associated with these evaluations are in the additional resource cited below.

Special Considerations: This LFA is listed as Tier I for solid, aerosol and water samples, and Tier II for all other environmental sample types. Crude preparations of ricin may also contain agglutinins that are unique to castor beans and that can cross-react in the immunoassays. A hook effect in the response of LFAs occurs when the amount of antigen in a sample overwhelms the amount of detector antibody present in the LFA. The resultant free antigen competes with the antigen-detector antibody complex for the capture antibody, which results in a decrease in the response and, under extreme conditions, can produce false-negative results. Like some other types of immunoassays, this assay is subject to the "hook effect," which is an interference that occurs when analyte is present in amounts significantly higher than the amounts for which the assay was designed. The end result is a decreased response and, under extreme conditions, a false-negative. False-negative results due to the hook effect were not observed with the commercial LFA evaluated, although a quantitative decrease in the response has been observed at high ricin concentrations. The incorporation of a serial dilution step in the sample protocol can eliminate such potential errors.

Source: Hodge, D.R., Prentice, K.W., Ramage, J.G., Prezioso, S., Gauthier, C., Swanson, T., Hastings, R., Basavanna, U., Datta, S., Sharma, S.K., Garber, E.A.E., Staab, A., Pettit, D., Drumgoole, R., Swaney, E., Estacio, P.L., Elder, I.A., Kovacs, G., Morse, B.S., Kellogg, R.B., Stanker, L., Morse, S. and Pillai, S.P. 2013. "Comprehensive Laboratory Evaluation of a Highly Specific Lateral Flow Assay for the Presumptive Identification of Ricin in Suspicious White Powders and Environmental Samples." Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science. 11(4): 237-250.
https://www.ncbi.nlm.nih.gov/pubmed/24320219

Additional Resource: Environmental Technology Verification (ETV) Report. 2004. Anthrax, Botulinum Toxin, and Ricin Immunoassay Test Strips. Cincinnati, OH: EPA.
http://www.epa.gov/sites/production/files/2015-07/documents/etv-biothreat092104.pdf

8.2.12.2 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Ricin in various foods and beverages
Method Selected for: This method has been selected for presumptive analysis of ricin in aerosol, solid, particulate and drinking water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercial antigen-capture ELISA detects antigens in samples by capturing them between a sandwich of antibodies. Positive and negative capture antibody reagents are applied to alternating wells of a 96-well plate, where they are passively adsorbed. Sample is then applied to the wells; if the target antigen is present in the sample, it will bind to the capture antibody. A detector antibody forms the top of the sandwich and binds to any bound antigen in the sample. The conjugate, to which the enzyme is covalently bound, is the third reagent added and binds to the detector antibody. The substrate, added after the conjugate, changes color in the presence of HRP. The amount of color change is directly proportional to the amount of HRP present, which correlates to the amount of ricin. Forty-eight samples can be processed in approximately 5 hours. The reference source reports an LOD of less than or equal to 0.02 µg/g. The additional resource provided below (ETV report 2004) describes the performance of this ELISA for water samples, and reports an LOD of 0.0075 mg/L.

Special Considerations: This method is listed as Tier II for presumptive analysis of ricin in aerosol, solid, particulate and water samples. Non-liquid samples such as soils, powders, and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay.

Source: Garber, E.A., Eppley, R.M., Stack, M.E., McLaughlin, M.A. and Park, D.L. 2005. "Feasibility of Immunodiagnostic Devices for the Detection of Ricin, Amanitin, and T-2 Toxin in Food." Journal of Food Protection. 68(6): 1294-1301. http://jfoodprotection.org/doi/abs/10.4315/0362-028X-68.6.1294

Additional Resource: James, R., Dinal, A., Willenberg, Z. and Riggs, K. Environmental Technology Verification (ETV) Report. 2004. "Anthrax, Botulinum Toxin, and Ricin Enzyme-Linked Immunosorbent Assay (ELISA)." Cincinnati, OH: EPA. https://archive.epa.gov/nrmrl/archive-etv/web/pdf/01_vr_elisa.pdf

8.2.12.3 Presumptive Analysis

Analytical Technique: Immunoassay (ECL)
Method Developed for: Ricin in various foods and beverages
Method Selected for: This method has been selected for presumptive analysis of ricin in aerosol, solid, particulate and drinking water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercial ECL-based immunoassay detects ricin in various food and beverage samples. The ECL immunoassay uses 96-well plates with integrated screen-printed carbon electrodes. The electrodes act both as solid phase supports to capture reagents used in the solid phase binding assays, and as the source of electrical energy for inducing ECL. The basis of the ECL measurement is the ability of these labels to emit light when oxidized at an electrode surface in the presence of tertiary amine (i.e., tripropylamine). During the ECL measurement, the plate reader applies a voltage to electrodes in the wells of the multi-array plates and measures the resulting ECL from labeled detector antibodies incorporated in sandwich complexes on each spot in the well with a cooled CCD sensor. The reference reports LODs of 0.04 ng/mL (sequential assay protocol) and 0.2−10 ng/mL (simultaneous assay protocol).

Special Considerations: This method is listed as Tier II for presumptive analysis of ricin in aerosol, solid, particulate and water samples. Sample preparation procedures used for food and beverages suggest that similar aqueous extraction procedures may be applicable to environmental samples. Soils, powders and aerosol filters may require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay.

Source: Garber, E.A.E. and O'Brien, T.W. 2008. **"**Detection of Ricin in Food Using Electrochemiluminescence-Based Technology." Journal of AOAC International. 91(2): 376-382. https://www.ncbi.nlm.nih.gov/pubmed/18476351

8.2.12.4 Presumptive Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Ricinine in drinking water samples
Method Selected for: This method has been selected for presumptive analysis of ricin by ricinine detection in aerosol, solid, particulate and water samples. Ricinine, an alkaloid component of castor beans, is found in crude preparations of ricin and may be an indicator of ricin contamination. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This method involves sample extraction by SPE, followed by LC-MS-MS analysis, using an isocratic LC gradient and detection by ESI-MS-MS. Samples are combined with isotopically-labeled internal standards, and sample extracts are concentrated to dryness

under nitrogen and heat, then adjusted to a 100-µL volume in HPLC-grade water. Accuracy and precision data are provided for application of the method to reagent water, finished ground water and surface waters containing residual chlorine and/or chloramine. The method has DL of 0.09 µg/mL and an MRL of 0.50 µg/mL for ricinine. Storage stability was tested at 4°C for up to 28 days for ricinine (50 µg/mL in ground water and surface water containing either chlorine or monochloramine). Analyte percent recoveries at five hours ranged from 94.2% (± 2) to 110.4% (± 9.5); percent recoveries at 28 days ranged from 86% (± 10) to 92% (± 7).

Special Considerations: This method is listed as Tier I for presumptive analysis of ricin (as ricinine) in drinking water and Tier II for presumptive analysis of ricin (as ricinine) in all other environmental sample types. Non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay. While ricinine can be used to indicate the presence of ricin, it can also be found alone, which is a limitation of this method.

Source: U.S. EPA and CDC. August 2013. "High Throughput Determination of Ricinine, Abrine, and Alpha-Amanitin in Drinking Water by Solid Phase Extraction and High Performance Liquid Chromatography Tandem Mass Spectrometry (HPLC/MS/MS)," Version 1.0. Cincinnati, OH: EPA/Atlanta, GA: CDC. EPA 600/R-13/022.
https://nepis.epa.gov/Exe/ZyPDF.cgi/P100I5I0.PDF?Dockey=P100I5I0.PDF

Additional Resource: Knaack, J.S., Pittman, C.T., Wooten, J.V., Jacob, J.T, Magnuson, M., Silvestri, E. and Johnson, R.C. 2013. "Stability of ricinine, abrine, and alpha-amanitin in finished tap water." Analytical Methods. 20(5): 5804-5811.
http://pubs.rsc.org/en/content/articlelanding/2013/ay/c3ay40304a#!divAbstract

8.2.12.5 Confirmatory Analysis

Analytical Technique: Immunocapture / LC-MS-MS

Method Developed for: Ricin in beverages and tap water
Method Selected for: These procedures have been selected for confirmatory analysis of ricin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water.

Description of Method: MS is used in a two-pronged approach for detection and quantification of ricin: LC-MS-MS or multiple reaction monitoring (MRM)-MS for absolute quantification of toxin using isotope dilution MS, combined with an enzymatic assay and MALDI-TOF-MS detection to determine functional activity. Both approaches include an antibody capture step (polyclonal anti-ricin antibodies immobilized on magnetic beads) for ricin extraction. Quantitative analysis is achieved by trypsin digestion to generate peptides that are analyzed by LC-MS-MS and quantified relative to isotope internal peptide standards. Ricin can be quantified down to 10 fmol/mL in tap water, milk, apple juice and orange juice.

Special Considerations: These procedures are listed as Tier I for confirmatory analysis of ricin in drinking water and Tier II for confirmatory analysis of ricin in all other environmental sample types. Non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay. Some information regarding the analysis of ricin in supernatants obtained from wipe samples is provided in the additional resource cited below. The quantification assay and enzymatic activity assay can be run either in parallel (to return results faster when sample is plentiful), or the quantification can be performed on ricin-bound beads after the enzymatic activity has been assessed (when sample is limited). It should be noted that the antibody capture step does not distinguish between ricin A chain and intact ricin (both A and B chains).

Source: McGrath, S.C., Schieltz, D.M., McWilliams, L.G., Pirkle, J.L. and Barr, J.R. 2011. "Detection and Quantification of Ricin in Beverages Using Isotope Dilution Tandem Mass Spectrometry." Analytical Chemistry. 83: 2897-2905.
http://pubs.acs.org/doi/abs/10.1021/ac102571f

8.2.12.6 Analysis of Biological Activity

Analytical Technique: Immunocapture / MALDI-TOF-MS

Method Developed for: Ricin activity in beverages and tap water
Method Selected for: These procedures have been selected for biological activity analysis of ricin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water.

Description of Method: MS is used in a two-pronged approach for detection and quantification of ricin: LC-MS-MS for absolute quantification of toxin, combined with an enzymatic assay and MALDI-TOF-MS detection to determine functional activity. The MS measurement of ricin's deadenylase activity is conducted using immunocaptured ricin. The magnetic bead/ricin complex is incubated with a synthetic deoxyribonucleic acid (DNA) mimic of the toxin's natural ribosomal ribonucleic acid (rRNA) target, and the product of the reaction is detected by MALDI-TOF-MS. Ricin deadenylase activity is determined by MALDI-TOF detection of the DNA oligomer reaction product. The immunopurification step prior to MS analysis allows application to complex matrices. The DL is approximately 1 pmol/mL (64 ng/mL) for the immunopurified ricin, using 500 pmol of DNA substrate and a reaction time of between 4–6 hours.

Special Considerations: These procedures are listed as Tier I for analysis of the biological activity of ricin in drinking water and Tier II for analysis of the biological activity of ricin in all other environmental sample types. Non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay. The additional resource cited below describes procedures for MALDI-TOF-MS analysis of ricin in supernatants obtained from wipe samples. Neither the antibody capture step or the deadenylase activity assay distinguish between ricin A chain and intact ricin (both A and B chains).

Source: McGrath, S.C., Schieltz, D.M., McWilliams, L.G., Pirkle, J.L. and Barr, J.R. 2011. "Detection and Quantification of Ricin in Beverages Using Isotope Dilution Tandem Mass Spectrometry." Analytical Chemistry. 83: 2897-2905.
http://pubs.acs.org/doi/abs/10.1021/ac102571f

Additional Resource: Schieltz, D.M., McGrath, S.C., McWilliams, L.G., Rees, J., Bowen, M.D., Kools, J.J., Dauphin, L.A., Gomez-Saladin, E., Newton, B.N., Stang, H.L., Vick, M.J., Thomas, J., Pirkle, J.L. and Barr, J.R. 2011. "Analysis of active ricin and castor bean proteins in a ricin preparation, castor bean extract, and surface swabs from a public health investigation." Forensic Science International. 209: 70-79.
http://www.sciencedirect.com/science/article/pii/S0379073810005554

8.2.13 Saxitoxins

CAS RNs: 35523-89-8 (STX) 64296-20-4 (NEO), 58911-04-9 (dcSTX), 68683-58-9 (dcNEOSTX), 143084-69-9 (doSTX), 77462-64-7 (GTX), 122075-86-9 (dcGTX)
Considered Variants: Saxitoxins (STX), Neosaxitoxins (NEO), Gonyautoxins (GTX)

Description: A suite of more than 50 structurally related neurotoxins produced by algae and cyanobacteria, including STX, NEO, GTX and decarbamoylsaxitoxin (dcSTX). Composed of 3,4-propinoperhydropurine tricyclic carbamates

Selected Methods	Analysis Type	Analytical Technique	Section
AOAC Method 2011.27	Presumptive	Receptor Binding Assay (RBA)	8.2.13.1
Toxicon. 2009. 54: 313-320	Presumptive	Immunoassay (ELISA)	8.2.13.2
Harmful Algae. 2016. 77-90	Presumptive	Immunoassay (ELISA)	8.2.13.3
J. Chromatogr. A. 2015. 1387: 1-12	Confirmatory	LC-MS-MS	8.2.13.4

8.2.13.1 Presumptive Analysis

Analytical Technique: RBA

Method Developed for: Determination of paralytic shellfish toxins (PSTs) in shellfish
Method Selected for: This method has been selected for presumptive analysis of saxitoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: An RBA is used to determine saxitoxins in shellfish tissue homogenates. The procedure is a competitive binding assay in which [^{3}H] STX competes with unlabeled STX standards or samples containing PSTs for a finite number of receptor sites in a rat brain membrane preparation. Tissue samples are extracted with 0.1 M hydrochloric acid at a pH of 3.0–4.0, followed by heating, cooling to room temperature, decantation and centrifugation to obtain clarified supernatant. Bound [^{3}H] STX is quantified by liquid scintillation counting. A standard curve is generated using unlabeled STX standards, which results in reduction in bound [^{3}H] STX that is proportional to the amount of unlabeled toxin. The concentration of STX in samples is determined in reference to the standard curve. Incubations are carried out in a microplate format to minimize sample handling and the amount of radioactivity used.

Special Considerations: This method is listed as Tier II for presumptive analysis of saxitoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types. Samples must be extracted under acidic (pH 3–4) conditions to prevent toxin degradation. Samples containing intact cyanobacteria must be treated to disrupt the cells in order to recover intracellular toxins.

Source: AOAC International. 2011. Official Method 2011.27: Paralytic Shellfish Toxins (PSTs) in Shellfish, Receptor Binding Assay, First Action.
http://www.eoma.aoac.org/methods/info.asp?ID=49771

8.2.13.2 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Determination of PSTs in shellfish
Method Selected for: This method has been selected for presumptive analysis of saxitoxins in aerosol, solid and particulate samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: A commercially available ELISA kit is used for detection of saxitoxins in shellfish. The test is a direct competitive ELISA based on the recognition of saxitoxin by specific antibodies. Shellfish homogenates are heat extracted in 1% acetic acid and diluted prior to assay. When present in the sample, saxitoxin and a saxitoxin-enzyme-conjugate compete for the binding sites of rabbit anti-saxitoxin antibodies in solution. The saxitoxin antibodies are then bound by a second antibody (sheep anti-rabbit) immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of the saxitoxin present in the sample. The color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run. The kit allows the determination of 42 samples in duplicate, with a total analysis time of 60 minutes. The assay LOD for saxitoxin in shellfish homogenates is reported as 0.02 ppb (0.02 µg/L). Cross-reactivity of other PSTs (e.g., NEO, GTX 1-4) are considerably less than STX in this ELISA, and their presence may be underestimated in samples containing complex toxin profiles.

Special Considerations: The ELISA assay is listed as Tier II for presumptive analysis of saxitoxins in aerosol, solid, particulate and water samples. The extraction procedure used for

shellfish may be applicable to non-aqueous environmental sample types. Samples containing intact cyanobacteria must be treated to disrupt the cells in order to recover intracellular toxins.

Source: Costa, P.R., Baugh, K.A., Wright, B., RaLonde, R., Nance, S.L., Tatarenkova, N., Etheridge, S.M. and Lefebvre, K.A. 2009. "Comparative determination of paralytic shellfish toxins (PSTs) using five different toxin detection methods in shellfish species collected in the Aleutian Islands, Alaska." Toxicon. 54(3): 313-320. http://www.sciencedirect.com/science/article/pii/S0041010109002402

8.2.13.3 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Determination of PSTs in water
Method Selected for: This method has been selected for presumptive analysis of saxitoxins in liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: A commercially available immunoassay (ELISA) is used to detect saxitoxins in water samples. The test is a direct competitive ELISA based on the recognition of saxitoxin by specific antibodies. Water samples are prepared by three successive freeze-thaw cycles followed by filtration prior to assay. Saxitoxin and a saxitoxin-enzyme-conjugate compete for the binding sites of rabbit anti-saxitoxin antibodies in solution. The saxitoxin antibodies are then bound by a second antibody (sheep anti-rabbit) immobilized on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of the saxitoxin present in the sample. The color is evaluated using an ELISA reader. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run. The kit allows the determination of 42 samples in duplicate, with a total analysis time of 60 minutes. The MRL reported for saxitoxin in water samples is 0.02 µg/L. Cross-reactivities of other PSTs (e.g., NEOSTX, GTX 1-4) are considerably less than STX in this ELISA.

Special Considerations: The ELISA method is listed as Tier I for presumptive analysis of saxitoxins in water samples. Non-aqueous samples require extraction prior to assay (see Section 8.2.13.2). Samples containing intact cyanobacteria must be treated to disrupt the cells in order to recover intracellular toxins.

Source: Loftin, K.A., Graham, J.L., Hilborn, E.D., Lehmann, S.C., Meyer, M.T., Dietze, J.E. and Griffith, C.B. 2016. "Cyanotoxins in inland lakes of the United States: Occurrence and potential recreational health risks in the EPA National Lakes Assessment 2007." Harmful Algae. 56: 77-90. http://www.sciencedirect.com/science/article/pii/S1568988315300883

8.2.13.4 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Determination of saxitoxins (STX, NEO, GTX, dcGTX, dcSTX) and saxitoxin analogues in shellfish
Method Selected for: These procedures have been selected for confirmatory analysis of saxitoxins and analogues in aerosol, solid, particulate and water samples. Modification and further research are needed to adapt and verify the procedures for environmental sample types.

Description of Method: This method uses multiple analogues of saxitoxin in shellfish are detected using LC-MS-MS. The method includes a single dispersive extraction of shellfish homogenates followed by SPE using graphitized carbon cartridges for sample cleanup and LC-MS-MS, with a hydrophilic interaction liquid chromatography (HILIC) column, for analysis. Validation study results included in Turner *et al*. (2015) are provided for specificity, linearity,

recovery, repeatability, and within-laboratory reproducibility. LOD and LOQ were significantly improved in comparison to other currently available fluorescence-based detection methods.

Special Considerations: This method is listed as Tier II for confirmatory analysis of saxitoxin in aerosol, solid, particulate and water samples. The sample extraction and cleanup (SPE) procedures described for shellfish homogenates may be suited for other sample types with slight modifications. For example, relatively clean samples (e.g., drinking water and liquid samples) may not require extensive extraction procedures prior to sample cleanup and analyte concentration using SPE. In addition, adaptation of online SPE in conjunction with LC-MS-MS, as reported by Bragg *et al*. (2015) for STX and NEO, and by Coleman *et al.* (2016) for tetrodotoxin analysis, may provide additional streamlining and high-throughput capacity for saxitoxin analysis.

Source: Boundy, M.J., Selwood, A.I., Harwood, D.T., McNabb, P.S. and Turner, A.D. 2015. "Development of a sensitive and selective liquid chromatography–mass spectrometry method for high-throughput analysis of paralytic shellfish toxins using graphitized carbon solid phase extraction." Journal of Chromatography A. 1387: 1-12. http://www.sciencedirect.com/science/article/pii/S0021967315001995

Additional Resources:

- Turner, A.D., McNabb, P.S., Harwood, A.J. and Boundy, M.J. 2015. "Single-Laboratory Validation of a Multitoxin Ultra-Performance LC-Hydrophilic Interaction LC-MS/MS Method for Quantitation of Paralytic Shellfish Toxins in Bivalve Shellfish." Journal of AOAC International. 98(3): 609-621. http://www.ingentaconnect.com/content/aoac/jaoac/2015/00000098/00000003/art00011
- Bragg, W.A., Lemire, S.W., Coleman, R.M., Hamelin, E. I. and Johnson, R.C. 2015. "Detection of human exposure to saxitoxin and neosaxitoxin in urine by online-solid phase extraction-liquid chromatography-tandem mass spectrometry." Toxicon. 99: 118-124. https://www.ncbi.nlm.nih.gov/pubmed/25817003
- Coleman, R., Lemire, S.W., Bragg, W., Garrett, A., Ojeda-Torres, G., Hamelin, E., Johnson, R. C. and Thomas, J. 2016. "Development and validation of a high-throughput online solid phase extraction-liquid chromatography-tandem mass spectrometry method for the detection of tetrodotoxin in human urine." Toxicon. 119: 64-71. http://www.sciencedirect.com/science/article/pii/S0041010116301386

8.2.14 Shiga and Shiga-like Toxins (Stx)

CAS RN: 75757-64-1 (Stx)
Considered Variants: Stx-1a, 1c, 1d and 1e, Stx-2a to 2g
Description: Protein produced by *Shigella dysenteriae* and the shiga toxin-producing *Escherichia coli* (STEC). Composed of one ~32 kDa A chain and five 7.7 kDa B chains

Selected Methods	Analysis Type	Analytical Technique	Section
Austin Immunology. 2016. 1(2), id1007: 1−7	Presumptive	Immunoassay (ELISA)	8.2.14.1
Analytical Chemistry. 2014. 86: 4698−4706	Confirmatory	LC-MS-MS	8.2.14.2

8.2.14.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Shiga-like toxins (Stx-1 and Stx-2) in foods and bacterial enrichment broth
Method Selected for: These procedures have been selected for presumptive analysis of Shiga and Shiga-like toxins in aerosol, solid, particulate and water samples. Further research is needed

to adapt and verify the procedures for these sample types.

