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Collection of Surface Samples Potentially Contaminated with Microbiological Agents Using Swabs, Sponge Sticks and Wipes

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Collection of Surface Samples Potentially Contaminated with Microbiological Agents Using Swabs, Sponge Sticks and Wipes

U.S. Environmental Protection Agency
Cincinnati, OH 45268

Disclaimer

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Not all information described in this document has been validated or verified at the time of publication. The document will be updated or replaced with validated steps for collection upon availability.

Questions concerning this document or its application should be addressed to:

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Foreword

The U.S. Environmental Protection Agency (EPA) is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The EPA's Center for Environmental Solutions and Emergency Response (CESER) within the Office of Research and Development conducts applied, stakeholder-driven research and provides responsive technical support to help solve the Nation's environmental challenges. The Center's research focuses on innovative approaches to address environmental challenges associated with the built environment. We develop technologies and decision-support tools to help safeguard public water systems and groundwater, guide sustainable materials management, remediate sites from traditional contamination sources and emerging environmental stressors, and address potential threats from terrorism and natural disasters. CESER collaborates with both public and private sector partners to foster technologies that improve the effectiveness and reduce the cost of compliance, while anticipating emerging problems. We provide technical support to EPA regions and programs, states, tribal nations, and federal partners, and serve as the interagency liaison for EPA in homeland security research and technology. The Center is a leader in providing scientific solutions to protect human health and the environment.

When an environmental contamination involving a microbiological agent occurs, whether resulting from intentional or an unintentional incident, collection and analysis of numerous numbers of environmental samples will be needed to determine the extent of contamination and to make informed decisions regarding remediation. Sample collection procedures can be used during site characterization and remediation activities in support of EPA's post-incident responsibilities in order to provide instructions regarding the collection of samples from indoor/outdoor environmental, building, and infrastructure materials that will be analyzed for contaminants. This document provides step-by-step instructions for the collection of select microbiological agents from non-porous surfaces using macrofoam swabs, cellulose sponge sticks and gauze wipes. This document provides information on the materials and equipment needed for sample collection; the assembly of sampling kits; step-by-step instructions for taking field and quality control (QC) samples; and information on sample packaging, storage, and transport. Use of the procedure by EPA, or EPA contracted sample collectors, will help ensure that samples are collected in a consistent manner prior to laboratory analysis.

Gregory Sales, Director
Center for Environmental Solutions and Emergency Response

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Executive Summary

This document provides step-by-step instructions for the collection of samples from surfaces potentially contaminated with pathogens. It is intended to be used in conjunction with the analytical methods listed in U.S. Environmental Protection Agency's (EPA's) *Selected Analytical Methods for Environmental Remediation and Recovery (SAM)2017* document[1] and in the Environmental Sampling and Analysis Method Program online query tools for SAM.[2] The instructions in this document are applicable to collection of pathogens using macrofoam swabs, cellulose sponge sticks and gauze wipes during site remediation and recovery following a contamination incident. Information is provided on the materials and equipment needed for sample collection, the assembly of sampling kits, step-by-step instructions for taking field and quality control samples, and sample packaging, storage, and transport. The approach described in this document is adapted from Centers for Disease Control and Prevention (CDC) protocols for sampling for *B. anthracis* spores[3] and CDC sampling videos.[4,5]

Product Development Quality Assurance

Literature used for this procedure came from recognized, reputable and credible secondary sources including: peer-reviewed journals, scientific manuals and other scientific publications; federal agency websites, publications and regulations; industry providers of equipment and materials (i.e., vendors); and nationally recognized scientific, technical or response organizations. Citations are provided throughout the document. Full citations and/or access to each source used are provided in the references section. No deficiencies were noted with this review.

The document completed several review cycles prior to publication including EPA project lead review, internal EPA technical review, Homeland Security and Materials Management Division (HSMMD) quality assurance and technical edit reviews, external technical review, and HSMMD management reviews. All comments from reviewers have been tracked and are maintained by EPA and General Dynamics Information Technology, along with the revisions and adjustments made to address the comments.

[1] U.S. Environmental Protection Agency (U.S. EPA). (2017). Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2017. U.S. Environmental Protection Agency: Washington, DC. EPA/600/R-17/356.

