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Sample Collection Information Document for Pathogens

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

SCIENCE

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Sample Collection Information Document for Pathogens

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

by

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Acronyms and Abbreviations

AAVLD	American Association of Veterinary Laboratory Diagnosticians
AOAC	Association of Official Analytical Chemists
APHIS	Animal and Plant Health Inspection Service
APHL	Association of Public Health Laboratories
ASM	American Society for Microbiology
BSL	biosafety level
CBR	chemical, biological, and radiological
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
Ch.	Chapter
COC	chain-of-custody
d_{50}	cut-off sizes correspond to 50% particle collection efficiency mark
DGR	Dangerous Goods Regulations
DHS	Department of Homeland Security (U.S.)
DOL	Department of Labor (U.S.)
DOT	Department of Transportation (U.S.)
DQO	data quality objectives
DWRPTB	Drinking Water Utility Response Protocol Toolbox
EMAC	Emergency Management Assistance Compact
EOC	EPA Emergency Operations Center
EPA	U.S. Environmental Protection Agency
ERLN	EPA's Environmental Response Laboratory Network
FBI	Federal Bureau of Investigation
FDA	Food and Drug Administration
FERN	Food Emergency Response Network
g	gram(s)
GPS	Global Positioning System
HASP	health and safety plan
HCV	Hepatitis C Virus
HEV	Hepatitis E virus
HFV	Hemorrhagic Fever Viruses
HSRP	Homeland Security Research Program
IATA	International Air Transportation Association
ICLN	Integrated Consortium of Laboratory Networks
ISO	International Organization for Standardization
L	liter
Lab Compendium	Compendium of Environmental Testing Laboratories
LRN	Laboratory Response Network
MCE	mixed cellulose ester
mL	Milliliter
MS/MSD	Matrix Spike/Matrix Spike Duplicates
NAHLN	National Animal Health Laboratory Network
NEMI	National Environmental Methods Index
NHSRC	National Homeland Security Research Center
NIFA	National Institute of Food and Agriculture
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
°C	degrees Celsius
OSHA	Occupational Safety and Health Administration
PPE	personal protective equipment
psi	Pound-force per square inch

PTFE	Polytetrafluoroethylene (Teflon®)
QA	quality assurance
QC	quality control
SAM	Selected Analytical Methods for Environmental Restoration Following Homeland Security Events
SCID	sample collection information document
spp.	species
UN	United Nations
URL	uniform resource locator
USAMRIID	United States Army Medical Research Institute of Infectious Diseases
USGS	United States Geological Survey
VCSB	voluntary consensus standards body
WCIT	Water Contaminant Information Tool
WLA	Water Laboratory Alliance
WWRPTB	Wastewater Utility Response Protocol Toolbox

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1.0 Background

The U.S. Environmental Protection Agency's (EPA's) Homeland Security Research Program (HSRP) has worked with experts from across EPA and other federal agencies since 2003 to develop a compendium of analytical methods to be used when responding to national homeland security related incidents. These sample collection methods are to be used by laboratories designated by EPA to perform the analyses of environmental samples following incidents resulting in the intentional or unintentional release of contaminants. Analytical methods have been selected for chemicals, radiochemicals, pathogens, and biotoxins for the types of environmental sample matrices that are anticipated in such incidents. The results of these efforts have been published in several revisions of EPA's *Selected Analytical Methods for Environmental Remediation and Recovery - 2017.* The HSRP periodically reviews and updates the Selected Analytical Methods document to address the needs of homeland security, reflect improvements in analytical methods and new technologies, and incorporate changes in target pathogens.

During development of the Selected Analytical Methods document, EPA recognized the need for a companion document to provide information regarding collection of samples for analysis by the listed methods. This Sample Collection Information Document (SCID) is intended to address this need, in part, by providing complementary information on sample collection, containers, preservation, size, and packaging, and by providing additional information sources to support the collection of samples to be analyzed for the selected pathogens, using the methods listed in *Selected Analytical Methods for Environmental Remediation and Recovery - 2017* (herein referred to as "the Selected Analytical Methods document"). As with the Selected Analytical Methods document, HSRP plans to update the information in this document periodically, to reflect changes to the list of pathogens and/or methods.

> The information contained in this document is intended to support and be used with the methods listed in *Selected Analytical Methods for Environmental Remediation and Recovery - 2017* for analysis of selected pathogens. The information will be reviewed and updated periodically, along with the Selected Analytical Methods document, to reflect advances in technologies, results of method evaluation and validation studies, and additional pathogens or matrices.

2.0 Scope and Application

This document provides general information for use by EPA and other users when collecting samples for pathogen analysis during environmental remediation following an intentional or unintentional release. The document is intended to be used with the Selected Analytical Methods document, and to provide information needed for collection of samples to be analyzed using the specific selected methods. Where possible, the information provided was obtained from the sample collection requirements and guidelines included in the *Selected Analytical Methods for Environmental Remediation and Recovery - 2017* analytical methods. Where this information was not available, additional sources were used (see Section 10.0 and additional resources).

A pathogen or infectious agent is a biological agent that causes disease or illness to its host. This document includes following pathogens: bacteria, viruses, protozoa, and helminths in a variety of environmental media (Table 1).

Table 1. Pathogens and Media Addressed in this Sample Collection Information Document

Pathogens (Size*) \ Media	Soil	Surface	Liquid	Aerosol
Bacteria  (0.2 – 5 µm)	• *Bacillus anthracis* • *Brucella* spp. • *Burkholderia mallei* • *Burkholderia pseudomallei* • *Campylobacter jejuni* • *Chlamydophila psittaci* • *Coxiella burnetii* • *Escherichia coli* • *Francisella tularensis*		• *Legionella pneumophila* • *Leptospira* spp. • *Listeria monocytogenes* • Non-typhoidal *Salmonella* • *Salmonella Typhi* • *Shigella* spp. • *Staphylococcus aureus* • *Vibrio cholerae* 01 and O139 • *Yersinia pestis*	
Viruses  (0.02 – 0.2 µm)	• Adenoviruses: enteric and non-enteric • Astroviruses • Caliciviruses: Norovirus and Sapovirus • Coronaviruses: SARS-associated human coronavirus • Hepatitis E virus (HEV) • Influenza H5N1 virus • Picornaviruses: Enteroviruses and Hepatitis A virus (HAV) • Reoviruses: Rotavirus (Group A)			
Protozoa  (4 – 20 µm)	• *Cryptosporidium* spp. • *Entamoeba histolytica* • *Giardia* spp. • *Naegleria fowleri* • *Toxoplasma gondii*			
Helminths  (40 – 100 µm)	• *Baylisascaris procyonis*			

* Sizes shown in the diagrams are not to scale.

The information in this document is intended to be used during site assessment, remediation, and clearance activities following an intentional or unintentional release of a contaminant; it assumes that samples will be collected by personnel trained in the collection of environmental samples containing the target pathogens, and trained in dealing with the corresponding health and safety concerns. Information is included regarding containers, collection volume or weight, sample preservation, sample holding times, and the packaging of samples representing the various matrices and pathogens of concern.

Certain information in this report may need to be modified to address site- or event-specific data needs; for example, additional sample volume may be needed for quality control (QC) or in cases when a low concentration of pathogen is suspected. Sample collection plans should be in place and consulted for specific sample collection requirements prior to initiation of sample collection activities. Site- or event-specific sample collection plans include information regarding laboratory capacity, the extent of contamination, target pathogens, data quality objectives (DQOs), sample locations, the number and type of samples needed, and other details.

2.1 Sample Collection Information Tables

This document contains the following tables listing information for collection of samples that will be analyzed for measurement of the selected pathogens.

- **Attachment A**: Sample collection information for pathogens in various environmental media (soil, surface, liquid, and aerosols). It should be noted that the surfaces include porous and non-porous surfaces; aerosols include natural aerosols and bioaerosols; solids include soils, granular and powder forms of debris and/or natural materials; and liquids include drinking water, surface water, and wastewater present at the pathogen impacted area.
- **Attachment B**: Sample collection information for pathogens.
- **Attachment C**: Holding times, packaging requirements, and shipping label requirements for samples.

Each table provides the sample size that should be collected to support sample analysis, the preservatives and/or temperature needed to maintain sample integrity prior to analysis, the maximum amount of time that should elapse between sample collection and the initiation of analytical procedures (e.g., sample analysis, digestion, inoculation), the appropriate type of container, the sample label and packaging procedures needed for sample shipment, and the source(s) used to provide the information. Unless otherwise specified, the following sample storage protocol may be followed:

- Ensure samples maintain integrity, and are not contaminated, lost, damaged.
- Samples requiring thermal preservation at other than ≤ 6°C shall be stored at ± 2° C of stated temperature.
- Samples are to be kept separate from reagents, standards, and other interfering items in refrigerators.

2.2 Document Development

EPA developed a hierarchy of references to prioritize the documents and resources that were used to identify the information that is included in this document. The first sources consulted were the methods listed in *Selected Analytical Methods for Environmental Remediation and Recovery - 2017*. If those methods included sample collection information, the information was evaluated and, if appropriate, included in the sample collection information tables. The second sources consulted were EPA procedures for collection of samples that address the specific pathogen/matrix pair. If there were no EPA procedures available, other federal agency or voluntary consensus standards body (VCSB) methods were consulted. If no procedures were identified for collection of a particular pathogen/matrix combination, methods for that pathogen in other matrices were considered, followed by procedures described and supported by data in peer-reviewed research literature, such as journal articles. The following agencies, organizations, and publications are representative examples:

- EPA – United States Environmental Protection Agency

- AOAC – AOAC International (formerly Association of Official Analytical Chemists)
- CDC – Centers for Disease Control and Prevention
- CFR – Code of Federal Regulations
- U.S. DHS – United States Department of Homeland Security
- U.S. DOL – United States Department of Labor
- U.S. DOT – United States Department of Transportation
- U.S. FDA – United States Food and Drug Administration
- USGS – United States Geological Survey
- IATA – International Air Transport Association
- ISO – International Organization for Standardization
- LRN – Laboratory Response Network
- NEMI – National Environmental Methods Index
- NIOSH – National Institute for Occupational Safety and Health
- OSHA – Occupational Safety and Health Administration
- Rice et al. 2017. *Standard Methods for the Examination of Water and Wastewater.* 23rd edition. Washington, DC: American Public Health Association
- Journals: *Analyst, Applied and Environmental Microbiology, Current Protocols in Microbiology, FEMS Microbiology Letters, Journal of Virological Methods, Public Health Reports*, and others.

2.3 Limitations

This document provides summary information only regarding collection of samples to be analyzed for selected target pathogens. This document includes the information based on the sampling protocols and analytical methods that were available at the time of publication. The document is expected to be updated with the advance of technologies. For example, research is needed to determine appropriate preservation and holding times for many of the biological agents. In addition, many of the pathogens listed in this document have only recently become an environmental concern, and EPA is actively pursuing development and validation of appropriate sample collection procedures.

Sample collection plans must be consulted for site- or event-specific requirements, including quality control (QC) and reporting. The information sources cited in this document also should be consulted for additional details regarding sample collection, including QC requirements, and sample handling, packaging, shipping, and safety procedures. Samplers should check with the incident commanders for special instructions regarding evidentiary matters prior to sample collection.

3.0 Health and Safety Considerations

This document assumes that a site- or event-specific health and safety plan (HASP) is in place that includes the safety concerns and requirements for the specific types of hazards that should be considered during a sample collection event. At a minimum, all sampling team members should be trained in Occupational Safety and Health Administration (OSHA) requirements for hazardous waste operations and emergency response (29 CFR 1910.120 or 29 CFR 1926.65) and should have current medical screening.

3.1 Health and Safety Plans

Health and safety plans (HASPs) will vary depending on the site, nature and extent of contamination, the sampling phase (site assessment, remediation, or final status determination), and the responsible organization. The purpose of these plans is to ensure maximum protection to workers, the environment, and surrounding communities, in a way that is consistent with requirements needed to perform operational activities.

> Sample collection and decontamination procedures should address personnel monitoring and decontamination during ingress and egress.

3.2 Personal Protective Equipment

Each site or event also will dictate the level of personal protective equipment (PPE) that will be required. Specific guidance for selection of PPE is provided in 29 CFR 1910.120, Appendix B. Factors that should be considered during selection include: contaminant identification, routes of exposure (i.e., inhalation, skin absorption, ingestion, and injection), performance of equipment in protecting against exposure, activity duration, and the stress that will be induced by work requirements.

3.3 Training

Sample collectors must be trained in collection and handling of samples suspected of containing the contaminants of concern, must be up to date regarding medical screening requirements, and must be approved for site entry. Additionally, sample collectors must be trained in the following:

- Ability to select and work with the appropriate level of PPE
- Decontamination procedures
- Prevention of sample cross-contamination.

4.0 Preparation for Sample Collection

During the early stages of an event, upon initial contact by the by the analytical services requester or other responsible party, coordination and communications with the primary responding laboratory may be performed to gather critical information pertaining to the nature of the samples to be collected, the number of samples required, prioritization of samples, and to alert member laboratories.

It is highly recommended that sampling kits be used during sample collection, and that these kits be properly equipped, maintained, and organized before deployment of sample collection personnel. Sample collectors should consult with project managers and the sample collection plan to determine what equipment and materials should be assembled. Sample kits should contain all sample containers, materials, supplies, and forms needed to perform sample collection, decontamination, documentation, and field packaging activities.

4.1 Field Sampling Equipment and Supplies

Before starting field sampling activities, all necessary equipment and supplies should be identified and available. The following is a preliminary list of equipment that needs to be specified and available:

- Sampling devices (e.g., air filters, soil samplers, water samplers, air filter samplers)
- Sample preservation equipment (e.g., acids, dechlorinating reagents)
- Sample volumetric measuring devices and/or weighing devices
- Sample containers and packaging equipment
- PPE
- Record keeping devices (e.g., logs, chain-of-custody [COC] forms, writing instruments)
- Site maps, Global Positioning System (GPS) recorders, etc.
- Sample location markers
- Pre-labeled and pre-weighed sampling containers
- Shipping containers, shipping forms, and shipping labels.

4.2 Field Data Documentation

All data collected in the field should be adequately documented. Documented information should include (for example):

- Names of field sampling personnel
- Sample collection plan
- Sample location(s)
- Sampling depth
- Physical and meteorological conditions
- Date and time of sampling
- Sample medium
- Expected radionuclides (if applicable)

- Sample identification number
- Sample size (weight, volume), sample duration (air filters), air volume
- Sample handling precautions.

4.3 Field Screening

Field screening procedures are typically qualitative or semi-quantitative in nature and are performed using special screening equipment or techniques, such as probes or portable hand-held instruments and meters. Some field screening is performed using field testing methods, and special kits that are designed for use in a field environment. Because the quality control and analytical sophistication of field screening is not as controlled as it is for laboratory testing, a representative set of split or duplicate samples should be submitted to a laboratory for comparison with the field results.

4.4 Quality Assurance/Quality Control

Sampling personnel should employ quality assurance/quality control (QA/QC) program requirements when collecting samples to include information on the collection of equipment blanks, field blanks, and field replicates, when available and as appropriate for the intended analyses. Field QA/QC requirements should be specified in sampling or site plans, and analytical support laboratories should be included in the discussion as analytical QA/QC requirements should greatly impact field sampling. The purpose of such QA/QC protocol is to ensure that (1) the laboratory receives samples that accurately represent the conditions existing at the sample site, (2) appropriate method-specific controls are provided to the analytical laboratory, and (3) the results of the analyses are traceable to the specific sample location or event. The following QC procedures should be included, as appropriate:

- **Decontamination of Sampling Equipment**: The field sampling plan should address the extent of decontamination and specify the procedures to prevent sample contamination that could be introduced from contaminated collection equipment. Sampling may be performed using separate laboratory-cleaned equipment for each sample location.
- **Sample Container Cleanliness Requirements**: The field sampling plan should also address the extent and type of sample container cleaning, to prevent sample contamination from containers. Pre-cleaned containers meeting EPA method-specific cleanliness protocols are available from many suppliers. If pre-cleaned containers are used, the serial number and QA batch number of each container should be recorded in the field log book/notes or field form. If sample containers are re-used, they should be decontaminated, and field blank samples should be submitted to the laboratory to verify container cleanliness.
- **Field Duplicates and Split Samples**: Field duplicates are two separate samples taken from the same source and are used to determine data repeatability based on field conditions. Field duplicate samples are assigned different sample numbers, specified in the field log book/notes or on the field form, distinguished from the regular field samples on the COC form, and often submitted blind to the laboratory to provide objectivity. The comparability of the results provides information on the repeatability of the field extraction and analytical procedures. Split samples are two or more representative portions taken from one sample and submitted to different

laboratories for identical analyses to obtain information on inter-laboratory repeatability.

- **Equipment Decontamination Blank**: These samples provide information on the levels of cross-contamination resulting from field or laboratory sample preparation actions. The equipment blank is reagent water that is free of the pathogen of interest, transported to the site, opened in the field, and poured over or through the sample collection device, collected in a sample container, and returned to the laboratory and analyzed. Equipment blanks are collected for each type of equipment used in sampling during the day. Equipment blanks are assigned sample numbers and are not distinguished from regular field samples on the COC form. To decontaminate, sampling equipment (e.g., scoops, spoons, bowls, etc.) will first be cleaned with a laboratory-grade detergent such as Alconox® using plastic brushes to remove soil and surface matter, and then rinsed with water to remove the remaining soapy material. The equipment will then be allowed to air dry. If the equipment is not to be used immediately, it will be wrapped with aluminum foil and stored in a clean, dry place. Verification of the effectiveness of the decontamination procedure will be acquired through equipment rinsate samples. Drill stems, rods, augers, tools, split spoons, sample barrels, and associated equipment will be cleaned prior to initial sampling and between sampling. Cleaning and decontamination of all equipment will occur at a designated area on the site. Equipment that is steam cleaned will be placed on racks or sawhorses at least two feet above the floor of the decontamination pad. After cleaning, all surfaces will be thoroughly rinsed. Cleaned equipment will be allowed to air dry.
- **Field Blanks**: Field blanks check the cleanliness of sample containers, for environmental contamination, for the purity of reagents, or for the purity of solvents used in the field. A sample container is filled with laboratory grade reagent water in the field, preserved, and submitted for analysis for the same parameters as the regular field sample.
- **Trip Blank**: A trip blank is a container of laboratory reagent water that is shipped, unopened, to and from the field, with empty and full sample containers. Its purpose is to identify contaminants introduced into samples during transit to and from the laboratory. At no time after their preparation are the sample containers opened before they reach the laboratory.
- **Matrix Spike/Matrix Spike Duplicates (MS/MSD)**: Some analytical methods require that the laboratory spike a portion or duplicate portions of the sample matrix with a predetermined quantity of analytes prior to sample extraction and analysis. A spiked sample is processed and analyzed in the same manner as the sample. The results of the spike compared with the non-spike sample indicate the ability of the test procedures to repeat recovery of the analyte from the matrix and also provides a measure of the performance of the analytical method. Additional containers may be specified to provide enough material for this procedure. The sample containers are assigned the same sample number as the regular field sample and are designated MS/MSD on the COC form.
- **Equipment Maintenance and Calibration**: All sampling equipment should be maintained on a regular basis, consistent with the documented criteria of the laboratory and normally accepted codes of practice/standards, which are well within the limits normally established and recommended for the care of the particular piece of equipment. Frequent checks on the reliability of

equipment and the calibration checks on all relevant equipment must be performed. Equipment calibration and maintenance records should be kept for all equipment, thus allowing the repair status of each piece of apparatus to be monitored. This reduces the likelihood that malfunctioning equipment will be used for sampling (thereby leading to poor bioassay data), and allows any problems with equipment to be more quickly diagnosed and corrected.

5.0 Sample Handling

A key aspect of biological research revolves around the gathering and collection of samples and their preservation for examination and analysis at a future date. Since time elapses between when a sample is collected and when it is analyzed, and biological samples often degrade over time, it is imperative to have a process of storage (short and long term) that is efficient and preserves sample integrity over time. Good storage practices of biological materials are essential component of any sampling activity. Biological samples often degrade over time when stored at room temperature, but some samples may also lose integrity at low temperatures if subjected to multiple freeze-thaw cycles. Many bio-specimens can be safely stored at a range between -20°C and 5°C, known as cold storage. Enzymes and antibodies can lose much of their functional activity if they are repeatedly frozen and thawed, so these samples are often refrigerated at around 2°C. Biological specimen storage in a range of 15°C to 27°C is known as room temperature storage. The best storage temperature for a given biological sample or reagent often varies depending on the type of biological material, the solution it is suspended in, the sample's intended use, and how long the material will be stored. Many variables go into making ideal storage temperature decisions for biological materials. For reagents and biological assays, it is often best to follow manufacturer/bioassay laboratory recommendations for both short-term and long-term storage temperatures. When storing samples, it is important to consider the sample's molecular structure (Holland et al., 2003; Budowle et al., 2006; NRC, 2014; Shabihkhani et al., 2015), the preservatives or solutions it is suspended with, and the degree of biological integrity required for analytical or research goals.

Samples that require low temperature preservation shall be considered acceptable if the arrival temperature of a representative sample container meets the method or mandated temperature requirement.

- Samples that are delivered to the laboratory on the same day they are collected may not meet the temperature or method requirements, if the time frame between collection and delivery is too short for the cooling process to complete. In these cases, the samples shall be considered acceptable if the samples were received nestled in ice with evidence that the cooling process has begun and the temperature of the sample(s) (or representative sample) is recorded upon receipt and is less than the temperature recorded at the time of sampling.
- Low temperature preservation along with temperature monitoring might not be required in the field if the laboratory receives the sample and either begins the analysis or refrigerates the sample within fifteen (15) minutes of collection.

Microbiological samples from known chlorinated sources, unknown sources where chlorine usage is suspected and all potable water supplies (including source water) shall be checked for absence of chlorine residual in the laboratory unless all of the following conditions are met:

- the laboratory can show that the received sample containers are from their laboratory or have been appropriately chlorine tested and documented;

- sufficient sodium thiosulfate was in each container before sample collection to neutralize at minimum 5 mg/L of chlorine for drinking water and 15 mg/L of chlorine for wastewater samples;
- one container from each batch of laboratory prepared containers or lot of purchased ready-to-use containers is checked to ensure efficacy of the sodium thiosulfate to 5 mg/L chlorine or 15 mg/L chlorine as appropriate and the check is documented;
- chlorine residual is checked in the field and actual concentration is documented with sample submission.