Description of Method: Shiga-like toxins (Stx-1, including 4 Stx-1a, 1c, 1d and 1e four subtypes and Stx-2, including Stx-2a to 2g seven subtypes) are produced by various STEC. Two commercial ELISA kits (Stx-1 ELISA and Stx-2 ELISA) are used to detect these toxins, using type-specific, polyclonal and mAbs to differentiate between the Stx-1 and Stx-2 toxin types. Samples are incubated with a medium that encourages *E. coli* (EC) growth and Stx production. If Stx-1or Stx-2 is present, it is bound by an immobilized polyclonal antibody on the wells of a microtiter plate. After a washing step, a mixture of mAbs is added which binds to the Stx-1 or Stx-2. A second washing step is followed by the addition of an HRP-labeled antibody that binds to the existing antigen/antibody complex in the wells. After a final wash and addition of substrate solution, a color signal is generated. The color intensity is evaluated using an ELISA plate reader and is proportional to the amount of Stx-1 or Stx-2 present. The result is compared to a known value to determine whether the sample is positive or below the limit of Stx-1 or Stx-2 detection (25 pg/mL). The additional source citation (below) describes the evaluation of these two ELISA kits for STEC-inoculated and enriched ground beef, romaine lettuce, recreational pond water, and pasteurized milk samples. Results indicate that Stx-1 and Stx-2 were readily detected and distinguished for all tested sample types.

Special Considerations: This assay is listed as Tier II for presumptive analysis of Shiga-like toxins in aerosol, solid, particulate and water samples. Appropriate standards are available. Sample preparation procedures used for foods and bacterial broth suggest that similar aqueous extraction procedures may be applicable to environmental samples. The Stx-1 ELISA may also be applicable for analysis of Stx produced by *Shigella* bacteria.

Source: Kong, Q., Patfield, S., Skinner, C., Stanker, L.H., Gehring, A. G., Fratamico, P.M., Rubio, F., Qi, W., and He, X. 2016. "Validation of Two New Immunoassays for Sensitive Detection of a Broad Range of Shiga Toxins." Austin Immunology. 1(2), id 1007: 1-7. http://austinpublishinggroup.com/austin-immunology/#

Additional Resource: Gehring, A.G., Fratamico, P.M., Lee, J., Ruth, L.E., He, X., He, Y., Paoli, G.C., Stanker, L.H. and Rubio, F.M. 2017. "Evaluation of ELISA Tests Specific for Shiga Toxin 1 and 2 in Food and Water Samples." Food Control. 77: 145-149. http://linkinghub.elsevier.com/retrieve/pii/S095671351730049X?showall=true

8.2.14.2 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: Stx-1 and Stx-2 in plasma and enrichment broth
Method Selected for: These procedures have been selected for confirmatory analysis of Shiga and Shiga-like toxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for these sample types.

Description of Method: This MRM method is based on analyzing conserved peptides, derived from the tryptic digestion of the toxin B subunits. Stable isotope-labeled analogues are prepared and used as internal standards to identify and quantify these characteristic peptides. The method detects and quantifies Shiga toxins (Stx) and Shiga-like toxins type 1 (Stx-1) and type 2 (Stx-2), and also distinguishes among most of the known Stx-1 and Stx-2 subtypes. The LOD for digested pure standards is approximately 10 attomole range/injection, which corresponds to a concentration of 1.7 femtomol/mL. Samples and standards are reduced using dithiothreitol, then alkylated using iodoacetamide, and finally subjected to proteolysis by the addition of a trypsin solution and incubation at 37°C for 16 hours. Digested samples are filtered through a 10000 molecular weight cut-off (MWCO) filter. For quantitative analysis, an aliquot of filter-sterilized sample is digested with trypsin and a fixed amount of the appropriate trypsin digested ^{15}N-labled internal standard is added. An MS equipped with a linear ion trap and a nanoelectrospray source was used to perform LC-MS-MS, operated in MRM mode, and alternating between detection of

the nine peptides and the corresponding ^{15}N-labeled internal standards. The additional source citation (see below) provides a more rapid (3-hour) approach to reduce/alkylate and trypsin digest serum samples, resulting in equivalent or improved detection compared to the procedure described above.

Special Considerations: This method is listed as Tier II for confirmatory analysis of Shiga-like toxins in aerosol, solid, particulate and water samples. Appropriate standards are available. Sample preparation procedures used for plasma and bacterial broth suggest that aqueous extraction may be applicable to environmental samples. Matrix effects were observed when dilute samples were digested in buffer, Luria broth, or mouse plasma (LOD ~30 attomol/injection = 5 femtomol/mL). This method may also be applicable for analysis of Stx produced by *Shigella* bacteria, using an appropriate standard.

Source: Silva, C.J., Erickson-Beltran, M.L., Skinner, C.B., Dynin, I., Hui, C., Patfield, S.A., Carter, J.M. and He, X. 2014. "Safe and Effective Means of Detecting and Quantitating Shiga-Like Toxins in Attomole Amounts." Analytical Chemistry. 86(10): 4698-4706. http://pubs.acs.org/doi/abs/10.1021/ac402930r

Additional Resource: Silva, C.J., Erickson-Beltran, M.L., Skinner, C.B., Patfield, S.A. and He, X. 2015. "Mass Spectrometry-Based Method of Detecting and Distinguishing Type 1 and Type 2 Shiga-Like Toxins in Human Serum." Toxins. 7(12): 5236-5253. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4690125/

8.2.15 Staphylococcal Enterotoxins (SETs)

CAS RNs: 37337-57-8 (staphylococcal enterotoxin type A [SEA]), 39424-53-8 (staphylococcal enterotoxin type B [SEB]), 39424-54-9 (staphylococcal enterotoxin type C [SEC]), 12788-99-7 (staphylococcal enterotoxin type D [SED]), (staphylococcal enterotoxin type E [SEE])
Considered Variants: SEA − SEE

Description: Heat stable, basic, single-chain proteins with molecular weights of 26,000 to 29,000 kDa, produced by certain *Staphylococcus* strains.

Selected Methods	Analysis Type	Analytical Technique	Section
AOAC Official Method 2007.06	Presumptive	Immunoassay (Enzyme-linked fluorescent immunoassay [ELFA])	8.2.15.1
Journal of AOAC International. 2014. 97(3): 862-867	Presumptive	Immunoassay (ECL)	8.2.15.2
Letters in Applied Microbiology. 2011. 52: 468-474	Confirmatory	Immunoassay (ELISA)	8.2.15.3

8.2.15.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELFA)

Method Developed for: Staphylococcal enterotoxins in selected foods
Method Selected for: This method has been selected for presumptive analysis of SETs in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercial test is an ELFA used with automated instrumentation for the specific detection of SEA − SEE. The solid-phase-receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and predispensed in the sealed reagent strips. The instrument performs all of the assay steps automatically. The user places the sample extract into the reagent strip, and the sample is cycled in and out of the SPR for a specific length of time. SET present in the sample will bind to the

anti-SET mAbs, which are coated on the interior of the SPR. Unbound sample components are washed away. Alkaline phosphatase-labeled antibodies are cycled in and out of the SPR and will bind to any SET captured on the SPR wall. Further wash steps remove unbound conjugate. During the final detection step, the substrate (4-methyl-umbelliferyl phosphate) is cycled in and out of the SPR. The bound enzyme conjugate catalyzes the hydrolysis of this substrate into a fluorescent product (4-methyl-umbelliferone), the fluorescence of which is measured at 450 nm. The test value is calculated by the instrument and is equal to the sample relative fluorescence value (RFV)/standard RFV. A "negative" result has a test value less than the threshold (0.13), indicating that the sample does not contain SET or contains SET at a concentration below the DL. A "positive" result has a test value equal to or greater than the threshold and indicates that the sample is contaminated with SET. DLs for SETs in various foods range from 0.25 ng/g to 0.5 ng/g (solids) or ng/mL (liquids).

Special Considerations: These procedures are listed as Tier II for presumptive analysis of staphylococcal neurotoxins in aerosol, solid, particulate and water samples. Non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay.

Source: AOAC International. 2008. "Method 2007.06: "VIDAS SET 2 for Detection of Staphylococcal Enterotoxins in Selected Foods" Journal of AOAC International. 91: 164. http://www.aoac.org/

8.2.15.2 Presumptive Analysis

Analytical Technique: Immunoassay (ECL)

Method Developed for: Determination of SEB in foods
Method Selected for: These procedures have been selected for presumptive analysis of SEB in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercial ECL immunoassay is used with automated instrumentation for the specific detection of SEB. The source reference describes a comparison of its use to ELISA and LFD assays, in conjunction with an outbreak of staphylococcal food poisoning, and notes demonstrated cross-reactivity with SED present in the food samples as well as interferences from the food matrices tested.

Special Considerations: These procedures are listed as Tier III for presumptive analysis of SEB in aerosol, solid, particulate and water samples. Non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay. Please consult the technical contacts listed in Section 4.0 for information regarding the status and availability of assay reagents and analytical instrumentation.

Source: Tallent, S. M., Hait, J., and Bennet, R., J. 2014. "Staphylococcal Enterotoxin B-Specific Electrochemiluminescence and Lateral Flow Device Assays Cross-React with Staphylococcal Enterotoxin D." Journal of AOAC International. 97(3): 862-867.

8.2.15.3 Confirmatory Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: Determination of SETs in naturally contaminated cheese
Method Selected for: These procedures have been selected for confirmatory analysis of SETs in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This commercially available sandwich type enzyme immunoassay (ELISA) detects the combined SET types SEA through SEE. The surface of a microtitre plate is

coated with specific purified antibodies that bind enterotoxins. The immobilized toxins are then bound by a mixture of toxin-specific detector antibodies (conjugated to biotin) forming a sandwich complex (antibody-antigen-antibody complex). Addition of specific antibodies forms a sandwich complex (antibody–antigen–antibody). The presence of enterotoxins is revealed by adding an enzyme substrate⁄chromogen solution containing tetramethylbenzidine, which results in a blue color in the presence of SETs. Addition of a sulphuric acid solution leads to a color change from blue to yellow, allowing for the confirmation of the presence of SEs using a spectrophotometer at a double wavelength of 450⁄630 nm. The sample is considered to be contaminated by SEs if the absorbance test value is greater than or equal to the threshold value (negative control value plus 0.15). Solid food samples (e.g., cheese) are homogenized, centrifuged and concentrated by dialysis prior to assay. DLs reported for cheese samples range from 0.012 ng/g (SEA) to 0.05 ng/g (SED).

Special Considerations: These procedures are listed as Tier II for confirmatory analysis of SETs in aerosol, solid, particulate and water samples. Non-liquid samples such as soils, powders and aerosol filters will require aqueous extraction followed by clarification (e.g., centrifugation) prior to assay. All samples may require a concentration step (e.g., dialysis) prior to assay.

Source: Ostyn, A., Fuillier, F., Prufer, A.L., Messio, S., Krys, S., Lombard, B. and Hennekinne, J.A. 2011. "Intra-laboratory validation of the Ridascreen® SET Total kit for detection staphylococcal enterotoxins SEA to SEE in cheese." Letters in Applied Microbiology. 52(5): 468-474. http://onlinelibrary.wiley.com/doi/10.1111/j.1472-765X.2011.03025.x/abstract

8.2.16 T-2 Mycotoxin

CAS RN: 21259-20-1 (T-2), 26934-87-2 (HT-2)
Considered Variants: T-2 and HT-2

Description: Trichothecene toxins produced by *Fusarium* spp.

Selected Methods	Analysis Type	Analytical Technique	Section
Journal of Food Protection. 2005. 68(6): 1294-1301	Presumptive	Immunoassay (ELISA)	8.2.16.1
Rapid Communications in Mass Spectrometry. 2006. 20(9): 1422-1428	Confirmatory	LC-MS	8.2.16.2

8.2.16.1 Presumptive Analysis

Analytical Technique: Immunoassay (ELISA)

Method Developed for: T-2 mycotoxin in food and beverages
Method Selected for: These procedures have been selected for presumptive analysis of α-amanitin (see Section 8.2.3.1) and T-2 toxin in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: A commercially available ELISA is used to detect T-2 mycotoxin at levels below those described as a health concern in food samples. Solid food samples are prepared by extracting the sample with methanol/water followed by dilution with phosphate-buffered saline. Liquid beverage samples are prepared by dilution in sodium phosphate buffer. The prepared samples are analyzed using commercially obtained ELISA kits according to the manufacturer's directions, except for the incorporation of an eight-point calibration curve and reading the plates at both 405 and 650 nm after 26 minutes of incubation at 37°C.

Special Considerations: This assay is listed as Tier II for presumptive analysis of T-2 mycotoxins in aerosol, solid, particulate and water samples. Non-aqueous samples will require extraction prior to analysis. The ELISA kit successfully detects T-2 toxin at targeted levels of 0.2

µg/g; the immunoassay for T-2 toxin, however, shows variable background responses up to 0.1 µg/g. Detection thresholds of 0.2 µg/g for T-2 toxin avoided the background problems but were low enough to allow detection at concentrations below those associated with serious health effects. The additional resource citation (Tima, 2016) describes the application of this ELISA for monitoring swine feedstuff for T-2 mycotoxin. The LOD for T-2 mycotoxin in this study was 12 µg/kg.

Source: Garber, E.A.E., Eppley, R.M., Stack, M.E., McLaughlin, M.A. and Park, D.L. 2005. "Feasibility of Immunodiagnostic Devices for the Detection of Ricin, Amanitin, and T-2 Toxin in Food." Journal of Food Protection. 68(6): 1294-1301. http://jfoodprotection.org/doi/abs/10.4315/0362-028X-68.6.1294?=

Additional Resource: Tima, H., Rácz, A., Guld, Z., Mohácsi-Farkas, C. and Kiskó, G. 2016. "Deoxynivalenol, zearalenone and T-2 in grain based swine feed in Hungary." Food Additives & Contaminants: Part B. 9(4):275-280. DOI: 10.1080/19393210.2016.1213318. http://dx.doi.org/10.1080/19393210.2016.1213318

8.2.16.2 Confirmatory Analysis

Analytical Technique: LC-MS

Method Developed for: DAS and mycotoxins in food
Method Selected for: These procedures have been selected for confirmatory analysis of T-2 and HT-2 mycotoxins in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: A LC-TOF-MS with atmospheric pressure chemical ionization (APCI) and time-of-flight (TOF)-MS with a real-time reference mass correction is used for simultaneous determination of *Fusarium* mycotoxins (including T-2 and HT-2 mycotoxins) in food. Samples are extracted and centrifuged, and the supernatant is applied to a MultiSep #226 column for cleanup. Prepared samples are separated by reversed-phase LC and detected by APCI-MS. LODs range from 0.1 to 0.3 ng/g and 0.5 to 0.9 ng/g for T-2 and HT-2 in analyzed foodstuffs, respectively. The additional resource citation below (Garcia-Moraleja, 2015) describes a liquid-liquid extraction procedure using either triple quadrupole or ion trap LC-MS-MS for determination of various mycotoxins in brewed coffee (see Section 8.2.8.2).

Special Considerations: This method is listed as Tier II for confirmatory analysis of T-2 mycotoxins in aerosol, solid, particulate and water samples. The procedures described may need to be modified for water, soils, air filters and particulates. It may be possible to analyze relatively clean water samples by direct injection into an LC-MS-MS. Dirty water samples or water samples with low concentrations of toxin may require cleanup and concentration.

Source: Tanaka, H., Takino, M., Sugita-Konishi, Y. and Tanaka, T. 2006. "Development of Liquid Chromatography/Time-of-Flight Mass Spectrometric Method for the Simultaneous Determination of Trichothecenes, Zearalenone, and Aflatoxins in Foodstuffs." Rapid Communications in Mass Spectrometry. 20(9): 1422-1428. http://onlinelibrary.wiley.com/doi/10.1002/rcm.2460/abstract

Additional Resource: Garcia-Moraleja, A., Font, G., Manes, J. and Ferrer, E. 2015. "Development of a new method for the simultaneous determination of 21 mycotoxins in coffee beverages by liquid chromatography tandem mass spectrometry." Food Research International. 72: 247-255. http://www.sciencedirect.com/science/article/pii/S0963996915001167

8.2.17 Tetrodotoxin (TTX)

CAS RN: 9014-39-5
Considered Variants: NA

Description: Neurotoxin produced by certain infecting or symbiotic bacteria like *Pseudomonas* and *Vibrio,* found in animals from the order Tetraodontiformes, which includes pufferfish, porcupinefish, ocean sunfish, and triggerfish. Composed of heterocyclic heat-stable (except in alkaline environments) and water-soluble neurotoxin.

Selected Methods	Analysis Type	Analytical Technique	Section
AOAC Official Method 2011.27. 2011	Presumptive	RBA	8.2.17.1
Toxicon. 2016. 119: 64-71	Confirmatory	LC-MS-MS	8.2.17.2

8.2.17.1 Presumptive Analysis

Analytical Technique: RBA

Method Developed for: PSTs in shellfish
Method Selected for: This method has been selected for confirmatory analysis of TTX in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This assay is used for determination of PSTs in shellfish tissue homogenates. The procedure is a competitive binding assay in which [^{3}H] STX competes with unlabeled standards (TTX) or PSTs in samples for a finite number of receptor sites in a rat brain membrane preparation. Tissue samples are extracted with 0.1 M hydrochloric acid at a pH of 3.0–4.0, followed by heating, cooling to room temperature, decantation and centrifugation to obtain clarified supernatant. Unbound [^{3}H] STX is removed by filtration and bound [^{3}H] STX is quantified by liquid scintillation counting. A standard curve is generated using unlabeled TTX standards, which results in reduction in bound [^{3}H] STX that is proportional to the amount of unlabeled toxin. The concentration of TTX in samples is determined in reference to the standard curve. Incubations are carried out in a microplate format to minimize sample handling and the amount of radioactivity used.

Special Considerations: This method is listed as Tier II for presumptive analysis of TTX in aerosol, solid, particulate and water samples. Samples must be extracted under acidic (pH 3–4) conditions to prevent toxin degradation. This method was originally developed for STX but can be used to quantify TTX in samples when TTX standards are used to develop a standard curve since both toxins compete for the same receptor.

Source: AOAC International. 2011. Official Method 2011.27: Paralytic Shellfish Toxins (PSTs) in Shellfish, Receptor Binding Assay, First Action.
http://www.eoma.aoac.org/methods/info.asp?ID=49771

8.2.17.2 Confirmatory Analysis

Analytical Technique: LC-MS-MS

Method Developed for: TTX in human urine
Method Selected for: These procedures have been selected for confirmatory analysis in aerosol, solid, particulate and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This procedure uses online SPE and LC-MS-MS is used for determination of TTX in human urine. Sample extraction involves online SPE using a mixed mode cartridge (strong cation exchange/C-18 reverse phase) followed by direct injection and detection of analytes by LC-MS-MS using a HILIC column. The reportable TTX concentration

range was 2.80–249 ng/mL in urine with a calculated LOD of 0.302 ng/mL.

Special Considerations: This method is listed as Tier II for confirmatory analysis of TTX in aerosol, solid, particulate and water samples. The procedures described may need to be modified for water, soil, air filter and particulate samples. Soil, air filter and particulate extracts may require cleanup and concentration by SPE. It may be possible to analyze relatively clean water samples by direct injection into an LC-MS-MS. Dirty water samples or water samples with low levels of TTX may also require cleanup and concentration using SPE. The additional resources cited below describe solvent or accelerated extraction of fish tissue and snail shells, followed by centrifugation or filtration. The resulting supernatant can then be cleaned up and concentrated by online SPE, and analyzed using LC-MS-MS.