[2] U.S. EPA. (2017). Environmental Sampling and Analytical Methods (ESAM) Program. U.S. Environmental Protection Agency. https://www.epa.gov/esam (Last accessed 04/29/2021)

[3] Centers for Disease Control and Prevention (2012). Emergency Response Resources: Surface sampling procedures for *Bacillus anthracis* spores from smooth, non-porous surfaces. Centers for Disease Control and Prevention: Atlanta, GA. https://www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthracis.html#e (last accessed 04/29/2021)

[4] CDC, National Institute for Occuapational Saftey and Health (NIOSH). (2015a). Anthrax surface sampling: How to sample with macrofoam swab on nonporous surfaces. Centers for Disease Control and Prevention, National Institute for Occuapational Saftey and Health. Available at: https://www.youtube.com/watch?v=95tTs0QNk0Y (last accessed 02/26/2021)

[5] CDC, National Institute for Occuapational Saftey and Health (NIOSH). (2015b). Anthrax surface sampling: How to sample with cellulose sponge on nonporous surfaces. Centers for Disease Control and Prevention, National Institute for Occuapational Saftey and Health. Available at: https://www.youtube.com/watch?v=dBEDs3XaqFQ (last accessed 02/26/2021)

Acronyms

CDC	U.S. Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
COC	Chain of Custody
DGR	Dangerous Goods Regulations
DOL	U.S. Department of Labor
DOT	U.S. Department of Transportation
DQO	Data quality objective
EPA	U.S. Environmental Protection Agency
ESAM	Environmental Sampling and Analysis Methods Program
GPS	Global Positioning System
HASP	Health and Safety Plan
HAZMAT	Hazardous Material
HAZWOPER	Hazardous Waste Operations and Emergency Response
HSMMD	Homeland Security and Materials Management Division
IATA	International Air Transport Association
ID	[Sample] Identification
I-WASTE DST	Incident Waste Decision Support Tool
LRN	Laboratory Response Network
NIOSH	National Institute for Occupational Safety and Health
OSHA	Occupational Safety and Health Administration
PBS	Phosphate Buffered Saline
PPE	Personal Protective Equipment
psi	pounds per square inch
QA	Quality Assurance
QAPP	Quality Assurance Project Plan
QC	Quality Control
SAM	Selected Analytical Methods for Environmental Remediation and Recovery
SAP	Sampling and Analysis Plan
SCID	Sample Collection Information Document
WMP	Waste Management Plan

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1.0 Scope and Application

When an environmental catastrophe resulting in contamination occurs, emergency responders and decision-makers need timely and accurate data as well as robust and well-defined methods for data collection. Catastrophic contamination can occur from an intentional incident such as a terrorist attack or an unintentional incident such as an industrial spill. Incidents can require the collection and analysis of numerous samples which will provide scientific data needed to make evidence-based decisions on the extent of contamination and subsequent remediation. The U.S. Environmental Protection Agency (EPA)'s Environmental Sampling & Analytical Methods (ESAM) Program (U.S. EPA 2017b) is intended to facilitate a coordinated response to a chemical, radiochemical, biotoxin or pathogen contamination incident by providing a comprehensive resource for sampling and analysis methods and guidance before, during and after a contamination incident. ESAM provides field- and laboratory-ready documents and web-based tools that focus on sample collection, processing, and analysis to facilitate site characterization, as well as remediation, waste disposal and clearance decisions.

As part of ESAM, Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2017 (U.S. EPA 2017a) provides a compendium of analytical methods that have been selected specifically for use during environmental response activities, by work groups consisting of methods experts from within EPA, as well as other federal, state and local agencies and public utilities.[6] SAM identifies a single selected method or suite of methods for each analyte/sample type. A SAM companion document Sample Collection Information Document [SCID] for Pathogens: Companion to Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2017 (SCID) (Chattopadhyay 2017) provides complementary information on sample containers, preservation, size and packaging, as well as additional resources that support collection of samples to be analyzed specifically for the selected pathogens, using the methods listed in SAM.