6.0 Sample Acceptance

Acceptance or rejection of samples may be based on individual samples (i.e., a laboratory can accept or reject samples at any time during the lifetime of the event). A laboratory's participation in a specific incident is at the discretion of the individual laboratory's management and may require consultation with higher level management in the parent organization before the laboratory agrees to provide analytical support. If samples are collected, shipped, and/or preserved in a manner that may affect sample integrity, the notification should be communicated as soon as possible. Consideration of possible impacts on data quality should be weighed against the monitoring objectives (e.g., the need to obtain rapid preliminary identification of the pathogen) before making a decision to accept or reject samples. Any results generated from analysis of samples with shipping or preservation issues should be appropriately qualified. Although sample acceptance (or rejection) is ultimately the laboratory director's or higher level management's prerogative, laboratories must consider the following before accepting samples:

- Sample integrity (i.e., condition)
- Sample packaging and preservation
- Sample volume
- Chain of custody provided
- Minimum documentation provided
- Potential sample hazards
- Field/safety screening results
- Law enforcement involvement or requirements
- Special instructions, if any
- Availability of additional, identical samples (splits)

Sample must be rejected if:

- Hold time is exceeded
- Improper preservation is noted
- Sample is in the wrong container
- Absence of chain of custody.

For those samples analyzed, all data must be reported with qualifiers. All associated results must be reported. Result qualification may be required when:

- Samples are improperly preserved

- The wrong container is used
- Holding time is exceeded
- Insufficient sample volume is available to perform analysis
- Known sampling errors are noted

The analytical laboratory may reject or require re-sample as alternative to qualification of sample results based on the appropriate bioassay qualification criteria established for the specific condition.

If discrepancies between sample collection records and sample receipts are noted, the laboratory must consult with the sample collector and other experts to determine if samples can still be analyzed and reported with qualification, or whether re-sampling is required.

7.0 Definitions

The following definitions are provided to describe the information listed in the sample collection tables:

- **Container** – The type of container (e.g., bottle, bag) that must be used to hold the sample. The container must be sufficient to maintain sample integrity and be composed of materials that will remain inert when in contact with the sample.
- **Holding Time** – The maximum amount of time allowable from sample collection until sample analysis, extraction, or inoculation.
- **Matrix** – The principal material of which the sample is composed.
- **Packaging** – Sample container packaging requirements for shipment of the sample to the laboratory.
- **Preservation** – Conditions and/or chemicals used to maintain the integrity of a sample (e.g., sodium thiosulfate and refrigeration at temperatures < 10°C but above freezing for biological samples).
- **Sample Size** – The minimum amount of sample that should be collected to support analysis of a single sample. Volume and weight requirements depend on the target pathogen(s), the analytical method that will be used, and the data requirements.
- **Shipping Label** – U.S. DOT shipping label requirements under 49 CFR 172 and 173.

8.0 Laboratory Support

8.1 Defining Analytical Support Requirements: Capabilities and Capacity

The inherent rigidity in a standard operating protocol for biological incident sampling and processing could be unwieldy and require consultation among the different entities involved in a response should provide best-practice options. If the pathogen incident fits a pattern or template for which a sample collection methodology and/or sampling strategy already has been validated, then the sampling activities could be well-defined and more focused. However, in most pathogenic incident cases, the sampling area, location, type of agent, substrates, and combinations of these variables are almost always novel (Budowle et al., 2006). A network of laboratories with technical infrastructure (centralized communication, personnel, standardized reagents and equipment and test

protocols, reporting policies, shipping and transportation guidelines assay development, and new or renovated facilities to increase levels of biosafety containment), provides the necessary infrastructure for a tiered capability of response to an event. Most field sample collectors might not be responsible for analyzing the samples. Water utilities, if involved, might have unique capabilities to collect samples and analyze them in their laboratories. For this reason, it is critical that the role of the microbiology laboratory in incident response be evaluated based on internal analytical capabilities and response capacity. Some contaminants (for example, select biological agents) should be analyzed by qualified laboratories using specialized or restricted analytical methods. It is important that utilities are familiar with analytical support networks. They are encouraged to look into the resources offered by EPA's Environmental Response Laboratory Network (ERLN) and Water Laboratory Alliance (WLA), such as the WLA response plan, as well as other members of the Integrated Consortium of Laboratory Networks (ICLN) including the CDC Laboratory Response Network (LRN). (Table 1 provides descriptions of these laboratory networks.) Internal and external analytical support networks should be in place and operational prior to initiating any baseline sampling and analysis activities, and in preparation for an event.

8.2 Establishing Analytical Support Networks

Establishing a support network of laboratory analytical capabilities and capacity should ensure that samples can be processed properly and expeditiously. To assist in locating laboratories capable of providing the necessary support, the EPA's Compendium of Environmental Testing Laboratories (Laboratory Compendium) provides users with real-time data related to laboratory contact, capability and capacity information, and ERLN/WLA Membership status, through a secure web-based tool. The Laboratory Compendium is available to emergency response, laboratory and water utility personnel, at the federal, state, and local levels. Access is secured through an application process at https://cfext.epa.gov/cetl.

Each EPA region maintains an EPA regional laboratory, which may be able to analyze samples or to help identify potential analytical support. Access the list of EPA regional laboratory contacts at http://www2.epa.gov/aboutepa/regional-science-and-technology-rst-organizations#branches.

LRN laboratories have response teams available 24 hours a day/7 days a week/365 days a year who may be able to assist with sample collection needs after routine business hours. Usually the closest LRN laboratory should be the state's department of health laboratory; also, consider contacting the local public health laboratory. For more information, CDC can be contacted at (800) CDC-INFO, (888) 232-6348 (TTY) or www.cdc.gov/info. More information is also available at: https://emergency.cdc.gov/lrn/biological.asp. Another resource for state laboratory contact information is maintained by Association of Public Health Laboratories (APHL) at https://www.aphl.org/membership/Pages/memberlabs.aspx.

EPA Headquarters might also be able to provide help in identifying support for analysis and collection of samples. The ERLN/WLA Helpline may be reached at (703) 461-2400, Monday-Friday from 8:30 AM to 5:00 PM ET, except for federal holidays. The WLA may also be reached at https://www.epa.gov/waterlabnetwork. Outside of regular business hours, the

EPA Emergency Operations Center (EOC) Hotline may be reached at (202) 564-3850.

8.3 Coordinating with Analytical Support Networks

Once appropriate analytical laboratory support has been identified, it is imperative to establish a chain of communication between and among the entity affected by a contamination incident and the supporting laboratories. Support laboratories should be consulted regarding specific sample collection, container, volume, preservation, holding time, and shipping requirements. In some cases, support laboratories should train sampling teams in specialized sample collection procedures. The support laboratory may also provide the affected entity with, or assist with the preparation of, sampling kits to ensure that the samples are properly prepared and preserved for the required analyses, particularly for sampling unknown or tentatively identified contaminants, as appropriate. It is important to follow specific laboratory requirements since this may impact the quality of the analytical results. Depending on the method and event, laboratories should request specific quality control (QC) samples such as field duplicates, field blanks, trip blanks, and field matrix spikes and may require specific chain of custody (COC), notification, and shipping procedures.

8.4 Laboratory Networks and Associations

Table 2 provides the key laboratory networks and associations.

Table 2. Key Laboratory Networks and Associations

Laboratory Networks/ Associations	Description	Additional Information Source*
Environmental Response Laboratory Network (ERLN)	EPA's ERLN is a national network of laboratories that provides analytical capability and enhanced capacity to meet project-specific data quality objectives on an as-needed basis. The ERLN integrates capabilities of existing public sector laboratories with accredited private sector laboratories to support environmental responses.	https://www.epa.gov/emergency-response/environmental-response-laboratory-network
CDC Laboratory Response Network (LRN)	In response to the threat of bioterrorism and following a presidential order, officials at the Centers for Disease Control and Prevention (CDC), Association of Public Health Laboratories (APHL), Federal Bureau of Investigation (FBI), and United States Army Medical Research Institute of Infectious Diseases (USAMRIID) established the Laboratory Response Network (LRN) in 1999. This national system is designed to link state and local public health laboratories with other advanced-capacity clinical, military, veterinary, agricultural, water, and food-testing laboratories, including those at the federal level. The LRN is a critical component of CDC's public health mission, enhancing U.S. readiness to detect and respond to bioterrorism incidents.	https://emergency.cdc.gov/lrn/
Water Laboratory Alliance (WLA)	The WLA provides the Water Sector with an integrated nationwide network of laboratories with the analytical capability and capacity to respond to intentional and unintentional drinking water contamination events involving chemical, biological, and radiochemical contaminants. The WLA structure consists of three tiers of laboratories: sentinel, confirmatory, and reference	https://www.epa.gov/waterlabnetwork

Laboratory Networks/ Associations	Description	Additional Information Source*
	laboratories. Sentinel labs will perform routine monitoring and surveillance and will rule out or refer samples to confirmatory labs for further analysis. Confirmatory labs will perform rapid, high-confidence presumptive and confirmatory identification of samples referred by sentinel labs. These labs generally have facilities with biosafety levels (BSLs) of 2 and 3. Reference labs will provide definitive characterization of agents and attribution of the source. These labs will also have highly specialized containment facilities (BSL levels of 3 and 4), and highly trained staff. Confirmatory and reference labs will likely participate in several laboratory networks including the LRN and the Environmental Response Laboratory Network (ERLN).	
Food Emergency Response Network (FERN)	FERN integrates the nation's food-testing laboratories at the local, state, and federal levels into a network that is able to respond to emergencies involving biological, chemical, or radiological contamination of food. The FERN structure is organized to ensure federal and state interagency participation and cooperation in the formation, development, and operation of the network. The FERN plays a number of critical roles related to food security and food defense, including prevention, preparedness, response, and recovery. FERN provides training, proficiency testing, method development and validation, surveillance, electronic communication, and laboratory outreach/cooperative agreements.	http://www.fernlab.org/
Association of Public Health Laboratories (APHL)	APHL promotes the role of public health laboratories in shaping national and global health objectives, and promotes policies, programs, and technologies which assure continuous improvement in the quality of laboratory practice and health outcomes. A membership must be purchased to access most APHL publications and services.	http://www.aphl.org/Pages/default.aspx
National Animal Health Laboratory Network (NAHLN)	The USDA's NAHLN is a network of laboratories that is organized and supported to have the capacity to respond to animal-disease outbreaks nationwide. The network is a cooperative effort between the USDA Animal and Plant Health Inspection Service (APHIS), the National Institute of Food and Agriculture (NIFA), and the American Association of Veterinary Laboratory Diagnosticians (AAVLD).	https://www.nahln.org/ and https://www.aphis.usda.gov/aphis/ourfocus/animalhealth/lab-info-services/nahln
Emergency Management Assistance Compact (EMAC)	EMAC is a congressionally ratified organization that provides form and structure to interstate mutual aid. The EMAC mutual aid agreement and partnership between member states exist because—from hurricanes to earthquakes, wildfires to toxic waste spills, and terrorist attacks to biological and chemical incidents—all states share a common enemy: the threat of disaster.	http://www.emacweb.org/

*Last accessed September 11, 2017.

9.0 Tools and Databases

Table 3 lists the representative tools and databases. Uniform resource locator (URL) can be accessed for additional information.

Table 3. Representative Tools and Databases

Tool/Database Name	Description	Additional Information Source*
Compendium of Environmental Testing Laboratories (Lab Compendium)	EPA's Lab Compendium is a secure Web-based system that provides users the ability to access and identify appropriate laboratories to support specific analytical needs. The Lab Compendium contains laboratory records for several hundred public and private sector environmental testing laboratories.	https://cfext.epa.gov/cetl/lblogin.cfm?action=None&CFID=32404&CFTOKEN=83271178
Drinking Water Utility Response Protocol Toolbox (DWRPTB) and Wastewater Utility Response Protocol Toolbox (WWRPTB)	Organized in modular format, this set of toolboxes assists with emergency response preparedness and is of value to drinking water and wastewater utilities, laboratories, emergency responders, state drinking water programs, technical assistance providers and public health and law enforcement officials. These modules provide emergency response planning tools that are designed to help the water sector to effectively and appropriately respond to intentional contamination threats and incidents.	https://www.epa.gov/waterutilityresponse/drinking-water-and-wastewater-utility-response-protocol-toolbox
Water Contaminant Information Tool (WCIT) for Priority Contaminants	WCIT is a secure on-line database with methods for more than 800 analytes, including detailed profiles for over 100 chemical, biological, and radiological (CBR) contaminants of concern for the water sector. It allows users to compare and contrast the performance, speed, and relative cost of analytical methods for response to all-hazard incidents from CBR type contaminants. This tool compiles drinking water and wastewater-specific data in a single location to help plan for and respond to drinking water contamination incidents. WCIT functionality and data were shaped and validated by water utility professionals, scientists, and public health experts. WCIT also features a search function capable of scanning searchable fields in the database. Users must apply to gain access to WCIT.	https://www.epa.gov/waterdata/water-contaminant-information-tool-wcit
WaterISAC	WaterISAC is a community of water sector professionals who share a common purpose: to protect public health and the environment. WaterISAC serves as a clearinghouse for government and private information that helps subscribers identify risks, prepare for emergencies and secure the nation's critical water infrastructure. Users must apply to gain access to WaterISAC.	http://www.waterisac.org/
Sampling Guidance for Unknown Contaminants in Drinking Water	This document provides comprehensive guidance that integrates recommendations for pathogen, toxin, chemical, and radiochemical sample collection, preservation, and transport procedures to support multiple analytical approaches for the detection and identification of potential contaminants in drinking water. The guidance is intended to support sampling for routine and baseline monitoring to determine background concentrations of naturally occurring pathogens, sampling in response to a triggered event, and sampling in support of remediation or decontamination efforts.	https://www.epa.gov/sites/production/files/2017-02/documents/sampling_guidance_for_unknown_contaminants_in_drinking_water_02152017_final.pdf
Pathogen Research Databases	Los Alamos National Laboratory maintains pathogen research databases including hepatitis C virus (HCV), hemorrhagic fever viruses (HFV)/Ebola, and human immunodeficiency virus.	http://lanl.gov/collaboration/pathogen-database/index.php

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10.0 Additional Resources

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Attachment A:
Sample Collection Information for the Environmental Media (Soil, Surface, Liquid, and Aerosol)

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1. Soil Sampling for Pathogens

This sample collection procedure describes the activities and considerations for the collection of pathogens from representative soil samples. There are a wide variety of reasons for collecting samples and various sampling strategies for different situations. Sample containers of the proper size/composition are shown in Table A-1 and identification/selection of sampling equipment/device are shown in Table A-2. Use of a device constructed of unsuitable material might compromise quality by the material leaching into the sample or sorbing materials from the sample. Even the most well designed, constructed and cleaned sampling device will yield a non-representative sample if used improperly. Identification of the physical environment is important in determining the potential distribution of pathogens at a given site. Pathogens can be deposited and distributed on the surface soil with greatest concentrations in the top few centimeters. If only a few large samples are taken at depth (e.g., 0 to 30 cm) to meet the soil volume requirements for testing, the pathogen concentrations in the test samples after homogenization will be diluted and probably not be representative of site conditions. A better approach may be to collect and composite many smaller samples at shallower depths (e.g., 0 to 5 cm).

Table A-1. Soil Sampling for Pathogens

Soil is a natural body comprised of solids (minerals and organic matter), liquid, and gases that occurs on the land surface, and is characterized by one or both of the following: horizons, or layers, that are distinguishable from the initial material as a result of additions, losses, transfers, and transformations of energy and matter or the ability to support rooted plants in a natural environment.

Soil Sampling Strategies: Size, Number and Type of Samples

Soil Sample Collection - Planning/ Preparation and Process

Sampling Materials

• Identification and selection of sampling equipment/device • Sample containers of the proper size and composition • Quality control samples (e.g., field and/or trip blanks, duplicates, performance evaluation samples) • Bound field logbook, writing instruments (pens, pencils and permanent markers), camera and extra charged batteries. • Appropriate paperwork (e.g., chain of custody, logging and calibration forms)	• Sample labels • Reagents, preservatives, coolers and a means to maintain sample temperature at 4ºC • Portable instrumentation and GPS unit • Decontamination equipment for personnel and/or equipment • Absorbent pads • Plastic bags for containerizing contaminated items • Packaging materials for sample shipment and custody seals, appropriate shipping containers that meet U.S. DOT/IATA or appropriate standards

Sampling Process Most pathogenic tests can be conducted with discreet soil samples or composite samples. The test end points measured are often the same, however, the test design (e.g., number of replicates, test species per replicate, volume of soil per test) can be different. Once composited samples (soil cores) are received at the testing facility, they should be stored immediately and remain undisturbed (to mimic the field conditions) until they are tested. Composited soil samples should be tested as soon as possible and may not be frozen as freezing and thawing can disrupt soil structure and could influence the biological activity.

Sample size: The minimum volume (or mass) of soil required depends on the overall objective, site conditions, and the tests to be conducted. A few examples of impact of soil and site characteristics are indicated below.

Bulk Density: Soil with high bulk density (e.g., sandy soil or clay rich subsurface soil) might require a greater mass of sample compared to low bulk density soil (e.g., peat or organic rich forest soil).

Moisture Content: Moisture content at the time of collection can influence sample quantity as soil mass requirements in a test method are recommended based on dry weight of the soil. If a site soil is very moist, more soil should be collected than if the soil at a site is dry.

Impurities: If the site soil contains significant amount of large (>6 cm) stones, industrial debris, or plant roots, then additional quantity of soil should be collected.

Nature, Extent, and Distribution of Pathogens: Pathogens may be deposited and distributed on the surface soil with greatest concentrations in the top few centimeters. If a few large samples are taken at depth (e.g., 0 to 30 cm) to meet the soil volume requirements for testing, after homogenization the pathogen concentrations in the test samples will be diluted and probably no longer represent the site. A better approach would be to collect many smaller samples at depths that represent the depth of contamination (e.g., 0 to 5 cm).

Number: The number of soil samples to collect depends on the study objectives, the DQOs, the desired level of certainty, and site-specific considerations such as predicted distribution of pathogens, the heterogeneity of the soil, test requirements, and the size of the site. The number and location of samples can be determined using two dimensional sampling patterns (random, transect, two-stage, and grid sampling) or three-dimensional sampling (information concerning depth is needed).

Type of Soil Samples - Point, Composite and Bulk: Point samples (or sample increments) are individual blocks of soil removed from one location by a sampling device. Composite samples are samples comprising two or more point samples. When point samples from different sampling locations are pooled together, the pooled sample is a composite sample. Bulk samples are large (e.g., >1L) point samples that consist of individual blocks of soil removed from one location by a sampling device and often collected to satisfy the large volume requirements for biological testing.

Surface soil: Bulk soil samples are easily obtained with a shovel or a soil auger. Soil augers can be more precise than simple shovels because they ensure that samples are taken to exactly the same depth on each occasion as several soil factors can vary considerably with depth. To minimize pathogen contamination a sterile spatula can be used to scrape away the outer layer of the core and use the inner part of the core for analysis. Pathogen cross-contamination can also occur between samples, which can be avoided by cleaning the auger after each sample is taken. The cleaning procedure involves washing the auger with water, then rinsing it with 75% ethanol or 10% bleach, and a final rinse with sterile water. Rhizosphere soil volumes are variable. Soil adhering to the plant roots is considered to be rhizosphere soil. Roots are normally excavated and shaken gently to remove bulk or non-rhizosphere soil. Surface soil samples usually undergo sieving through a 2-mm mesh to remove large stones and debris. Prior to separation, air drying may need to be performed to facilitate sieving. However, care should be taken so that the soil moisture content does not become too low to reduce microbial populations.

Subsurface soil: Subsurface soil samples generally have lower pathogen contents and microbial contamination from extraneous sources during sample collection may significantly affect the numbers counted. Mechanical approaches (such as drill rigs) may be necessary for collecting deep or shallow subsurface samples. Air rotary drilling can be used for unsaturated systems; however, if the core barrel overheats, pathogens within the sample may be effectively sterilized rendering the sample unrepresentative and unusable. To avoid potential contamination from water and surfactants that are normally injected to control dust and prevent overheating, coring can be performed slowly to avoid the need for these additives. To limit or prevent contamination from air, all air used in the coring process can be pre-filtered through a 0.3 µm high-efficiency particulate air (HEPA) filter. Immediately following core collection, the surface layer from the core can be scraped away with a sterile spatula, and sub-cores can then be taken using a sterile plastic (e.g. 60-mL) syringe with the end removed. The sample can subsequently be placed in a sterile plastic bag or sleeve and either analyzed immediately or frozen for future analysis.

Sample Storage: Preservation Method and Maximum Holding Time

- Pathogen analyses should be performed as soon as possible (dependent on the specified holding times for the pathogen of interest) after collection of a soil to minimize the effects of storage on pathogens. Once removed from the field, pathogen populations within a sample can and will change regardless of the method of storage. If immediate testing is not possible, guidance needs to be obtained for storage and holding times allowed for the specific pathogen of interest.
- Samples should be stored in darkness (to avoid growth of algae) with free access to air (to avoid development of anaerobic conditions).
- Samples should not be stacked, nor be too large as anaerobic conditions might develop. If samples are stored, care should be taken to ensure that samples do not dry out and that anaerobic conditions do not develop at the bottom of the sample.
- Samples must not dry out or become waterlogged during storage.
- Samples that are to be tested for pathogenic DNA/RNA or enzyme activity should be tested immediately. If this is not possible, samples for DNA and phospholipids fatty acid analyses and dehydrogenase activity analyses can be stored at -20°C for 1 to 2 years. Samples for RNA analyses can be stored at -80°C for 1 to 2 years after an initial shock-freezing with liquid nitrogen.

Containers for Soil Samples Collected for Pathogen Testing			
Container Material of Construction and Type	**Sample Volume (L)**	**Advantages**	**Disadvantages**
HDPE bucket	10 - 20	• Widely available • Inexpensive • Rugged • Suitable for long-term storage	• Can influence organic co-contaminants analyses
SS bucket with push-fit lids	5 – 20	• Commercially available • Reasonably priced • Rugged • Suitable for VOCs • Suitable for long-term storage	• Need specialized equipment to seal buckets
Polyethylene bag	Up to 60	• Usable as a bucket liner for samples contaminated with inorganics	• Not rugged
Teflon bag	Up to 60	• Chemically inert and solvent resistant to most chemicals • Can be used as a bucket liner or as a sample container by itself	• Not rugged
Glass wide-mouthed jars with polyethylene/polypropylene caps or HDPE lids	0.125 - 2	• Widely available • Inexpensive • Suitable for long-term storage	• Not rugged • Can only contain small sample volumes
Plastic* wide-mouthed jars with plastic caps and HDPE lids	0.125 - 4	• Widely available • Inexpensive • Rugged • Suitable for long-term storage	• Can only contain small sample volumes • Not suitable for non-weathered organics

HDPE, high density polyethylene; SS, stainless steel; VOC, volatile organic compound
*Plastic materials include polypropylene, polystyrene, HDPE, and polystyrene.