Source: Coleman, R., Lemire, S.W., Bragg, W., Garrett, A., Ojeda-Torres, G., Hamelin, E., Johnson, R.C. and Thomas, J. 2016. "Development and validation of a high-throughput online solid phase extraction-liquid chromatography-tandem mass spectrometry method for the detection of tetrodotoxin in human urine." Toxicon. 119: 64-71.
http://www.sciencedirect.com/science/article/pii/S0041010116301386

Additional Resources:

- Rodríguez, P., Alfonso, A., Otero, P., Katikou, P., Georgantelis, D.B and Botana, L.M. 2012. "Liquid chromatography–mass spectrometry method to detect Tetrodotoxin and Its analogues in the puffer fish *Lagocephalus sceleratus* (Gmelin, 1789) from European waters." Food Chemistry. 132(2): 1103-1111.
http://www.sciencedirect.com/science/article/pii/S0308814611016633
- Nzoughet, J.K., Campbell, K., Barnes, P., Cooper, K.M., Chevallier, O.P. and Elliott, C.T. 2013. "Comparison of sample preparation methods, validation of an UPLC–MS/MS procedure for the quantification of tetrodotoxin present in marine gastropods and analysis of pufferfish." Food Chemistry. 136(3-4): 1584-1589.
http://www.sciencedirect.com/science/article/pii/S0308814612001616

Section 9.0: Conclusions

The information in this document is intended for use by EPA and EPA-contracted and -subcontracted laboratories; it also can be used by other agencies and laboratory networks, such as the ICLN. The information also can be found on the SAM website (https://www.epa.gov/homeland-security-research/sam), which provides searchable links to supporting information based on the analytes and the analytical methods listed. Methods listed in Appendix A (chemical methods), Appendix B (radiochemical methods), Appendix C (pathogen methods), and Appendix D (biotoxin methods) are recommended for use in assessment of the extent of contamination and the effectiveness of decontamination following an intentional or unintentional contamination incident.

The primary objective of this document is to identify appropriate methods that represent a balance between providing existing, documented, techniques and providing consistent and valid analytical results. The method selected for each analyte/sample type combination was deemed the most general, appropriate, and broadly applicable of available methods by a group of technical experts in each appropriate field. The selected methods are subject to change following further research to improve methods or following the development of new methods. The contacts listed in Section 4.0 encourage the scientific community to inform them of any such method improvements.

Since publication of Revision 1.0 in September 2004, NHSRC has continued to convene technical work groups to evaluate and, if necessary, update the analytes and methods that are listed. Details regarding changes that have been incorporated into each revision are provided in Attachment 1. This current revision (2017) includes the addition of outdoor building and infrastructure materials as sample types in the radiochemistry sections; the addition of solids as a sample type in the pathogens sections; the assignment of method applicability tiers for methods selected for pathogen and biotoxin analytes; several new methods added or replaced for chemical, radiochemical, pathogen and biotoxin analytes; the addition of several new analytes to each of these technical sections; and considerations regarding the potential impacts of decontamination agents on the analytical performance of selected radiochemical methods.

Appendix A: Selected Chemical Methods

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SAM 2017 — Appendix A: Selected Chemical Methods

The fitness of a method for an intended use is related to site-specific data quality objectives (DQOs) for a particular environmental remediation activity. These selected chemical methods have been assigned tiers (below) to indicate a level of method usability for the specific analyte and sample type. The assigned tiers reflect the conservative view for DQOs involving timely implementation of methods for analysis of a high number of samples (such that multiple laboratories are necessary), low limits of identification and quantification, and appropriate quality control. Assigned usability tiers are indicated next to each method or method combination throughout this appendix.

Tier I: Analyte/sample type is a target of the method(s). Data are available for all aspects of method performance and quality control measures supporting its use for analysis of environmental samples following a contamination event. Evaluation and/or use of the method(s) in multiple laboratories indicate that the method can be implemented with no additional modifications for the analyte/sample type

Tier II: (1) The analyte/sample type is a target of the method(s) and the method(s) has been evaluated for the analyte/sample type by one or more laboratories, or (2) the analyte/sample type is not a target of the method(s), but the method has been used by laboratories to address the analyte/sample type. In either case, available data and/or information indicate that modifications will likely be needed for use of the method(s) to address the analyte/sample type (e.g., due to potential interferences, alternate matrices, the need to address different DQOs).

Tier III: The analyte/sample type is not a target of the method(s), and/or no reliable data supporting the method's fitness for its intended use are available. Data from other analytes or sample types, however, suggest that the method(s), with significant modification, may be applicable.

***Note*: Column headings are defined in Section 5.0.**

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Acephate	30560-19-1	LC-MS-MS	Sample Prep	Adapted from J. Chromatogr. A, (2007) 1154(1): 3-25	II	Adapted from Chromatographia, 63(5/6): 233-237	II	538 (EPA OW)	I	Adapted from J. Chromatogr. A, (2007) 1154(1): 3-25	III	Adapted from J. Chromatogr. A, (2007) 1154(1): 3-25	III
			Determinative										
Acrylamide	79-06-1	HPLC-UV	Sample Prep	Water extraction	III	8316 (EPA SW-846)	II	8316 (EPA SW-846)	II	PV2004 (OSHA)	I	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8316 (EPA SW-846)								8316 (EPA SW-846)	
Acrylonitrile	107-13-1	HPLC-UV / GC-MS	Sample Prep	5035A (EPA SW-846)	II	524.2[1] (EPA OW)	II	524.2[1] (EPA OW)	II	PV2004 (OSHA)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8260D (EPA SW-846)								8260D (EPA SW-846)	
Aldicarb (Temik)	116-06-3	HPLC-UV / HPLC-FL / LC-MS-MS	Sample Prep	8318A (EPA SW-846)	II	D7645-16 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846)	III
			Determinative									8318A (EPA SW-846)	
Aldicarb sulfone	1646-88-4	HPLC-UV / HPLC-FL / LC-MS-MS	Sample Prep	8318A (EPA SW-846)	II	D7645-16 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative									8318A (EPA SW-846)	
Aldicarb sulfoxide	1646-87-3	HPLC-UV / HPLC-FL / LC-MS-MS	Sample Prep	8318A (EPA SW-846)	III	D7645-16 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative									8318A (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Allyl alcohol	107-18-6	GC-MS	Sample Prep	5035A (EPA SW-846)	II	5030C (EPA SW-846)	II	5030C (EPA SW-846)	II	TO-15[2] (EPA ORD)	III	Not of concern	NA
			Determinative	8260D (EPA SW-846)		8260D (EPA SW-846)		8260D (EPA SW-846)					
4-Aminopyridine	504-24-5	HPLC-UV	Sample Prep	8330B (EPA SW-846)	III	3535A/8330B (EPA SW-846)	III	3535A/8330B (EPA SW-846)	III	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative			8330B (EPA SW-846)		8330B (EPA SW-846)				8330B (EPA SW-846)	
Ammonia	7664-41-7	Visible spectrophotometry	Sample Prep	Not of concern	NA	4500-NH_3 B (SM)	I	350.1 (EPA OW)	I	6015 (NIOSH)	I	Not of concern	NA
			Determinative			4500-NH_3 G (SM)							
Ammonium metavanadate (analyze as total vanadium)	7803-55-6	ICP-AES / ICP-MS	Sample Prep	3050B/3051A (EPA SW-846)	I	3015A (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010D/6020B (EPA SW-846)		6010D/6020B (EPA SW-846)				IO-3.4/IO-3.5 (EPA ORD)		6010D/6020B (EPA SW-846)	
Arsenic, Total	7440-38-2	ICP-AES / ICP-MS	Sample Prep	3050B/3051A (EPA SW-846)	I	3015A (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010D/6020B (EPA SW-846)		6010D/6020B (EPA SW-846)				IO-3.4/IO-3.5 (EPA ORD)		6010D/6020B (EPA SW-846)	
Arsenic trioxide (analyze as total arsenic)	1327-53-3	ICP-AES / ICP-MS	Sample Prep	3050B/3051A (EPA SW-846)	I	3015A (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010D/6020B (EPA SW-846)		6010D/6020B (EPA SW-846)				IO-3.4/IO-3.5 (EPA ORD)		6010D/6020B (EPA SW-846)	
Arsine (analyze as total arsenic in non-air samples)	7784-42-1	GFAA / ICP-AES / ICP-MS	Sample Prep	3050B/3051A (EPA SW-846)	I	3015A (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	6001 (NIOSH)	I	9102 (NIOSH)	I
			Determinative	6010D/6020B (EPA SW-846)		6010D/6020B (EPA SW-846)						6010D/6020B (EPA SW-846)	
Asbestos	1332-21-4	TEM	Sample Prep	D5755-03 (soft surfaces-microvac) (ASTM)	III	Not of concern	NA	Not of concern	NA	10312:1995 (ISO)	I	D6480-05 (hard surfaces-wipes) (ASTM)	I
			Determinative										
Boron trifluoride	7637-07-2	ISE	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	ID216SG (OSHA)	I	Not of concern	NA
			Determinative										
Brodifacoum	56073-10-0	LC-MS-MS	Sample Prep	3541/3545A (EPA SW-846)	III	D7644-16 (ASTM)	II	D7644-16 (ASTM)	II	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	D7644-16 (ASTM)								D7644-16 (ASTM)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Bromadiolone	28772-56-7	LC-MS-MS	Sample Prep	3541/3545A (EPA SW-846)	III	D7644-16 (ASTM)	II	D7644-16 (ASTM)	II	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	D7644-16 (ASTM)								D7644-16 (ASTM)	
BZ [Quinuclidinyl benzilate]	6581-06-2	HPLC-UV / LC-MS-MS	Sample Prep	3541/3545A (EPA SW-846)	III	Adapted from J. Chromatogr. B (2008) 874: 42-50	III	Adapted from J. Chromatogr. B (2008) 874: 42-50	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	Adapted from J. Chromatogr. B (2008) 874: 42-50								Adapted from J. Chromatogr. B (2008) 874: 42-50	
Calcium arsenate (analyze as total arsenic)	7778-44-1	ICP-AES / ICP-MS	Sample Prep	3050B/3051A (EPA SW-846)	I	3015A (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010D/6020B (EPA SW-846)		6010D/6020B (EPA SW-846)				IO-3.4/IO-3.5 (EPA ORD)		6010D/6020B (EPA SW-846)	
Carbofuran (Furadan)	1563-66-2	HPLC-UV / HPLC-FL / LC-MS-MS	Sample Prep	8318A (EPA SW-846)	II	D7645-16 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846)	III
			Determinative									8318A (EPA SW-846)	
Carbon disulfide	75-15-0	GC-MS	Sample Prep	5035A (EPA SW-846)	I	5030C (EPA SW-846)	I	524.2[1] (EPA OW)	I	TO-15 (EPA ORD)	I	Not of concern	NA
			Determinative	8260D (EPA SW-846)		8260D (EPA SW-846)							
Carfentanil	59708-52-0	LC-MS-MS	Sample Prep	3541/3545A (EPA SW-846)	III	3520C/3535A (EPA SW-846)	III	3520C/3535A (EPA SW-846)	III	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	Adapted from J. Chromatogr. B (2014) 962: 52-58		Adapted from J. Chromatogr. B (2014) 962: 52-58		Adapted from J. Chromatogr. B (2014) 962: 52-58				Adapted from J. Chromatogr. B (2014) 962: 52-58	
Chlorfenvinphos	470-90-6	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	3520C/3535A (EPA SW-846)	I	3520C/3535A (EPA SW-846)	I	TO-10A (EPA ORD)	II	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative			8270E (EPA SW-846)		8270E (EPA SW-846)					
Chlorine	7782-50-5	Visible spectrophotometry	Sample Prep	Not of concern	NA	4500-Cl G (SM)	I	4500-Cl G (SM)	I	Adapted from Analyst (1999) 124(12):1853-1857	II	Not of concern	NA
			Determinative							4500-Cl G (SM)			

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
2-Chloroethanol	107-07-3	GC-MS / GC-FID	Sample Prep	5035A (EPA SW-846)	II	5030C (EPA SW-846)	II	5030C (EPA SW-846)	II	2513 (NIOSH)	I	Not of concern	NA
			Determinative	8260D (EPA SW-846)		8260D (EPA SW-846)		8260D (EPA SW-846)					
3-Chloro-1,2-propanediol	96-24-2	GC-MS	Sample Prep	Adapted from Eur. J. Lipid Sci. Technol. (2011) 113: 345-355	II	Adapted from J. Chromatogr. A (2000) 866: 65-77	II	Adapted from J. Chromatogr. A (2000) 866: 65-77	II	TO-10A[3] (EPA ORD)	III	Adapted from Eur. J. Lipid Sci. Technol. (2011) 113: 345-355	III
			Determinative										
Chloropicrin	76-06-2	GC-MS / GC-ECD	Sample Prep	EPA/600/R-16/114[15] (EPA NHSRC)	II	551.1 (EPA OW)	I	551.1 (EPA OW)	I	PV2103 (OSHA)	I	EPA/600/R-16/114[15] (EPA NHSRC)	II
			Determinative										
Chlorosarin	1445-76-7	GC-MS	Sample Prep	EPA/600/R-16/115 (EPA NHSRC)	*III	EPA/600/R-16/115 (EPA NHSRC)	*III	EPA/600/R-16/115 (EPA NHSRC)	*III	TO-17[3] (EPA ORD)	*III	EPA/600/R-16/115 (EPA NHSRC)	*III
			Determinative										
Chlorosoman	7040-57-5	GC-MS	Sample Prep	EPA/600/R-16/115 (EPA NHSRC)	*III	EPA/600/R-16/115 (EPA NHSRC)	*III	EPA/600/R-16/115 (EPA NHSRC)	*III	TO-17[3] (EPA ORD)	*III	EPA/600/R-16/115 (EPA NHSRC)	*III
			Determinative										
2-Chlorovinyl-arsonic acid (CVAOA) (degradation product of Lewisite)	64038-44-4	LC-MS-MS / ICP-AES / ICP-MS	Sample Prep	EPA/600/R-15/258[4] (EPA NHSRC)	*II	EPA/600/R-15/258[4] (EPA NHSRC)	*II	EPA/600/R-15/258[4] (EPA NHSRC)	*II	IO-3.1[5] (EPA ORD)	*I	EPA/600/R-15/258[4] (EPA NHSRC)	*II
			Determinative							IO-3.4/IO-3.5[5] (EPA ORD)			
2-Chlorovinylarsonous acid (CVAA) (degradation product of Lewisite)	85090-33-1	LC-MS-MS / ICP-AES / ICP-MS	Sample Prep	EPA/600/R-15/258[4] (EPA NHSRC)	*II	EPA/600/R-15/258[4] (EPA NHSRC)	*II	EPA/600/R-15/258[4] (EPA NHSRC)	*II	IO-3.1[5] (EPA ORD)	*I	EPA/600/R-15/258[4] (EPA NHSRC)	*II
			Determinative							IO-3.4/IO-3.5[5] (EPA ORD)			
Chlorpyrifos	2921-88-2	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	EPA/600/R-16/114 (EPA NHSRC)	II	525.2[6] (EPA OW)	I	TO-10A (EPA ORD)	I	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative										
Chlorpyrifos oxon	5598-15-2	GC-MS / LC-MS-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	III	540 (EPA OW)	I	540 (EPA OW)	I	TO-10A (EPA ORD)	III	EPA/600/R-16/114 (EPA NHSRC)	III
			Determinative										
Crimidine	535-89-7	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	EPA/600/R-16/114 (EPA NHSRC)	II	EPA/600/R-16/114 (EPA NHSRC)	II	Not of concern	NA	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative										
Cyanide, Amenable to chlorination	NA	Visible spectrophotometry	Sample Prep	3135.2I (EPA RLAB)	I	3135.2I[7] (EPA RLAB)	I	3135.2I[7] (EPA RLAB)	I	Not of concern	NA	3135.2I (EPA RLAB)	III
			Determinative										
Cyanide, Total	57-12-5	Visible spectrophotometry	Sample Prep	ISM02.3 CN (EPA CLP)	I	ISM02.3 CN[8] (EPA CLP)	I	335.4 (EPA OW)	I	6010 (NIOSH)	I	ISM02.3 CN (EPA CLP)	III
			Determinative										

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Cyanogen chloride	506-77-4	GC-MS / GC-ECD	Sample Prep	Adapted from Encyclopedia of Anal. Chem. (2006) DOI: 10.1002/9780 470027318.a0809	II	Adapted from Encyclopedia of Anal. Chem. (2006) DOI: 10.1002/9780 470027318.a0809	II	Adapted from Encyclopedia of Anal. Chem. (2006) DOI: 10.1002/9780 470027318.a0809	II	TO-15 (EPA ORD)	III	Not of concern	NA
			Determinative										
Cyclohexyl sarin (GF)	329-99-7	GC-MS	Sample Prep	EPA/600/R-16/115 (EPA NHSRC)	*I	EPA/600/R-16/115 (EPA NHSRC)	*I	EPA/600/R-16/115 (EPA NHSRC)	*I	TO-17 (EPA ORD)	III	EPA/600/R-16/115 (EPA NHSRC)	*I
			Determinative										
1,2-Dichloroethane (degradation product of HD)	107-06-2	GC-MS	Sample Prep	5035A (EPA SW-846)	I	5030C (EPA SW-846)	I	524.2[1] (EPA OW)	I	TO-15 (EPA ORD)	I	Not of concern	NA
			Determinative	8260D (EPA SW-846)		8260D (EPA SW-846)							
Dichlorvos	62-73-7	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	3535A (EPA SW-846)	I	525.2[6] (EPA OW)	I	TO-10A (EPA ORD)	I	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative			8270E (EPA SW-846)							
Dicrotophos	141-66-2	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	3535A (EPA SW-846)	I	3535A (EPA SW-846)	I	TO-10A (EPA ORD)	I	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative			8270E (EPA SW-846)		8270E (EPA SW-846)					
Diesel range organics	NA	GC-FID	Sample Prep	3541/3545A (EPA SW-846)	I	3520C/3535A (EPA SW-846)	I	3520C/3535A (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative	8015D (EPA SW-846)		8015D (EPA SW-846)		8015D (EPA SW-846)				8015D (EPA SW-846)	
Diisopropyl methylphosphonate (DIMP) (degradation product of GB)	1445-75-6	HPLC-UV / LC-MS-MS	Sample Prep	E2866-12 (ASTM)	II	D7597-16 (ASTM)	II	538 (EPA OW)	I	TO-10A[3] (EPA ORD)	III	EPA/600/R-13/224 (EPA NHSRC)	II
			Determinative										
Dimethylphosphite	868-85-9	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	Not of concern	NA	Not of concern	NA	TO-10A (EPA ORD)	II	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative										
Dimethylphosphoramidic acid (degradation product of GA)	33876-51-6	HPLC-UV / LC-MS-MS	Sample Prep	E2866-12 (ASTM)	III	D7597-16 (ASTM)	III	D7597-16 (ASTM)	III	TO-10A (EPA ORD)	III	EPA/600/R-13/224 (EPA NHSRC)	III
			Determinative										
Diphacinone	82-66-6	LC-MS-MS	Sample Prep	3541/3545A (EPA SW-846)	III	D7644-16 (ASTM)	II	D7644-16 (ASTM)	II	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	D7644-16 (ASTM)								D7644-16 (ASTM)	
Disulfoton	298-04-4	GC-MS / GC-FPD	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	525.2[6,9] (EPA OW)	II	525.2[6,9] (EPA OW)	II	5600 (NIOSH)	I	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative										