This document provides step-by-step instructions for the use of macrofoam swabs, cellulose sponge sticks and gauze wipes to collect samples from non-porous surfaces potentially contaminated with selected pathogens and has been adapted from Centers for Disease Control and Prevention (CDC) protocols (CDC 2012; CDC NIOSH 2015a; CDC NIOSH 2015b). This document is intended to be used in conjunction with the analytical methods listed in SAM, as well as the corresponding SCID for Pathogens. It provides information on the materials and equipment needed for sample collection and the assembly of sampling kits; step-by-step instructions for taking field and quality control (QC) samples; and information on sample packaging, storage, and transport.

> This document is applicable to collection of pathogens from non-porous surfaces using macrofoam swabs, cellulose sponge sticks and gauze wipes for sampling activities involving *B. anthracis* spores and its surrogates. Although testing has not been completed and efficiencies are unknown, these procedures may also be applicable to other microbiological agents (See Appendix A for a list of SAM microbiological agents this document is applicable for). In addition, it should be noted that wetting buffers might inactivate viruses during collection.

In summary, this document:

- Is applicable for collection of samples from non-porous surfaces potentially contaminated with the microbiological agents listed in EPA's SAM (see Appendix A).
- Addresses sample collection only and is intended for use by sampling personnel who have been sufficiently trained in sampling techniques for microbiological agents and corresponding safety

[6] For more information on SAM, methods and workgroups see: https://www.epa.gov/esam/selected-analytical-methods-environmental-remediation-and-recovery-sam (last accessed 04/29/2021)

protocols.

- Is intended to support collection of environmental samples at the point where remediation activities are turned over to the EPA and is applicable to the following sampling phases of a remediation event: site characterization and post-decontamination sampling. While this document was not specifically developed to support sample collection during the public health response, it could be used for that purpose.
- Assumes that collected samples will be analyzed using analytical methods and protocols consistent with those listed in EPA's SAM. The laboratory(ies) analyzing samples should be consulted prior to preparation of the Sampling and Analysis Plan (SAP) and prior to sample collection to ensure they will accept samples, the number of samples they will accept and whether they can process and analyze according to the SAM.

SAPs (and other site- and incident-specific plans and procedures) should be consulted to determine additional procedures and data needs beyond what is discussed in this document, including, but not limited to, whether additional sampling is needed for QC, if low concentrations of the microbiological agent are suspected. In addition, SAPs should be consulted to determine if additional modifications to plans and procedures are needed to accommodate laboratory capacity, target agent, incident background information, data quality objectives, and sample locations and amounts. This document ***does not*** provide information that is typically included in the following documents, which are described briefly in Appendix B:

- Sampling and analysis plan (SAP)
- Quality assurance project plan (QAPP)
- Health and safety plan (HASP)
- Analytical methods
- Waste management plan (WMP)

2.0 Limitation and Interferences

This document includes information based on sampling techniques that were available at the time of publication; not all information has been verified or validated. In addition, more research is needed to determine appropriate preservation and holding times for many of the microbiological agents, as well as collection efficiencies for agents other than *Bacillus anthracis*. The document is expected to be updated to include advances in technologies and results of validation studies on a periodic basis. Factors that can influence collection of microbiological agents from non-porous surfaces include concentration, other particulates (e.g., dust, dirt), and irregular surfaces (e.g., rough, textured).

3.0 Health and Safety Considerations

This document does not address all health and safety issues associated with sample collection. The importance of training, medical monitoring, required vaccinations (if applicable), and information included in the site- or incident-specific HASP should be emphasized. Sampling personnel can refer to their site-specific HASP for health, safety, and personal protective equipment (PPE) considerations specific to the sample collection event. The HASP should include a job hazard analysis for the site-specific sampling procedures that will be conducted. In addition to potential harm posed to the individuals involved, unsafe conditions in the field can indirectly impact the ability to collect representative samples which may affect resulting analytical data. A summary of health and safety considerations are included below:

- Training –Training is critical, and in some cases mandatory in order to ensure appropriate safety and health conditions for sampling personnel. Training requirements for Hazardous Waste Operations and Emergency Response (HAZWOPER) are outlined in the Occupational Safety and Health Administration's (OSHA's) HAZWOPER standard 29 Code of Federal Regulations [CFR] 1910.120 (U.S. Department of Labor (DOL) and OSHA 2013). Training elements to be covered are specified in 1910.120(e)(2) and include specific training on biohazards and microbiological agent awareness. Please consult the safety officer and/or sampling lead for required training that will be needed to operate equipment and techniques prior to their use in the field, methods to minimize cross-contamination, and appropriate donning and doffing requirements for PPE. Training requirements for respiratory protection can be found in OSHA's Respiratory Protection standard 1910.134 (U.S. DOL and OSHA 2011). Training requirements for packaging, documenting, and shipping infections substances can be found in the U.S. Department of Transportations (DOT's) Transporting Infectious Substances Safely (U.S. DOT 2020).
- Safety Officer – The safety officer must be appropriately trained and is responsible for: development and implementation of safety requirements and the HASP; assessing all site activities for potential safety concerns; ensuring that personnel are informed as to the potential hazards in a sampling area and dictating the requirements for safely working in the area; and stopping any sampling activity if necessary to protect personnel from a dangerous situation.
- Medical Examination – Medical examinations are performed to assess fitness to conduct sampling. Fitness for sampling includes clearance for wearing respiratory protection (see OSHA's Respiratory Protection standard 1910.134 (U.S. DOL and OSHA 2011), clearance for working with specific microbiological agents, receiving vaccinations if applicable, and use of prophylactics if available for the microbiological agent. Sampling personnel will be monitored for fatigue, stress, behavior, and general health during sampling events.
- First Aid – First aid kits must be available at all times during a sampling event. At least one kit should be available to sampling team at the primary sampling site. HASPs require that all injuries be reported and, if necessary, examined by medical personnel.
- PPE – PPE will be used during all sample collection and equipment decontamination activities, as required in the HASP. The type and level of PPE will be selected based on the potential hazard to provide the optimal personal protection and mobility for the task being performed. Sampling personnel must familiarize themselves with the HASP and SAP for required PPE. Sampling personnel can also review specific guidance for levels of protection and protective gear developed by OSHA provided in Appendix B of 29 CFR 1910.120 (U.S. DOL and OSHA 2013). The National Institute for Occupational Safety and Health (NIOSH) has also developed recommendations (Interim Recommendations for the Selection and Use of Respirators and Protective Clothing for Protection Against Biological Agents; NIOSH 2009) for selection and use

of respirators and PPE for protection against biological agents (NIOSH 2009). Incident-specific PPE requirements will be included in the HASP and SAP. General overarching considerations include the following:

  - In all cases, new powder-free disposable nitrile gloves are worn to protect hands and to protect samples from contact with potential contamination from surfaces and when using swabs, wipes, or sponge sticks to collect samples. Two pairs of new gloves are worn by the sample collector. The outer gloves are changed between samples or whenever they become visibly contaminated or the integrity of the gloves is compromised (torn, etc).
  - Care is taken to ensure that PPE is not compromised. If PPE is suspect or is compromised, sample collection must be stopped. Compromised PPE can result in contamination of personnel or contamination of collected samples.
  - After use, PPE (e.g., gloves, protective clothing) is appropriately disposed of or decontaminated.

- Medical Monitoring – Medical examinations include clearance for work with specific microbiological agents, and administration of appropriate vaccinations and prophylactic antibiotics. Sampling personnel are monitored for fatigue, stress, and general health during sampling events.

4.0 General Considerations for Collection of Surface Samples

Selection of the technique required for surface sampling is based primarily on site-specific sampling objectives and strategies, the analyses to be performed, conditions of the environment, surface type, fate and transport of the microbiological agent, and the physiological characteristics of the agent including agent size. Other aspects that might be considered include concentrations of microbiological agent and other particulates (high levels may overload some sampling devices); comprehensive quantitative and qualitative analysis (which might require the use of multiple sampling devices and analytical methods); and practical constraints (such as surface contours, proximity to the source, and other logistical considerations).

In addition to the information included in this document, sampling personnel should consult the site- and incident-specific SAP for sampling and laboratory requirements, which might include:

- Number, type, location, and area of samples that will be needed to support QC requirements
- Appropriate sample containers, preservation and holding times
- Sample packaging requirements (e.g., primary and secondary containment)
- Sample receipt requirements.