Table A-2. Representative Soil Sampling Devices

Device	Sample Type	Soil Type	Soil Sample Area/Volume	Penetration Depth	Advantage	Disadvantage	References
Shovel. Scoop, Spoon, Trowel, Spade	U	All soil types including non-cohesive sandy or loose soils	0.5 to 4 L	Surface, shallow subsurface	• Collection of large volumes of soil can be done quickly and easily • Collects blocks of soil • Easy to decontaminate	• Samples can be biased because of shape and imprecise volume. Bias can be minimized by careful sample collection.	Prévost and Antoun, 2008
Cutting/ Sampling Frame	U	Organic horizon(s), mineral A horizon(s)	100 to 900 cm^3	Surface	• Efficient way to collect representative bulk sample	• Can be difficult to remove all soil within frame	Bélanger and Van Rees, 2008
Ring Sampler	C or U	Cohesive soils	0.5 to 20 cm diameter	Surface	• Easy to use • Precise core	• Not as useful for unconsolidated soils or hard clay	ISO, 2002
Bulb Planter	C or U	Cohesive soils	1.5 L	Surface (0 to 15 cm)	• Large core – higher volume	• Not useful for hard soils	Dalpé and Hamel, 2008
Cutting Cylinder (Soil Punch)	C or U	Organic, A horizon	59 to 556 cm^2	Surface	• Soil cores are large and can efficiently collect large volume	• Can compress soil samples	Bélanger and Van Rees, 2008
Soil Corer	C or U	Cohesive soils	2.5 to 10 cm (dia.) 30 to 60 cm (height)	0 to 60 cm	• Easy to use • Precise core • Easy to clean • Can use liner or sample tube	• Compaction when driving corer into soil • Cores not truly disturbed unless linear used	USEPA, 2006
Slide-hammer Core Sampler	Co or U	Cohesive soils	2.5 to 10 cm (dia.) 30 to 60 cm (height)	0 to 60 cm	• Easy to use • Precise core • Easy to clean • Can use liner or sample tube	• Compaction when driving corer into soil • Cores not truly disturbed unless linear used	EC and SRC, 2007
Auger	U	Cohesive soils	2.5 to 15 cm long	0 to 60 cm	• Easy to use • Can handle various types of soils	• Less precise sample than coring device • Hard to decontaminate • Modifies soil matrix • Can introduce artifacts into soil sample	Mason, 1992

Split Spoon/ Tube Sampler	C or U	Cohesive soils and hard soils	Variable (up to 10 cm (dia.) and up to 2 kg sample		• Easy to use • Precise core • Large cores • Can use liner	• Deep cores can only be obtained using drilling rig	Weinfurtner and Kördel, 2007
Shelby Tube Sampler	C or U	Cohesive soils and hard soils	Variable (up to 10 cm (dia.)	0 to 40 cm or 0 cm to bedrock	• Easy to use • Precise core • Large cores • Can use liner	• Deep cores can only be obtained using drilling rig • Not durable in hard soils	CCME, 1993
Piston Samplers	C or U	Non-cohesive soils, wet soils, wet clay, dry and wet peat	Variable	Surface Shallow subsurface	• Holds moisture and fine materials in place in sample	• Can be difficult to operate	Mason, 1992
Direct Push Corer (GeoProbe™)	C	Cohesive soils	Tubes: 5 or 7 cm (dia.) and 1.2 m long Size of probes and liners vary	Surface Subsurface	• Saturated sands and silts can be collected • Consolidated samples used to classify soils	• Must use a drill rig • Not optimal in wet condition with stony soils or soils with high clay content	ASTM, 2008
Rotary Auger with lined or unlined core barrels	C	Cohesive soils and soft bedrock	Variable	Surface to bedrock	• Saturated sands and silts can be collected	• Must use a drill rig • Not suitable for stony soils • Modified soil matrix • Can introduce artifacts	ASTM, 2009
Rotary (solid stem) Auger	U	Cohesive soils, frozen soils, and soft bedrock	15 cm and larger	Surface to bedrock	• Easy to use • Faster than hollow stem • Provides continuous lithology information	• Must use a drill rig • Limited by stony soils • Sample depth determination can be imprecise due to auger sample spin up • Modified soil matrix • Can introduce artifacts	ASTM, 2009
PPE and Emergency Equipment							
Depending on site and pathogen specific health and safety plan (HASP) to be followed.							

C, consolidated; U, unconsolidated

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2. Surface Samples

Surface sampling involves collecting microbial contaminants from a surface using an appropriate sampling device to determine the presence of pathogens. Swabs, wipes, Sponge-Sticks (3M, Solar-Cult, or equivalent), and vacuum filter, socks or cassettes are the primary collection devices for spores on surfaces and are used during all phases (identification, characterization, decontamination, and clearance) of a response (CDC 2012).

Determining the most appropriate type of surface sample collection method depends on whether porous or non-porous surfaces are to be sampled. Wipes and swabs should be used on non-porous surfaces while vacuum socks or filter cassettes should be used on porous surfaces (Raber, 2006). Examples of non-porous surfaces include: stainless steel, painted wallboard, glass, floor tile, and wood laminate. Examples of porous surfaces include: ceiling tile, fabrics, carpet, clothing, rugs, and upholstered furniture.

When collecting samples for pathogen on porous surfaces, use of wipes can be considered, because some studies have demonstrated higher recovery efficiencies when wipes were used to sample carpet and upholstery than when vacuum methods were used (Buttner et al. 2004, Estill et al. 2009, Valentine et al. 2008). Rayon/polyester or cellulose/polyester blends are superior to cotton wipes (Valentine et al. 2008). Vacuum sampling is also effective for spore collection from carpet or upholstery and could be used on these surfaces if high concentrations ($> 10^2$ spores/cm^2) are expected (Brown et al. 2007).

Certain solutions (wetting agents) can be used to pre-moisten biological collection devices to enhance their overall performance. Common solutions include sterile water, sterile saline, neutralizing buffer, sterile phosphate buffer, and peptone buffer. In addition, surfactants (such as Tween® 80, Tween® 20, or Pluronic®) can be added to these pre-moistening solutions to improve removal of spores from surfaces. Neutralizing solutions block the continued action of a disinfectant after sampling. These neutralizing solutions are important during post-decontamination activities (verification and clearance sampling) to ensure that samples, when analyzed properly, are not falsely negative due to the presence of residual disinfectant. Among available neutralizing solutions are:

- Butterfield's buffer with 0.02% Tween 80 (Tween 80 is effective in neutralizing phenolic compounds and acting as a surfactant)
- Day Engley broth (Becton Dickinson, Sparks, MD) [neutralizes chlorine compounds and iodine, but may encourage growth during transport]
- Neutralizing Buffer (Becton Dickinson) [contains sodium thiosulfate to neutralize chlorine compounds and aryl sulfonate complex to neutralize quaternary ammonium compounds]
- Neutralizing Buffer (Hardy Diagnostics) [contains aryl sulfonate complex to neutralize quaternary ammonium compounds, sodium thiosulfate to neutralize chlorine compounds, potassium phosphate to maintain the pH, and sodium hydroxide]

- Letheen broth (Becton Dickinson [neutralizes quaternary ammonium compounds, but may encourage growth during transport]
- Phosphate Buffered Saline, pH 7.2 with 0.02% Tween 80 [Tween 80 is effective at neutralizing phenolic compounds at appropriate concentrations and acts as a surfactant]

Similar recovery efficiencies (26.8 ─ 39.0%) have been obtained with wipes pre-moistened with each of these neutralizing buffers that were processed by the LRN processing procedure. The choice of neutralizing solution depends on the disinfectant used. During the initial identification and characterization of a contaminated building, collection devices with a neutralizing solution are less important.

There are factors that will affect the choice of which wetting solutions to use for pre-moistening swabs and wipes for sampling. For example, phosphate-containing solutions (e.g., Butterfield's buffer and phosphate buffered saline [PBS]) may inhibit polymerase chain reaction (PCR) assays if appropriate DNA extraction and purification is not performed; the use of Dey Engley or Letheen broth may encourage germination and growth during transport. Sterile saline will not neutralize the action of a sporicide or chemical. However, neutralization may not be a concern during characterization sampling (on surfaces that do not already contain sporicides).

Some of the sampling devices can be purchased pre-moistened or they can be pre-moistened prior to collecting a sample. The Centers for Disease Control and Prevention (CDC) recommends the use of a neutralizing buffer as the pre-moistening solution in their validated swab and wipe-sampling and analysis methods (CDC 2012). The CDC developed methods for processing macrofoam swab and cellulose sponge wipe samples collected on environmental surfaces. These processing protocols use traditional culture methods and yield semi-quantitative estimates of the amount of pathogen contamination in a sample. The CDC collection procedures for the validated swab and wipe method and a non-validated gauze method are provided on the CDC website at http://www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthracis.html.

3. Swab Samples

Swabs are appropriate for sampling small [26 square centimeters (cm^2)] non-porous surfaces. Swabs work best for small areas like crevices, corners, supply air diffusers, air return grills, and hard-to-reach places. The CDC currently recommends using macrofoam swabs for the collection of *Bacillus anthracis* spores on smooth, non-porous surfaces (CDC 2012). The Laboratory Response Network (LRN) laboratories are capable of processing samples collected in accordance with this sample collection protocol using the prescribed swab type.

4. Wipe Samples

Wipes are appropriate for sampling larger (e.g., 645 cm^2 per CDC sampling method) non-porous surfaces, such as walls, desks, and non-carpeted floors. Wipe sampling can be performed using either cellulose sponges or gauze. Sponge-Sticks (3M, Solar-Cult, or equivalent) are sponge wipes with handle and are therefore preferred for surface sampling. The CDC currently recommends using a cellulose sponge wipe for the collection of *B. anthracis* spores on smooth, non-porous surfaces (CDC 2012). The LRN laboratory or laboratories that will be analyzing the sponge wipe samples should be consulted prior to using this collection method to determine if that laboratory is capable of processing and analyzing the sample.

5. Vacuum Samples

The primary sample collection method for sampling large porous surfaces (> 600 cm^2) for *B. anthracis* spores is vacuum sampling using filter socks or cassettes. Collecting samples by vacuuming is advantageous for covering large, non-porous and porous surfaces such as carpeting, ceiling tiles, ventilation systems filters, and upholstered furniture. This type of sampling also works well for capturing bulk powder or dust in hard-to-reach places. Vacuum sampling is also the best choice if sensitive items such as electronics and personal items are a concern, since it is less likely to cause damage compared to pre-moistened swabs and wipes. The laboratories analyzing the vacuum filter socks or cassettes should be consulted prior to using this collection method to determine if that laboratory is capable and willing to process this sample type, since at this time there are no LRN-approved processing methods for either device. Vacuum sampling and analysis methods have been evaluated for their performance to collect a surrogate spore (*B. atrophaeus*) contamination from carpet, concrete, upholstery and HVAC filters (USEPA 2013).

During vacuum sampling, bulk material is trapped by the dry collection media/filter by utilizing a small, HEPA vacuum cleaner or a small sampling pump to draw air through the filter. A number of sampling devices can be used to collect samples from porous materials including filter socks, 3M Forensics Vacuum filters, or 37 mm cassettes. The filter sock method utilizes a filter sock and attachment nozzle that fits onto the inlet nozzle of a HEPA vacuum hose. The 3M Forensics Vacuum filter is favored by law enforcement groups due to its ease of use in evidence collection protocols. This filter also attaches to a HEPA vacuum cleaner hose for sampling, though care should be exercised to regulate the power of the vacuum so the filter integrity is not compromised during sampling. The last option uses micro-vacuuming techniques to collect a sample using personal sampling pumps or carbon vane pumps. These pumps utilize a suitable filter contained in a closed-face, conductive sampling cassette to which a short section of plastic tubing cut at a 45° angle is added to the inlet. In the EPA comparison (USEPA 2013) the 37mm vacuum cassettes were found to be more efficient than the vacuum socks at collecting the spores from multiple surfaces. Filter cassettes were also determined to be safer for samplers and laboratorians to handle because the filter is sealed within a plastic case, thus reducing potential for exposures. The EPA methods for collecting vacuum filter sock samples and 37 mm vacuum cassettes samples (USEPA 2013) are described in Attachment C. Information on proper packaging and shipping of vacuum socks can be found on the CDC website (CDC 2012).

Vacuum sock samples must be collected using only HEPA filtered vacuum pumps. Conventional home or industrial vacuum cleaners should not be used for sample collection, because they can further disperse spores as filtration is not highly efficient.

Three of the CDC surface sampling procedures (macrofoam swab, Cellulose Sponge, and gauze) for *Bacillus anthracis* spores from smooth, non-porous surfaces are indicated as examples in the following sections.

6. Macrofoam Swab Procedure

Swab Materials

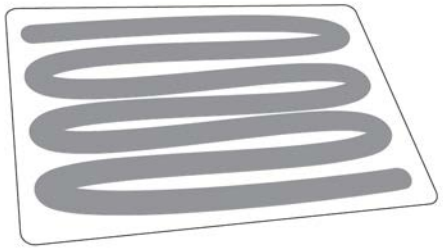
1. Gloves, nitrile
2. Ruler, disposable, and masking tape or sample template, disposable, sample area size 4 in^2 (26 cm^2)
3. Macrofoam swab, sterile, 3/16-inch thick medical-grade polyurethane foam head, 100 pores per inch, thermally bonded to a polypropylene stick (such as the Sterile Foam Tipped Applicators Scored with Thumb Stop [Puritan, Guilford, Maine; catalog number 25-1607 1PF SC] or equivalent)
4. General neutralizing buffer that will inactivate halogen disinfectants and quaternary ammonium compounds, 10 milliliter (mL), sterile (such as the Neutralizing Buffer [Hardy Diagnostics, Santa Maria, California; catalog number K105] or equivalent)
5. Screw-cap centrifuge tubes, sterile, 15 mL (such as 15 mL High-Clarity Polypropylene Conical Centrifuge Tube [Becton Dickinson, Franklin Lakes, New Jersey; catalog number 352097] or equivalent)
6. Sample labels or permanent marker
7. Re-sealable plastic bag, 1-quart or smaller
8. Re-sealable plastic bag, 1-gallon or larger

Swab Sampling Procedure

1. Wearing a clean pair of gloves over existing gloves, place the disposable template over the area to be sampled and secure it. If the template cannot be used, measure the sampling area with a disposable ruler, and delineate the area to be sampled with masking tape.
2. Remove the sterile swab from its package. Grasp the swab near the top of the handle. Do not handle below the thumb stop.
3. If the sterile swab is not pre-moistened, moisten the sterile swab by dipping it in the 10 mL container of neutralizing buffer solution. Remove any excess liquid by pressing the swab head on the inside surface of the neutralizing buffer solution container.

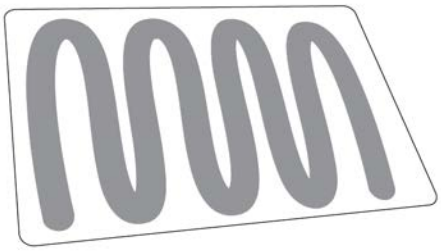
   Note: Once a sterile swab has been moistened, the remaining neutralizing buffer solution and container must be discarded.

4. Swab the surface to be sampled using the moistened sterile swab. Use an overlapping 'S' pattern to cover the entire surface with horizontal strokes.

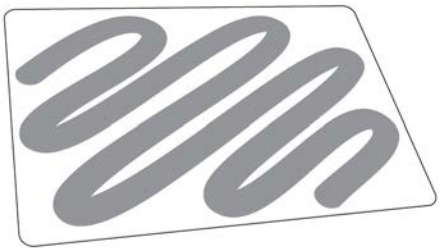


   Note: Depending on the design of the swab, a rolling motion can be used when swabbing the surface to maximize swab contact with the surface.

5. Rotate the swab and swab the same area again using vertical 'S'-strokes.



6. Rotate the swab once more and swab the same area using diagonal 'S'-strokes.



7. Place the head of the swab directly into a sterile screw-capped centrifuge tube. Break off the head of the swab by bending the handle. The end of the swab handle, touched by the collector, should not touch the inside of the tube. Securely tighten the screw-cap and label the tube (e.g., unique sample identifier, sample location, initials of collectors and date and time sample was collected). Collection tubes and re-sealable bags may be pre-labeled to assist with sampling efficiency.
8. Place the sample container in a re-sealable 1-quart plastic bag. Securely seal and label the bag (e.g., sample location, date and time sample was collected, and name of individual collecting the sample).

   Note: Remove excessive air from the re-sealable plastic bags to increase the number of samples that can be shipped in one container.

9. Dispose of the template, if used.
10. Remove outer gloves and discard. Clean gloves must be worn for each new sample.

7. Cellulose Sponge Procedure

Cellulose Sponge Materials

1. Gloves, nitrile
2. Ruler, disposable, and masking tape or sample template, disposable, sample area size 100 in^2 (645 cm^2)
3. Sponge, sterile, pre-moistened with 10 mL neutralizing buffer solution, 1.5 by 3 inches cellulose sponge folded over a handle (such as the 3M™ Sponge-Stick [3M, St. Paul, Minnesota; catalog number SSL-10NB] or equivalent)[a] or sponge, sterile, dry, 1.5 by 3 inches cellulose sponge folded over a handle (such as the 3M™ Sponge-Stick [3M, St. Paul, Minnesota; catalog number SSL-100] or equivalent) and general neutralizing buffer that will inactivate halogen disinfectants and quaternary ammonium compounds, sterile, 10 mL (such as the Neutralizing Buffer [Hardy Diagnostics, Santa Maria, California; catalog number K105] or equivalent)
4. Screw-cap specimen container, sterile, individually wrapped 4 ounce (such as General Purpose Specimen Container [Kendall Healthcare, Mansfield, Massachusetts; catalog number 8889-207026] or equivalent)
5. Sample labels or permanent marker
6. Re-sealable plastic bag, 1-quart or smaller
7. Re-sealable plastic bag, 1-gallon or larger

Cellulose Sponge Sampling Procedure

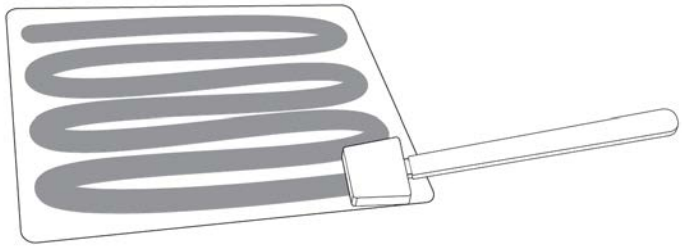
1. Wearing a clean pair of gloves over existing gloves, place the disposable template over the area to be sampled and secure it. If a template cannot be used, measure the sampling area with a disposable ruler, and delineate the area to be sampled with masking tape. The surface area sampled should be less than or equal to 100 in^2 (645 cm^2).
2. Remove the sterile sponge from its package. Grasp the sponge near the top of the handle. Do not handle below the thumb stop.
3. If the sterile sponge is not pre-moistened, moisten the sponge by pouring the 10 mL container of neutralizing buffer solution over the dry sponge.

   Note: The moistened sponge should not be dripping neutralizing buffer solution.

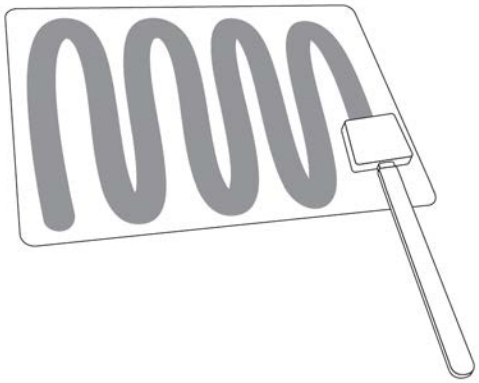
   Note: Any unused neutralizing buffer solution **must** be discarded.
4. Wipe the surface to be sampled using the moistened sterile sponge by laying the widest part of the sponge on the surface, leaving the leading edge slightly lifted. Apply gentle but firm pressure and use an overlapping ‘S’ pattern to cover the entire surface with

[a] Additional sponges with limited recovery efficiency data available include the Versalon Non-Woven All-Purpose Gauze Sponge (Kendall Healthcare, Mansfield, Massachusetts; catalog number 8042), Bacti-Sponge (Hardy Diagnostics, Santa Maria, California; catalog number SK711), Cellulose Sponge with DE Broth (Solar Biological, Ogdensburg, New York; catalog number BS-10BPB-1), and Sponge-Wipe (Micronova, Torrance, California; catalog number SWU-99 [cut into 2 by 2 inches).

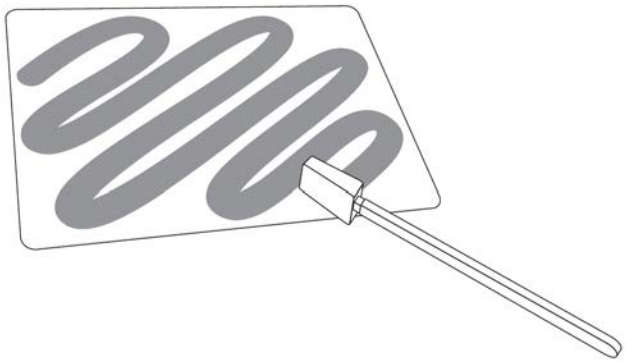
horizontal strokes.



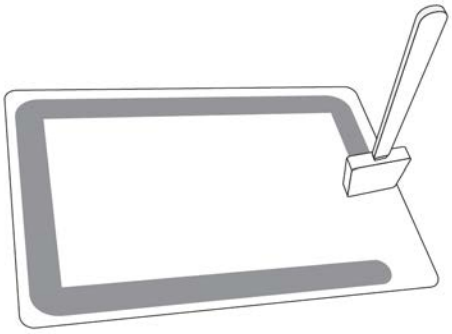
5. Turn the sponge over and wipe the same area again using vertical ‘S’-strokes.



6. Use the edges of the sponge (narrow sides) to wipe the same area using diagonal ‘S’-strokes.



7. Use the tip of the sponge to wipe the perimeter of the sampling area.



8. Place the head of the sponge directly into a sterile specimen container. Break off the head of the sponge by bending the handle. The end of the sponge handle, touched by

the collector, should not touch the inside of the specimen container. Securely seal and label the container (e.g., unique sample identifier, sample location, initials of collector and date and time sample was collected).

9. Place the sample container in a re-sealable 1-quart plastic bag. Securely seal and label the bag (e.g., sample location, date and time sample was collected, and name of individual collecting the sample). Specimen containers and re-sealable bags may be pre-labeled to assist with sampling efficiency.

    Note: Remove excessive air from the re-sealable plastic bags to increase the number of samples that can be shipped in one container.

10. Dispose of the template, if used.
11. Remove outer gloves and discard. Clean gloves should be worn for each new sample.

8. Gauze Procedure

Gauze Materials

Note: This sampling and analytical method has not been validated by CDC. A standard sampling procedure is provided in the event that the macrofoam swab or cellulose sponge methods cannot be utilized.