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Disulfoton sulfone oxon	2496-91-5	GC-MS / GC-FPD	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	III	525.2[6,9] (EPA OW)	III	525.2[6,9] (EPA OW)	III	5600 (NIOSH)	III	EPA/600/R-16/114 (EPA NHSRC)	III
			Determinative										
Disulfoton sulfoxide	2497-07-6	GC-MS / GC-FPD	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	III	525.2[6,9] (EPA OW)	II	525.2[6,9] (EPA OW)	II	5600 (NIOSH)	III	EPA/600/R-16/114 (EPA NHSRC)	III
			Determinative										
Disulfoton sulfoxide oxon	2496-92-6	GC-MS / GC-FPD	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	III	525.2[6,9] (EPA OW)	III	525.2[6,9] (EPA OW)	III	5600 (NIOSH)	III	EPA/600/R-16/114 (EPA NHSRC)	III
			Determinative										
1,4-Dithiane (degradation product of HD)	505-29-3	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	EPA/600/R-16/114 (EPA NHSRC)	II	EPA/600/R-16/114 (EPA NHSRC)	II	Not of concern	NA	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative										
EA 2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid] (hydrolysis product of VX)	73207-98-4	HPLC-UV / LC-MS-MS	Sample Prep	3541/3545A (EPA SW-846)	III	EPA/600/R-15/097 (EPA NHSRC)	II	EPA/600/R-15/097 (EPA NHSRC)	II	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	EPA/600/R-15/097 (EPA NHSRC)								EPA/600/R-15/097 (EPA NHSRC)	
Ethyl methylphosphonic acid (EMPA) (degradation product of VX)	1832-53-7	HPLC-UV / LC-MS-MS	Sample Prep	E2866-12 (ASTM)	II	D7597-16 (ASTM)	II	D7597-16 (ASTM)	III	TO-10A (EPA ORD)	III	EPA/600/R-13/224 (EPA NHSRC)	II
			Determinative										
Ethyldichloroarsine (ED)	598-14-1	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III	3535A (EPA SW-846)	III	3535A (EPA SW-846)	III	TO-15 (EPA ORD)	III	9102 (NIOSH)	III
			Determinative	8270E (EPA SW-846)		8270E (EPA SW-846)		8270E (EPA SW-846)				8270E (EPA SW-846)	
N-Ethyldiethanolamine (EDEA) (degradation product of HN-1)	139-87-7	LC-MS-MS / IC- conductivity detection	Sample Prep	3541/3545A (EPA SW-846)	III	D7599-16 (ASTM)	II	D7599-16 (ASTM)	III	3509 (NIOSH)	III	EPA/600/R-11/143 (EPA NHSRC)	II
			Determinative	EPA/600/R-11/143 (EPA NHSRC)									
Ethylene oxide	75-21-8	GC-MS	Sample Prep	5035A (EPA SW-846)	II	5030C (EPA SW-846)	II	5030C (EPA SW-846)	II	TO-15 (EPA ORD)	I	Not of concern	NA
			Determinative	8260D (EPA SW-846)		8260D (EPA SW-846)		8260D (EPA SW-846)					
Fenamiphos	22224-92-6	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	EPA/600/R-16/114 (EPA NHSRC)	II	525.2[6] (EPA OW)	I	TO-10A (EPA ORD)	II	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative										

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Fentanyl	437-38-7	LC-MS-MS	Sample Prep	3541/3545A (EPA SW-846)	III	3520C/3535A (EPA SW-846)	III	3520C/3535A (EPA SW-846)	III	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	Adapted from J. Chromatogr. A (2011) 1218: 1620 - 1649		Adapted from J. Chromatogr. A (2011) 1218: 1620 - 1649		Adapted from J. Chromatogr. A (2011) 1218: 1620 - 1649				Adapted from J. Chromatogr. A (2011) 1218: 1620 - 1649	
Fluoride	16984-48-8	IC-conductivity detection	Sample Prep	Not of concern	NA	300.1, Rev 1.0 (EPA OW)	I	300.1, Rev 1.0 (EPA OW)	I	Not of concern	NA	Not of concern	NA
			Determinative										
Fluoroacetamide	640-19-7	GC-MS	Sample Prep	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	II	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	II	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	II	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	III	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	III
			Determinative										
Fluoroacetic acid and fluoroacetate salts (analyze as fluoroacetate ion)	NA	LC-MS / LC-MS-MS	Sample Prep	Adapted from J. Chromatogr. A (2007) 1139: 271-278	III	Adapted from J. Chromatogr. B (2010) 878: 1045-1050	III	Adapted from J. Chromatogr. B (2010) 878: 1045-1050	III	S301-1 (NIOSH)	III	Adapted from J. Chromatogr. A (2007) 1139: 271-278	III
			Determinative							Adapted from J. Chromatogr. A (2007) 1139: 271-278			
2-Fluoroethanol	371-62-0	GC-MS / GC-FID	Sample Prep	5035A (EPA SW-846)	III	5030C (EPA SW-846)	III	5030C (EPA SW-846)	III	2513 (NIOSH)	III	Not of concern	NA
			Determinative	8260D (EPA SW-846)		8260D (EPA SW-846)		8260D (EPA SW-846)					
Fluorosilicic acid (analyze as fluoride)	16961-83-4	IC-conductivity detection	Sample Prep	Not of concern	NA	300.1, Rev 1.0 (EPA OW)	I	300.1, Rev 1.0 (EPA OW)	I	Not of concern	NA	Not of concern	NA
			Determinative										
Formaldehyde	50-00-0	FGC-ECD / HPLC-UV	Sample Prep	8315A (EPA SW-846)	I	8315A (EPA SW-846)	I	556.1 (EPA OW)	I	2016 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846)	III
			Determinative									8315A (EPA SW-846)	
Gasoline range organics	NA	GC-FID	Sample Prep	5035A (EPA SW-846)	I	5030C (EPA SW-846)	I	5030C (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative	8015D (EPA SW-846)		8015D (EPA SW-846)		8015D (EPA SW-846)				8015D (EPA SW-846)	
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4	HPLC-UV	Sample Prep	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative			8330B (EPA SW-846)		8330B (EPA SW-846)				8330B (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Hexamethylenetriperoxidediamine (HMTD)	283-66-9	LC-MS	Sample Prep	8330B (EPA SW-846)	II	3535A/8330B (EPA SW-846)	II	3535A/8330B (EPA SW-846)	II	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	Adapted from Analyst (2001) 126:1689-1693		Adapted from Analyst (2001) 126:1689-1693		Adapted from Analyst (2001) 126:1689-1693				Adapted from Analyst (2001) 126:1689-1693	
Hydrogen bromide	10035-10-6	IC-conductivity detection	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	7907 (NIOSH)	I	Not of concern	NA
			Determinative										
Hydrogen chloride	7647-01-0	IC-conductivity detection	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	7907 (NIOSH)	I	Not of concern	NA
			Determinative										
Hydrogen cyanide	74-90-8	Visible spectrophotometry	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	6010 (NIOSH)	I	Not of concern	NA
			Determinative										
Hydrogen fluoride	7664-39-3	IC-conductivity detection	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	7906 (NIOSH)	I	Not of concern	NA
			Determinative										
Hydrogen sulfide	7783-06-4	IC-conductivity detection	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	6013 (NIOSH)	I	Not of concern	NA
			Determinative										
Isopropyl methylphosphonic acid (IMPA) (degradation product of GB)	1832-54-8	HPLC-UV / LC-MS-MS	Sample Prep	E2866-12 (ASTM)	II	D7597-16 (ASTM)	II	D7597-16 (ASTM)	III	TO-10A (EPA ORD)	III	EPA/600/R-13/224 (EPA NHSRC)	II
			Determinative										
Kerosene	64742-81-0	GC-FID	Sample Prep	3541/3545A (EPA SW-846)	I	3520C/3535A (EPA SW-846)	I	3520C/3535A (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative	8015D (EPA SW-846)		8015D (EPA SW-846)		8015D (EPA SW-846)				8015D (EPA SW-846)	
Lead arsenate (analyze as total arsenic)	7645-25-2	ICP-AES / ICP-MS	Sample Prep	3050B/3051A (EPA SW-846)	I	3015A (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010D/6020B (EPA SW-846)		6010D/6020B (EPA SW-846)				IO-3.4/IO-3.5 (EPA ORD)		6010D/6020B (EPA SW-846)	
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]	541-25-3	ICP-AES / ICP-MS / LC-MS-MS	Sample Prep	EPA/600/R-15/258[4] (EPA NHSRC)	*II	EPA/600/R-15/258[4] (EPA NHSRC)	*II	EPA/600/R-15/258[4] (EPA NHSRC)	*II	IO-3.1[5] (EPA ORD)	*I	EPA/600/R-15/258[4] (EPA NHSRC)	*II
			Determinative							IO-3.4/IO-3.5[5] (EPA ORD)			
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]	40334-69-8	ICP-AES / ICP-MS / LC-MS-MS	Sample Prep	EPA/600/R-15/258[4] (EPA NHSRC)	*III	EPA/600/R-15/258[4] (EPA NHSRC)	*III	EPA/600/R-15/258[4] (EPA NHSRC)	*III	IO-3.1[5] (EPA ORD)	*I	EPA/600/R-15/258[4] (EPA NHSRC)	*III
			Determinative							IO-3.4/IO-3.5[5] (EPA ORD)			

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]	40334-70-1	ICP-AES / ICP-MS / LC-MS-MS	Sample Prep Determinative	EPA/600/R-15/258[4] (EPA NHSRC)	*III	EPA/600/R-15/258[4] (EPA NHSRC)	*III	EPA/600/R-15/258[4] (EPA NHSRC)	*III	IO-3.1[5] (EPA ORD) IO-3.4/IO-3.5[5] (EPA ORD)	*I	EPA/600/R-15/258[4] (EPA NHSRC)	*III
Lewisite oxide (degradation product of Lewisite)	1306-02-1	ICP-AES / ICP-MS / LC-MS-MS	Sample Prep Determinative	EPA/600/R-15/258[4] (EPA NHSRC)	*III	EPA/600/R-15/258[4] (EPA NHSRC)	*III	EPA/600/R-15/258[4] (EPA NHSRC)	*III	IO-3.1[5] (EPA ORD) IO-3.4/IO-3.5[5] (EPA ORD)	*I	EPA/600/R-15/258[4] (EPA NHSRC)	*III
Mercuric chloride (analyze as total mercury)	7487-94-7	Visible spectrophotometry / CVAA / CVAFS	Sample Prep Determinative	7473[10] (EPA SW-846)	I	245.1[11] (EPA OW)	I	245.1 (EPA OW)	I	Not of concern	NA	9102 (NIOSH) 7473[10] (EPA SW-846)	I
Mercury, Total	7439-97-6	Visible spectrophotometry / CVAA / CVAFS	Sample Prep Determinative	7473[10] (EPA SW-846)	I	245.1[11] (EPA OW)	I	245.1 (EPA OW)	I	IO-5 (EPA ORD)	I	9102 (NIOSH) 7473[10] (EPA SW-846)	I
Methamidophos	10265-92-6	LC-MS-MS	Sample Prep Determinative	Adapted from J. Chromatogr. A (2007) 1154(1): 3-25	II	Adapted from Chromatographia (2006) 63(5/6): 233-237	II	538 (EPA OW)	I	Adapted from J. Chromatogr. A (2007) 1154(1): 3-25	III	Adapted from J. Chromatogr. A (2007) 1154(1): 3-25	III
Methomyl	16752-77-5	HPLC-UV / HPLC-FL / LC-MS-MS	Sample Prep Determinative	8318A (EPA SW-846)	II	D7645-16 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846) 8318A (EPA SW-846)	III
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2	Visible spectrophotometry / CVAA / CVAFS	Sample Prep Determinative	7473[10] (EPA SW-846)	I	245.1[11] (EPA OW)	I	245.1 (EPA OW)	I	IO-5 (EPA ORD)	I	9102 (NIOSH) 7473[10] (EPA SW-846)	I
Methyl acrylonitrile	126-98-7	HPLC-UV / GC-MS	Sample Prep Determinative	5035A (EPA SW-846) 8260D (EPA SW-846)	II	524.2[1] (EPA OW)	II	524.2[1] (EPA OW)	II	PV2004 (OSHA)	III	3570/8290A Appendix A (EPA SW-846) 8260D (EPA SW-846)	III
Methylamine	74-89-5	HPLC-FL / HPLC-vis	Sample Prep Determinative	Not of concern	NA	Not of concern	NA	Not of concern	NA	OSHA 40	I	Not of concern	NA
N-Methyldiethanolamine (MDEA) (degradation product of HN-2)	105-59-9	LC-MS-MS / IC-conductivity detection	Sample Prep Determinative	3541/3545A (EPA SW-846) EPA/600/R-11/143 (EPA NHSRC)	III	D7599-16 (ASTM)	II	D7599-16 (ASTM)	III	3509 (NIOSH)	III	EPA/600/R-11/143 (EPA NHSRC)	II
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3	GC-MS	Sample Prep Determinative	EPA/600/R-16/115 (EPA NHSRC)	*III	EPA/600/R-16/115 (EPA NHSRC)	*III	EPA/600/R-16/115 (EPA NHSRC)	*III	TO-17[3] (EPA ORD)	*III	EPA/600/R-16/115 (EPA NHSRC)	*III

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
3-Methyl fentanyl	42045-87-4	LC-MS-MS	Sample Prep	3541/3545A (EPA SW-846)	III	3520C/3535A (EPA SW-846)	III	3520C/3535A (EPA SW-846)	III	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	Adapted from J. Chromatogr. B (2014) 962: 52-58		Adapted from J. Chromatogr. B (2014) 962: 52-58		Adapted from J. Chromatogr. B (2014) 962: 52-58				Adapted from J. Chromatogr. B (2014) 962: 52-58	
Methyl fluoroacetate (analyze as fluoroacetate ion)	453-18-9	LC-MS	Sample Prep	J. Chromatogr. A (2007) 1139: 271-278	III	J. Chromatogr. B (2010) 878: 1045-1050	III	J. Chromatogr. B (2010) 878: 1045-1050	III	S301-1 (NIOSH)	III	J. Chromatogr. A (2007) 1139: 271-278	III
			Determinative							J. Chromatogr. A (2007) 1139: 271-278			
Methyl hydrazine	60-34-4	Visible spectrophotometry / HPLC-UV	Sample Prep	3541/3545A (EPA SW-846)	III	J. Chromatogr. (1993) 617: 157-162	III	J. Chromatogr. (1993) 617: 157-162	III	3510 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	J. Chromatogr. (1993) 617: 157-162								J. Chromatogr. (1993) 617: 157-162	
Methyl isocyanate	624-83-9	HPLC-UV	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	OSHA 54	I	Not of concern	NA
			Determinative										
Methyl paraoxon	950-35-6	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	III	3535A (EPA SW-846)	III	3535A (EPA SW-846)	III	TO-10A (EPA ORD)	III	EPA/600/R-16/114 (EPA NHSRC)	III
			Determinative			8270E[9] (EPA SW-846)		8270E[9] (EPA SW-846)					
Methyl parathion	298-00-0	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	3535A (EPA SW-846)	I	3535A (EPA SW-846)	I	TO-10A (EPA ORD)	I	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative			8270E (EPA SW-846)		8270E (EPA SW-846)					
Methylphosphonic acid (MPA) (degradation product of VX, GB, & GD)	993-13-5	HPLC-UV / LC-MS-MS	Sample Prep	E2866-12 (ASTM)	II	D7597-16 (ASTM)	II	D7597-16 (ASTM)	III	TO-10A (EPA ORD)	III	EPA/600/R-13/224 (EPA NHSRC)	II
			Determinative										
Mevinphos	7786-34-7	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	3535A (EPA SW-846)	I	525.2[6] (EPA OW)	I	TO-10A (EPA ORD)	II	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative			8270E (EPA SW-846)							
Monocrotophos	6923-22-4	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	I	3535A (EPA SW-846)	I	3535A (EPA SW-846)	I	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8270E (EPA SW-846)		8270E (EPA SW-846)		8270E (EPA SW-846)				8270E (EPA SW-846)	
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8	GC-MS	Sample Prep	EPA/600/R-12/653 (EPA NHSRC)	*II	EPA/600/R-12/653 (EPA NHSRC)	*II	EPA/600/R-12/653 (EPA NHSRC)	*II	TO-17 (EPA ORD)	*III	EPA/600/R-12/653 (EPA NHSRC)	*II
			Determinative										

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine]	51-75-2	GC-MS	Sample Prep	EPA/600/R-12/653 (EPA NHSRC)	*III	EPA/600/R-12/653 (EPA NHSRC)	*III	EPA/600/R-12/653 (EPA NHSRC)	*III	TO-17 (EPA ORD)	*III	EPA/600/R-12/653 (EPA NHSRC)	*III
			Determinative										
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1	GC-MS	Sample Prep	EPA/600/R-12/653 (EPA NHSRC)	*II	EPA/600/R-12/653 (EPA NHSRC)	*II	EPA/600/R-12/653 (EPA NHSRC)	*II	TO-17 (EPA ORD)	*III	EPA/600/R-12/653 (EPA NHSRC)	*II
			Determinative										
Mustard, sulfur / Mustard gas (HD)	505-60-2	GC-MS	Sample Prep	EPA/600/R-16/115 (EPA NHSRC)	*I	EPA/600/R-16/115 (EPA NHSRC)	*I	EPA/600/R-16/115 (EPA NHSRC)	*I	TO-17 (EPA ORD)	III	EPA/600/R-16/115 (EPA NHSRC)	*I
			Determinative										
Nicotine compounds (analyze as nicotine)	54-11-5	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	3535A (EPA SW-846)	II	3535A (EPA SW-846)	II	Not of concern	NA	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative			8270E (EPA SW-846)		8270E (EPA SW-846)					
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0	HPLC-UV	Sample Prep	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative			8330B (EPA SW-846)		8330B (EPA SW-846)				8330B (EPA SW-846)	
Osmium tetroxide (analyze as total osmium)	20816-12-0	ICP-AES / ICP-MS	Sample Prep	3051A (EPA SW-846)	II	3015A (EPA SW-846)	II	3015A (EPA SW-846)	II	IO-3.1 (EPA ORD)	II	3051A (EPA SW-846)	III
			Determinative	6010D/6020B (EPA SW-846)		6010D/6020B (EPA SW-846)		6010D/6020B (SW-846)		IO-3.4 (EPA ORD)		6010D/6020B (EPA SW-846)	
Oxamyl	23135-22-0	HPLC-UV / HPLC-FL / LC-MS-MS	Sample Prep	8318A (EPA SW-846)	II	D7645-16 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846)	III
			Determinative									8318A (EPA SW-846)	
Paraoxon	311-45-5	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	III	3520C/3535A (EPA SW-846)	III	3520C/3535A (EPA SW-846)	III	TO-10A (EPA ORD)	III	EPA/600/R-16/114 (EPA NHSRC)	III
			Determinative			8270E (EPA SW-846)		8270E (EPA SW-846)					
Paraquat	4685-14-7	HPLC-UV / LC-MS-MS	Sample Prep	Adapted from J. Chromatogr. A (2008) 1196-1197: 110-116	II	549.2 (EPA OW)	I	549.2 (EPA OW)	I	Not of concern	NA	Adapted from J. Chromatogr. A (2008) 1196-1197: 110-116	III
			Determinative										
Parathion	56-38-2	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	3520C/3535A (EPA SW-846)	I	3520C/3535A (EPA SW-846)	I	TO-10A (EPA ORD)	III	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative			8270E (EPA SW-846)		8270E (EPA SW-846)					

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Pentaerythritol tetranitrate (PETN)	78-11-5	HPLC-UV	Sample Prep	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative			8330B (EPA SW-846)		8330B (EPA SW-846)				8330B (EPA SW-846)	
Phencyclidine	77-10-1	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	EPA/600/R-16/114 (EPA NHSRC)	II	EPA/600/R-16/114 (EPA NHSRC)	II	TO-10A (EPA ORD)	II	9106/9109 (NIOSH)	II
			Determinative										
Phorate	298-02-2	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	3535A (EPA SW-846)	I	3535A (EPA SW-846)	I	TO-10A (EPA ORD)	II	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative			8270E[9] (EPA SW-846)		8270E[9] (EPA SW-846)					
Phorate sulfone	2588-04-7	GC-MS / LC-MS-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	III	540 (EPA OW)	I	540 (EPA OW)	I	TO-10A (EPA ORD)	III	EPA/600/R-16/114 (EPA NHSRC)	III
			Determinative										
Phorate sulfone oxon	2588-06-9	GC-MS / LC-MS-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	III	540 (EPA OW)	III	540 (EPA OW)	III	TO-10A (EPA ORD)	III	EPA/600/R-16/114 (EPA NHSRC)	III
			Determinative										
Phorate sulfoxide	2588-03-6	GC-MS / LC-MS-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	III	540 (EPA OW)	I	540 (EPA OW)	I	TO-10A (EPA ORD)	III	EPA/600/R-16/114 (EPA NHSRC)	III
			Determinative										
Phorate sulfoxide oxon	2588-05-8	GC-MS / LC-MS-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	III	540 (EPA OW)	III	540 (EPA OW)	III	TO-10A (EPA ORD)	III	EPA/600/R-16/114 (EPA NHSRC)	III
			Determinative										
Phosgene	75-44-5	GC-NPD	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	OSHA 61	I	Not of concern	NA
			Determinative										
Phosphamidon	13171-21-6	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	3520C/3535A (EPA SW-846)	I	525.3 (EPA OW)	I	TO-10A (EPA ORD)	II	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative			8270E (EPA SW-846)							
Phosphine	7803-51-2	Visible spectrophotometry	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	6002 (NIOSH)	I	Not of concern	NA
			Determinative										
Phosphorus trichloride	7719-12-2	Visible spectrophotometry	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	6402 (NIOSH)	I	Not of concern	NA
			Determinative										
Pinacolyl methyl phosphonic acid (PMPA) (degradation product of GD)	616-52-4	HPLC-UV / LC-MS-MS	Sample Prep	E2866-12 (ASTM)	II	D7597-16 (ASTM)	II	D7597-16 (ASTM)	III	TO-10A (EPA ORD)	III	EPA/600/R-13/224 (EPA NHSRC)	II
			Determinative										

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Propylene oxide	75-56-9	GC-MS / GC-FID	Sample Prep	5035A (EPA SW-846)	II	5030C (EPA SW-846)	II	5030C (EPA SW-846)	II	1612 (NIOSH)	I	Not of concern	NA
			Determinative	8260D (EPA SW-846)		8260D (EPA SW-846)		8260D (EPA SW-846)					
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4	GC-MS	Sample Prep	EPA/600/R-12/653 (EPA NHSRC)	*II	EPA/600/R-12/653 (EPA NHSRC)	*II	EPA/600/R-12/653 (EPA NHSRC)	*II	TO-17 (EPA ORD)	*III	EPA/600/R-12/653 (EPA NHSRC)	*II
			Determinative										
Sarin (GB)	107-44-8	GC-MS	Sample Prep	EPA/600/R-16/115 (EPA NHSRC)	*I	EPA/600/R-16/115 (EPA NHSRC)	*I	EPA/600/R-16/115 (EPA NHSRC)	*I	TO-17[3] (EPA ORD)	*III	EPA/600/R-16/115 (EPA NHSRC)	*I
			Determinative										
Sodium arsenite (analyze as total arsenic)	7784-46-5	ICP-AES / ICP-MS	Sample Prep	3050B/3051A (EPA SW-846)	I	3015A (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010D/6020B (EPA SW-846)		6010D/6020B (EPA SW-846)				IO-3.4/IO-3.5 (EPA ORD)		6010D/6020B (EPA SW-846)	
Sodium azide (analyze as azide ion)	26628-22-8	IC-conductivity detection	Sample Prep	Adapted from J. Forensic Sci. (1998) 43(1): 200-202[12]	II	Adapted from J. Forensic Sci. (1998) 43(1): 200-202[12]	II	Adapted from J. Forensic Sci. (1998) 43(1): 200-202[12]	II	ID-211 (OSHA)	I	ID-211 (OSHA)	I
			Determinative	300.1, Rev 1.0[13] (EPA OW)		300.1, Rev 1.0[13] (EPA OW)		300.1, Rev 1.0[13] (EPA OW)					
Soman (GD)	96-64-0	GC-MS	Sample Prep	EPA/600/R-16/115 (EPA NHSRC)	*I	EPA/600/R-16/115 (EPA NHSRC)	*I	EPA/600/R-16/115 (EPA NHSRC)	*I	TO-17[3] (EPA ORD)	*III	EPA/600/R-16/115 (EPA NHSRC)	*I
			Determinative										
Strychnine	57-24-9	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	3535A (EPA SW-846)	I	3535A (EPA SW-846)	I	Not of concern	NA	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative			8270E (EPA SW-846)		8270E (EPA SW-846)					
Tabun (GA)	77-81-6	GC-MS	Sample Prep	EPA/600/R-12/653 (EPA NHSRC)	*II	EPA/600/R-12/653 (EPA NHSRC)	*II	EPA/600/R-12/653 (EPA NHSRC)	*II	TO-17 (EPA ORD)	*III	EPA/600/R-12/653 (EPA NHSRC)	*II
			Determinative										
Tetraethyl pyrophosphate (TEPP)	107-49-3	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	3511 (EPA SW-846)	II	3511 (EPA SW-846)	II	TO-10A (EPA ORD)	II	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative			8270E (EPA SW-846)		8270E (EPA SW-846)					
Tetramethylenedisulfotetramine (TETS)	80-12-6	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)	II	EPA/600/R-16/114 (EPA NHSRC)	I	EPA/600/R-16/114 (EPA NHSRC)	I	TO-10A (EPA ORD)	II	EPA/600/R-16/114 (EPA NHSRC)	II
			Determinative										

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
Thallium sulfate (analyze as total thallium)	10031-59-1	ICP-AES / ICP-MS	Sample Prep	3050B/3051A (EPA SW-846)	I	3015A (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010D/6020B (EPA SW-846)		6010D/6020B (EPA SW-846)				IO-3.4/IO-3.5 (EPA ORD)		6010D/6020B (EPA SW-846)	
Thiodiglycol (TDG) (degradation product of HD)	111-48-8	HPLC / LC-MS-MS	Sample Prep	E2787-11 (ASTM)	II	D7598-16 (ASTM)	II	D7598-16 (ASTM)	III	TO-10A (EPA ORD)	III	E2838-11 (ASTM)	II
			Determinative										
Thiofanox	39196-18-4	HPLC-UV / LC-MS-MS	Sample Prep	3541/3545A (EPA SW-846)	III	D7645-16 (ASTM)	II	538 (EPA OW)	I	5601 (NIOSH)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	D7645-16 (ASTM)								D7645-16 (ASTM)	
1,4-Thioxane (degradation product of HD)	15980-15-1	GC-MS	Sample Prep	EPA/600/R-16/114 (EPA NHSRC)[14]	II	EPA/600/R-16/114 (EPA NHSRC)[14]	II	EPA/600/R-16/114 (EPA NHSRC)[14]	II	Not of concern	NA	EPA/600/R-16/114 (EPA NHSRC)[14]	II
			Determinative										
Titanium tetrachloride (analyze as total titanium)	7550-45-0	ICP-AES / ICP-MS	Sample Prep	3051A (EPA SW-846)	I	Not of concern	NA	Not of concern	NA	Not of concern	NA	3051A (EPA SW-846)	III
			Determinative	6010D/6020B (EPA SW-846)								6010D/6020B (EPA SW-846)	
Triethanolamine (TEA) (degradation product of HN-3)	102-71-6	LC-MS-MS / IC-conductivity detection	Sample Prep	3541/3545A (EPA SW-846)	III	D7599-16 (ASTM)	II	D7599-16 (ASTM)	III	3509 (NIOSH)	II	EPA/600/R-11/143 (EPA NHSRC)	II
			Determinative	EPA/600/R-11/143 (EPA NHSRC)									
Trimethyl phosphite	121-45-9	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III	Not of concern	NA	Not of concern	NA	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8270E[15] (EPA SW-846)								8270E[15] (EPA SW-846)	
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4	HPLC-UV	Sample Prep	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative			8330B (EPA SW-846)		8330B (EPA SW-846)				8330B (EPA SW-846)	
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7	HPLC-UV	Sample Prep	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative			8330B (EPA SW-846)		8330B (EPA SW-846)				8330B (EPA SW-846)	
Vanadium pentoxide (analyze as total vanadium)	1314-62-1	ICP-AES / ICP-MS	Sample Prep	3050B/3051A (EPA SW-846)	I	3015A (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010D/6020B (EPA SW-846)		6010D/6020B (EPA SW-846)				IO-3.4/IO-3.5 (EPA ORD)		6010D/6020B (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Non-Drinking Water Samples		Drinking Water Samples		Air Samples		Wipes	
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0	GC-MS	Sample Prep Determinative	EPA/600/R-16/116 (EPA NHSRC)	*III	EPA/600/R-16/116 (EPA NHSRC)	*III	EPA/600/R-16/116 (EPA NHSRC)	*III	TO-17 (EPA ORD)	*III	EPA/600/R-16/116 (EPA NHSRC)	*III
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5	GC-MS	Sample Prep Determinative	EPA/600/R-16/116 (EPA NHSRC)	*III	EPA/600/R-16/116 (EPA NHSRC)	*III	EPA/600/R-16/116 (EPA NHSRC)	*III	TO-17 (EPA ORD)	*III	EPA/600/R-16/116 (EPA NHSRC)	*III
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5	GC-MS	Sample Prep Determinative	EPA/600/R-16/116 (EPA NHSRC)	*III	EPA/600/R-16/116 (EPA NHSRC)	*III	EPA/600/R-16/116 (EPA NHSRC)	*III	TO-17 (EPA ORD)	*III	EPA/600/R-16/116 (EPA NHSRC)	*III
VX [O-ethyl-S-(2-diisopropylaminoethyl)methyl-phosphonothiolate]	50782-69-9	GC-MS	Sample Prep Determinative	EPA/600/R-16/116 (EPA NHSRC)	*II	EPA/600/R-16/116 (EPA NHSRC)	*I	EPA/600/R-16/116 (EPA NHSRC)	*I	TO-17 (EPA ORD)	*III	EPA/600/R-16/116 (EPA NHSRC)	*II
White phosphorus	12185-10-3	GC-NPD / GC-FPD	Sample Prep Determinative	7580 (EPA SW-846)	I	7580 (EPA SW-846)	I	7580 (EPA SW-846)	I	7905 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846) 7580 (EPA SW-846)	III

* Only laboratories approved under the ERLN umbrella are designated for handling the CWA standards needed for this method. For access to the nearest ERLN laboratory specially trained and equipped for CWA analysis, contact the EPA Headquarters Emergency Operations Center (EPA/HQ-EOC) at 202-564-3850.

Footnotes

[1] Methods 524.3 or 524.4 may be used in place of Method 524.2 provided the laboratory has the necessary equipment and expertise.

[2] If problems occur when using this method, TO-10A should be used.

[3] If problems occur when using this method, Method TO-15 should be used.

[4] The following methods can be used to analyze these compounds as total arsenic in situations where high throughput analysis is needed or where standards are not available for the specific compounds: ICP AES/MS Methods 200.7/200.8 for drinking water; Methods 3015A/6010D/6020B for aqueous liquid samples; Methods 3050B/3051A/6010D/6020B for solid samples; and Methods 9102/6010D/6020B for wipes.

[5] TO Methods IO-3.1, IO-3.4 and IO-3.5 address these compounds as total arsenic in air samples.

[6] Method 525.3 may be used in place of Method 525.2 provided the laboratory has the necessary equipment and expertise.

[7] Standard Method 4500-CN-G may be used in place of RLAB Method 3135.2I for the analysis of cyanide amenable to chlorination in aqueous liquid or drinking water samples.

[8] The inline distillation method, EPA-821-B-01-009, may be used to prepare and analyze for total cyanide in aqueous liquid samples.

[9] If problems occur during measurement of oxon compounds, analysts should consider use of procedures included in Kamal, A. et al ., "Oxidation of selected organophosphate pesticides during chlorination of simulated drinking water." Water Research. 2009. 43(2): 522–534. http://www.sciencedirect.com/science/journal/00431354.

[10] If equipment is not available or problems occur when analyzing solid and wipe samples, use CVAA Method 7471B (EPA SW-846).

[11] If problems occur when using EPA Method 245.1 for these analytes during preparation and analysis of aqueous liquid samples, refer to EPA Method 7470A (SW-846).

[12] Water extraction, filtration and acidification steps from the Journal of Forensic Science. 1998. 43(1): 200-202 should be used for the preparation of solid samples. Filtration and acidification steps from this journal should be used for preparation of aqueous liquid and drinking water samples.

[13] If analyses are problematic, refer to column manufacturer for alternate conditions.

[14] If problems occur when using this method, SW-846 Method 8260D and appropriate corresponding sample preparation procedures (i.e., 5035A for solid samples, and 5030C for aqueous liquid and drinking water samples) should be used.

[15] If problems occur with analyses, lower the injection temperature.

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Appendix B1: Selected Radiochemical Methods for Environmental Samples

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SAM 2017 — Appendix B1: Selected Radiochemical Methods for Environmental Samples

***Note*: Column headings are defined in Section 6.0.**

Analyte Class	Determinative Technique	Drinking Water	Aqueous and Liquid Phase	Soil and Sediment	Surface Wipes	Air Filters	Vegetation
Gross Alpha	Alpha / Beta counting	900.0 (EPA)	7110 B (SM)	AP1 (ORISE)	FRMAC, Vol 2, pg. 33 (DOE)	FRMAC, Vol 2, pg. 33 (DOE)	AP1 (ORISE)
Gross Beta	Alpha / Beta counting	900.0 (EPA)	7110 B (SM)	AP1 (ORISE)	FRMAC, Vol 2, pg. 33 (DOE)	FRMAC, Vol 2, pg. 33 (DOE)	AP1 (ORISE)
Gamma	Gamma spectrometry	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Select Mixed Fission Products[1]	Gamma spectrometry	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Total Activity Screening	Liquid scintillation	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)

Analyte(s)	CAS RN	Determinative Technique	Drinking Water		Aqueous and Liquid Phase		Soil and Sediment		Surface Wipes		Air Filters		Vegetation	
			Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory
Americium-241[3]	14596-10-2	Alpha spectrometry	Rapid Radiochemical Method for Am-241[4] (EPA)	Am-04-RC (HASL-300)	D3084-05 (2012) (ASTM)	Am-04-RC (HASL-300)	Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	Am-01-RC[5] (HASL-300)	Rapid methods* for acid or fusion digestion (EPA)	Am-04-RC (HASL-300)	Rapid methods* for acid or fusion digestion (EPA)	Am-04-RC (HASL-300)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	Am-06-RC (HASL-300)
		Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Californium-252[3]	13981-17-4	Alpha spectrometry	D3084-05 (2012) (ASTM)	Rapid Radiochemical Method for Californium-252 (EPA)	D3084-05 (2012) (ASTM)	Rapid Radiochemical Method for Californium-252 (EPA)	D3084-05 (2012) (ASTM)	Rapid Radiochemical Method for Californium-252 (EPA)	D3084-05 (2012) (ASTM)	Rapid Radiochemical Method for Californium-252 (EPA)	D3084-05 (2012) (ASTM)	Rapid Radiochemical Method for Californium-252 (EPA)	D3084-05 (2012) (ASTM)	Am-06-RC (HASL-300)
Cesium-137	10045-97-3	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Cobalt-60	10198-40-0	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Curium-244[3]	13981-15-2	Alpha spectrometry	D3084-05 (2012) (ASTM)	Rapid Radiochemical Method for Curium-244 in Water (EPA)	D3084-05 (2012) (ASTM)	Rapid Radiochemical Method for Curium-244 in Water (EPA)	D3084-05 (2012) (ASTM)	Rapid Radiochemical Method for Curium-244 in Air Particulate Filters, Swipes and Soil (EPA)	D3084-05 (2012) (ASTM)	Rapid Radiochemical Method for Curium-244 in Air Particulate Filters, Swipes and Soil (EPA)	D3084-05 (2012) (ASTM)	Rapid Radiochemical Method for Curium-244 in Air Particulate Filters, Swipes and Soil (EPA)	D3084-05 (2012) (ASTM)	Am-06-RC (HASL-300)
Europium-154	15585-10-1	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Indium-111	15750-15-9	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Iodine-125	14158-31-7	Gamma spectrometry	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9[6] (ORISE)	Procedure #9[6] (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)

Analyte(s)	CAS RN	Determinative Technique	Drinking Water		Aqueous and Liquid Phase		Soil and Sediment		Surface Wipes		Air Filters		Vegetation	
			Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory
Iodine-131	10043-66-0	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R[6] (HASL-300)	Ga-01-R[6] (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Iridium-192	14694-69-0	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Molybdenum-99	14119-15-4	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Neptunium-237	13994-20-2	Alpha spectrometry	907.0 (EPA)	907.0 (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	SOP for Actinides in Environmental Matrices (EPA-NAREL)
Neptunium-239	13968-59-7	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Phosphorus-32	14596-37-3	Liquid scintillation / Beta counting	Rapid Radiochemical Method for P-32 in water[4] (EPA)	R4-73-014 (EPA)	R4-73-014 (EPA)	R4-73-014 (EPA)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)
Plutonium-238[3]	13981-16-3	Alpha spectrometry	Rapid Radiochemical Method for Pu-238 and -239/240[4] (EPA)	EMSL-33 (EPA)	D3084-05 (2015) (ASTM)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	Am-06-RC (HASL-300)
Plutonium-239[3]	15117-48-3	Alpha spectrometry	Rapid Radiochemical Method for Pu-238 and 239/240[4] (EPA)	EMSL-33 (EPA)	D3084-05 (2012) (ASTM)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	Am-06-RC (HASL-300)
Polonium-210	13981-52-7	Alpha spectrometry	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Method 111 (EPA)	Method 111 (EPA)	Method 111 (EPA)	Method 111 (EPA)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)
Radium-223	15623-45-7	Alpha spectrometry	Rapid Radiochemical Method for Ra-226[4] (EPA)		Rapid Radiochemical Method for Ra-226 (EPA)		Rapid Radiochemical Method for Ra-226 (EPA)		Rapid Radiochemical Method for Ra-226 (EPA)		Rapid Radiochemical Method for Ra-226 (EPA)		Rapid Radiochemical Method for Ra-226 (EPA)	
Radium-226	13982-63-3	Alpha spectrometry / Alpha counting / Gamma spectrometry	Rapid Radiochemical Method for Ra-226[4] (EPA)	903.1 (EPA)	7500-Ra B (SM)	7500-Ra C (SM)	Rapid Method for Radium in Soil (EPA)	AP7 (ORISE)	Rapid methods* for acid or fusion digestion (EPA)	Rapid Method for Radium-226 in Building Materials (EPA)	Rapid methods* for acid or fusion digestion (EPA)	Rapid Method for Radium-226 in Building Materials (EPA)	Ra-03-RC (HASL-300)	Ra-03-RC (HASL-300)
Rhenium-188	14378-26-8	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Rubidium-82	14391-63-0	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Ruthenium-103	13968-53-1	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)

Analyte(s)	CAS RN	Determinative Technique	Drinking Water		Aqueous and Liquid Phase		Soil and Sediment		Surface Wipes		Air Filters		Vegetation	
			Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory
Ruthenium-106	13967-48-1	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Selenium-75	14265-71-5	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Strontium-89	14158-27-1	Beta counting	905.0 (EPA)	905.0 (EPA)	905.0 (EPA)	905.0 (EPA)	Actinides and Sr-89/90 in Soil Samples (DOE SRS)	Strontium in Food and Bioenvironmental Samples (EPA)	Strontium in Food and Bioenvironmental Samples (EPA)		Strontium in Food and Bioenvironmental Samples (EPA)		Actinides and Sr-89/90 in Vegetation (DOE SRS)	Strontium in Food and Bioenvironmental Samples (EPA)
Strontium-90	10098-97-2	Beta counting / alpha spectrometry	Rapid Radiochemical Method for Radiostrontium[4] (EPA)	905.0 (EPA)	D5811-08 (2013) (ASTM)	D5811-08 (2013) (ASTM)	Rapid Method for Sodium Carbonate Fusion of Soil and Soil-Related Matrices (EPA)	Sr-03-RC (HASL-300)	Rapid methods* for acid or fusion digestion (EPA)	Sr-03-RC (HASL-300)	Rapid methods* for acid or fusion digestion (EPA)	Sr-03-RC (HASL-300)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	Sr-03-RC (HASL-300)
Technetium-99	14133-76-7	Liquid scintillation / beta counting	Tc-02-RC (HASL-300)	Tc-02-RC (HASL-300)	D7168-16 (ASTM)	D7168-16 (ASTM)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	Tc-01-RC (HASL-300)
Technetium-99m	378784-45-3	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Thorium-227	15623-47-9	Alpha spectrometry	907.0 (EPA)	907.0 (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)	
Thorium-228	14274-82-9	Alpha spectrometry	907.0 (EPA)	907.0 (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)	
Thorium-230	14269-63-7	Alpha spectrometry	907.0 (EPA)	907.0 (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)	
Thorium-232	7440-29-1	Alpha spectrometry	907.0 (EPA)	907.0 (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)		SOP for Actinides in Environmental Matrices (EPA-NAREL)	
Tritium (Hydrogen-3)	10028-17-8	Liquid scintillation	906.0 (EPA)	906.0 (EPA)	906.0 (EPA)	906.0 (EPA)	AP2 (ORISE)	AP2 (ORISE)	AP2 (ORISE)	AP2 (ORISE)	Not applicable[7]	Not applicable[7]	AP2 (ORISE)	AP2 (ORISE)
Uranium-234[3]	13966-29-5	Alpha spectrometry	Rapid Radiochemical Method for Isotopic Uranium in Water[4] (EPA)	D3972-09 (ASTM)	7500-U B[8] (SM)	7500-U C (SM)	Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	U-02-RC (HASL-300)
Uranium-235[3]	15117-96-1	Alpha spectrometry	Rapid Radiochemical Method for Isotopic Uranium in Water[4] (EPA)	D3972-09 (ASTM)	7500-U B[8] (SM)	7500-U C (SM)	Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	U-02-RC (HASL-300)

Analyte(s)	CAS RN	Determinative Technique	Drinking Water		Aqueous and Liquid Phase		Soil and Sediment		Surface Wipes		Air Filters		Vegetation	
			Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory
Uranium-238[3]	7440-61-1	Alpha spectrometry	Rapid Radiochemical Method for Isotopic Uranium in Water[4] (EPA)	D3972-09 (ASTM)	7500-U B[8] (SM)	7500-U C (SM)	Rapid Method for Fusion of Soil and Soil-Related Matrices (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Rapid methods* for acid or fusion digestion (EPA)	SOP for Actinides in Environmental Matrices (EPA-NAREL)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	U-02-RC (HASL-300)

* These rapid methods describe wipe and air filter digestion procedures, and include references to the analyte-specific separation procedures listed for rapid analysis of drinking water samples, to be used to complete analysis of the digested samples.