1. Gloves, nitrile
2. Gloves, sterile, nitrile
3. Ruler, disposable, and masking tape or sample template, disposable, sample area between 144 in^2 (929 cm^2)
4. Gauze, sterile, non-cotton, polyester blend sponge or rayon/polyester blend, 2 inches × 2 inches (such as the Versalon All-Purpose Sponge [Kendall Healthcare, Mansfield, Massachusetts; catalog number 8042; includes two gauze squares/packet] or equivalent)
5. General neutralizing buffer that will inactivate halogen disinfectants and quaternary ammonium compounds solution, 10 mL, sterile (such as the Neutralizing Buffer [Hardy Diagnostics, Santa Maria, California; catalog number K105] or equivalent)
6. Pipette, 5 mL, sterile, individually wrapped (such as the Greenwood Products' Sterile 5mL Standard Transfer Pipette [Greenwood Products, Inc., Middlesex, New Jersey; catalog number GS137038] or equivalent)
7. Screw-cap specimen container, 4-ounce, sterile, individually wrapped (such as General Purpose Specimen Container [Kendall Healthcare, Mansfield, Massachusetts; catalog number 8889-207026] or equivalent)
8. Sample labels or permanent marker
9. Re-sealable plastic bag, 1-quart or smaller
10. Re-sealable plastic bag, 1-gallon or larger

Gauze Sampling Procedure

1. Wearing a pair of gloves over existing gloves, place the disposable template over the area to be sampled and secure it. If the template cannot be used, measure the sampling area (144 in^2) with a disposable ruler, and delineate the area to be sampled with masking tape.
2. Partially peel open the sterile gauze package carefully exposing the gauze.

Note: The sterile gauze should not be touched without sterile gloves.

3. Measure 5 mL of neutralizing buffer solution from the 10 mL container using a disposable pipette and apply to sterile gauze in its original packaging. Remove outer gloves.

   Note: The moistened gauze should not be dripping neutralizing buffer solution.

   Note: Any unused neutralizing buffer solution and the pipette **must** be discarded.

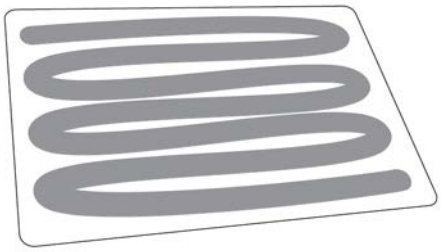
4. Don a pair of sterile gloves.

   Note: Sterile gloves are required when sampling with gauze because of the direct contact with the sampling media.

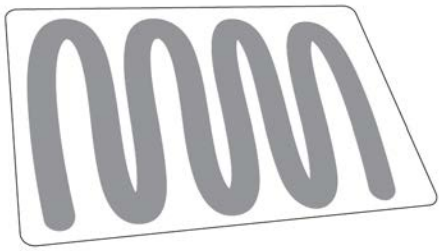
5. Remove one of the sterile gauze (if two per package) and dispose of or retain the other gauze as a field blank (see section 4.1).

6. Completely unfold the remaining moistened sterile gauze, and then fold in half.

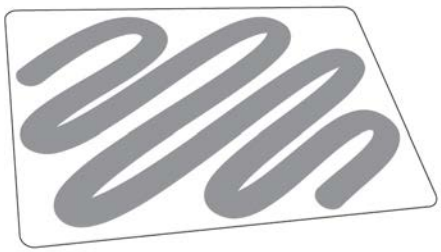
7. Wipe the surface to be sampled using the moistened sterile gauze, fingertips should be held together and apply gentle but firm pressure. Use an overlapping ‘S’ pattern to cover the entire surface with horizontal strokes.



8. Fold the exposed side of the gauze in and wipe the same area again using vertical ‘S’-strokes.



9. Fold the exposed side of the gauze in once more and wipe the same area using diagonal ‘S’-strokes.



10. Fold the gauze, exposed side in, and place it into a sterile screw-cap specimen container.

11. Securely tighten the screw-cap and label the container (e.g., unique sample identifier, sample location, initials of the collectors and date and time sample was collected).
12. Place the sample container into a re-sealable 1-quart plastic bag. Securely seal and label the bag (e.g., sample location, date and time sample was collected, and name of individual collecting the sample). Specimen containers and re-sealable bags may be pre-labeled to assist with sampling efficiency.

    Note: Remove excessive air from the re-sealable plastic bags to increase the number of samples that can be shipped in one container.

13. Dispose of the template, if used.
14. Remove outer gloves and discard. Clean sterile gloves should be worn for each new sample.

9. Liquid Sampling for Pathogens

Liquids are often easier to collect but obtaining representative samples may still be difficult. Density, solubility, temperature, and other factors/properties can cause changes in the composition of a liquid in both time and space. Sampling must be responsive to these dynamics to ensure collection of representative samples. The objective prior to sample collection must always be clear. Indoor (e.g., small fish tank in an office to large storage tank or indoor pool in multistoried building) or outdoor settings may include a variety of liquids: surface water, wastewater, and containerized liquids. Liquid sampling in a flowing indoor conduit/channel should proceed from downstream locations to upstream locations so that disturbances related to sampling do not affect sampling quality. The opening of the sampling device or container should face upstream. If water and solid samples need to be collected during the same sampling event, they must be co-located, and the aqueous samples should be collected first. When possible, sumps and monitoring manholes at which sampling is required should be suctioned to remove any accumulated silt or floating layer, then allowed to refill before sampling begins. It is essential to prevent accidental intake of such material into a sampler when intending to assess qualities of bulk liquids. When taking a grab sample, the entire mouth of the container should be submerged below the surface of the liquid. A wide mouth bottle with an opening of at least two inches can be used for this type of sampling.

For shallow waters, samples may be collected by directly filling the sample bottle. For deeper water layers, below about 0.5 m, these methods may not work, so dedicated water samplers can be used. They are lowered in an open condition on a rope or steel cable and remotely triggered to close. A third option is the use of pumps (e.g., peristaltic pumps offer the option of collecting larger amounts of water). For example, a biological agent grab sample can be obtained in the following manner:

- Take a bacteriological sample container and remove the covering and closure (protect from contamination).
- Grasp the container at the base with one hand and plunge the container (opening down) into the water to avoid introducing surface scum.
- Do not rinse the container.
- Position the mouth of the container into the current away from the hand of the collector and away from the sampler location.
- The sampling depth could be 15 to 30 cm (6 to 12 inches) below the water surface under certain conditions. If the water is static, an artificial current can be created by moving the container horizontally in the direction it is pointed and away from the sampler.

- Tip the container slightly upward to allow air to exit and the container to fill.
- After removal of the container from the water, pour out a small portion of the sample to allow an air space of 2 to 3 cm (1 inch) above the sample for proper mixing of the sample before analysis.
- Tightly close and label the container.

When collecting a sample at a depth greater than an arm's reach use a Kemmerer or weighted container sampler. The devices are lowered into the water in the open position, and a water sample is collected in the device. A drop messenger closes the sampler. Appropriate sterilization and cleaning protocols should be followed. Sample collection frequency for pathogens should be appropriate for the investigation objectives.

Table A-3 provides representative liquid samplers for a variety of environmental settings, the procedures, advantages and disadvantages. Appropriate sampling methods and sampling devices should be determined based on the site specific conditions. Appropriate care should be taken to avoid limitations such as (a) spot water sampling that reflect residue composition only at the moment of sampling and may fail to detect episodic contamination; (b) quality control issues when, for example, large volumes of water must be collected and extracted for quantifying and assessing biological pathogens. An ideal sampling device for water should be one that is:

- Made of materials that are inert to or non-interfering with the pathogen detection method
- Able to deliver sample without causing biological, chemical or physical alteration
- Compatible with the bioassay sensitivity
- Easily operated under the indoor settings
- Easily disassembled for cleaning and maintenance
- Easily transported to indoor locations
- Reliable and durable to use and able to withstand potentially hostile environments

Table A-3. Liquid Sampling for Pathogen

Designation	Typical Setting	Salient Features/Procedure	Advantage	Disadvantage
Dipper/Pond sampler/ Swing sampler	Water/wastewater from aquarium, pits, or other reservoirs	• Assemble the pond sampler to be performed by making sure that the sampling container and fixtures are secured to the pole. • Slowly submerge the container with minimal surface disturbance. Retrieve the sampler from the surface water with minimal disturbance. • Remove the cap from the sample bottle and slightly tilt the mouth of the bottle below the dipper/device edge. • Empty the sampler slowly, allowing the stream to flow gently down the inside of the bottle with minimal entry turbulence. • Repeat above three steps until sufficient sample volume is acquired. Dismantle the sampler, if applicable and store in plastic bags for subsequent decontamination.	• Relatively inexpensive to fabricate • Can sample depths or distances up to 3.5m	• Difficult to obtain representative samples in stratified liquids • Difficult to decontaminate when handling viscous liquids
Weighted Bottle Sampler	Tanks, wells, sumps, or other reservoirs	• Sampler consists of a bottle, usually glass or plastic, a weight sinker, and a bottle stopper. • Assemble the weighted bottle sampler. Lower the sampling device to the predetermined depth. • When the sampler is at the required depth, pull out the bottle stopper with a sharp jerk of the sampler line and allow the bottle to fill completely (usually evidenced by the cessation of air bubbles) • Retrieve sampler. Transfer sample into laboratory cleaned sample bottles, if applicable. Follow procedures for preservation and transport.	• Sampler remains unopened until at sampling depth	• Laboratory supplied bottle may not fit into sampler, thus requiring additional equipment. • Some mixing of sample may occur when retrieving the sampler from depth.
Open Tube Thief Sampler	Versatile, e.g. may be used to sample water from sump areas in homeowner basements	• A hollow glass or rigid plastic tube, which is anywhere from four to five feet in length. It generally has an inside diameter of ¼-inch or ½-inch.	• Inexpensive • Simplicity of operation • Small puddle of liquid can be collected, which other samplers may not • Disposable	• Sample leakage • Small sample volume

Syringe	Collects representative small volume liquid samples in puddles	• Use the syringe to draw the sample from the top of the container or puddle by pulling the plunger. Syringe plunger may become difficult to push while handling slurry due to clogging. Once you encounter moderate resistance, do not push harder and you may have to start again. Syringes should be kept in clean containers or original packaging until ready for use to prevent contamination (e.g., keep both wrapped in original package or in new/clean plastic baggies until actually collecting and/or filtering the sample). Under certain indoor conditions, accessory equipment may be necessary for operation of syringe sampler is a hand pump and a length of tubing to supply negative/positive pressure to the syringe to actuate the piston.	• Samples does not come in contact with atmospheric gas and is subjected to a negative pressure, thus neither aeration nor degassing of the sample occurs • Syringes are or can be made inert or nearly inert materials • Syringe can be utilized as sample container, thus removing the possibility of cross-contamination • Inexpensive, highly portable and simple to operate	• Inefficient to collect large volume of samples • Limited to water with a low suspended solids content • Leakage may occur around the plunger when syringes are used to sample high levels of suspended solids.
Kemmerer Depth Sampler/ Van Dorn sampler/ Niskin bottle	Liquid samples in storage tank, tank trailer, vacuum tanks, or other situations where collection depth prevents use of other sampling devices	• Sampling device consists of an open tube with two sealing end pieces. Niskin sampler has the same design as the Van Dorn sampler except that it can be cast in a series on a single line for simultaneous sampling at multiple depths with the use of auxiliary messengers. • Set the sampling device so that the sealing end pieces are pulled away from the sampling tube, allowing the substance to pass through the tube. • Lower the pre-set sampling device to the predetermined depth. • When the sample is at the required depth, send down the messenger, closing the sampling device. • Retrieve sampler. Transfer sample into laboratory cleaned sample bottles (if applicable) and follow procedures for preservation and transport	• Able to sample at discrete depths • Able to sample great depths	• Open sampling tube is exposed while traveling down to sampling depth • Transfer of sample into sample bottle may be difficult

Bailer	Well, deep sump pit	• Bailer should be cleaned and handled with surgical gloves to prevent cross contamination. Surgical gloves must be changed between each sample location. • Lower bailer slowly until it contacts the water surface. Allow bailer to sink and fill with a minimum of disturbance to the sample. Slowly raise the bailer to the surface. Avoid contact of the bailer line to the well casing and/or ground. Tip the bailer to allow a slow discharge from the top gently down the side of the sample bottle to minimize turbulence. • Repeat above steps until a sufficient sample volume is acquired. • Place used bailer in bag for return to lab for decontamination and dispose of polyethylene line.	• No external power source required • Economical enough that a separate laboratory cleaned bailer may be utilized for each sampling to eliminate cross contamination • PTFE (polytetrafluoroethylene) or stainless steel construction available • Simple to use, lightweight, portable	• Limited volume of sample collected • Unable to collect discrete samples from a depth below the water surface • Leakage due to wear, dimension distortion and silt buildup may aerate succeeding sample and may gather unwanted material. • Aeration and turbidity may bias the result.
Suction-lift mechanisms	Well, deep sump pit, large storage tank	• Low volume pump that, by applying vacuum, causes water to be drawn upward through a suction line. Two types of suction-lift pumps are generally available for shallow water sampling: centrifugal pumps and peristaltic pumps.	• Flow rate of suction-lift pumps is easily controlled • Highly portable and readily available.	• A drop in pressure due to negative pressure (suction) causes degassing of the sample • Where the sample comes in contact with pump rotating parts or tubing, the choice of appropriate material for impeller or flexible pump tubing may be restrictive.
Liquid Grab Sampler	Collect liquid and slurry samples from surface impoundments, pool or containers.	• Grab samples can be obtained at discrete depths. The sample bottle might be attached to the end of a 6-ft. long handle. The control valve is operated from the top of the handle once the sampler is at the desired depth. The general procedure would be: • Assemble the sampler. Operate the sampler several times to ensure proper adjustment, tightness of the cap, etc. Submerge sampler into liquid to be sampled. When the desired depth is reached, pull valve finger ring to open control valve and allow sample to enter container. • Retrieving sampler by closing valve. Transfer sample into laboratory cleaned sample bottles and follow procedures for preservation and transport.	• Allows discrete samples to be taken at depth	• Depth of sampling is limited by length of pole • Hard to decontaminate

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10. Sampling of Bioaerosols

The term *biological aerosol particle* is defined as a solid airborne particle derived from biological organisms, including microorganisms and fragments of biological materials such as plant debris and animal dander (IGAP, 1992). The term primary biological aerosol is more or less equivalent to the term *bioaerosol* (Reponen et al., 1995; Hinds, 1999). The term bioaerosol is used in a broad sense to include any particle with biological activity/toxicity (Hirst, 1995). This document uses the term bioaerosol to include airborne particles (dead or alive), large molecules or volatile compounds that are or were derived from living organisms, including micro-organisms and fragments of all varieties of living materials (viruses [0.02 to 0.3µm], bacterial cells [0.5 to 30µm], fungal spores [0.5 to 30µm], pollen [10 to 100µm], and protozoa [>10µm]). Physical characterization of bioaerosols is the concentration of pathogens that can be cultured, which is expressed as the number of colony forming units per unit volume of air (cfu/m^3). A schematic diagram of bioaerosol sampling procedure is shown in Figure A-1, and examples of sources of bioaerosols are shown in Table A-4.

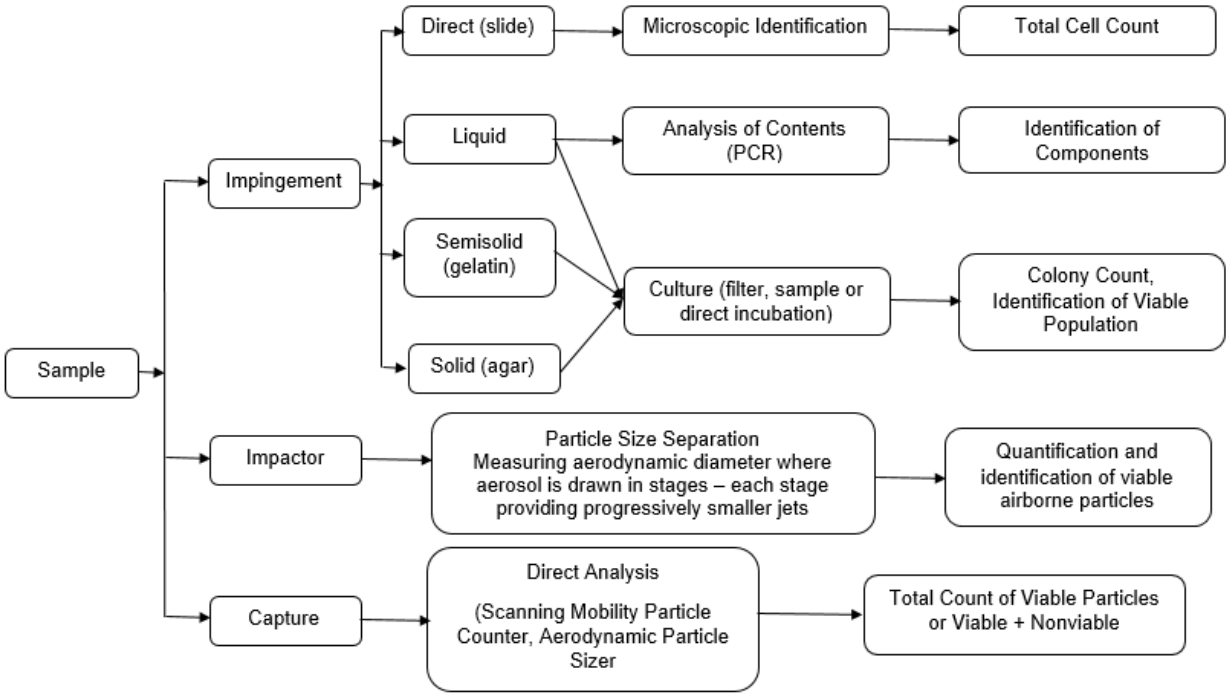

Figure A-1. Schematic diagram of bioaerosol sampling procedure.

Table A-4. Sources and Particle Size Distribution of Bioaerosols

Typical Bioaerosol Source/Activity	Particle Size Distribution[1,2]	Reference
Surgical/dental procedure	Up to 50 µm	Jewett et al. 1992; Szymańska, 2007
Hospital air	<2 µm (22%), 2 to 6 µm (30%), >5 µm (48%)	Greene et al., 1962
Mechanical ventilators, bed making, resuspension on dust or skin squamae	0.3 µm to >5 µm	Tang et al., 2006; Roberts et al., 2006
Cooling tower	<5 µm up to > 100 µm (bimodal peaks at <5 µm and 20-40 µm)	Rothman et al., 1975
Wastewater irrigation	1.0 to 5.9 µm	Bausum et al., 1982
Grain harvesting, food processing, animal farming activities	0.9 to 18.9 µm (0.5 to >5 µm)	Lee at al., 2006; Olsen et al., 2009
Mail sorting and opening	0.3 µm to >5 µm; 19.6-fold increase in particles >5 µm	Brandl et al., 2005
Mist machine	Between 40 and 70 µm	Barrabeig et al., 2010
Whirlpools	<1 and 15 µm depending on turbulence	Baron et al., 1986
Breathing	<0.8 to 2 µm	Morawska et al., 2009
Speaking	16 to 125 µm	Chao et al., 2009; Xie et al. 2009
Shouting	0.5 to 10 µm (mean = 1.0 µm)	Lai et al. 2011
Sneezing	7 to 125 µm	Duguid et al. 1945; Jennison et al., 1942
Showering	Hot water 5.2 to 7.5 µm Cold water 2.5 to 3.1 µm	Zhou et al. 2007; Chattopadhyay et al. 2017

1: aerodynamic diameter
2: distribution should be considered with caution as often tests used samplers with cut off limits <15 µm and therefore were preferentially selective for particles smaller than this size.

References for Table A-4

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Bioaerosol samplers are designed for sampling biological aerosols under various conditions such as short sampling cycles, long sampling cycles, high temperature, and low temperature. Knowledge and use of efficient air samplers enhance the ability to protect users, first responders, and the general public from airborne agents. Sampling devices and detection systems need to be tested and their performance efficiencies determined so that they can be appropriately matched for various challenges. Each air sampler has multiple components such as an inlet, transmission tubes, a pre-separator skimmer to reject large particles, aerosol concentrating stages, and a collector such as an impactor. The performance of an aerosol sampler, or the sampling efficiency, is the overall end-to-end ratio of the amount of aerosol contained in the sample produced by the sampler to the amount of aerosol contained in the volume of ambient air sampled by the system's inlet. In a well-designed, well-fabricated, well-assembled system, it is the product of the performance efficiencies of the sampler's individual components, variously: aspiration, transmission, collection, retention, and recovery efficiencies. The aspiration efficiency of a sampler's inlet describes the efficiency with which particles are extracted from the air and transmitted through the sampler inlet and is dependent on particle aerodynamic size and wind speed. Transmission efficiency describes the efficiency with which particles are transported from the intake of a component to its collector, and the collection

efficiency describes the efficiency with which particles are captured by the collector. Retention efficiency indicates how efficiently particles are retained by the sampler during a long sampling time, e.g., either in an impinger or in a wetted cyclone that stores the collected particles in the active collection fluid throughout the collection time. Particles in the collection fluid can escape into the air (reaerosolization) and be ejected with the exhaust. The collected particles are recovered for assay, and the efficiency with which they are recovered is indicated by the recovery efficiency. These efficiencies described above can depend on particle size, density, charge, composition, and biological factors. Organisms have two additional issues: survival fraction and culturable fraction. Survival of an organism can be measured by flow cytometry using different dyes that reveal viable versus non-viable organisms, and by other life function measures such as ATP. The culturability is determined by plating. These are reported as fractions rather than efficiencies because they are characteristics of the aerosol in the sample not the amount of aerosol in the sample.

The key factors affecting aerosol characteristics during sampling include:

- Aspiration efficiency and deposition in the sampling inlet
- Deposition during transport
- Extremes or inhomogeneity in the ambient aerosol concentration
- Agglomeration of particles during transport
- Evaporation and/or condensation of aerosol material during transport
- Retainment of deposited aerosol back into the sample flow
- High local deposition causing flow restriction or plugging

Desirable sampling conditions are:

- Constant free stream flow rate during sampling
- Stable aerosol condition during sampling
- Sufficiently low sampling gas velocity so that the sampled particles can accommodate themselves to the sampling gas flow within a distance comparable to the inlet diameter (inertial condition)
- Sufficiently high sampling gas velocity so that the sampled particles do not settle appreciably (gravitational settling condition)
- Application of larger inlet diameters (of the order of a centimeter) as they are less susceptible to deposition caused by free-stream turbulence

11. Instrument and System Calibration

Instrument and system calibration are essential for successful measurement of bioaerosol properties in a sampling environment. Calibration can be conducted via direct measurement or using primary standards, e.g. latex spheres size calibration; currently no concentration standards are available; gravimetric techniques are applicable for larger particles only. Reliable and accurate calibration requires:

- A proper selection of a desired test aerosol
- A complete understanding of the principles and procedures of operation
- A thorough investigation of the relevant parameters
- A sufficient knowledge of the capabilities and limitations of the instrument

Before setting up of a sampling system, it should be determined whether standard procedures for this type of sampling are available. There are prescribed standard sampling procedures for certain types of measurements, such as:

- ASTM E2720 – 16: Standard Practice for Evaluation of Effectiveness of Decontamination Procedures for Air-Permeable Materials when Challenged with Biological Aerosols Containing Human Pathogenic Viruses.
- NIST Technical Note 1737: Challenges in Microbial Sampling in the Indoor Environment. National Institute of Standards and Technology.
- NIOSH Manual of Analytical Methods: Bioaerosol Sampling (Indoor Air); Sampling and Characterization of Bioaerosols.