Footnotes

[1] Please note that this category does not cover all fission products. In addition to the specific radionuclides listed in this appendix, gamma-ray spectrometry with a high resolution HP(Ge) detector will identify and quantify fission products with gamma rays in the energy range of 30 keV to 2000 keV. The sensitivity will be dependent on the detector efficiency and the gamma-ray emission probabilities (branching ratio) for the specific radionuclide.

[2] In those cases where the same method is listed for qualitative determination and confirmatory analysis, qualitative determination can be performed by application of the method over a shorter count time than that used for confirmatory analysis.

[3] If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 for qualitative determination or confirmatory analysis of alpha radioactivity.

[4] This method is listed for rapid qualitative screening of drinking water samples. The method is not intended for use in compliance monitoring of drinking water.

[5] In cases where only small sample volumes (≤100 g) are available, use HASL-300 Method Pu-12-RC.

[6] This procedure should be used only for filters specifically designed for iodine.

[7] Because tritium is not sampled using traditional air filters, this matrix is not applicable.

[8] This method was developed for measurement of total uranium and does not distinguish between uranium isotopes.

Appendix B2: Selected Rapid Radiochemical Methods for Outdoor Building and Infrastructure Materials

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SAM 2017 — Appendix B2: Selected Rapid Radiochemical Methods for Outdoor Building and Infrastructure Materials

***Note*: Column headings are defined in Section 6.0.**

Analyte(s)	CAS RN	Determinative Technique	Asphalt Shingles		Asphalt Matrices (Paving Materials)		Concrete		Brick	
			Sample Preparation	Confirmatory Analysis	Sample Preparation	Confirmatory Analysis	Sample Preparation	Confirmatory Analysis	Sample Preparation	Confirmatory Analysis
Americium-241	14596-10-2	Alpha spectrometry	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Am-241 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Am-241 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Americium-241 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Americium-241 in Building Materials (EPA)
Plutonium-238	13981-16-3	Alpha spectrometry	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Pu-238 and Pu-239/240 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Pu-238 and Pu-239/240 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Plutonium-238, -239/240 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Plutonium-238, -239/240 in Building Materials (EPA)
Plutonium-239	15117-48-3	Alpha spectrometry	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Pu-238 and Pu-239/240 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Pu-238 and Pu-239/240 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Plutonium-238, -239/240 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Plutonium-238, -239/240 in Building Materials (EPA)
Radium-226	13982-63-3	Alpha spectrometry	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Ra-226 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Ra-226 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Radium-226 in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Radium 226 in Building Materials (EPA)
Strontium-90	10098-97-2	Beta counting	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Total Radiostrontium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Total Radiostrontium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Total Radiostrontium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Total Radiostrontium in Building Materials (EPA)
Uranium-234	13966-29-5	Alpha spectrometry	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)
Uranium-235	15117-96-1	Alpha spectrometry	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)
Uranium-238	7440-61-1	Alpha spectrometry	Rapid Method for Sodium Hydroxide Fusion of Asphalt Roofing Materials (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Asphalt Matrices (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)	Rapid Method for Sodium Hydroxide Fusion of Concrete and Brick (EPA)	Rapid Method for Isotopic Uranium in Building Materials (EPA)

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Appendix C: Selected Pathogen Methods

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SAM 2017 — Appendix C: Selected Pathogen Methods

Not all methods have been evaluated for each pathogen/sample type/environmental matrix combination in Appendix C. Each laboratory using these methods must operate a formal quality assurance program and, at a minimum, analyze appropriate quality control (QC) samples (Section 7.1.2). Also, if required, a modification or an appropriate replacement method may be warranted for a specific pathogen/sample type/environmental matrix or a combination thereof. Additionally, the SAM Pathogen primary and alternate points of contact should be consulted for additional guidance (Section 4.0, Points of Contact).

The fitness of a method for an intended use is related to site-specific data quality objectives (DQOs) for a particular environmental remediation activity. These selected pathogen methods have been assigned tiers (below) to indicate a level of method usability for the specific analyte and sample type. The assigned tiers pertain only to technical aspects of method usability, and do not pertain to aspects such as cost, equipment availability, and sample throughput. Assigned usability tiers are indicated next to each method or method combination throughout this appendix.

Tier I: The method was developed for the pathogen and sample type. The method has been evaluated by multiple laboratories, a detailed protocol has been developed, and suitable QC measures and checks are provided. (Examples: EPA Method 1623.1 [Cryptosporidium in drinking water]; Standard Methods 9260 E [Shigella culture method])

Tier II: The pathogen is not the target of the method, and the method has been evaluated by one or more laboratories. The available data and/or information indicate that additional testing and/or modifications will likely be needed. (Example: Cunningham et al. 2010. [Shigella molecular method])

Tier III: The pathogen is not the target of the method but the method is for the specific sample type and the pathogen is similar to the target of the method (i.e. vegetative bacteria, spore-forming bacteria, virus or protozoan). Data and expert opinion suggest, however, that the method(s) may be applicable with modifications. (Example: EPA Yersinia pestis protocol for Salmonella enterica serovar Typhi [S . Typhi] in post decontamination waste water.)

***Notes*:**
Samples should not be stored indefinitely, and should be processed and analyzed as soon as possible upon receipt.

If viability determinations are needed (e.g., for post decontamination phase samples), a viability-based procedure (such as culture) should be used. Rapid analysis techniques (such as PCR, immunoassays) without culture are preferred for determination of the extent and magnitude of contamination (e.g., for site characterization phase samples). Please see Figure 7-1.

Column headings are defined in Section 7.0.

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method									
			Aerosol (growth media, filter, liquid)		Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)		Soil		Drinking Water		Post Decontamination Waste Water	
Bacteria												
Bacillus anthracis [Anthrax]	NA	Sample Preparation	EPA *Bacillus anthracis* (BA) Protocol (EPA/600/R-12/577)	I	EPA BA Protocol (EPA/600/R-12/577)	I	Silvestri *et al.* 2016. J. of Microbiol. Methods. 130: 6-13	II	EPA BA Protocol (EPA/600/R-12/577)	I	EPA BA Protocol (EPA/600/R-12/577)	I
	Culture	Analytical Technique	EPA BA Protocol (EPA/600/R-12/577)	I	EPA BA Protocol (EPA/600/R-12/577)	I	EPA BA Protocol (EPA/600/R-12/577)	I	EPA BA Protocol (EPA/600/R-12/577)	I	EPA BA Protocol (EPA/600/R-12/577)	I
	Real-time PCR/ RV-PCR											
Brucella spp. (*B. abortus, B. melitensis, B. suis*) [Brucellosis]	NA	Sample Preparation	EPA *Yersinia pestis* (YP) Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Silvestri *et al.* 2016. J. of Microbiol. Methods. 130: 6-13	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III
	Culture	Analytical Technique	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species	I
	Real-time PCR	Analytical Technique	Hinić *et al* . 2008. J. Microbiol. Methods. 75(2): 375-378	II	Hinić *et al* . 2008. J. Microbiol. Methods. 75(2): 375-378	II	Hinić *et al* . 2008. J. Microbiol. Methods. 75(2): 375-378	II	Hinić *et al* . 2008. J. Microbiol. Methods. 75(2): 375-378	II	Hinić *et al* . 2008. J. Microbiol. Methods. 75(2): 375-378	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method									
			Aerosol (growth media, filter, liquid)		Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)		Soil		Drinking Water		Post Decontamination Waste Water	
Burkholderia mallei [Glanders] and *Burkholderia pseudomallei* [Melioidosis]	NA	Sample Preparation	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Limmathurotsakul *et al*. 2013. PLoS Negl. Trop. Dis. 7(3): e2105	II	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III
	Culture	Analytical Technique	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*	I
	Real-time PCR	Analytical Technique	Tomaso *et al*. 2006. Clin. Chem. 52(2): 307-310 and Novak *et al*. 2006. J. Clin. Microbiol. 44(1): 85-90	II	Tomaso *et al*. 2006. Clin. Chem. 52(2): 307-310 and Novak *et al*. 2006. J. Clin. Microbiol. 44(1): 85-90	II	Tomaso *et al*. 2006. Clin. Chem. 52(2): 307-310 and Novak *et al*. 2006. J. Clin. Microbiol. 44(1): 85-90	II	Tomaso *et al*. 2006. Clin. Chem. 52(2): 307-310 and Novak *et al*. 2006. J. Clin. Microbiol. 44(1): 85-90	II	Tomaso *et al*. 2006. Clin. Chem. 52(2): 307-310 and Novak *et al*. 2006. J. Clin. Microbiol. 44(1): 85-90	II
Campylobacter jejuni [Campylobacteriosis]	NA	Sample Preparation	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Silvestri *et al.* 2016. J. of Microbiol. Methods. 130: 6-13	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III
	Culture	Analytical Technique	ISO 17995	I	ISO 17995	I	ISO 17995	I	ISO 17995	I	ISO 17995	I
	Real-time PCR	Analytical Technique	Cunningham *et al*. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham *et al*. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham *et al*. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham *et al*. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham *et al*. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II
Chlamydophila psittaci (formerly known as *Chlamydia psittaci*) [Psittacosis]	NA	Sample Preparation	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Silvestri *et al.* 2016. J. of Microbiol. Methods. 130: 6-13	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III
	Tissue culture	Analytical Technique	Madico *et al*. 2000. J. Clin. Microbiol. 38(3): 1085-1093	II	Madico *et al*. 2000. J. Clin. Microbiol. 38(3): 1085-1093	II	Madico *et al*. 2000. J. Clin. Microbiol. 38(3): 1085-1093	II	Madico *et al*. 2000. J. Clin. Microbiol. 38(3): 1085-1093	II	Madico *et al*. 2000. J. Clin. Microbiol. 38(3): 1085-1093	II
	PCR											

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method									
			Aerosol (growth media, filter, liquid)		Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)		Soil		Drinking Water		Post Decontamination Waste Water	
Coxiella burnetii [Q-fever]	NA	Sample Preparation	EPA BA Protocol (EPA/600/R-12/577)	III	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-12/577)	III	Silvestri *et al.* 2016. J. of Microbiol. Methods. 130: 6-13	III	EPA and CDC Ultrafiltration (UF) Report (EPA 600/R-11/103)	III	EPA and CDC UF Report (EPA 600/R-11/103)	III
	Tissue Culture	Analytical Technique	Raoult *et al*. 1991. Antimicrob. Agents Chemother. 35(10): 2070-2077	II	Raoult *et al*. 1991. Antimicrob. Agents Chemother. 35(10): 2070-2077	II	Raoult *et al*. 1991. Antimicrob. Agents Chemother. 35(10): 2070-2077	II	Raoult *et al*. 1991. Antimicrob. Agents Chemother. 35(10): 2070-2077	II	Raoult *et al*. 1991. Antimicrob. Agents Chemother. 35(10): 2070-2077	II
	Real-time PCR	Analytical Technique	Panning *et al*. 2008. BMC Microbiol. 8:77	II	Panning *et al*. 2008. BMC Microbiol. 8:77	II	Panning *et al*. 2008. BMC Microbiol. 8:77	II	Panning *et al*. 2008. BMC Microbiol. 8:77	II	Panning *et al*. 2008. BMC Microbiol. 8:77	II
Escherichia coli O157:H7	NA	Sample Preparation	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Silvestri *et al. 2* 016. J. of Microbiol. Methods. 130: 6-13	III	EPA *Eschericia coli* O157:H7 (EC) Protocol (EPA/600/R-10/056) or EPA YP Protocol (EPA/600/R-16/109)	I/III	EPA YP Protocol (EPA/600/R-16/109)	III
	Culture	Analytical Technique	EPA EC Protocol (EPA/600/R-10/056)	I	EPA EC Protocol (EPA/600/R-10/056)	I	EPA EC Protocol (EPA/600/R-10/056)	I	EPA EC Protocol (EPA/600/R-10/056)	I	EPA EC Protocol (EPA/600/R-10/056)	I
	Real-time PCR	Analytical Technique	Sen *et al*. 2011. Environ. Sci. Technol. 45(7): 2250-2256	II	Sen *et al*. 2011. Environ. Sci. Technol. 45(7): 2250-2256	II	Sen *et al*. 2011. Environ. Sci. Technol. 45(7): 2250-2256	II	Sen *et al*. 2011. Environ. Sci. Technol. 45(7): 2250-2256	II	Sen *et al*. 2011. Environ. Sci. Technol. 45(7): 2250-2256	II
Francisella tularensis [Tularemia]	NA	Sample Preparation	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Silvestri *et al.* 2016. J. of Microbiol. Methods. 130: 6-13	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III
	Culture	Analytical Technique	CDC, ASM and APHL: Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Francisella tularensis*	I	CDC, ASM and APHL: Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Francisella tularensis*	I	CDC, ASM and APHL: Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Francisella tularensis*	I	CDC, ASM and APHL: Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Francisella tularensis*	I	CDC, ASM and APHL: Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Francisella tularensis*	I
	Real-time PCR	Analytical Technique	Versage *et al*. 2003. J. Clin. Microbiol. 41(12): 5492–5499	II	Versage *et al*. 2003. J. Clin. Microbiol. 41(12): 5492–5499	II	Versage *et al*. 2003. J. Clin. Microbiol. 41(12): 5492–5499	II	Versage *et al*. 2003. J. Clin. Microbiol. 41(12): 5492–5499	II	Versage *et al*. 2003. J. Clin. Microbiol. 41(12): 5492–5499	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method									
			Aerosol (growth media, filter, liquid)		Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)		Soil		Drinking Water		Post Decontamination Waste Water	
Legionella [Legionellosis]	NA	Sample Preparation	US DHHS. 2005. Procedures for the Recovery of *Legionella* from the Environment	I	Kozak *et al*. 2013. Identification of *Legionella* in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak *et al*. 2013. Identification of *Legionella* in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak *et al*. 2013. Identification of *Legionella* in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak *et al*. 2013. Identification of *Legionella* in the Environment. Methods Mol. Biol. 954: 3-25	I
	Culture	Analytical Technique	Kozak *et al*. 2013. Identification of *Legionella* in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak *et al*. 2013. Identification of *Legionella* in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak *et al*. 2013. Identification of *Legionella* in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak *et al*. 2013. Identification of *Legionella* in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak *et al*. 2013. Identification of *Legionella* in the Environment. Methods Mol. Biol. 954: 3-25	I
	Real-time PCR	Analytical Technique	Benitez and Winchell. 2013. J. Clin. Microbiol. 51(1): 348-351	II	Benitez and Winchell. 2013. J. Clin. Microbiol. 51(1): 348-351	II	Benitez and Winchell. 2013. J. Clin. Microbiol. 51(1): 348-351	II	Benitez and Winchell. 2013. J. Clin. Microbiol. 51(1): 348-351	II	Benitez and Winchell. 2013. J. Clin. Microbiol. 51(1): 348-351	II
Leptospira [Leptospirosis]	NA	Sample Preparation	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Silvestri *et al.* 2016. J. of Microbiol. Methods. 130: 6-13	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III
	Culture	Analytical Technique	Standard Method 9260 I: *Leptospira*	I	Standard Method 9260 I: *Leptospira*	I	Standard Method 9260 I: *Leptospira*	I	Standard Method 9260 I: *Leptospira*	I	Standard Method 9260 I: *Leptospira*	I
	Real-time PCR	Analytical Technique	Palaniappan *et al*. 2005. Mol. Cell Probes. 19(2): 111-117	II	Palaniappan *et al*. 2005. Mol. Cell Probes. 19(2): 111-117	II	Palaniappan *et al*. 2005. Mol. Cell Probes. 19(2): 111-117	II	Palaniappan *et al*. 2005. Mol. Cell Probes. 19(2): 111-117	II	Palaniappan *et al*. 2005. Mol. Cell Probes. 19(2): 111-117	II
Listeria monocytogenes [Listeriosis]	NA	Sample Preparation	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Silvestri *et al.* 2016. J. of Microbiol. Methods. 130: 6-13	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III
	Culture	Analytical Technique	Hitchins *et al*. 2003. Bacteriological Analytical Manual Online	I	Hitchins *et al.* 2003. Bacteriological Analytical Manual Online	I	Hitchins *et al.* 2003. Bacteriological Analytical Manual Online	I	Hitchins *et al.* 2003. Bacteriological Analytical Manual Online	I	Hitchins *et al.* 2003. Bacteriological Analytical Manual Online	I
	Real-time PCR	Analytical Technique	USDA, FSIS. 2009. Microbiology Laboratory Guidebook MLG 8A.04	I	USDA, FSIS. 2009. Microbiology Laboratory Guidebook MLG 8A.04	I	USDA, FSIS. 2009. Microbiology Laboratory Guidebook MLG 8A.04	I	USDA, FSIS. 2009. Microbiology Laboratory Guidebook MLG 8A.04	I	USDA, FSIS. 2009. Microbiology Laboratory Guidebook MLG 8A.04	I

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method									
			Aerosol (growth media, filter, liquid)		Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)		Soil		Drinking Water		Post Decontamination Waste Water	
Non-typhoidal *Salmonella* (Not applicable to *S.* Typhi) [Salmonellosis]	NA	Sample Preparation	EPA YP Protocol (EPA/600/R-16/109)	**III**	EPA YP Protocol (EPA/600/R-16/109)	**III**	EPA Method 1682 (EPA-821-R-06-14)	**I**	EPA Method 1200 (EPA 817-R-12-004) or Standard Method 9260 B: *Salmonella* or EPA YP Protocol (EPA/600/R-16/109)	**I/I/III**	EPA Method 1200 (EPA 817-R-12-004) or EPA Method 1682 (EPA-821-R-06-14) or Standard Method 9260 B: *Salmonella* or EPA YP Protocol (EPA/600/R-16/109)	**I/I/I/III**
	Culture	Analytical Technique	EPA Method 1682 (EPA-821-R-06-14) or EPA Method 1200 (EPA 817-R-12-004)	**I**	EPA Method 1682 (EPA-821-R-06-14) or EPA Method 1200 (EPA 817-R-12-004)	**I**	EPA Method 1682 (EPA-821-R-06-14) or EPA Method 1200 (EPA 817-R-12-004)	**I**	EPA Method 1682 (EPA-821-R-06-14) or EPA Method 1200 (EPA 817-R-12-004)	**I**	EPA Method 1682 (EPA-821-R-06-14) or EPA Method 1200 (EPA 817-R-12-004)	**I**
	Real-time PCR	Analytical Technique	Jyoti *et al*. 2011. Environ. Sci. Technol. 45(20): 8996-9002	**II**	Jyoti *et al*. 2011. Environ. Sci. Technol. 45(20): 8996-9002	**II**	Jyoti *et al*. 2011. Environ. Sci. Technol. 45(20): 8996-9002	**II**	Jyoti *et al*. 2011. Environ. Sci. Technol. 45(20): 8996-9002	**II**	Jyoti *et al*. 2011. Environ. Sci. Technol. 45(20): 8996-9002	**II**
Salmonella Typhi [Typhoid fever]	NA	Sample Preparation	EPA YP Protocol (EPA/600/R-16/109)	**III**	EPA YP Protocol (EPA/600/R-16/109)	**III**	Silvestri *et al.* 2016. J. of Microbiol. Methods. 130: 6-13	**III**	EPA *Salmonella* Typhi (ST) Protocol (EPA 600/R-10/133) or Standard Method 9260 B: *Salmonella* or EPA YP Protocol (EPA/600/R-16/109)	**I/I/III**	Standard Method 9260 B: *Salmonella* or EPA YP Protocol (EPA/600/R-16/109)	**I/III**
	Culture	Analytical Technique	EPA ST Protocol (EPA 600/R-10/133)	**I**	EPA ST Protocol (EPA 600/R-10/133)	**I**	EPA ST Protocol (EPA 600/R-10/133)	**I**	EPA ST Protocol (EPA 600/R-10/133)	**I**	EPA ST Protocol (EPA 600/R-10/133)	**I**
	Real-time PCR	Analytical Technique	CDC Laboratory Assay	**I**	CDC Laboratory Assay	**I**	CDC Laboratory Assay	**I**	CDC Laboratory Assay	**I**	CDC Laboratory Assay	**I**
Shigella spp. [Shigellosis]	NA	Sample Preparation	EPA YP Protocol (EPA/600/R-16/109)	**III**	EPA YP Protocol (EPA/600/R-16/109)	**III**	Silvestri *et al. 2* 016. J. of Microbiol. Methods. 130: 6-13	**III**	EPA YP Protocol (EPA/600/R-16/109)	**III**	EPA YP Protocol (EPA/600/R-16/109)	**III**
	Culture	Analytical Technique	Standard Method 9260 E: *Shigella*	**I**	Standard Method 9260 E: *Shigella*	**I**	Standard Method 9260 E: *Shigella*	**I**	Standard Method 9260 E: *Shigella*	**I**	Standard Method 9260 E: *Shigella*	**I**
	Real-time PCR	Analytical Technique	Cunningham *et al*. 2010. J. Clin. Microbiol. 48(8): 2929-2933	**II**	Cunningham *et al*. 2010. J. Clin. Microbiol. 48(8): 2929-2933	**II**	Cunningham *et al*. 2010. J. Clin. Microbiol. 48(8): 2929-2933	**II**	Cunningham *et al*. 2010. J. Clin. Microbiol. 48(8): 2929-2933	**II**	Cunningham *et al*. 2010. J. Clin. Microbiol. 48(8): 2929-2933	**II**

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method									
			Aerosol (growth media, filter, liquid)		Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)		Soil		Drinking Water		Post Decontamination Waste Water	
Staphylococcus aureus	NA	Sample Preparation	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Silvestri *et al.* 2016. J. of Microbiol. Methods. 130: 6-13	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III
	Culture	Analytical Technique	Standard Method 9213 B: *Staphylococcus aureus*	I	Standard Method 9213 B: *Staphylococcus aureus*	I	Standard Method 9213 B: *Staphylococcus aureus*	I	Standard Method 9213 B: *Staphylococcus aureus*	I	Standard Method 9213 B: *Staphylococcus aureus*	I
	Real-time PCR	Analytical Technique	Chiang *et al*. 2007. J. Food Prot. 70(12): 2855-2859	II	Chiang *et al*. 2007. J. Food Prot. 70(12): 2855-2859	II	Chiang *et al*. 2007. J. Food Prot. 70(12): 2855-2859	II	Chiang *et al*. 2007. J. Food Prot. 70(12): 2855-2859	II	Chiang *et al*. 2007. J. Food Prot. 70(12): 2855-2859	II
Vibrio cholerae [Cholera]	NA	Sample Preparation	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Silvestri et al. 2016. J. of Microbiol. Methods. 130: 6-13	III	EPA *Vibrio cholerae* (VC) Protocol (EPA 600/R-10/139) or EPA YP Protocol (EPA/600/R-16/109)	I/III	EPA VC Protocol (EPA 600/R-10/139) or EPA YP Protocol (EPA/600/R-16/109)	I/III
	Culture	Analytical Technique	EPA VC Protocol (EPA 600/R-10/139)	I	EPA VC Protocol (EPA 600/R-10/139)	I	EPA VC Protocol (EPA 600/R-10/139)	I	EPA VC Protocol (EPA 600/R-10/139)	I	EPA VC Protocol (EPA 600/R-10/139)	I
	Real-time PCR	Analytical Technique	Blackstone *et al*. 2007. J. Microbiol. Methods. 68(2): 254-259	II	Blackstone *et al*. 2007. J. Microbiol. Methods. 68(2): 254-259	II	Blackstone *et al*. 2007. J. Microbiol. Methods. 68(2): 254-259	II	Blackstone *et al*. 2007. J. Microbiol. Methods. 68(2): 254-259	II	Blackstone *et al*. 2007. J. Microbiol. Methods. 68(2): 254-259	II
Yersinia pestis [Plague]	NA	Sample Preparation	EPA YP Protocol (EPA/600/R-16/109)	I	EPA YP Protocol (EPA/600/R-16/109)	I	Silvestri *et al.* 2016. J. of Microbiol. Methods. 130: 6-13	III	EPA YP Protocol (EPA/600/R-16/109)	I	EPA YP Protocol (EPA/600/R-16/109)	I
	Culture	Analytical Technique	EPA YP Protocol (EPA/600/R-16/109)	I	EPA YP Protocol (EPA/600/R-16/109)	I	EPA YP Protocol (EPA/600/R-16/109)	I	EPA YP Protocol (EPA/600/R-16/109)	I	EPA YP Protocol (EPA/600/R-16/109)	I
	Real-time PCR/ RV-PCR											

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method									
			Aerosol (growth media, filter, liquid)		Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)		Soil		Drinking Water		Post Decontamination Waste Water	
Viruses												
Adenoviruses: Enteric and non-enteric (A-F)	NA	Sample Preparation	Cao *et al*. 2011. J. Environ. Monit. 13: 3321-3328 (NIOSH biosampler)	**III**	Park *et al*. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	**III**	Williamson *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3119-3125	**III**	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	**III**	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	**III**
	Tissue Culture	Analytical Technique	Boczek *et al.* 2016. J. Microbiol. Methods. 122: 43-49 or Green and Loewenstein. 2005. Curr. Protoc. Microbiol. 14C.1.1-14C.1.19	**II**	Boczek *et al.* 2016. J. Microbiol. Methods. 122: 43-49 or Green and Loewenstein. 2005. Curr. Protoc. Microbiol. 14C.1.1-14C.1.19	**II**	Boczek *et al.* 2016. J. Microbiol. Methods. 122: 43-49 or Green and Loewenstein. 2005. Curr. Protoc. Microbiol. 14C.1.1-14C.1.19	**II**	Boczek *et al.* 2016. J. Microbiol. Methods. 122: 43-49 or Green and Loewenstein. 2005. Curr. Protoc. Microbiol. 14C.1.1-14C.1.19	**II**	Boczek *et al.* 2016. J. Microbiol. Methods. 122: 43-49 or Green and Loewenstein. 2005. Curr. Protoc. Microbiol. 14C.1.1-14C.1.19	**II**
	Real-time PCR	Analytical Technique	Jothikumar *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3131-3136	**II**	Jothikumar *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3131-3136	**II**	Jothikumar *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3131-3136	**II**	Jothikumar *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3131-3136	**II**	Jothikumar *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3131-3136	**II**
Astroviruses	NA	Sample Preparation	Cao *et al*. 2011. J. Environ. Monit. 13: 3321-3328 (NIOSH biosampler)	**III**	Park *et al*. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	**III**	Williamson *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3119-3125	**III**	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	**III**	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	**III**
	Integrated Cell Culture	Analytical Technique	Grimm *et al*. 2004. Can. J. Microbiol. 50(4): 269-278	**II**	Grimm *et al*. 2004. Can. J. Microbiol. 50(4): 269-278	**II**	Grimm *et al*. 2004. Can. J. Microbiol. 50(4): 269-278	**II**	Grimm *et al*. 2004. Can. J. Microbiol. 50(4): 269-278	**II**	Grimm *et al*. 2004. Can. J. Microbiol. 50(4): 269-278	**II**
	Real-time reverse transcription-PCR											
Caliciviruses: Noroviruses	NA	Sample Preparation	Cao *et al*. 2011. J. Environ. Monit. 13: 3321-3328 (NIOSH biosampler)	**III**	Park *et al*. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	**III**	Williamson *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3119-3125	**III**	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	**III/I**	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	**III/I**
	Real-time reverse transcription-PCR	Analytical Technique	EPA Method 1615 (EPA/600/R-10/181)	**I**	EPA Method 1615 (EPA/600/R-10/181)	**I**	EPA Method 1615 (EPA/600/R-10/181)	**I**	EPA Method 1615 (EPA/600/R-10/181)	**I**	EPA Method 1615 (EPA/600/R-10/181)	**I**

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method									
			Aerosol (growth media, filter, liquid)		Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)		Soil		Drinking Water		Post Decontamination Waste Water	
Caliciviruses: Sapovirus	NA	Sample Preparation	Cao *et al*. 2011. J. Environ. Monit. 13: 3321-3328 (NIOSH biosampler)	III	Park *et al*. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Williamson *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3119-3125	III	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	III	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	III
	Tissue Culture	Analytical Technique	Parwani *et al*. 1991. Arch. Virol. 120(1-2): 115-122	II	Parwani *et al*. 1991. Arch. Virol. 120(1-2): 115-122	II	Parwani *et al*. 1991. Arch. Virol. 120(1-2): 115-122	II	Parwani *et al*. 1991. Arch. Virol. 120(1-2): 115-122	II	Parwani *et al*. 1991. Arch. Virol. 120(1-2): 115-122	II
	Real-time reverse transcription-PCR	Analytical Technique	Oka *et al*. 2006. J. Med. Virol. 78(10): 1347-1353	II	Oka *et al*. 2006. J. Med. Virol. 78(10): 1347-1353	II	Oka *et al*. 2006. J. Med. Virol. 78(10): 1347-1353	II	Oka *et al*. 2006. J. Med. Virol. 78(10): 1347-1353	II	Oka *et al*. 2006. J. Med. Virol. 78(10): 1347-1353	II
Coronaviruses: SARS-associated human coronavirus	NA	Sample Preparation	Cao et al. 2011. J. Environ. Monit. 13: 3321-3328 (NIOSH biosampler)	III	Park *et al*. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Williamson *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3119-3125	III	EPA and CDC UF Report (EPA 600/R-11/103)	III	EPA and CDC UF Report (EPA 600/R-11/103)	III
	Tissue Culture	Analytical Technique	Pagat *et al*. 2007. Applied Biosafety 12(2): 100-108	II	Pagat *et al*. 2007. Applied Biosafety 12(2): 100-108	II	Pagat *et al*. 2007. Applied Biosafety 12(2): 100-108	II	Pagat *et al*. 2007. Applied Biosafety 12(2): 100-108	II	Pagat *et al*. 2007. Applied Biosafety 12(2): 100-108	II
	Reverse transcription-PCR	Analytical Technique	Adachi *et al*. 2004. J. Virol. Methods. 122(1): 29-36	II	Adachi *et al*. 2004. J. Virol. Methods. 122(1): 29-36	II	Adachi *et al*. 2004. J. Virol. Methods. 122(1): 29-36	II	Adachi *et al*. 2004. J. Virol. Methods. 122(1): 29-36	II	Adachi *et al*. 2004. J. Virol. Methods. 122(1): 29-36	II
Hepatitis E virus (HEV)	NA	Sample Preparation	Cao *et al*. 2011. J. Environ. Monit. 13: 3321-3328 (NIOSH biosampler)	III	Park *et al*. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Williamson *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3119-3125	III	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	III	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	III
	Tissue Culture	Analytical Technique	Zaki *et al*. 2009. FEMS Immunol. Med. Mic. 56: 73-79	II	Zaki *et al*. 2009. FEMS Immunol. Med. Mic. 56: 73-79	II	Zaki *et al*. 2009. FEMS Immunol. Med. Mic. 56: 73-79	II	Zaki *et al*. 2009. FEMS Immunol. Med. Mic. 56: 73-79	II	Zaki *et al*. 2009. FEMS Immunol. Med. Mic. 56: 73-79	II
	Real-time reverse transcription-PCR	Analytical Technique	Jothikumar *et al*. 2006. J. Virol. Methods. 131(1): 65-71	II	Jothikumar *et al*. 2006. J. Virol. Methods. 131(1): 65-71	II	Jothikumar *et al*. 2006. J. Virol. Methods. 131(1): 65-71	II	Jothikumar *et al*. 2006. J. Virol. Methods. 131(1): 65-71	II	Jothikumar *et al*. 2006. J. Virol. Methods. 131(1): 65-71	II
Influenza H5N1 virus	NA	Sample Preparation	Hermann *et al*. 2006. Appl. Environ. Microbiol. 72(7): 4811-4818	III	Park *et al*. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Williamson *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3119-3125	III	EPA and CDC UF Report (EPA 600/R-11/103)	III	EPA and CDC UF Report (EPA 600/R-11/103)	III
	Tissue Culture	Analytical Technique	Krauss *et al*. 2012. Influeza Virus Isolation. Methods Mol. Biol. 865: 11-24	II	Krauss *et al*. 2012. Influeza Virus Isolation. Methods Mol. Biol. 865: 11-24	II	Krauss *et al*. 2012. Influeza Virus Isolation. Methods Mol. Biol. 865: 11-24	II	Krauss *et al*. 2012. Influeza Virus Isolation. Methods Mol. Biol. 865: 11-24	II	Krauss *et al*. 2012. Influeza Virus Isolation. Methods Mol. Biol. 865: 11-24	II
	Real-time reverse transcription-PCR	Analytical Technique	Ng *et al*. 2005. Emerg. Infect. Dis. 11(8): 1303-1305	II	Ng *et al*. 2005. Emerg. Infect. Dis. 11(8): 1303-1305	II	Ng *et al*. 2005. Emerg. Infect. Dis. 11(8): 1303-1305	II	Ng *et al*. 2005. Emerg. Infect. Dis. 11(8): 1303-1305	II	Ng *et al*. 2005. Emerg. Infect. Dis. 11(8): 1303-1305	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method									
			Aerosol (growth media, filter, liquid)		Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)		Soil		Drinking Water		Post Decontamination Waste Water	
Picornaviruses: Enteroviruses	NA	Sample Preparation	Cao *et al*. 2011. J. Environ. Monit. 13: 3321-3328 (NIOSH biosampler)	III	Park *et al*. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Williamson *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3119-3125	III	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	III/I	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	III/I
	Tissue Culture	Analytical Technique	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I
	Reverse transcription-PCR											
Picornaviruses: Hepatitis A virus (HAV)	NA	Sample Preparation	Cao *et al*. 2011. J. Environ. Monit. 13: 3321-3328 (NIOSH biosampler)	III	Park *et al*. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Williamson *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3119-3125	III	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	III	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	III
	Integrated Cell Culture	Analytical Technique	Hyeon *et al*. 2011. J. Food Prot. 74(10):1756-1761	II	Hyeon *et al*. 2011. J. Food Prot. 74(10):1756-1761	II	Hyeon *et al*. 2011. J. Food Prot. 74(10):1756-1761	II	Hyeon *et al*. 2011. J. Food Prot. 74(10):1756-1761	II	Hyeon *et al*. 2011. J. Food Prot. 74(10):1756-1761	II
	Real-time Reverse Transcription-PCR											
Reoviruses: Rotavirus (Group A)	NA	Sample Preparation	Cao *et al*. 2011. J. Environ. Monit. 13: 3321-3328 (NIOSH biosampler)	III	Park *et al*. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Williamson *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3119-3125	III	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	III	EPA and CDC UF Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	III
	Tissue Culture	Analytical Technique	EPA Method 1615 (EPA/600/R-10/181)	III	EPA Method 1615 (EPA/600/R-10/181)	III	EPA Method 1615 (EPA/600/R-10/181)	III	EPA Method 1615 (EPA/600/R-10/181)	III	EPA Method 1615 (EPA/600/R-10/181)	III
	Real-time reverse transcription-PCR	Analytical Technique	Jothikumar *et al*. 2009. J. Virol. Methods. 155(2): 126-131	II	Jothikumar *et al*. 2009. J. Virol. Methods. 155(2): 126-131	II	Jothikumar *et al*. 2009. J. Virol. Methods. 155(2): 126-131	II	Jothikumar *et al*. 2009. J. Virol. Methods. 155(2): 126-131	II	Jothikumar *et al*. 2009. J. Virol. Methods. 155(2): 126-131	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method									
			Aerosol (growth media, filter, liquid)		Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)		Soil		Drinking Water		Post Decontamination Waste Water	
Protozoa												
Cryptosporidium spp. [Cryptosporidiosis]	NA	Sample Preparation	EPA BA Protocol (EPA/600/R-12/577)	III	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-12/577)	III	Liang and Keeley. 2011. Appl. Environ. Microbiol. 77(18): 6476-6485	II	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001) or EPA and CDC UF Report (EPA 600/R-11/103)	I	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001) or EPA and CDC UF Report (EPA 600/R-11/103)	I
	Cell Culture Immunofluorescence Procedure	Analytical Technique	Bukhari *et al*. 2007. Can. J. Microbiol. 53(5): 656-663	II	Bukhari *et al*. 2007. Can. J. Microbiol. 53(5): 656-663	II	Bukhari *et al*. 2007. Can. J. Microbiol. 53(5): 656-663	II	Bukhari *et al*. 2007. Can. J. Microbiol. 53(5): 656-663	II	Bukhari *et al*. 2007. Can. J. Microbiol. 53(5): 656-663	II
	IMS/FA	Analytical Technique	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001)	I
	Real-time PCR	Analytical Technique	Guy *et al*. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185 and Jiang *et al*. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141	II	Guy *et al*. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185 and Jiang *et al*. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141	II	Guy *et al*. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185 and Jiang *et al*. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141	II	Guy *et al*. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185 and Jiang *et al*. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141	II	Guy *et al*. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185 and Jiang *et al*. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141	II
Entamoeba histolytica	NA	Sample Preparation	EPA BA Protocol (EPA/600/R-12/577)	III	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-12/577)	III	Liang and Keeley. 2011. Appl. Environ. Microbiol. 77(18): 6476-6485	III	EPA and CDC UF Report (EPA 600/R-11/103)	III	EPA and CDC UF Report (EPA 600/R-11/103)	III
	Cell Culture	Analytical Technique	Stringert *et al*. 1972. J Parasitol. 58(2): 306-310	II	Stringert *et al*. 1972. J Parasitol. 58(2): 306-310	II	Stringert *et al*. 1972. J Parasitol. 58(2): 306-310	II	Stringert *et al*. 1972. J Parasitol. 58(2): 306-310	II	Stringert *et al*. 1972. J Parasitol. 58(2): 306-310	II
	Real-time PCR	Analytical Technique	Roy *et al*. 2005. J. Clin. Microbiol. 43(5): 2168-2172	II	Roy *et al*. 2005. J. Clin. Microbiol. 43(5): 2168-2172	II	Roy *et al*. 2005. J. Clin. Microbiol. 43(5): 2168-2172	II	Roy *et al*. 2005. J. Clin. Microbiol. 43(5): 2168-2172	II	Roy *et al*. 2005. J. Clin. Microbiol. 43(5): 2168-2172	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method									
			Aerosol (growth media, filter, liquid)		Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)		Soil		Drinking Water		Post Decontamination Waste Water	
Giardia spp. [Giardiasis]	NA	Sample Preparation	EPA BA Protocol (EPA/600/R-12/577)	III	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-12/577)	III	Liang and Keeley. 2011. Appl. Environ. Microbiol. 77(18): 6476-6485	III	EPA Method 1623.1 (EPA 816-R-12-001) or EPA and CDC UF Report (EPA 600/R-11/103)	I	EPA Method 1623.1 (EPA 816-R-12-001) or EPA and CDC UF Report (EPA 600/R-11/103)	I
	Cell Culture	Analytical Technique	Keister. 1983. T. Roy. Soc. Trop. Med. H. 77(4): 487-488	II	Keister. 1983. T. Roy. Soc. Trop. Med. H. 77(4): 487-488	II	Keister. 1983. T. Roy. Soc. Trop. Med. H. 77(4): 487-488	II	Keister. 1983. T. Roy. Soc. Trop. Med. H. 77(4): 487-488	II	Keister. 1983. T. Roy. Soc. Trop. Med. H. 77(4): 487-488	II
	IMS/FA	Analytical Technique	EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1623.1 (EPA 816-R-12-001)	I
	Real-time PCR	Analytical Technique	Guy *et al*. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185	II	Guy *et al*. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185	II	Guy *et al*. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185	II	Guy *et al*. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185	II	Guy *et al*. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185	II
Naegleria fowleri [Naegleriasis]	NA	Sample Preparation	EPA BA Protocol (EPA/600/R-12/577)	III	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-12/577)	III	Mull *et al*. 2013. J. Parasitol. Res. 2013: 1-8	II	Cope *et al*. 2015. Clin. Infect. Dis. 60(8): e36-42 or EPA and CDC UF Report (EPA 600/R-11/103)	II/III	Cope *et al*. 2015. Clin. Infect. Dis. 60(8): e36-42 or EPA and CDC UF Report (EPA 600/R-11/103)	II/III
	Cell Culture	Analytical Technique	Mull *et al*. 2013. J. Parasitol. Res. 2013: 1-8	II	Mull *et al*. 2013. J. Parasitol. Res. 2013: 1-8	II	Mull *et al*. 2013. J. Parasitol. Res. 2013: 1-8	II	Mull *et al*. 2013. J. Parasitol. Res. 2013: 1-8	II	Mull *et al*. 2013. J. Parasitol. Res. 2013: 1-8	II
	Real-time PCR											

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method									
			Aerosol (growth media, filter, liquid)		Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)		Soil		Drinking Water		Post Decontamination Waste Water	
Toxoplasma gondii [Toxoplasmosis]	NA	Sample Preparation	EPA BA Protocol (EPA/600/R-12/577)	**III**	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-12/577)	**III**	Liang and Keeley. 2011. Appl. Environ. Microbiol. 77(18): 6476–6485	**III**	Villegas *et al*. 2010. J. Microbiol. Methods. 81(3): 219-225 or EPA Method 1623.1 (EPA 816-R-12-001)	**II/III**	Villegas *et al*. 2010. J. Microbiol. Methods. 81(3): 219-225 or EPA Method 1623.1 (EPA 816-R-12-001)	**II/III**
	Cell Culture	Analytical Technique	Villegas *et al*. 2010. J. Microbiol. Methods. 81(3): 219-225	**II**	Villegas *et al*. 2010. J. Microbiol. Methods. 81(3): 219-225	**II**	Villegas *et al*. 2010. J. Microbiol. Methods. 81(3): 219-225	**II**	Villegas *et al*. 2010. J. Microbiol. Methods. 81(3): 219-225	**II**	Villegas *et al*. 2010. J. Microbiol. Methods. 81(3): 219-225	**II**
	Real-time PCR	Analytical Technique	Yang *et al*. 2009. Appl. Environ. Microbiology. 75(11): 3477-3483	**II**	Yang *et al*. 2009. Appl. Environ. Microbiology. 75(11): 3477-3483	**II**	Yang *et al*. 2009. Appl. Environ. Microbiology. 75(11): 3477-3483	**II**	Yang *et al*. 2009. Appl. Environ. Microbiology. 75(11): 3477-3483	**II**	Yang *et al*. 2009. Appl. Environ. Microbiology. 75(11): 3477-3483	**II**
Helminths												
Baylisascaris procyonis [Raccoon roundworm infection]	NA	Sample Preparation	EPA BA Protocol (EPA/600/R-12/577)	**III**	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-12/577)	**III**	Liang and Keeley. 2011. Appl. Environ. Microbiol. 77(18): 6476-6485	**III**	EPA and CDC UF Report (EPA 600/R-11/103) or Gatcombe *et al*. 2010. Parasitol. Res. 106: 499-504	**III/II**	EPA and CDC UF Report (EPA 600/R-11/103) or Gatcombe *et al*. 2010. Parasitol. Res. 106: 499-504	**III/II**
	Real-time PCR	Analytical Technique	Gatcombe *et al*. 2010. Parasitol. Res. 106: 499-504	**II**	Gatcombe *et al*. 2010. Parasitol. Res. 106: 499-504	**II**	Gatcombe *et al*. 2010. Parasitol. Res. 106: 499-504	**II**	Gatcombe *et al*. 2010. Parasitol. Res. 106: 499-504	**II**	Gatcombe *et al*. 2010. Parasitol. Res. 106: 499-504	**II**
	Embryonation of Eggs and Microscopy	Analytical Technique	Control of Pathogens and Vector Attraction in Sewage Sludge (EPA/625/R-92/013)	**II**	Control of Pathogens and Vector Attraction in Sewage Sludge (EPA/625/R-92/013)	**II**	Control of Pathogens and Vector Attraction in Sewage Sludge (EPA/625/R-92/013)	**II**	Control of Pathogens and Vector Attraction in Sewage Sludge (EPA/625/R-92/013)	**II**	Control of Pathogens and Vector Attraction in Sewage Sludge (EPA/625/R-92/013)	**II**

Appendix D: Selected Biotoxin Methods

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SAM 2017 — Appendix D: Selected Biotoxin Methods

The fitness of a method for its intended use is related to data quality objectives (DQOs) for a particular environmental remediation activity. The tiers below have been assigned to the methods selected for each biotoxin/sample type pair to indicate a level of method usability for the specific biotoxin and sample type for which it has been selected. The assigned tiers reflect the conservative view for DQOs involving timely implementation of methods for analysis of a high number of samples (such that multiple laboratories are necessary), and appropriate quality control. The sample types indicated reflect respresentative examples and are not necessarily inclusive of all sample types that might be encountered by laboratories following a contamination incident. Assigned usability tiers are indicated next to each method or method combination throughout this appendix.