The sampling methods and sampling devices available today are shown in Table A-5 with the mechanisms involved, ability, availability, advantages and disadvantages. The selection criteria of sampling devices for pathogens are dependent on the needs of post-sampling analysis method, the fate and transport of and exposure to the bioaerosols through size resolved measurements, and conditions dictated by the indoor environment. Generally, the desired properties exist in the variety of aerosol samplers, but rarely in a single sampler. There is lack of standard protocols for aerosol sampling and sample preparation. Without standard protocols that contain information on efficiencies associated with sample collection and sample preparation, quantitative bioaerosol data may lack both accuracy and precision. Standards are necessary to provide consistency in investigations in order to compare data sets. Challenges with bioaerosol sampling technology include the need for compact and portable sampling devices, and the significant contamination issues association with high volume liquid impingers. Regarding the application of molecular techniques, many of the sampling techniques provide sufficient material for PCR-based analysis, but significant limitations still occur in concentrating the samples into small volumes, and collecting sufficient samples for non-PCR based analyses. Table A-6 provides a comparison of commercially available representative aerosol samplers.

Bioaerosol sampling aims to take a sample that is physically and biologically representative of the indoor environment. Air will often contain microorganisms such as viruses, bacteria, spores, and other microorganisms. Airborne spores can remain viable for much longer periods, even at low relative humidity and high or low temperature extremes. A proper sampling process includes determining location and number of sampling locations, selecting an appropriate sampler or sampling system, and determining sampling duration and frequency. A bioaerosol sampling plan should begin by determining the purpose of sampling. Sampling objectives may include verification and quantification of pathogen present, identification of sources that could lead to control and mitigation, and subsequent monitoring to ensure the effectiveness of control measures implemented. Sampling parameters that may be considered include type of sample, duration of samples, potential interferences and expected co-contaminant concentrations in the indoor environment. The sampling media should be specifically identified, e.g., pore size and type of filter, concentration and amount of liquid media required, and specific type and amount of solid sorbent. The sampling pump used to collect the sample must also be compatible with the sampling needs and the media used. The pump should be capable of maintaining the desired flow rate over the time period needed using the sampling media specified. Certain pumps may not be able to handle the large pressure drop due to media, fine mesh (smaller than 40 mesh) solid sorbent tubes, small pore size filters or when attempting to take a short-term sample on a sorbent tube of a higher than normal pressure drop at a flow rate of 1 L/min or greater. Factors that can influence collection of pathogens in indoor environments include relative humidity, temperature, oxygen, indoor pollutants, sampling flow rate/face velocity, concentration (breakthrough capacity/breakthrough volume), and indoor atmospheric stability

(degree to which the atmosphere can dampen vertical and horizontal motion) – stable atmospheric conditions result in low dispersion, and unstable atmospheric conditions (for example, hot conditions) result in higher dispersion. The flow rate recommended for a specific device/method can be used for the desired sampling period considering total sample volume, sampling time, and limit of quantitation. Some of these variables will be fixed by sampling needs, e.g., sampling time or by the measurement method of choice (limit of quantitation or maximum sampling volumes).

Bioaerosol concentrations generally have considerable temporal and spatial variation because pathogen sources may not generate aerosols continuously. The time and space dependent characteristics in bioaerosol concentrations have a significant effect on determining the optimal sampling duration and location. The overall performance of an aerosol sampler can be determined by two factors: physical factors (inlet sampling efficiency and collection efficiency) and biological factors (preserving biological characteristics of pathogens during sampling and accurate analysis for identification and quantification). There can be challenges that may be addressed when determining an appropriate sampling protocol: (a) level of concentrations of pathogen as high levels may overload some samplers, which may lead to shortened sampling time or use of a diluter system; (b) comprehensive quantitative and qualitative analysis may require the use of multiple sampling and analysis methods; and (c) practical constraints (such as spatial restrictions, proximity to the source, proximity to the ventilation systems, and other logistical considerations). The number and location of sampling points may be selected according to the variability, or sensitivity, of the sampling and analytical methods being utilized, the variability of contaminant concentration over time at the site, and the level of precision required. The number of locations and placement of samplers can be determined by considering the nature of the response, indoor location (with respect to other conflicting background sources), size of the concerned area, and the number, size, and relative proximity of separate on-site emission sources. The duration of sampling activities should be considered when choosing the location and number of samples to be collected. Air quality dispersion models may be used to place samplers in areas of maximum predicted concentrations. Sampling duration and flow rate dictate the volume of air collected, and to a major degree, the detection limit. The analytical method selected will provide a reference to flow rate and volume. Flow rates are limited to the capacity of the pumps being employed and the contact time required by the collection media. The duration or period of air sampling is commonly divided into two categories: (a) samples collected over a brief time period are referred to as instantaneous or grab samples and are usually collected in less than five minutes and (b) average or integrated samples are collected over a significantly longer period of time. Integrated samples provide an average concentration over the entire sampling period. The typical optimal sampling times for representative commercially available bioaerosol samplers are illustrated in Figure A-2. Case studies on bioaerosol sampling frequency, layout, and estimates of collectable biological particle are performed by various researchers (LaForce, 1990; Fennelly et al. 2004; Hwang et al., 2011)

Once the pathogen sample has been collected, it must be conditioned and transported to a laboratory for further analysis. Appropriate care should be taken so the physical and biological properties of the sample are preserved (i.e., refrigeration, observing sample holding times).

12. Optimal Sampling Time Determination

The concentrations of bioaerosols can vary with time. Sufficiently long collection times or multiple samples with short collection times may be required during periods of changing concentration so that collected sample(s) may properly represent the average environmental

concentration over some time period. During a sampling process within a sampling period t (start time = t_s and final time = t_f), the number of particles per unit area varies with bioaerosol particle concentration in the sampled air. This results in a change in surface density (δ) of the sample, which equates to the number of particles on the surface per viewing area (A), i.e., microbial colonies on a petri dish. The surface density of a bioaerosol sample is determined by the following equation:

$$\delta = \frac{N}{A} = \frac{C \times Q}{A} \times t \qquad \text{Equation (1)}$$

Where δ is the surface density of a bioaerosol sample in cfu/m^2, A is the viewing area (i.e., petri dish) in m^2; C is the average concentration of bioaerosols in cfu/m^3, N is the number of viable bioaerosol particles collected on the impaction substrate, in cfu, Q is the flow rate of the sampling system in m^3/min, and t is the sampling time in minutes.

In general, post analyses of bioaerosol samples include viewing, counting, and identifying the particles within the sample. This can occur following collection by viewing the collected particles under a microscope, or it may occur after an incubation period, which allows the colonies to grow to sufficient size so they can be counted without magnification. An accurate quantification of bioaerosols in a sample may only be obtained if the surface density of organisms is optimal, δ_o. If the sample surface density is very low, $\delta << \delta_o$ sampling and counting errors may be high. As a result, the calculated concentration may not be accurate and may misrepresent the true concentration in the original air sampled. On the other hand, if the sample surface density is very high, $\delta >> \delta_o$, the particles may be located in close proximity to each other, whereby the collected organisms may grow together or may inhibit each other's growth such that accurate counting and identification may not be possible. As shown in equation 1, the surface density of a bioaerosol sample collected on a substrate is linearly related to sampling time. To avoid insufficiently-loaded samples ($\delta << \delta_o$) and overloaded samples ($\delta >> \delta_o$), the sampling time should be adjusted accordingly. The optimal sampling time for a given bioaerosol concentration depends upon sampler flow rate and collection surface area as demonstrated by the following equation:

$$t_o = \frac{A}{Q \times C} \times \delta_o \qquad \text{Equation (2)}$$

The calculated optimal sampling times for representative commercially available bioaerosol samplers are illustrated in Figure A-2. Impinger samples are not sensitive to under- or overloading during sampling because the liquid sample can be diluted or concentrated following sample collection, depending on the concentration of collected bioaerosol particles in the liquid. However, evaporation of sampling liquid and reaerosolization of prior-collected particles limit the sampling time for most impingers.

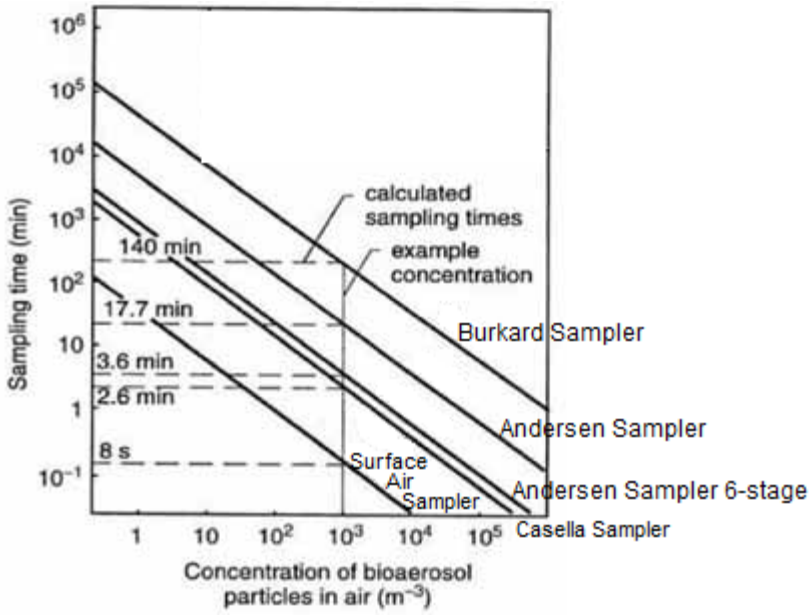

Figure A-2. Typical sampling times for representative bioaerosol samplers. (Modified after Baron, P. A., and K. Willeke. 2001. Aerosol Measurement: Principles, Techniques, and Applications, 2nd ed. John Wiley & Sons.)

In general, impactors can be used for cut-off sizes (d_{50}) in the range from 0.1 to 50 µm (cut-size of the impactor stage corresponds to the 50% particle collection efficiency mark), flow rates from a few cm^3/min to 1000s of m^3/min, and sampling times from minutes to hours. Scanning mobility particle sizer measures the particle size distribution in the range of 5 to 1000 nm, measurement cycle time 60 to 500 s, and concentration range 20 to 1×10^7 particles/cm^3. The aerodynamic particle sizer measures particle aerodynamic diameter in real time (1 s to 18 hrs) within the size range 0.5 to 30 µm.

Table A-5. Bioaerosol Samplers – Common Devices and Mechanisms Involved (Chattopadhyay et al., 2017*)

Device	Mechanism	Typical Model/ Materials	Sampling Rate	Sampling Approach	Viability	Advantage	Disadvantage
Cascade Impactor 	Sampling air stream makes a sharp bend and particles are stripped based on their aerodynamic diameter.	Anderson, MOUDI, BGI, or equivalent	10–28 L/min (typical) >500 L/min (high volume)	Provides the best size distribution information. 1 and 12 stages for aerosols with aerodynamic diameters from 10 nm to >18 µm.	Only at 28 L/min collection rates and requires direct sampling onto agar plates.	• Widely used to define particle size distributions • Models available to perform culturing	• High cost, especially for high volume • Inefficiencies due to particle bounce • Not sensitive as total sampled mass is divided among multiple stages.
Liquid Impingement 	Sampling air passes through a small opening and captured into a liquid medium.	SKC swirl, Omni, or equivalent	14 L/min for glass impingers >100 L/min (high volume)	Efficiency drops in low volume glass impingers below aerodynamic diameters of 1 µm.	Impingers are flexible since pathogens are impinged into liquid media or buffer and can be used for culturing or molecular analysis.	• Sample is collected into liquid and does not require extraction from solid • Low cost of low flow glass impingers	• Impacts on pathogen viability due to evaporation of fluid and collection efficiency are concerns if an extended sample collection is desired • Effective decontamination the equipment is a concern.
Filtration 	Aerosols are captured on filters by impaction or diffusional forces.	Anderson, SKC IMPACT, or equivalent	Ranges from 4 to 1000 L/min	Typical for ≤10 µm and ≤2.5 µm size fractions. High diffusional forces, filters are efficient at sampling sizes down to the 20 nm	Not recommended for viability due to high stresses from impaction and desiccation	• Available for high sampling rates • Common and robust form of high volume sampling and low cost	• No possibility for viable determination • Limited ability of particle size distributions

* The evaluations are based on tests performed using selected bioaerosol samplers and selected vegetative bacteria and spores.

Table A-6. Comparison of Commercially Available Representative Aerosol Samplers

Bioaerosol Reference Sampler Name	Able to Effectively Sample Spores	Able to Provide Bioaerosol Concentration Data	Able to Provide Bioaerosol Size Distribution Data	Remarks
AGI-30	Yes	Yes	No	AGI-30 has been used as a standard bioaerosol sampler for several decades and its use has been widely published.
SKC BioSampler®	Yes	Yes	No	SKC sampler is similar in size and operation to the AGI-30.
Gelatin Filter	Yes	Yes	No	The use of gelatin filters for sampling spore-forming bacterial bioaerosols is well-documented. These filters (in a 47-mm format) can be used for sampling spores because of their excellent total efficiency and ease of use.
WWC	Yes	Yes	No	The use of somewhat-unique high-volume cyclones is supported in the literature, though there are no well-documented, commercially available high-volume cyclones. It has the potential to provide much better detection limits than the low-volume impingers and filters (approximately two orders-of-magnitude better detection limit due to its high sampling rate).
ACI	Yes	Yes	Yes	ACI has been used as a standard bioaerosol sampler for several decades and its use has been widely published. The information can be used to provide both bioaerosol concentration and size distribution information. Since particles are impacted directly into the agar, this sampler provides data about the number of bioaerosol particles, rather than the total number. The size distribution information should be expressed in terms of the number size distribution, rather than a mass-weighted distribution.
BCI	Yes	Yes	Yes	BCI provides good data on the effective mass-weighted size distribution of bioaerosols, and thus these data complement the ACI data well.
MLI	Yes	Yes	Yes	There are publications that cite the use of the MLI for sampling bioaerosols. It has good potential for providing mass-weighted size distribution information.
ELPI®	TBD	Yes	Yes	Limited publications available regarding the use of the ELPI for characterization. It has potential for providing both real-time and culturable mass-weighted size distribution information.

Note: Mention of trade names, products, or services does not convey official Agency approval, endorsement, or recommendation. The models, trade names are indicated as examples.

13. Air Impactor Samples

Below is procedure for collecting air impactor samples with petri dishes specific to the contaminant being sampled.

Materials and Equipment

- Calibrated high-flow sampling pump (28.3 liters/minute [LPM])
- Rotameter (air flow meter) or dry cell calibrator
- Calibration adapter for impactors
- Sterile single or six stage impactor
- Sterile Petri dish and agent-specific agar for each stage
- Flexible Tygon™ tubing
- Sterile non-powdered sampling gloves
- Sealable plastic bags
- Parafilm M® wax strips
- Sample labels and wax pencil
- Documentation materials, digital camera, indelible ink pen, and logbook
- Custody seals and tags
- Chain-of-custody forms and shipping paperwork

Procedure

1. For each sample collected, ensure that a new pair of sterile gloves is worn.
2. Set the pump flow rate to 28.3 LPM or as specified in the analytical method, and turn it on.
3. To calibrate the impactor, aseptically remove the lids from the calibration set of Petri dish(es) and keep lids in a clean sealable plastic bag. For the single stage impactor, place each one calibration Petri dish on the stage and reassemble the impactor. For the 6 stage impactor, place one of the calibration Petri dishes on each of the impactor stages and reassemble the stages in the correct numerical order. Attach the calibration adapter to the top of the impactor. Attach flexible Tygon™ tubing from the impactor calibration adapter to the calibrator or rotameter inlet. Attach the second piece of tubing from the outlet of the impactor to the inlet of the sample pump. Turn on the calibrator and record the initial flow rate in the logbook.
4. Calibration of the sampling train can be performed outside the hot zone such as in the sample preparation area. If using a rotameter for calibration, then it should be calibrated with a primary standard such as the dry cell calibrator. Rotameters are considered secondary standards.
5. After calibration, remove the calibration Petri dishes from each stage of the impactor and cover with a lid. These can be reused for calibration several times until they begin to dry out and not more than one day.
6. In preparation to sample, aseptically remove lids from the sample Petri dish(es) and keep in a clean sealable plastic bag. For the single stage impactor, place one Petri dish on the stage and reassemble the impactor. For the 6 stage impactor, place on of the 6 Petri dishes on each impactor stage and reassemble the impactor ensuring that the stages are in

the correct numerical order. Connect the Tygon™ from the outlet of the impactor to the inlet of the pump.

7. Place the impactor and pump in desired sample location and photo document and map the location.
8. Start the pump and record the time sampling began and the time the sampling is completed. Sampling times should be between 10 to 15 minutes. At completion of sample time, don sterile gloves and aseptically remove the petri dish(es), cover with lids and seal each dish with Parafilm M® to secure, label each dish with the wax pencil including the stage number and place into sterile zippered sample bag upside down (agar oriented up).
9. Double bag each sample.
10. Decontaminate outer bag prior to leaving hot zone. This is usually done at the entrance of the personnel decontamination line.
11. For post sampling calibration, aseptically remove lids from each of the pre-calibration sample Petri dishes and place on the impactor stages. Attach the tubing to the calibrator and the pump as in the initial calibration.
12. Turn on pump and record the post sampling flow rate in the log book. Pre- and post-calibration flow rates are very important in determining final contaminate concentration.
13. Pre and post sampling train calibration can be done either inside or outside the hot zone. For calibration outside the hot zone the sampling equipment must be protected from contamination or easily decontaminated. Otherwise, pre and post sampling train calibration should be done in the hot zone.
14. Package samples for transport.
15. Fill out chain-of-custody form, and make a copy.
16. Refrigerate samples or package with ice, ensuring agar does not freeze.
17. Secure samples in shipping container with chain-of-custody and attach custody seals.
18. Fill out shipping manifest or contact courier.
19. Prior to use to collect another sample, the impactor must be autoclaved.

14. Impinger (Wet Method) Air Samples

Below is procedure for collecting air samples with an impinger using a wet method.

Materials and Equipment

- High Flow Sampling Pump
- Dry cell calibrator and stand
- Two sterile impinger, pump attachment, and sterile impinger fluid
- Teflon or Parafilm M® tape
- Flexible Tygon tubing
- Sterile sample container bottle
- Sterile non-powdered sample gloves
- Documentation materials, digital camera and logbook
- Custody seals, sealable plastic bags, and tags
- Sample labels, documentation forms, permanent marker(s)

- Chain-of-custody forms and shipping paperwork

Procedure

1. Aseptically fill an impinger with appropriate sterile fluid and attach to pump. This should be done outside the hot zone in a clean area.
2. Set up the sampling train by attaching Tygon™ tubing to outlet of impinger and the other end to inlet of the sample pump.
3. In a clean area, calibrate the sample train by attaching another piece of Tygon ™ tubing to the outlet of the impinger and the other end to a rotameter or dry cell calibrator. Adjust pump to the desired flow rate of 12.5 LPM. If using a rotameter for calibration, then it should be calibrated with a primary standard such as the dry cell calibrator before using. Rotameters are considered secondary standards.
4. After pre-sampling calibration, remove impinger, place caps or Parafilm M® over both the inlet and outlet of the impinger and set aside to use to check the flow rate after the sample is collected.
5. Don a new pair of sterile gloves and attach a second sterile impinger, filled with appropriate sterile fluid, to the sampling train.
6. Place sampling train in desired sample location and turn on pump.
7. Photo document sample location, draw map and record sample start time in the log book.
8. After sampling time has elapsed, turn off pump, don sterile gloves and aseptically remove the impinger.
9. Ascetically transfer impinger fluid to sample container bottle can be done either inside or outside the hot zone. If done outside the hot zone, place a cap or Parafilm M® over the inlet and outlet of the impinger. It is important to keep impingers upright to prevent loss of fluid due to leaking or spillage. Fluid transfer done outside the hot zone must be done in an appropriate fume hood. If impinger fluid will be transferred to sample container bottle in the hot zone, don sterile gloves and aseptically remove the impinger, transfer fluid to labeled, sterile sample container and seal the lid with Teflon or Parafilm M® tape.
10. Double bag the sample.
11. For post sampling train calibration, don sterile gloves and attach a fluid filled calibration impinger to the sample train as described in Step 4. Turn on pump and record flow rate. Record flow rate in log book.
12. Pre and post sampling train calibration can be done either inside or outside the hot zone. For calibration outside the hot zone the sampling equipment must have be protected from contamination or easily decontaminated. Otherwise, pre and post sampling train calibration should be done in the hot zone.
13. Decontaminate sample bag before leaving hot zone. This is usually done at the entrance of the personnel decontamination line.
14. Package samples for shipment including ice, if needed.
15. Complete chain-of-custody form and place in sample shipment container.
16. Secure shipment container and complete shipping manifest.
17. Prior to another use, the impinger used to collect the sample must be autoclaved.

    Note: For each sample collected, ensure that a new pair of sterile gloves is worn.

15. Passive Samplers

Selecting pathogen samplers and sampling methods depends on the site-specific questions that need to be addressed. Since samples for active pathogen sampling methods, described in previous sections, are collected from single points in time, the data are representative "snapshots" of the pathogens. Thus, multiple sampling might be used to describe how pathogen conditions vary over time. Passive pathogenic sampling devices are incubated within the sampled environment for weeks (typically 15 - 90 days) and depend on the formation and collection of biofilms that grow on surfaces or within a solid matrix. The passive samplers provide a more time-integrated sample of pathogens from the sampled environment. In active monitoring a pathogenic air sampler physically draws a known volume of air through or over a particle collection device which can be a liquid or a solid culture media or a nitrocellulose membrane and the quantity of pathogens present is measured (for example in CFU/m^3 of air). Passive monitoring uses settle plates, which are standard Petri dishes containing culture media, that are exposed to the air for a given time in order to collect biological particles, which settle out and are then incubated. Results are expressed in CFU/plate/time or in CFU/m2/hour. Passive sampling provides a valid risk assessment as it measures the harmful part of the airborne population that falls onto a critical surface (French et al. 1980; Matysik et al. 2009; Napoli et al. 2012; Mills et al. 2014). Table A-9 provides advantages and challenges of commonly used passive samplers.

Table A-7. Advantages and Challenges of Passive Samplers

Advantages	Challenges
• Sampling devices are relatively easy to deploy and recover. • Sample collection over an extended period of time might be desirable at certain conditions compared to single, grab-sample collection of pathogen. • Passive sampling devices can concentrate contaminants.	• Sampling devices require several days of placement in the sampled environment and require two mobilizations to the site to install and then retrieve the sampling devices. • The solid matrix of most passive microbial sampling devices is a surrogate; thus, differences may exist between pathogens colonizing the sampling device and native material.

Even though the implementation might vary between different types of passive samplers, nearly all share certain common characteristics, the most important of which is the presence of a barrier between the sampled medium and the collecting medium. The barrier defines the rate at which analytes are collected at a given concentration, which is crucial for quantitative analysis. An effective sampler should eliminate or minimize the effects of external factors (such as the velocity of the sampled medium at the face of the sampler, humidity, and temperature) on the sampling rate. In practice, the barrier usually falls into one of two categories: (1) diffusion or (2) permeation. Schematic diagrams of the two types of samplers are given in Figure A-3. The sampling process is similar for both categories of samplers.

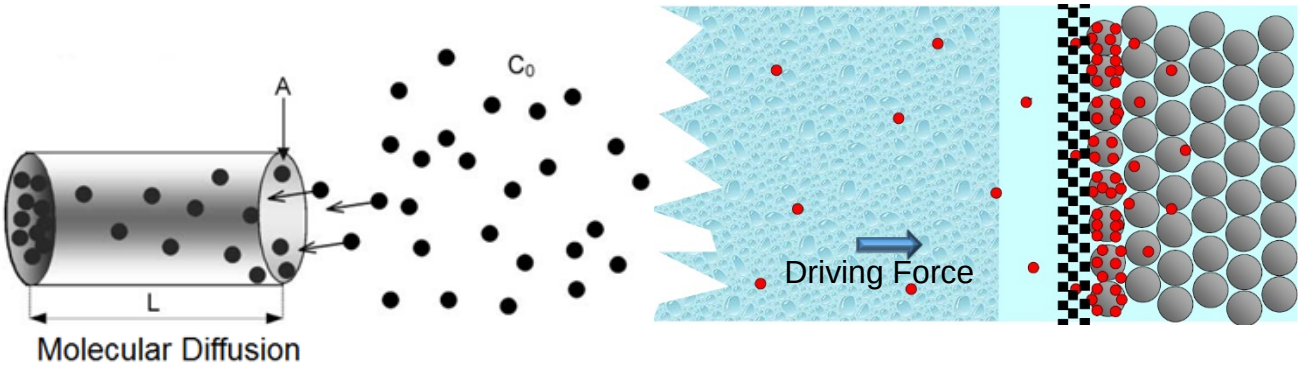

Figure A-3. Schematic diagram of passive samplers: (a) Diffusion, (b) Permeation.

For example, the Rutgers Electrostatic Passive Sampler is comprised of a permanently polarized ferroelectric polymer film, which electrostatically attracts bioaerosol and other particles from the air onto its surface. Airborne bioaerosol particles are particularly well suited to this electrostatic collection method because they carry a relatively high electrical charge. Captured particles are easily washed from the film and assayed. The advantages of this passive sampler are its small size, customizable shape, ease of use, and the fact that it does not inactivate sampled microbes. This device does not require a device to pull air through the sample, does not require external power, and can be placed anywhere for any length of time. It can be easily applied anywhere in indoor and outdoor environments providing representative data on ambient levels of bioaerosols and also other particulate matter. The sampler can be used in any area and for personal applications, where it can be worn by clipping it onto a shirt collar for applications such as widespread airport bioterrorism monitoring.

Commercially available membranes (such as Zetapor®, gauze, nylon, low-density polyethylene, or polyvinylidene difluoride) are also used as passive samplers to improve the detection of various types of pathogens including viruses in water and wastewater systems. These passive samplers are valuable tools for microbiome analysis with new-generation sequencing. The sorption of pathogens on membranes is influenced by several parameters including characteristics of the pathogens (i.e., isoelectric point, pH, particle size), membrane properties (i.e., electric charge, hydrophobicity) and aqueous solution characteristics (pH, ionic strength). Field applications of these passive samplers has revealed that short-term exposure allows for qualitative detection, and long-term exposure gives an integrated concentration over a period of time.

Most traditional methods for the sampling and analysis of bioaerosols are offline and involve the collection of the investigated particles on solid deposition substrates (membrane or fiber filters, inertial impaction plates, thermal or electrostatic precipitation plates) or in a liquid (wetted wall cyclone, impinger, or washing bottle) and intermediate steps of sample storage, transport, and preparation before analysis. These methods are prone to artifacts caused by evaporation of particle components, sorption of additional gas phase components, and reaction/alteration during sample collection, storage, transport, and preparation. The potential for measurement artifacts for bioaerosols can be minimized or at least quantify the effects outlined above by using elaborate sampling techniques combining parallel or consecutive trains of denuders, filters, and adsorbent cartridges. Substantial progress has been made in the development of aerosol mass spectrometers for real-time measurements of size-selected particles. As the methods of vaporization, ionization, calibration, and data analysis are improved, these instruments promise reliable quantitative analyses by allowing differentiation between surface and bulk composition. A particularly interesting application of aerosol mass spectrometry with high relevance is the identification of biological particles and pathogens (bacteria, viruses, spores, etc.). Alternative concepts for online monitoring of bioaerosols are based on aerodynamic sizing and fluorescence spectroscopy, whereas most other applicable techniques are offline and highly labor intensive (cultivation, staining, fluorescence and electron microscopy, enzyme and immunoassays, DNA analysis, etc.). The key features of bioaerosol sampling are shown in Table A-7. Table A-8 lists manufacturers of representative aerosol samplers.

Table A-8. Key Features of Bioaerosol Sampling

Passive Sampling[1]	**Settle plates** o Consider using the 1/1/1 scheme (for 1 h, 1 m from the floor, at least 1 m away from walls or any obstacle - standard index of microbial air contamination) with 90mm plates **Surface sampling** o Consider using membranes (e.g., nitrocellulose) as an alternative to contact plates on curved surfaces o Surface and aerial contamination may have different sources
Active Sampling(1)	**Impactors** o Collection on to agar plates o Collection efficiency highly dependent on particle size (should be sieve-like in performance) o Ideal as a particle size classifier o Loss of bioefficiency: shear forces, desiccation, particle bounce, and deposition build-up **Virtual impactors** o Collection into liquid, thus minimizing risk of desiccation o Collection efficiency dependent on particle size o Useful as particle concentrators **Slit impactors** o Collection on to agar plates o Loss of bioefficiency: shear forces, desiccation, particle bounce, and deposition build-up o Records variation in bioaerosol concentration over a specified time-period **Impingers** o Collection into liquid, thus minimizing risk of desiccation o Loss of bioefficiency: shear forces, re-aerosolization, evaporation, adherence to device walls o Collection efficiency dependent on particle size **Cyclones (wetted)** o Collection into liquid, thus minimizing risk of desiccation o Loss of bioefficiency: shear forces, liquid carryover, evaporation, adherence to device walls o May be used as pre-classifiers for particle size o Collection efficiency dependent on particle size o Vary considerably in size and airflow rate **Filters** o Small, portable personal samplers o Loss of bioefficiency: desiccation o Collection efficiency dependent on particle size (sample head, foam, or cyclone being used as pre-selectors)
Laboratory Testing	*Calibrate the flow rate of the active sampler* o Ensures the maximum collection efficiency o Influences the size of particles collected *Determine the bioefficiency of the sampler against the target pathogen* o Test in air conditions expected in the field (relative humidity and temperature) o Spike sampler with known concentration of the target pathogen o Each type of pathogen has a unique response to conditions experienced o Surrogate viruses may be used in place of pathogens; however, response may differ from target pathogen o Check that bio-efficiency is maintained throughout planned sampling time **Determine errors** in numeration when sampling from a known, repeatable concentration of the target pathogen

	Ensure that the sampler exhaust is not a source of pathogen contamination to the environment **Test the storage**, enumeration, and identification procedure
Field Testing	**Position of the inlet sampler** o Avoid strong airflows around the inlet of the sampler o If using an inlet nozzle, position horizontally o Ensure that the sample position is beyond the range of droplet fallout from a source **Aerial microbial concentration** o Expect non-uniformed concentration in the area studied (expect associated sampling errors) o Consider taking samples at various locations in the area studied o Be aware of airflow patterns due to HVAC and natural ventilation o Note air quality: relative humidity, temperature, and particle dust o There may be seasonal variation in concentration of the pathogen **Active samplers: quantification of pathogens** o Expressed as enumeration per cubic meters of air o Need to know the collection time and flow rate of the sampler.

(1) Results from passive and active samplers should not be assumed comparable.

Table A-9. Manufacturers of Representative Aerosol Samplers

Impaction Samplers

Andersen 6-Stage, 2-Stage, and 1-Stage
Graseby Andersen
500 Technology Court
Smyrna, GA 30082-5211
(404) 319-9999
(800) 241-6898

SAS, and Compact SAS
Spiral Biotech, Inc.
7830 Old Georgetown Road
Bethesda, MD 20814
(301) 657-1620

Allergenco MK-2
Allergenco/Blewstone Press
P.O. Box 8571
Wainwright Station
San Antonio, TX 78208
(210) 822-4116

Casella Slit Sampler
BGI Incorporated
58 Guinan Street
Waltham, MA 02154
(617) 891-9380

Reuter Centrifugal Sampler
BIOTEST Diagnostics Corp.
66 Ford Road, Suite 131
Denville, NJ 07834
(201) 625-1300
(800) 522-0090

Mattson-Garvin Slit-to-Agar
Barramundi Corporation
P.O. Drawer 4259
Homosassa Springs, FL 32647
(904) 628-0200

Aeroallergen Rotorod®
Sampling Technologies, Inc.
26338 Esperanza Drive
Los Altos, CA 94022
(415) 941-1232

Volumetric Spore Traps (Indoor/Outdoor, 1- & 7-day; Personal)
Burkard Manufacturing Co. Ltd.
Woodcock Hill Industrial Estate
Rickmansworth, Hertfordshire
WD3 1PJ
England
0923-773134

SKC Biosampler®
SKC, Inc.
863 Valley View Rd.
Eighty Four, PA 15330
(724) 941-9701

Biocapture™, BioBadge™
MesoSystems Technology, Inc.
1021 N. Kellogg Street
Kennewick, WA 99336
(509) 737-8383

Filtration Samplers

Samplers and Supplies
Costar Nuclepore™
One Alewife Center
Cambridge, MA 02140
(617) 868-6200
(800) 492-1110

Gelman Sciences Inc.
600 South Wagner Road
Ann Arbor, MI 48106
(313) 665-0651

Millipore Corporation
80 Ashby Road
Bedford, MA 01730
(617) 275-9200
(800) 225-1380

Sandia Met-One Sampler
Sandia National Laboratories
1515 Eubank Blvd. SE
Albuquerque, NM 87123
(505) 845-0011

Impingement Samplers

All Glass Impinger-30 and -4 (AGI-30 & AGI-4)
Ace Glass Incorporated
P.O. Box 688
1430 Northwest Blvd.
Vineland, NJ 08360
(609) 692-3333

Multi-Stage Liquid Impinger (May)
Burkard Manufacturing Co. Ltd.
Woodcock Hill Industrial Estate
Rickmansworth, Hertfordshire
WD3 1PJ
England
0923-773134

General Air Sampling Equipment Vendors

Industrial Hygiene News
Buyer's Guide
Circulation Department
8650 Babcock Blvd.
Pittsburgh, PA 15237
(412) 364-5366
(800) 245-3182

American Chemical Society
Environmental Buyer's Guide
1155 16th Street, NW
Washington, DC 20036
(202) 872-4600

Dycor Technologies Ltd.
1851 94 St NW, Edmonton, AB
T6N 1E6, Canada
(780) 486-0091

EMD Chemicals, Inc.
480 S Democrat Rd.
Gibbstown, NJ 08027
(856) 224-0094

BioGuardian®
InnovaTek
350 Hills Street, # 104
Richland, WA 99352
(509) 375-1093

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Attachment B-1:
Sample Collection Information
for Pathogens (Bacteria, Viruses, Protozoa, and Helminths)
in Solids (Soil, Powder)

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Attachment B-1: Sample Collection Information for Pathogens in Solid Samples

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Additional Source[4]
Solid Bacteria				
Bacillus anthracis **[Anthrax]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer. Care should be taken to avoid freezing the samples.	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	U.S. EPA/USGS, 2014; Mott et al., 2017; Olm et al., 2017
Brucella **spp. [Brucellosis]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer.	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Scholz et al., 2008; USAMRIID, 2016
Burkholderia mallei **[Glanders][3]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer.	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Velasco et al., 1998; Prakash et al., 2014; U.S. EPA/USGS, 2014; USAMRIID, 2016
Burkholderia pseudomallei **[Melioidosis][3]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer.	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Velasco et al., 1998; Prakash et al., 2014; EPA/USGS, 2014; USAMRIID, 2016
Campylobacter jejuni **[Campylobacteriosis][3]**	Sterile, leak-proof container	Keep on ice (secure double-bagged ice).	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Rivoal et al., 2005; Carrillo et al., 2017; Hiett, 2017
Chlamydophila psittaci **[Psittacosis][3]**	Sterile, leak-proof container	Keep on ice (secure double-bagged ice).	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Hulin et al., 2016; Koskela, 2017
Coxiella burnetii **[Q-fever][3]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer.	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Fitzpatrick et al., 2010; Bruin et al., 2013; Duncan et al., 2013; Hong et al., 2013
Escherichia coli **O157:H7[3]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer.	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Gagliardi and Karns, 2000; Jiang et al., 2002; Park et al., 2015

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Additional Source[4]
Francisella tularensis **[Tularemia][3]**	Sterile, leak-proof container	Room temperature if held for 1 hour or less; keep on ice (e.g., secure double-bagged ice) if longer.	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Barns et al., 2005; Petersen et al., 2009; Berrada and Telford, 2010; Baird et al., 2012
Legionella pneumophila **[Legionellosis – a) Pontiac fever; and b) Legionnaires' disease]**	Sterile, leak-proof container. Water and swab samples must be packed into a container that protects the samples from exposure to light and temperature fluctuation.	Do not pack any samples with chilled or frozen ice packs or chiller packs. Samples must reach the laboratory within 24 hours of collection.	100 g (gravimetric) Fill ≥120mL mL (volumetric)	Steele et al., 1990; Yang, 2004; Kuroki et al., 2007; Environmental Microbiology Laboratory, 2014
Leptospira **spp. (*L. interrogans* serovars: *L. icteroheamorrhagiae, L. autralis, L. balum, L. bataviae, L. sejro, L. pomona*) [Leptospirosis]**	Small, tightly sealed sterile bottle or plastic bag. A small amount of sterile deionized water may be added to prevent drying.	A small amount of sterile deionized water should be present in container to prevent drying. Room temperature within 72 hours of collection; if longer, keep on ice packs (or secure double-bagged ice).	10 – 20 g (gravimetric)	Benacer et al., 2013; Saito et al., 2013
Listeria monocytogenes **[Listeriosis][3]**	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice). If sample is already frozen, do not thaw until analysis.	At least 100 g (gravimetric)	Beuchat and Ryu, 1997; Locatelli et al., 2013; U.S. FDA, 2016
Non-typhoidal *Salmonella* **[Salmonellosis][3]**	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice).	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Hutchison et al., 2004; Boes et al., 2005; Courty et al., 2008
Salmonella **Typhi [Typhoid fever][3]**	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice).	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Hutchison et al., 2004; Boes et al., 2005; Courty et al., 2008

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Additional Source[4]
***Shigella* spp. [Shigellosis][3]**	Sterile plastic bags or glass or plastic bottles	Keep on ice packs (or secure double-bagged ice).	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Alvarez et al., 1995; U.S. EPA/USGS, 2014; Stanley et al., 2015; Steiner-Asiedu et al., 2016
***Staphylococcus aureus*[3]**	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice) if longer.	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Rusin et al., 2003; Chaudhary et al., 2013; Mohammed and Sheikh, 2010
***Vibrio cholerae* 01 and O139 [Cholera][3]**	Sterile, leak-proof container	Store at room temperature. Do not ship on ice.	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Santamaria and Toranzos, 2003; Huq et al., 2012; Djaouda et al., 2013; Menezes et al., 2014
***Yersinia pestis* [Plague][3]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer.	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Pohanka and Skladal, 2009; U.S. EPA/USGS, 2014; U.S. EPA, 2016
Solid Viruses				
Adenoviruses: Enteric and non-enteric (A-F)[3]	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice).	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Horswell et al., 2010; Rigotto et al., 2010; Ahmed et al., 2015; ASTM, 2016
Astroviruses[3]	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice).	50 – 100 g (gravimetric) Fill 50 mL sample tube to at least 40 mL mark (volumetric)	Rodríguez et al., 2009; ASTM, 2016; Amoah et al., 2017
Caliciviruses: Norovirus[3]	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice).	50 – 100 g (gravimetric)	Jones et al., 2007; La Rosa et al., 2010; Bibby and Peccia, 2013; Boehm et al., 2016
Caliciviruses: Sapovirus[3]	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice).	50 – 100 g (gravimetric)	Jones et al., 2007; La Rosa et al., 2010; Bibby and Peccia, 2013; Boehm et al., 2016

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Additional Source[4]
Coronaviruses: SARS-associated human coronavirus[3]	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice).	50 – 100 g (gravimetric)	Derbyshire and Brown, 1978; De Paoli, 2005; Staggemeier et al., 2015
Hepatitis E virus (HEV)[3]	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice).	50 – 100 g (gravimetric)	Sobsey et al., 1986; Rigotto et al., 2010; Parashar et al., 2011
Influenza H5N1 virus[3]	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice).	50 – 100 g (gravimetric)	Vong et al., 2008; Gutiérrez and Buchy, 2012; Horm et al., 2012
Picornaviruses: Enteroviruses[3]	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice).	50 – 100 g (gravimetric)	Spilki et al., 2013; Faleye et al., 2016
Picornaviruses: Hepatitis A virus (HAV)[3]	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice).	50 – 100 g (gravimetric)	Rodrıguez-Lazaro et al., 2012; Xagoraraki et al., 2014; Adefisoye et al., 2016
Reoviruses: Rotavirus (Group A)	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice).	50 – 100 g (gravimetric)	Horswell et al., 2010; Spilki et al., 2013; Trubl et al., 2016
Solid Protozoa				
***Cryptosporidium* spp. [Cryptosporidiosis]**	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice); do not freeze.	50 – 100 g (gravimetric)	Prystajecky et al., 2014; Bonilla et al., 2015
***Entamoeba histolytica*[3]**	Sterile, sealed, leak-proof container	Keep on ice packs (or secure double-bagged ice); do not freeze.	50 – 100 g (gravimetric)	Branco et al., 2012; Calegar et al., 2016
***Giardia* spp. [Giardiasis][3]**	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice); do not freeze.	50 – 100 g (gravimetric)	Covert et al., 1999; Olson et al., 1999; Guy et al., 2003
***Naegleria fowleri* [Naegleriasis - primary amoebic meningoencephalitis (PAM)/ amebic encephalitis]**	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice); do not freeze.	100 g (gravimetric) 250 mL-10 L (volumetric)	Mull et al., 2013; Moussa et al., 2013; Mahittikorn et al., 2015; Morgan et al., 2016

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Additional Source[4]
***Toxoplasma gondii* [Toxoplasmosis][3]**	Sterile, sealed, leak-proof container	Keep on ice packs (or secure double-bagged ice); do not freeze.	50 – 100 g	Afonso et al., 2008; Sroka and Szymanska, 2012; Krueger et al., 2014
Solid Helminths				
***Baylisascaris procyonis* [Raccoon roundworm infection]**	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice). Store at 2 – 5°C at laboratory; do not freeze samples.	300 – 600 g (gravimetric)	Gavin et al., 2005; Gatcombe et al., 2010; Collender et al., 2015; Amoah et al., 2017

Footnotes:

(1) Any sample collected for cultivation-based analysis must not be allowed to freeze.

(2) The sample sizes listed are based on the amount needed for analysis of a single sample. If requested by the laboratory, additional sample(s) must be collected for laboratory quality control analyses (e.g., duplicates, matrix spikes). It is also recommended that additional sample(s) be collected in case of the need for reanalysis due to sample spillage or unforeseen analytical difficulties.

(3) Currently, no information is available for this analyte in this sample type. Until such time that analyte-specific information is available, collection procedures described for a similar analyte/sample type are considered to be appropriate.

(4) References for these sources are provided at the end of this attachment.