Tier I: The biotoxin and sample type are both targets of the method(s). Data are available for all aspects of method performance and QC measures supporting its use without modifications.

Tier II: The biotoxin is a target of the method, and the method has been evaluated by one or more laboratories. The sample type may or may not be a target of the method, and available data and/or information regarding sample preparation indicate that analyses of similar sample types were successful. However, additional testing and/or modifications may be needed.

Tier III: The sample type is not a target of the method, and no reliable data supporting the method's fitness for its intended use are available. Data suggest, however, that the method(s) may be applicable with significant modification.

Notes :
The presence of disinfectants (e.g., chlorine) and/or preservatives added during water sample collection to slow degradation (e.g., pH adjustors, de-chlorinating agents) could possibly affect analytical results. When present, the impact of these agents on method performance should be evaluated if not previously determined.

Column headings are defined in Section 8.0.

Analyte(s)	CAS RN	Analysis Type	Analytical Technique	Aerosol (filter/cassette, liquid impinger)		Solid (soil, powder)		Particulate (swabs, wipes, dust socks)		Non-Drinking Water (surface water, waste water)		Drinking Water	
Abrin	Abrin (1393-62-0) Abrine (526-31-8)	Presumptive	Immunoassay (LFA)	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	II	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	II	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	II
		Presumptive (Abrine)	LC-MS-MS	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	I
		Confirmatory	Immunoassays (ELISA and ECL)	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II
		Biological Activity	Enzyme activity	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II
Aflatoxins	B1 (27261-02-5) B2 (22040-96-6) G1 (1385-95-1) G2 (7241-98-7)	Presumptive and Confirmatory (B1, B2, G1, G2)	Immunoaffinity (column) purification / LC-FL (detection)	Adapted from 991.31 (AOAC)	II	Adapted from 991.31 (AOAC)	II	Adapted from 991.31 (AOAC)	II	Adapted from 991.31 (AOAC)	II	Adapted from 991.31 (AOAC)	II
α-Amanitin	23109-05-9	Presumptive	Immunoassay (ELISA)	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II
		Confirmatory	LC-MS-MS	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	I
Anatoxin-a	64285-06-9	Presumptive	Immunoassay (ELISA)	See summary in Section 8.2.4.1	III	See summary in Section 8.2.4.1	III	See summary in Section 8.2.4.1	III	See summary in Section 8.2.4.1	III	See summary in Section 8.2.4.1	III
		Confirmatory	LC-MS-MS	Method 545 (EPA)	II	Method 545 (EPA)	II	Method 545 (EPA)	II	Method 545 (EPA)	II	Method 545 (EPA)	I

Analyte(s)	CAS RN	Analysis Type	Analytical Technique	Aerosol (filter/cassette, liquid impinger)		Solid (soil, powder)		Particulate (swabs, wipes, dust socks)		Non-Drinking Water (surface water, waste water)		Drinking Water	
Botulinum neurotoxins (Serotoypes A, B, C, D, E, F, and G)	Type A (93384-43-1) Type B (93384-44-2) Type C (93384-45-3) Type D (93384-46-4) Type E (93384-47-5) Type F (107231-15-2) Type G (107231-16-3)	Presumptive (Types A and B)	Immunoassay (LFA)	Adapted from EPA Environmental Technology Verification report	II	Adapted from EPA Environmental Technology Verification report	II	Adapted from EPA Environmental Technology Verification report	II	Adapted from EPA Environmental Technology Verification report	II	Adapted from EPA Environmental Technology Verification report	I
		Presumptive (Types A, B, D, E, F, and G)	Immunocapture Forster Resonance Energy Transfer (FRET)-based activity assay	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II
		Presumptive (Types A-G)	Immunoassay (fluorescent bead-based)	See summary in Section 8.2.5.3	II	See summary in Section 8.2.5.3	II	See summary in Section 8.2.5.3	II	See summary in Section 8.2.5.3	II	See summary in Section 8.2.5.3	II
		Presumptive (Type A)	Immunoassay (ECL)	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II
		Confirmatory (Types A-G)	LC-MS-MS (Types A, B, E and F)	Adapted from J. Agric.Food Chem. 63(4): 1133-1141	II	Adapted from J. Agric.Food Chem. 63(4): 1133-1141	II	Adapted from J. Agric.Food Chem. 63(4): 1133-1141	II	Adapted from J. Agric.Food Chem. 63(4): 1133-1141	II	Adapted from J. Agric.Food Chem. 63(4): 1133-1141	II
			MALDI-TOF MS (Types A-G)										
		Biological Activity (Total)	Mouse Bioassay	APHA Press Compendium of Methods, Chapter 32	I	APHA Press Compendium of Methods, Chapter 32	I	APHA Press Compendium of Methods, Chapter 32	I	APHA Press Compendium of Methods, Chapter 32	I	APHA Press Compendium of Methods, Chapter 32	I
Brevetoxins	98112-41-5 (A form) 79580-28-2 (B form)	Presumptive (B forms)	Immunoassay (ELISA)	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II
		Confirmatory (A and B forms)	LC-MS	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II
Cylindrospermopsin	143545-90-8	Presumptive	Immunoassay (ELISA)	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II
		Confirmatory	LC-MS-MS	Method 545 (EPA)	II	Method 545 (EPA)	II	Method 545 (EPA)	II	Method 545 (EPA)	II	Method 545 (EPA)	I
Diacetoxyscirpenol (DAS)	2270-40-8	Presumptive	Immunoassay (ELISA)	See summary in Section 8.2.8.1	III	See summary in Section 8.2.8.1	III	See summary in Section 8.2.8.1	III	See summary in Section 8.2.8.1	III	See summary in Section 8.2.8.1	III
		Confirmatory	LC-MS-MS	Adapted from Food Research International (2015) 72: 247-255	II	Adapted from Food Research International (2015) 72: 247-255	II	Adapted from Food Research International (2015) 72: 247-255	II	Adapted from Food Research International (2015) 72: 247-255	II	Adapted from Food Research International (2015) 72: 247-255	II

Analyte(s)	CAS RN	Analysis Type	Analytical Technique	Aerosol (filter/cassette, liquid impinger)		Solid (soil, powder)		Particulate (swabs, wipes, dust socks)		Non-Drinking Water (surface water, waste water)		Drinking Water	
Domoic acid (DA)	14277-97-5	Presumptive	Immunoassay (ELISA)	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II
		Presumptive	Immunoassay (ELISA)	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II
		Confirmatory	LC-UV	Adapted from Journal of the Mexican Chemical Society (2011) 55(2): 65-71	II	Adapted from Journal of the Mexican Chemical Society (2011) 55(2): 65-71	II	Adapted from Journal of the Mexican Chemical Society (2011) 55(2): 65-71	II	Adapted from Journal of the Mexican Chemical Society (2011) 55(2): 65-71	II	Adapted from Journal of the Mexican Chemical Society (2011) 55(2): 65-71	II
Microcystins	96180-79-9 (LA) 154037-70-4 (LF) 101043-37-2 (LR) 123304-10-9 (LY) 111755-37-4 (RR) 101064-48-6 (YR)	Presumptive (Total Adda-containing microcystins)	Immunoassay (ELISA)	Method 546 (EPA)	II	Method 546 (EPA)	II	Method 546 (EPA)	II	Method 546 (EPA)	I	Method 546 (EPA)	I
		Confirmatory (LA, LF, LR, LY, RR, YR)	LC-MS-MS	Method 544 (EPA)	III	Method 544 (EPA)	III	Method 544 (EPA)	III	Method 544 (EPA)	II	Method 544 (EPA)	I
Picrotoxin	124-87-8	Presumptive	TBD	TBD		TBD		TBD		TBD		TBD	
		Confirmatory	LC-UV	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II
Ricin	Ricin (9009-86-3) Ricinine (5254-40-3)	Presumptive	Immunoassay (LFA)	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I
		Presumptive	Immunoassay (ELISA)	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II
		Presumptive	Immunoassay (ECL)	Adapted from Journal of AOAC International (2008) 91(2): 376-382	II	Adapted from Journal of AOAC International (2008) 91(2): 376-382	II	Adapted from Journal of AOAC International (2008) 91(2): 376-382	II	Adapted from Journal of AOAC International (2008) 91(2): 376-382	II	Adapted from Journal of AOAC International (2008) 91(2): 376-382	II
		Presumptive (Ricinine)	LC-MS-MS	EPA 600/R-13/022 (EPA/CDC)	II	EPA 600/R-13/022 (EPA/CDC)	II	EPA 600/R-13/022 (EPA/CDC)	II	EPA 600/R-13/022 (EPA/CDC)	II	EPA 600/R-13/022 (EPA/CDC)	I
		Confirmatory	Immunocapture / LC-MS-MS	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from-Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	I
		Biological Activity	Immuncapture / MALDI-TOF MS	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	I

Analyte(s)	CAS RN	Analysis Type	Analytical Technique	Aerosol (filter/cassette, liquid impinger)		Solid (soil, powder)		Particulate (swabs, wipes, dust socks)		Non-Drinking Water (surface water, waste water)		Drinking Water	
Saxitoxins	35523-89-8 (STX) 64296-20-4 (NEO) 58911-04-9 (dcSTX) 68683-58-9 (dcNEOSTX) 143084-69-9 (doSTX) 77462-64-7 (GTX 1 - 6) 122075-86-9 (dcGTX 1 - 4)	Presumptive (Total)	Receptor Binding Assay	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II
		Presumptive (Total)	Immunoassay (ELISA)	Adapted from Toxicon (2009) 54: 313-320	II	Adapted from Toxicon (2009) 54: 313-320	II	Adapted from Toxicon (2009) 54: 313-320	II	Adapted from Harmful Algae (2016) 56: 77-90	I	Adapted from Harmful Algae (2016) 56: 77-90	I
		Confirmatory (STXs and GTXs)	LC-MS-MS	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II
Shiga and Shiga-like Toxins	Stx (75757-64-1)	Presumptive (Stx, Stx-1 and Stx-2)	Immunoassay (ELISA)	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	II	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	II	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	II	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	II	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	II
		Confirmatory (Stx, Stx-1 and Stx-2)	LC-MS-MS	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II
Staphylococcal enterotoxins	37337-57-8 (SEA) 39424-53-8 (SEB) 39424-54-9 (SEC) 12788-99-7 (SED) 39424-55-0 (SEE)	Presumptive (SEA - SEE)	Enzyme Immunoassay (ELFA)	2007.06 (AOAC)	II	2007.06 (AOAC)	II	2007.06 (AOAC)	II	2007.06 (AOAC)	II	2007.06 (AOAC)	II
		Presumptive (SEB)	Immunoassay (ECL)	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III
		Confirmatory (SEA - SEE)	Immunoassay (ELISA)	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II
T-2 Mycotoxin	21259-20-1 (T-2) 26934-87-2 (HT-2)	Presumptive (T-2)	Immunoassay (ELISA)	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II
		Confirmatory (T-2 and HT-2)	LC-MS	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II
Tetrodotoxin	9014-39-5	Presumptive	Receptor Binding Assay	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II
		Confirmatory	LC-MS-MS	Adapted from Toxicon (2016) 119: 64-71	II	Adapted from Toxicon (2016) 119: 64-71	II	Adapted from Toxicon (2016) 119: 64-71	II	Adapted from Toxicon (2016) 119: 64-71	II	Adapted from Toxicon (2016) 119: 64-71	II

Attachment 1:
SAM Revisions and Supporting Documents

The information in this document is updated periodically to incorporate revisions to the list of target analytes and sample types, and to provide the most recent analytical methods and procedures. The table below provides information regarding additional changes that were incorporated into each revision since publication of Revision 1.0 in September 2004.

SAM Revisions Tracking Table		
SAM Revision	**Publication Date**	**Changes incorporated summary**
Revision 1.0 EPA/600/R-04/126	September 2004	*Standardized Analytical Methods for Use During Homeland Security Events (SAM) 1.0* Included chemical and biological contaminants
Revision 2.0 EPA/600/R-04/126B	September 2005	• Added radiochemicals • Added several persistent chemical warfare agent (CWA) degradation products • Added separate drinking water sample type for chemicals and radiochemicals • Added viability determination methods for pathogens • Added separate section for biotoxins
Revision 3.0 EPA/600/R-07/015	February 2007	• Added explosive chemicals • Combined identification and viability methods for pathogens • Added drinking water sample type for pathogens • Title changed to: *Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events (SAM) 3.0*
Revision 3.1 EPA/600/R-07/136	November 2007	Developed a SAM website, to provide the SAM document and a format for searching and linking to SAM methods by analyte and sample type. (See https://www.epa.gov/homeland-security-research/sam)
Revision 4.0 EPA/600/R-04/126D	September 2008	• Added wipe samples for chemistry analytes • Added PCR methods for pathogens
Revision 5.0 EPA/600/R-04/126E	September 2009	Added separate drinking water sample type to biotoxins
2010 (Revision 6.0) EPA/600/R-10/122	October 2010	• Removed non-aqueous liquid sample type from chemistry • Temporary removal of pathogens

SAM Revisions Tracking Table		
SAM Revision	**Publication Date**	**Changes incorporated summary**
2012 (Revision 7.0) EPA/600/R-12/555	July 2012	• Changed title to: *Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2012* • Added vegetation s*ample type, newly available rapid methods, and total activity screenin*g procedure for radiochemistry analytes • Re-introduced pathogen methods with restructuring to clarify method applications for site characterization and post remediation • Assigned applicability tiers to chemistry methods
2017 (Revision 8.0) EPA/600/R-17/356	October 2017	• Added outdoor building and infrastructure material sample types for radiochemistry analytes • Added soil sample type for pathogens • Assigned applicability tiers to pathogen and biotoxin methods • Added analytes to chemistry, radiochemistry, pathogen and biotoxin sections • Added considerations regarding the potential impacts of decontamination agents on the analytical performance of selected radiochemical methods • Changed the names of “aqueous liquid” (chemistry methods sample type) and “liquid water” (biotoxin methods sample type) to “non-drinking water” to clarify that the sample type applies to all non-drinking water aqueous sample matrices

The following documents and tools have been developed by EPA to provide information regarding a contamination incident. The information included in these documents is intended to be complementary to information provided in the analytical methods that are listed in SAM. As additional documents containing similar complementary information become available, they will be added to the list contained in this Attachment.

- Searchable SAM website at: https://www.epa.gov/homeland-security-research/sam

- U.S. EPA. 2009. "Guide for Development of Sample Collection Plans for Radiochemical Analytes in Environmental Matrices Following Homeland Security Events." Cincinnati, OH: U.S. EPA. EPA/600/R-08/128.

  This document provides a framework to assist incident commanders, project managers, state and local authorities, contractors and enforcement divisions in developing and implementing an approach for sample collection during the cleanup of an urban environment after a radiological contamination incident. Information in this document can be used to develop a systematic and integrated methodology for sample collection to meet data use needs and site disposition objectives. https://www.epa.gov/sites/production/files/2015-07/documents/guide_for_developing_sample_collection_plans_for_radiochemical_analytes.pdf

- U.S. EPA. 2010. "Sample Collection Information Document for Pathogens – Companion to SAM 2017." Cincinnati, OH: U.S. EPA. EPA/600/R-17/374.

  This document provides information regarding sample containers/media, preservation, holding time, sizes, packaging and shipping, pertaining to collection of environmental samples to be analyzed for the pathogens in SAM 2017. https://www.epa.gov/homeland-security-research/sam-companion-documents-and-sample-collection-procedures

- U.S. EPA. 2010. "Rapid Screening and Preliminary Identification Techniques and Methods – Companion to SAM Revision 5.0." Cincinnati, OH: U.S. EPA. EPA/600/R-10/090.

  This document provides information regarding procedures for use when multiple laboratories are needed to perform rapid preliminary analysis of environmental samples following a contamination incident. The information is intended to support the analytical methods listed for chemical and radiochemical analytes in SAM Revision 5.0. https://www.epa.gov/sites/production/files/2015-07/documents/rapid_screening_and_preid.pdf

- U.S. EPA. 2010. "Laboratory Environmental Sample Disposal Information Document – Companion to SAM Revision 5.0." Cincinnati, OH: U.S. EPA. EPA/600/R-10/092.

  This document addresses laboratory disposal of samples and associated analytical waste unique to remediation activities following a contamination incident, and assumes specific environmental sample types (i.e., water, soil, particulates and air collection media) that will be analyzed by laboratories using the methods listed in SAM. https://www.epa.gov/sites/production/files/2015-06/documents/lesdid.pdf

- U.S. EPA. 2012. "Sample Collection Procedures for Radiochemistry Analytes in Environmental Matrices." Cincinnati, OH: U.S. EPA. EPA/600/R-12/566.

  This document focuses on the Site Characterization, Remediation, and Final Status Survey (site release) phases of a contamination incident and is not intended to address sample collection needs

during Initial Response. The procedures are intended for collection of environmental samples in response to a radiological contamination incident at the point where Federal Radiological Monitoring and Assessment Center (FRMAC) activities are turned over to EPA.
https://www.epa.gov/sites/production/files/2015-07/documents/sample_collection_procedures_for_radiochemical_analytes.pdf

- U.S. EPA. 2017. "Sample Collection Information Document for Chemicals, Radiochemicals and Biotoxins – Companion to SAM 2017." Cincinnati, OH: U.S. EPA. EPA/600/R-17/389.

  This document provides information regarding sample containers/media, preservation, holding time, sizes, packaging and shipping, pertaining to collection of samples to be analyzed for the chemical, radiochemical and biotoxin analytes listed in SAM 2017.
  https://www.epa.gov/homeland-security-research/sam-companion-documents-and-sample-collection-procedures

- U.S. EPA. 2016. "Sample Collection Procedures for Radiochemistry Analytes in Outdoor Building and Infrastructure Materials." Cincinnati, OH: U.S. EPA. EPA/600/R-16/128.

  This document provides instructions regarding the collection of samples from outdoor building and infrastructure materials to be analyzed for radiological contaminants following a contamination incident. The document focuses on the Site Characterization, Remediation and Final Status Survey (site release) phases of an incident and is not intended to address sample collection needs during Initial Response. The procedures are intended for collection of samples to be analyzed using the methods in SAM 2017.
  https://cfpub.epa.gov/si/si_public_record_report.cfm?dirEntryId=335065

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