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Attachment B-2:
Sample Collection Information for
Pathogens (Bacteria, Viruses, Protozoa, and Helminths) in Surfaces
(Swab, Wipe, Dust Socks)

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Attachment B-2: Sample Collection Information for Pathogens in Surfaces (Swab, Wipe, Dust Socks)

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
Surfaces (Swab, Wipe, Dust Socks) Bacteria				
Bacillus anthracis **[Anthrax]**	Sterile, leak-proof container	Room temperature if held for 1 hour or less; keep on ice (e.g., secure double-bagged ice) if longer. Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	ASTM, 2010; Brown et al., 2007a; Brown et al., 2007b; Hodges et al., 2010; Rose et al., 2011; CDC, 2012; Piepel et al., 2015; Hutchison et al., 2015
Brucella **spp. [Brucellosis]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer. Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	USAMRIID, 2016; Arizona Department of Health Services, 2017; Ohio Department of Health, 2013
Burkholderia mallei **[Glanders][4]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer. Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	USAMRIID, 2016; Arizona Department of Health Services, 2017; Downey et al., 2012
Burkholderia pseudomallei **[Melioidosis][4]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer. Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	USAMRIID, 2016; Arizona Department of Health Services, 2017; Downey et al., 2012; Hong-Geller et al., 2010
Campylobacter jejuni **[Campylobacteriosis][4]**	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice). Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Vidal et al., 2016; Arizona Department of Health Services, 2017; Standard Methods, 2006; Standard Methods, 2007

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
Chlamydophila psittaci **[Psittacosis][4]**	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice). Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	U.S. EPA, 2015; Hulin et al., 2016; NRC, 2014; Madico et al., 2000
Coxiella burnetii **[Q-fever][4]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer. Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	USAMRIID, 2016; Arizona Department of Health Services, 2017; Kersch et al., 2010
Escherichia coli **O157:H7[4]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer. Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Ismaïl et al., 2013; Downey et al., 2012; Arizona Department of Health Services, 2017
Francisella tularensis **[Tularemia][4]**	Sterile, leak-proof container	Room temperature if held for 1 hour or less; keep on ice (e.g., secure double-bagged ice) if longer. Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	USAMRIID, 2016; Arizona Department of Health Services, 2017; U.S. EPA/CDC, 2012; U.S. Army Test and Evaluation Command, 2016; Rastogi et al., 2008
Legionella pneumophila **[Legionellosis – a) Pontiac fever; and b) Legionnaires' disease]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer. Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Arizona Department of Health Services, 2017; OSHA, 2016

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
***Leptospira* spp. (*L. interrogans* serovars: *L. icteroheamorrhagiae, L. autralis, L. balum, L. bataviae, L. sejro, L. pomona*) [Leptospirosis]**	Sterile, leak-proof container. A small amount of sterile deionized water may be added to prevent drying.	Ambient temperature within 72 hours of collection; keep on ice (e.g., secure double-bagged ice) if longer. Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	U.S. EPA, 2008; U.S. EPA, 1978; Firth et al., 2014; Burroughs et al., 2007; Riediger et al., 2016
***Listeria monocytogenes* [Listeriosis][4]**	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice). If frozen, do not thaw until analysis.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Lahou and Uyttendaele, 2014; Gómez. et al., 2012; Zhu et al., 2012; Downey et al., 2012; Lim et al., 2005;
Non-typhoidal *Salmonella* [Salmonellosis][4]	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice).	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Williams et al., 2015; Tu et al., 2015; Rose et al., 2004
***Salmonella* Typhi [Typhoid fever][4]**	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice).	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Weir, 2016; U.S. EPA, 2010; Zewde et al., 2009; Rusin et al., 2002
***Shigella* spp. [Shigellosis][4]**	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice).	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Lim et al., 2005; Sehulster and Chinn, 2003; Rusin et al., 2002; Page et al., 2014
***Staphylococcus aureus*[4]**	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice).	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Lutz et al., 2013; Landers et al., 2010
***Vibrio cholerae* 01 and O139 [Cholera][4]**	Sterile, leak-proof container	Store at room temperature. Do not ship in ice.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Ley et al., 2012; Lim et al., 2005; Page et al., 2014; U.S. EPA, 1978

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
***Yersinia pestis* [Plague][4]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Silvestri et al., 2016; AFQTP, 2015; Gilbert et al., 2014; Da Silva et al., 2012; Dauphin et al., 2010; Petrovick et al., 2007
Surfaces (Swab, Wipe, Dust Socks) Viruses				
Adenoviruses: Enteric and non-enteric (A-F)[4]	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice).	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Williams et al., 2001; ASTM 2016; Xagoraraki et al., 2014; Tuladhar et al., 2012
Astroviruses[4]	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice).	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Williams et al., 2001; U.S. EPA, 2015; ASTM 2016; Scherer et al., 2009; Tuladhar et al., 2012
Caliciviruses: Norovirus[4]	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice).	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Williams et al., 2006; U.S. EPA, 2015; Kimmitt and Redway 2016; Tuladhar et al., 2012
Caliciviruses: Sapovirus[4]	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice).	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Williams et al., 2006; U.S. EPA, 2015; Kimmitt and Redway, 2016; Tuladhar et al., 2012
Coronaviruses: SARS-associated human coronavirus[4]	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice).	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Weir, 2016; Julian et al., 2011; Casanova et al., 2010
Hepatitis E virus (HEV)[4]	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice).	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Givens et al., 2016; Julian et al., 2011
Influenza H5N1 virus[4]	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice).	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Ip et al., 2012; Indriani et al., 2010

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
Picornaviruses: Enteroviruses[4]	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice).	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Rönnqvist 2014; Tuladhar et al., 2012; Sanderson et al., 2010
Picornaviruses: Hepatitis A virus (HAV)[4]	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice).	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Rönnqvist, 2014; Tuladhar et al., 2012
Reoviruses: Rotavirus (Group A)	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice).	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Savage and Jones, 2003
Surfaces (Swab, Wipe, Dust Socks) Protozoa				
***Cryptosporidium* spp. [Cryptosporidiosis]**	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice). Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Edmonds et al., 2009; McDermott, 2004; Carlsen et al., 2001
***Entamoeba histolytica*[4]**	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice). Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Miller et al., 2010
***Giardia* spp. [Giardiasis][4]**	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice). Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Rhodes et al., 2012; Palomar Health, 2014
***Naegleria fowleri* [Naegleriasis - primary amoebic meningoencephalitis (PAM)/ amebic encephalitis]**	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice). Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	NIST, 2012; Khan, 2008
***Toxoplasma gondii* [Toxoplasmosis][4]**	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice). Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Hoorfar, 2011; NHANES, 2006; Dumètre. and Dardé, 2003

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
Surfaces (Swab, Wipe, Dust Socks) Helminths				
Baylisascaris procyonis **[Raccoon roundworm infection]**	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice). Samples may be stored at 2°C–5°C in the laboratory. Care should be taken to avoid freezing the samples.	At least 2 sterile, synthetic, and moistened wipes, swabs, or dust socks	Ogdee et al., 2016; Hernandez et al., 2013; Sorvillo et al., 2002; Gavin et al., 2005

Footnotes:

(1) Any sample collected for cultivation-based analysis must not be allowed to freeze.

(2) The sample sizes listed are based on the amount needed for analysis of a single sample. If requested by the laboratory, additional sample(s) must be collected for laboratory quality control analyses (e.g., duplicates, matrix spikes). It is also recommended that additional sample(s) be collected in case of the need for reanalysis due to sample spillage or unforeseen analytical difficulties.

(3) Additional resources. References for these sources are provided at the end of this attachment.

(4) Currently, no information is available for this analyte in this sample type. Until such time that analyte-specific information is available, collection procedures described for a similar analyte/sample type are considered to be appropriate.

Notes:

- Sample transport containers are packed outside the contaminated area. Samples must be packed in a manner that protects the integrity of the sample containers and provides temperature conditions required for sample preservation. Primary receptacles should be leak-proof with a volumetric capacity of not more than 500 mL (liquid) or 4 kilograms (solid). If several individual primary containers are placed in a single secondary packaging, they must be individually wrapped or separated so as to prevent contact between them. Secondary packaging should be leak-proof and surrounded by shock- and water-absorbent packing materials or ice (if required for preservation) and shipped in a cooler to ensure sample temperatures do not exceed preservation requirements. Ice should be placed in separate plastic bags or cold packs should be used to avoid leakage, and the bags placed around, among, and on top of the secondary sample containers. Further guidance can be obtained from 49 CFR 173.199 (https://www.gpo.gov/fdsys/pkg/CFR-2006-title49-vol2/pdf/CFR-2006-title49-vol2-sec173-199.pdf) and 42 CFR 72 and 73 (http://oig.hhs.gov/authorities/docs/05/032905FRselectagents.pdf).
- U.S. Department of Transportation (DOT) and International Air Transportation Association (IATA) labeling requirements apply to materials that are known to contain, or are suspected of containing, an infectious substance and reflect the most recent changes, effective October 1, 2006. Further guidance on these changes and lists of substances considered to be either category A (not listed in this document) or category B can be obtained from the U.S. Department of Transportation, Pipeline and Hazardous Materials Safety Administration (DOT, PHMSA) at http://www.phmsa.dot.gov/staticfiles/PHMSA/DownloadableFiles/Files/Transporting_Infectious_Substances_brochure.pdf. Definitions and exceptions for Class 6, Division 6.2 infectious substances are described in 49 CFR 173.134.
- For collection of aqueous samples containing residual chlorine, add a stock solution of filter-sterilized 10% sodium thiosulfate at 0.5 mL/L.
- If using impingers that do not replenish the liquid as it is evaporated by the air stream, the maximum recommended sampling volume is 200 L (, Duchaine et al., 2001, Applied and Environmental Microbiology 67(6): 2775-2780).
- Mixed cellulose ester (MCE) and polytetrafluoroethylene (PTFE) filters are available as cassettes.

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Attachment B-3:
Sample Collection Information for
Pathogens (Bacteria, Viruses, Protozoa, and Helminths) in Liquids
(Water and Wastewater)

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Attachment B-3: Sample Collection Information for Pathogens in Water (Water, Wastewater)

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
Liquid (Water, Wastewater) Bacteria				
Bacillus anthracis **[Anthrax]**	Sterile, leak-proof container	Room temperature if held for 1 hour or less; keep on ice (e.g., secure double-bagged ice) if longer. Care should be taken to avoid freezing the samples.	200 mL (minimum)	Celebi et al., 2016; Singh et al., 2015; U.S. EPA, 2012; Létant et al., 2011; Perez et al., 2005
Brucella **spp. [Brucellosis]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer.	100 mL (minimum)	Saraswathy et al., 2015; Goenka et al., 2012; Martin et al., 2012; Corbel 2006
Burkholderia mallei **[Glanders][4]**	Sterile, leak-proof container	Room temperature if held for 1 hour or less; keep on ice (e.g., secure double-bagged ice) if longer.	100 mL (minimum)	Prakash et al., 2014; Thaipadungpanit et al., 2014; Vongphayloth et al., 2012; Baker et al., 2011; Lever et al., 2003
Burkholderia pseudomallei **[Melioidosis][4]**	Sterile, leak-proof container	Room temperature if held for 1 hour or less; keep on ice (e.g., secure double-bagged ice) if longer.	100 mL (minimum)	Delgado-Gardea et al., 2016; Limmathurotsakul et al., 2013; Limmathurotsakul et al., 2012; Vongphayloth et al., 2012
Campylobacter jejuni **[Campylobacteriosis][4]**	Sterile, leak-proof container	Keep on ice (secure double-bagged ice).	1 – 5 L	Khan et al., 2009; Pitkänen et al., 2009; ISO, 2005; Hänninen et al., 2003
Chlamydia psittaci **(formerly *Chlamydophila psittaci*) [Psittacosis][4]**	Sterile, leak-proof container	Keep on ice (secure double-bagged ice).	100 mL (minimum)	Hulin et al., 2015; USDA, 2014b
Coxiella burnetii **[Q-fever][4]**	Sterile, leak-proof container	Room temperature if held for 1 hour or less; keep on ice (e.g., secure double-bagged ice) if longer.	500 mL (minimum)	Deshmukh et al., 2016; Schets et al., 2013

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
***Escherichia coli* O157:H7**[4]	Sterile, leak-proof container	Room temperature if held for 1 hour or less; keep on ice (e.g., secure double-bagged ice) if longer.	100 mL (minimum)	U.S. EPA, 2010; Brewster, 2009
***Francisella tularensis* [Tularemia]**[4]	Sterile, leak-proof container	Room temperature if held for 1 hour or less; keep on ice (e.g., secure double-bagged ice) if longer.	100 mL (minimum)	U.S. EPA, 2015; Forsman, 1995
***Legionella pneumophila* [Legionellosis – a) Pontiac fever; and b) Legionnaires' disease]**	Sterile, leak-proof container. Water and swab samples must be packed into a container that protects the samples from exposure to light and temperature fluctuation.	Do not pack any samples with chilled or frozen ice packs or chiller packs. All samples other than compost material must reach the laboratory within 24 hours of collection. Compost material to be reached within three days to the laboratory. Avoid sampling for at least 72 hours after on-line disinfection or system decontamination or cleaning.	100 mL (minimum)	ASHRAE, 2015; AS/NZS, 2011a; AS/NZS, 2011b; Flanders et al., 2014
***Leptospira* spp. (*L. interrogans* serovars: *L. icteroheamorrhagiae, L. autralis, L. balum, L. bataviae, L. sejro, L. pomona*) [Leptospirosis]**	Sterile, leak-proof container	A small amount of sterile deionized water should be present in container to prevent drying. Room temperature within 72 hours of collection; if longer, keep on ice packs (or secure double-bagged ice).	100 mL – 1000 mL	Riediger et al., 2016; Wójcik-Fatla et al., 2014; Benacer et al., 2013; U.S. EPA, 2008
***Listeria monocytogenes* [Listeriosis]**[4]	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice). If sample is already frozen, do not thaw until analysis.	100 mL (minimum)	Gorski et al., 2014; USDA, 2014; Taherkhani et al., 2013
Non-typhoidal *Salmonella* [Salmonellosis][4]	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice).	1000 mL and above	Cabral, 2010; Obi et al., 2004
***Salmonella* Typhi [Typhoid fever]**[4]	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice).	1000 mL. Smaller volumes may be appropriate for highly contaminated waters.	McEgan et al., 2012; Kumar et al., 2006; Standing Committee of Analysts, 2006

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
***Shigella* spp. [Shigellosis][4]**	Sterile plastic bags or glass or plastic bottles	Keep on ice packs (or secure double-bagged ice).	1000 mL. Smaller volumes may be appropriate for highly contaminated waters.	Standing Committee of Analysts, 2006; Faruque et al., 2003
***Staphylococcus aureus*[4]**	Sterile, leak-proof container	Keep on ice (e.g., secure double-bagged ice) if longer.	100 mL (minimum)	Plano et al., 2011; Lechevallier and Seidler, 1980
***Vibrio cholerae* 01 and O139 [Cholera][4]**	Sterile, leak-proof container	Store at room temperature. Do not ship on ice.	100 mL (minimum)	Huq et al., 2012; Schauera et al., 2012; CDC, 2010
***Yersinia pestis* [Plague][4]**	Sterile, leak-proof container	Room temperature if held for 2 hours or less; keep on ice (e.g., secure double-bagged ice) if longer.	100 mL (minimum)	Deshmukh et al., 2016; U.S. EPA, 2015; Simon et al., 2013
Liquid (Water, Wastewater) Viruses				
Adenoviruses: Enteric and non-enteric (A-F)[4]	Positively charged 1MDS cartridge filter	Keep on ice packs (or secure double-bagged ice).	2 – 20 L (wastewater); 200 – 300 L (surface/recreational water); 1500 – 2000 L (drinking water/groundwater).	Xagoraraki et al., 2014; Cashdollar and Wymer, 2013; Ikner et al., 2011; Williams et al., 2001
Astroviruses[4]	Positively charged 1MDS cartridge filter	Keep on ice packs (or secure double-bagged ice).	2 – 20 L (wastewater); 200 – 300 L (surface/recreational water); 1500 – 2000 L (drinking water/groundwater) Filter apparatus should be allowed to run overnight.	Cashdollar and Wymer, 2013; Rodríguez-Lázaro et al., 2012; Espinosa et al., 2009; Williams et al., 2001
Caliciviruses: Norovirus[4]	Positively charged 1MDS cartridge filter	Keep on ice packs (or secure double-bagged ice).	2 – 20 L (wastewater); 200 – 300 L (surface/recreational water); 1500 – 2000 L (drinking water/groundwater)	Gabrieli et al., 2009; Karim et al., 2009; USGS, 2001; Williams et al., 2001
Caliciviruses: Sapovirus[4]	Positively charged 1MDS cartridge filter	Keep on ice packs (or secure double-bagged ice).	2 – 20 L (wastewater); 200 – 300 L (surface/recreational water); 1500 – 2000 L (drinking water/groundwater)	Hata et al., 2015; Williams et al., 2001

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
Coronaviruses: SARS-associated human coronavirus[4]	Positively charged 1MDS cartridge filter	Keep on ice packs (or secure double-bagged ice).	2 – 20 L (wastewater); 200 – 300 L (surface/recreational water); 1500 – 2000 L (drinking water/groundwater)	AWWA, 2007; Williams et al., 2001
Hepatitis E virus (HEV)[4]	Double layer 142 mm diameter 1MDS cartridge filter	Keep on ice packs (or secure double-bagged ice).	2 – 20 L (wastewater); 200 – 300 L (surface/recreational water); 1500 – 2000 L (drinking water/groundwater)	Williams et al., 2001; Jothikumar et al., 1993; Rose et al., 1984
Influenza H5N1 virus[4]	Positively charged 1MDS cartridge filter	Keep on ice packs (or secure double-bagged ice).	2 – 20 L (wastewater); 200 – 300 L (surface/recreational water); 1500 – 2000 L (drinking water/groundwater)	Deboosere et al., 2011; Nazir et al., 2011; Williams et al., 2001
Picornaviruses: Enteroviruses[4]	Positively charged 1MDS cartridge filter	Keep on ice packs (or secure double-bagged ice).	2 – 20 L (wastewater); 200 – 300 L (surface/recreational water); 1500 – 2000 L (drinking water/groundwater) Filter apparatus should be allowed to run overnight.	Faleye et al., 2016; CDC/WHO, 2015; Spilki et al., 2013; Williams et al., 2001
Picornaviruses: Hepatitis A virus (HAV)[4]	Positively charged 1MDS cartridge filter	Keep on ice packs (or secure double-bagged ice).	2 – 20 L (wastewater); 200 – 300 L (surface/recreational water); 1500 – 2000 L (drinking water/groundwater) Filter apparatus should be allowed to run overnight.	Adefisoye et al., 2016; Xagoraraki et al., 2014; Rodríguez-Lázaro et al., 2012; Fout et al., 2003; Williams et al., 2001
Reoviruses: Rotavirus (Group A)	Positively charged 1MDS cartridge filter	Keep on ice packs (or secure double-bagged ice).	2 – 20 L (wastewater); 200 – 300 L (surface/recreational water); 1500 – 2000 L (drinking water/groundwater) Filter apparatus should be allowed to run overnight.	Trubl et al., 2016; Spilki et al., 2013; USGS/U.S. EPA, 2004; Fout et al., 2003; Williams et al., 2001

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
Liquid (Water, Wastewater) Protozoa				
***Cryptosporidium* spp. [Cryptosporidiosis]**	Sterile, leak-proof container or Filter in sterile leak-proof container	Keep on ice (e.g., secure double-bagged ice); do not freeze	10 L – 15 L	Bonilla et al., 2015; Prystajecky et al., 2014; U.S. EPA, 2005
***Entamoeba histolytica*[4]**	Polypropylene carboys	Keep on ice packs (or secure double-bagged ice); do not freeze.	10L – 50 L	Skotarczak, 2009; Guy et al., 2003
***Giardia* spp. [Giardiasis][4]**	Sterile, leak-proof container/ Polypropylene carboys	Keep on ice packs (or secure double-bagged ice); do not freeze.	100 L – ≥1000 L through cartridge filtration	Skotarczak, 2009; U.S. EPA, 2005; Guy et al., 2003; McCuin and Clancy, 2003
***Naegleria fowleri* [Naegleriasis - primary amoebic meningoencephalitis (PAM)/ amebic encephalitis]**	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice); do not freeze.	250 mL - 10 L	Morgan et al., 2016; Mahittikorn et al., 2015; Moussa et al., 2013; Mull et al., 2013
***Toxoplasma gondii* [Toxoplasmosis][4]**	Sterile, sealed, leak-proof container/Filter in sterile leak-proof container/Polypropylene carboys	Keep on ice packs (or secure double-bagged ice); do not freeze.	100 L (ten 10 L containers)/4650 L for filter cartridge	Krueger et al., 2014; Sroka and Szymańska, 2012; Villena et al., 2004
Liquid (Water, Wastewater) Helminths				
***Baylisascaris procyonis* [Raccoon roundworm infection]**	Sterile, leak-proof container	Keep on ice packs (or secure double-bagged ice). Store at 2 – 5°C at laboratory; do not freeze samples.	1 L (minimum)	Graeff-Teixeira et al., 2016; Gatcombe et al., 2010

Footnotes:

(1) Any sample collected for cultivation-based analysis must not be allowed to freeze.

(2) The sample sizes listed are based on the amount needed for analysis of a single sample. If requested by the laboratory, additional sample(s) must be collected for laboratory quality control analyses (e.g., duplicates, matrix spikes). It is also recommended that additional sample(s) be collected in case of the need for reanalysis due to sample spillage or unforeseen analytical difficulties.

(3) Additional resources. References for these sources are provided at the end of this attachment.

(4) Currently, no information is available for this analyte in this sample type. Until such time that analyte-specific information is available, collection procedures described for a similar analyte/sample type are considered to be appropriate.

Notes:

- Sample transport containers are packed outside the contaminated area. Samples must be packed in a manner that protects the integrity of the sample containers and provides temperature conditions required for sample preservation. Primary receptacles should be leak-proof with a volumetric capacity of not more than 500 mL (liquid) or 4 kilograms (solid). If several individual primary containers are placed in a single secondary packaging, they must be individually wrapped or separated so as to prevent contact between them. Secondary packaging should be leak-proof and surrounded by shock- and water-absorbent packing materials or ice (if required for preservation) and shipped in a cooler to ensure sample temperatures do not exceed preservation requirements. Ice should be placed in separate plastic bags or cold packs should be used to avoid leakage, and the bags placed around, among, and on top of the secondary sample containers. Further guidance can be obtained from 49 CFR 173.199 (https://www.gpo.gov/fdsys/pkg/CFR-2006-title49-vol2/pdf/CFR-2006-title49-vol2-sec173-199.pdf) and 42 CFR 72 and 73 (http://oig.hhs.gov/authorities/docs/05/032905FRselectagents.pdf).
- U.S. DOT and IATA labeling requirements apply to materials that are known to contain, or are suspected of containing, an infectious substance and reflect the most recent changes, effective October 1, 2006. Further guidance on these changes and lists of substances considered to be either category A (not listed in this document) or category B can be obtained from the U.S. Department of Transportation, Pipeline and Hazardous Materials Safety Administration (DOT, PHMSA) at http://www.phmsa.dot.gov/staticfiles/PHMSA/DownloadableFiles/Files/Transporting_Infectious_Substances_brochure.pdf. Definitions and exceptions for Class 6, Division 6.2 infectious substances are described in 49 CFR 173.134.
- For collection of aqueous samples containing residual chlorine, add a stock solution of filter-sterilized 10% sodium thiosulfate at 0.5 mL/L.
- If using impingers that do not replenish the liquid as it is evaporated by the air stream, the maximum recommended sampling volume is 200 L (Applied and Environmental Microbiology, Duchaine et al., 2001, 67(6): 2775-2780).
- Mixed cellulose ester (MCE) and polytetrafluoroethylene (PTFE) filters are available as cassettes.

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Attachment B-4:
Sample Collection Information for
Pathogens (Bacteria, Viruses, Protozoa, and Helminths) in Aerosols

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Attachment B-4: Sample Collection Information for Pathogens in Aerosols

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
Aerosol Bacteria				
Bacillus anthracis **[Anthrax]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Room temperature if held for 2 hours or less; keep on ice (e.g., ice packs, secure double-bagged ice) if longer.	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Teshale et al., 2002; Estill et al., 2009; NIST, 2012; U.S. EPA, 2012; U.S. EPA, 2013; Xu et al., 2013; Clauss, 2015; Grinshpun et al., 2016; Haig et al., 2016
Brucella **spp. [Brucellosis]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Room temperature if held for 15 minutes or less; keep on ice (e.g., ice packs, secure double-bagged ice) if longer.	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Fatah et al., 2007; NIST, 2012; Dybwad, 2014
Burkholderia mallei **[Glanders][4]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Room temperature if held for 15 minutes or less; keep on ice (e.g., ice packs, secure double-bagged ice) if longer.	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Fatah et al., 2007; Blatny et al., 2008; Dabisch et al., 2012; U.S. EPA, 2013; Grinshpun et al., 2016
Burkholderia pseudomallei **[Melioidosis][4]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Room temperature if held for 15 minutes or less; keep on ice (e.g., ice packs, secure double-bagged ice) if longer.	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Fatah et al., 2007; Dabisch et al., 2012; U.S. EPA, 2013; Grinshpun et al., 2016
Campylobacter jejuni **[Campylobacteriosis][4]**	Sterile MCE/PTFE filter[6], gel filter, impinger	Keep on ice (e.g. ice packs, secure double bagged ice)	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L	Zhao et al., 2011a; Zhao et al., 2011b; Dybwad et al., 2014
Chlamydia psittaci **(formerly *Chlamydophila psittaci*) [Psittacosis][4]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice (e.g. ice packs, secure double bagged ice)	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Van Droogenbroeck, et al., 2009; NIST, 2012; Dybwad, 2014
Coxiella burnetii **[Q-fever][4]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Room temperature if held for 15 minutes or less; keep on ice (e.g., ice packs, secure double-bagged ice) if longer.	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	NIST, 2012; Aarnink et al., 2015; Núñez et al., 2016

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
***Escherichia coli* O157:H7[4]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice (e.g. ice packs, secure double bagged ice)	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Kesavan et al., 2008; Riemenschneider et al., 2010; NIST, 2012; Xu et al., 2013; Grinshpun et al., 2016
***Francisella tularensis* [Tularemia][4]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Room temperature if held for 2 hours or less; keep on ice (e.g., ice packs, secure double-bagged ice) if longer.	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Burton et al., 2007; Srikanth et al., 2008; Dabisch et al., 2012
***Legionella pneumophila* [Legionellosis – a) Pontiac fever; and b) Legionnaires' disease]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep frozen at ≤-20°C (dry ice or super cold packs rated for temps below -70°C)	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	CDC, 2015; AS/NZS, 2011c; Mandal and Brandl, 2011; CDC, 2003; Ishimatsu et al., 2001
***Leptospira* spp. (*L. interrogans* serovars: *L. icteroheamorrhagiae, L. autralis, L. balum, L. bataviae, L. sejro, L. pomona*) [Leptospirosis]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice (e.g. ice packs, secure double bagged ice)	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	VanDyke-Gonnerman, 2013; Li et al., 2012
***Listeria monocytogenes* [Listeriosis][4]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice (e.g. ice packs, secure double bagged ice). If sample is already frozen do not thaw until analysis.	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Kretzer et al., 2008; Srikanth et al., 2008; Pillai and Ricke, 2002
Non-typhoidal *Salmonella* [Salmonellosis][4]	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Room temperature if held for 15 minutes or less; keep on ice (e.g., ice packs, secure double-bagged ice) if longer.	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Adell et al., 2014; Riemenschneider et al., 2010; Barker and Jones, 2005
***Salmonella* Typhi [Typhoid fever][4]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice (e.g. ice packs, secure double bagged ice).	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	NIST, 2012; Woodword et al., 2004; Pillai and Ricke, 2002

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
***Shigella* spp. [Shigellosis][4]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice (e.g. ice packs, secure double bagged ice).	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Srikanth et al., 2008; Morey, 2007; Kalogerakis et al., 2005
***Staphylococcus aureus*[4]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice (e.g. ice packs, secure double bagged ice).	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Haig et al., 2016; Chang and Wang, 2015; Tseng et al., 2014
***Vibrio cholerae* 01 and O139 [Cholera][4]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Store at room temperature. Do not ship on ice. Note: unlikely to be viable – samples should be collected only for PCR analysis.	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Blatny et al., 2008; Crook, 1996
***Yersinia pestis* [Plague][4]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Room temperature if held for 2 hours or less; keep on ice (e.g., ice packs, secure double-bagged ice) if longer.	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Dybwad et al., 2014; Cooper, 2010; Burton et al., 2007; Bergman et al., 2005
Aerosol — Viruses				
Adenoviruses: Enteric and non-enteric (A-F)[4]	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice packs (or secure double-bagged ice).	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Kienlen, 2015; Ge et al., 2014; Cooper, 2010
Astroviruses[4]	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice packs (or secure double-bagged ice).	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	D'Arcy, 2014; Carducci, 2013; Uhrbrand et al., 2012
Caliciviruses: Norovirus[4]	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice packs (or secure double-bagged ice).	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Ge et al., 2014; Carducci, 2013; Grinshpun et al., 2007
Caliciviruses: Sapovirus[4]	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice packs (or secure double-bagged ice).	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Ge et al., 2014; Carducci, 2013; Grinshpun et al., 2007
Coronaviruses: SARS-associated human coronavirus[4]	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice packs (or secure double-bagged ice).	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Kienlen, 2015; Xu et al., 2013; Wu et al., 2013; Verreault et al., 2008

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
Hepatitis E virus (HEV)[4]	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice packs (or secure double-bagged ice).	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Aarnink et al., 2015; Verreault et al., 2008
Influenza H5N1 virus[4]	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice packs (or secure double-bagged ice).	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Lednicky et al., 2016; Fennelly et al., 2015; Tang et al., 2015; Cooper 2010
Picornaviruses: Enteroviruses[4]	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice packs (or secure double-bagged ice).	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Kienlen, 2015; Verreault et al., 2008; Sattar et al., 1987
Picornaviruses: Hepatitis A virus (HAV)[4]	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice packs (or secure double-bagged ice).	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Kienlen, 2015; Verreault et al., 2008; Burton et al., 2007; Sattar et al., 1987
Reoviruses: Rotavirus (Group A)	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice packs (or secure double-bagged ice).	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Fronczek and Yoon, 2015; Johnson et al., 2013; Riemenschneider et al., 2010; Verreault et al., 2008; Gerone et al., 1966
Aerosol — Protozoa				
***Cryptosporidium* spp. [Cryptosporidiosis]**	Unlikely to be found.			
***Entamoeba histolytica*[4]**	Unlikely to be found.			
***Giardia* spp. [Giardiasis][4]**	Unlikely to be found.			
***Naegleria fowleri* [Naegleriasis - primary amoebic meningoencephalitis (PAM)/ amebic encephalitis]**	Sterile MCE/PTFE filter[6], gel filter, impinger, and/or impactor (agar plate)	Keep on ice packs (or secure double-bagged ice).	MCE/PTFE filter: 120 – 960 L; gel filter: 40 – 135 L; impinger[5]: 750 – 6000 L; impactor: 84.9 – 849 L	Srikanth et al., 2008; Fink and Gilman, 2000

Analyte(s) [Disease]	Container	Preservation[1]	Sample Size[2]	Source[3]
Toxoplasma gondii **[Toxoplasmosis][4]**	Unlikely to be found.			
Aerosol — Helminths				
Baylisascaris procyonis **[Raccoon roundworm infection]**	Unlikely to be found.			

Footnotes:

(1) Any sample collected for cultivation-based analysis must not be allowed to freeze.
(2) The sample sizes listed are based on the amount needed for analysis of a single sample. If requested by the laboratory, additional sample(s) must be collected for laboratory quality control analyses (e.g., duplicates, matrix spikes). It is also recommended that additional sample(s) be collected in case of the need for reanalysis due to sample spillage or unforeseen analytical difficulties.
(3) Additional resources. References for these sources are supplied at the end of this attachment.
(4) Currently, no information is available for this analyte in this sample type. Until such time that analyte-specific information is available, collection procedures described for a similar analyte/sample type are considered to be appropriate.
(5) If using impingers that do not replenish the liquid as it is evaporated by the air stream, the maximum recommended sampling volume is 200 L (Applied and Environmental Microbiology, Duchaine et al., 2001, 67(6): 2775-2780).
(6) Mixed cellulose ester (MCE) and polytetrafluoroethylene (PTFE) filters are available as cassettes.

Notes:

- U.S. DOT and IATA labeling requirements apply to materials that are known to contain, or are suspected of containing, an infectious substance and reflect the most recent changes, effective October 1, 2006. Further guidance on these changes and lists of substances considered to be either category A (not listed in this document) or category B can be obtained from the U.S. Department of Transportation, Pipeline and Hazardous Materials Safety Administration (DOT, PHMSA) (http://www.phmsa.dot.gov/staticfiles/PHMSA/DownloadableFiles/Files/Transporting_Infectious_Substances_brochure.pdf). Definitions and exceptions for Class 6, Division 6.2 infectious substances are described in 49 CFR 173.134.
- For collection of aqueous samples containing residual chlorine, add a stock solution of filter-sterilized 10% sodium thiosulfate at 0.5 mL/L.
- Sample transport containers are packed outside the contaminated area. Samples must be packed in a manner that protects the integrity of the sample containers and provides temperature conditions required for sample preservation. Primary receptacles should be leak-proof with a volumetric capacity of not more than 500 mL (liquid) or 4 kilograms (solid). If several individual primary containers are placed in a single secondary packaging, they must be individually wrapped or separated so as to prevent contact between them. Secondary packaging should be leak-proof and surrounded by shock- and water-absorbent packing materials or ice (if required for preservation) and shipped in a cooler to ensure sample temperatures do not exceed preservation requirements. Ice should be placed in separate plastic bags or cold packs should be used to avoid leakage, and the bags placed around, among, and on top of the secondary sample containers. Further guidance can be obtained from 49 CFR 173.199 (https://www.gpo.gov/fdsys/pkg/CFR-2006-title49-vol2/pdf/CFR-2006-title49-vol2-sec173-199.pdf) and 42 CFR 72 and 73 (http://oig.hhs.gov/authorities/docs/05/032905FRselectagents.pdf).

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Attachment C:

Holding Time, Packaging Requirements, and Shipping Label of Sample

Attachment C: Table of Contents

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Attachment C: Holding Time, Packaging Requirements, and Shipping Label of Sample

Sample Holding time, packaging requirements, and shipping label of the samples discussed in this document will follow the following protocol, unless otherwise specified.

C.1 Holding time

Maximum holding time is the time between sample collection and analysis, which is the sum of the time to transport the sample from the field and storage time at the laboratory. When samples are to be analyzed for more than one microbiological parameter, due regard must be given to the appropriate storage conditions. The terms microbial testing can include a wide range of organisms, some of which may be more or less sensitive to storage times or temperature. Sample analysis should be prioritized such that the organisms most susceptible to change are analyzed first. Samples should be shipped to the laboratory without delay so that analysis can be completed quickly after collection. Samples should be kept in the dark and measures should be taken to avoid changes in sample moisture content.

Holding Time

Minimize transport and storage time. Analyze or extract immediately upon receipt at the laboratory.

None of the standards provide published evidence to support the recommended sample handling guidance and the holding times can appear arbitrary when a single set of instructions is applied to a large group of organisms. The terms *microbial testing* or *bacteriological examination* can include a wide range of organisms, some of which may be more or less sensitive to storage times or temperature. A criticism that has been levelled at standards is that sample holding times were originally established for aqueous media and then blindly applied to other media (USEPA, 2005).

C.2 Packaging Requirements

This section provides packaging requirements biological materials as needed to safely move the material from one location to another. Packaging, transportation, and shipping should be in accordance with:

- U.S. Department of Transportation (DOT) Hazardous Materials Regulations (HMR) for movement of biological materials in public right-of-ways within the U.S.
- International Air Transport Association (IATA) Dangerous Goods Regulations (DGR) for shipment of biological materials (e.g., infectious substances) by air.

Table C-1 lists the desired transportation modes that should be considered while shipping pathogen contaminated samples.

Table C-1. Transportation Modes and Pathogenic Samples Not Allowed

General Transport Mode	Specific Transport Mode	Pathogenic Samples That Are Not Allowed
Personal Transportation	Hand carry between laboratories	No restrictions on types of biological materials
	Hand carry between buildings	No restrictions on types of biological materials
	Personal motor vehicle*	Regulated biological materials are not allowed except for regulated materials being transported for research, diagnosis, investigational activities, or disease treatment or prevention; or that are biological products. Samples containing "Category A" infectious substances are not allowed.
	Public transportation	Regulated biological materials or other biological materials that may present a detrimental risk to the health of humans or other organisms either directly through infection or indirectly through damage to the environment are not allowed.
Licensed Transporter	Common carrier	No restrictions on types of biological materials unless restricted by the carrier.

* Personal transport in a motor vehicle means transportation in a private or government passenger vehicle such as a car, van, or pickup truck.

Using the proper packing materials, package, and labels incorrectly can cause the package to be out of compliance. Proper packaging is the responsibility of the sender. The sender assumes sole responsibility for compliance with all governmental regulations. Receiving drivers have the authority and responsibility to refuse any biological substance shipment that does not meet minimum packaging requirements.

Use well-constructed packaging to cushion the inner containers and enough absorbent material to absorb the entire contents of the inner packages should they break open during transport. Inner containers can be glass or plastic with the closure held securely in place (taped closed). The outer container can be a cardboard box.

Packages may be re-used if they are in good condition and have been disinfected. If packages are used for items other than infectious substances, all labels and marks for infectious substances must be removed or completely covered.

C.2.1 Primary Receptacle Requirements

Primary receptacles must be able to be secured with a lid or sealed with a screw top lid or with tape or Parafilm®. Each of the containers must have the container's content, hazards, and ownership on or with the container.

- **Inner containers:**
  - Use break-resistant (e.g., plastic) containers, if possible.

- Liquids must be in a leak-proof container. Lids on inner containers must have a positive means of closure. For example, a screw cap should be used instead of Parafilm, aluminum foil, or a stopper.
- Container(s) must be disinfected as needed for safety and should be placed in a Ziploc® bag or an equivalent secondary spill container.
- Information must be placed on or with the container(s) as needed to clearly communicate the container's contents, hazards, and ownership. Each individual container must be labeled with enough information to identify its contents. In addition, the container(s) or secondary bag(s) must also be labeled with the identity of the material, the name and phone number of the sender, the recipient's name and phone number if they are different from the sender's, and hazard information. Hazard information includes a biohazard label if the material is biohazardous, any words needed to explain the hazard, or words indicating the material is not hazardous.
- Containers for sharps (i.e., sharps container) must be constructed of a rigid material resistant to punctures and securely closed to prevent leaks or punctures. If several fragile primary receptacles are placed in a single secondary packaging, they must be individually wrapped or separated so as to prevent contact between them.

C.2.2 Secondary Packaging Requirements

When placing multiple primary glass receptacles in the same secondary package, each primary glass receptacle must be wrapped or separated from each other. This will prevent them from breaking or becoming damaged during transport. The secondary package must be sealed so that it will not open and spill the contents during transport. See Figure C-1, below.

- **Outer Container Requirements**

To prevent a release or leak of the pathogen contaminated substance, place sorbent material between the primary containers and secondary package. Use enough sorbent material to absorb the entire contents of the primary containers if they should break. In addition, the secondary package must fit in the outer package, and it must fit as close as possible to prevent the secondary package from moving too much during transport.

The outer container should meet the following criteria:

- Must be capable of surviving a drop test at a height of 1.2 meters without leakage from the primary receptacle. The primary receptacles must remain protected in the secondary packaging.
- Be adequate in strength
- Have a secure lid (e.g. plastic box, insulated cooler).
- Be rigid so as to retain its original shape and dimensions at all times under all conditions of transportation.
- Have at least one surface with a minimum dimension of 100-mm X 100-mm (4-inches x 4-inches).
- Allow the secondary container to fit as closely as possible to prevent excessive movement during transport, which could damage the primary containers.

NOTE: If there is space between the secondary container and outer container, place padding between the two containers to prevent the inner container from shifting.

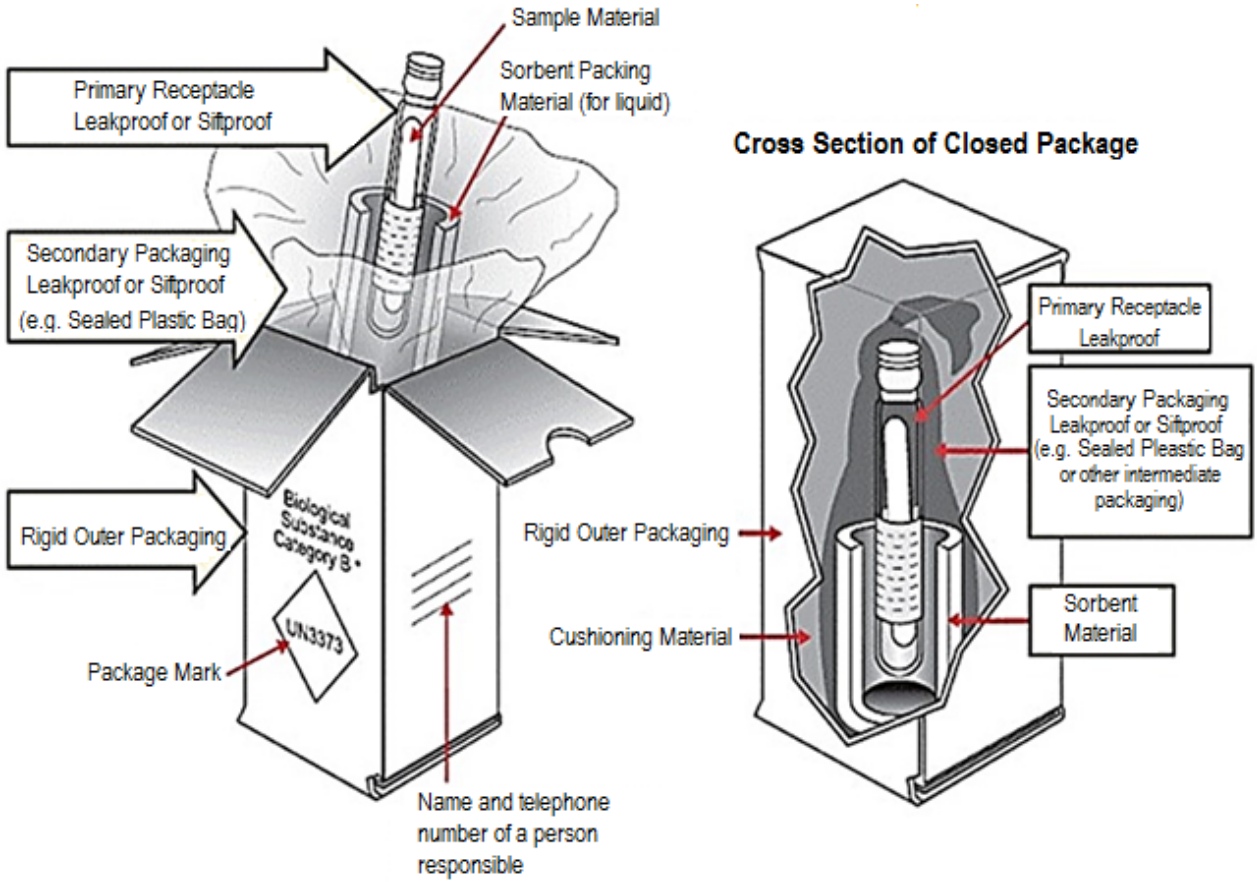

Figure C-1. Secondary packaging.

C.2.3 Manufacturer's packaging.

When applicable, each regulated biological material must be contained and packaged in the manufacturer's original container and packaging, or a container and packaging of equal or greater strength and integrity.

C.2.4 Markings

Markings refer to the information on the outer package and airway bills.

- The marking must be 2 inches × 2 inches (minimum)
- A diamond marking with the appropriate UN number (the four-digit United Nations number, which identify dangerous goods for transportation purposes)
- The proper shipping name to the marking
- The name, address and phone number of a responsible person must be on the air waybill or marked on the package.
- If an airway bill is used, the "Nature and Quantity of Goods" box must show the text "**Biological Substance, Category B**" and "**UN 3373**".

C.2.5 Refrigerants

All refrigerants must be placed outside the secondary packaging.

- Gel packs: Use gel-packs in place of wet ice. There are no requirements for marking or labeling the outer package for use of gel packs. It is difficult to achieve and maintain lower temperatures using gel packs.
- Dry ice: Class 9 Dangerous Good.

C.2.6 Packaging Requirements for Dry Ice

Dry ice is a hazardous material and is regulated by both the DOT) and the IATA. Specific procedures are required for handling, packaging, and shipping materials refrigerated with dry ice, if applicable. In addition, refer the IATA/DOT Requirements for Packing Instructions (PI) 904 and the document ACCEPTANCE CHECKLIST FOR DRY ICE (https://www.iata.org/whatwedo/cargo/dgr/Documents/acceptance-checklist-dry-ice-en.pdf) for more information.

- Contact the carrier to ensure proper ventilation will be available for the package and to determine if the carrier has additional requirements from those specified in the IATA PI 904 regulations.
- Coordinate logistics of the shipment with the recipient. Take into account local holidays or closings that might delay package receipt.

Refer to package manufacturer's recommendations to determine the correct amount of dry ice to include in your shipment. The actual time will vary depending on the package used and the volume and density of the dry ice. In general, however, dry ice will sublimate from a solid to a gas at a rate of 5-10 pounds (2.27-4.54 kg) per 24 hours when shipped in an appropriate insulated cooler.

C.3 IATA/DOT Marking and Labeling Requirements

The outermost container must be labeled with a hazard Class 9 Miscellaneous Dangerous Good label, UN 1845, and net weight of dry ice in kilograms.

FedEx has no additional restrictions for shipping dry ice. UPS requires the UPS Blue Dry Ice label in addition to the IATA/DOT requirements for marking and labeling:

Shipments of dry ice and other dangerous goods without an approved contract with UPS are prohibited.

C.4 Chain of Custody

A Chain of Custody (CoC) form documents transfer of sample custody from one individual to another, from the time the sample is collected until final analytical disposition. Each individual in possession of the sample must be noted by recording their signature on the form. The CoC record should include instructions for the laboratory technician as to analytical methods, potential dangers, and any pertinent handling procedures that should be observed. The CoC form should be kept separate from the sample (i.e., should not be placed with the sample) in order to preserve appropriate CoC. The CoC record must include at least the following information:

- All available information regarding the potential hazards associated with the agent;
- Handling procedures associated with the samples;
- Sample identification number;
- Sample concentration, if known;
- Sampling location;
- Collection date and time;
- Sample matrix;
- Names and signatures of the samplers; and
- Signatures of all individuals who had custody of the samples.

An unbroken COC must be maintained for all samples from collection through analysis and archiving. In order to maintain COC, the form must be readily accessible when transferring samples from one individual to another. Therefore, COC forms should not be placed inside the primary sample containment. A copy of the record will be kept with the samples until they are analyzed and returned with the analytical results or will be maintained on site at the laboratory if samples are archived for later use or collection by law enforcement.

C.5 Background References

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