4.1 General Considerations

Wipe sampling can be performed using either cellulose sponges or non-cotton gauze wipes, while macrofoam swabs are used for swab sampling. Swabs, gauze wipes, and sponge sticks are typically used on non-porous surfaces such as stainless steel, painted wallboard, glass, floor tile, and wood laminate. Swabs are generally used for crevices and hard to reach surfaces, sponge sticks and gauze wipes are used for flat smooth surfaces. Gauze wipes and sponge sticks are of limited use for sampling porous surfaces, crevices, and depressions.

Swabs, sponge sticks, and gauze wipes should be pre-moistened, using a wetting solution (i.e., wetting agents, neutralizing buffers) prior to sample collection to enhance overall performance. Example wetting solutions are provided in Section 7.5. The CDC recommends the use of a neutralizing buffer as a pre-moistening solution in their validated swab and wipe sampling and analytical methods (CDC 2012). Note: Selection of a wetting solution will depend on the microbiological agent(s) to be collected, as well as the presence of disinfectant residuals which can require the use of a neutralizing buffer. The SAP should be consulted to determine the appropriate wetting solution and the optimal amount of wetting solution to be used.

4.2 Sampling Techniques

Table 4-1 provides information regarding the sampling techniques discussed in this document, along with their potential uses and some potential problems or considerations. In general, swabs are used to sample small (2” × 2”) non-porous surface areas (e.g., crevices, keyboards) while sponge sticks and gauze wipes are typically used to sample larger (10” × 10” to 12” × 12”) non-porous surface areas (e.g., walls, desktops). In all cases, multiple types of sampling devices (swabs, sponge sticks or wipes) can be used to cover larger areas than those recommended. Sponge sticks and wipes also can be used to collect composite samples, using the same device to collect sample material from multiple areas or combining multiple sticks or wipes towards one analytical sample. When composite sampling, Tuft et al 2014 combined up to four equivalent sampling areas (12” × 12”) using different sides of the same sponge-stick to reduce spore transfer of contamination to other surfaces.

Table 4-1. Surface Sampling Techniques

Sampling Device	Description and Potential Uses	Potential Problems or Considerations
Macrofoam swab	• Validated for *Bacillus anthracis* on steel surfaces and a preferred CDC sample collection protocol • Used to sample small (4 inch^2 [26 cm^2]) non-porous surfaces and hard to reach locations such as crevices, supply air diffusers, corners, air return grills • Commonly processed by CDC Laboratory Response Network (LRN) laboratories	• Visible amounts of particulates (e.g., dust) can saturate the surface of the swab and negatively impact collection and analytical results; the sampling area should be reduced in these instances • Swabs may be damaged or have difficulty collecting samples from irregular or non-porous surfaces • The wetting solution is dependent on the microbiological agent and decontamination status of the surface to be sampled
Cellulose sponge sticks	• Validated for *B. anthracis* on steel surfaces and a preferred CDC sample collection protocol • Used to sample flat non-porous surfaces, such as walls, floors, table-tops • Sampling area is 100 inch^2 (645 cm^2) per sponge stick; multiple sponge sticks can be used to sample an entire surface • A 10" ×10" template is recommended to standardize the sampling area	• Visible amounts of particulates (e.g., dust) can saturate the surface of the sponge stick and negatively impact collection and analytical results; the sampling area should be reduced in these instances • Limited use for sampling porous surfaces, crevices, and depressions • The wetting solution is dependent on the microbiological agent and decontamination status of the surface to be sampled
Gauze wipes	• Generally, more durable than swabs or sponge sticks • Used to sample flat non-porous surfaces, such as walls, floors, table-tops • Sampling area is 144 inch^2 (1 foot^2 [929 cm^2]) per gauze wipe; multiple wipes can be used to sample an entire surface • A 12" × 12" template is recommended to standardizing the sampling area	• Visible amounts of particulates (e.g., dust) can impact the analytical results; the sampling area should be reduced in these instances • Limited use for sampling porous surfaces, crevices, and depressions • The wetting solution is dependent on the microbiological agent and decontamination status of the surface to be sampled • Requires sample collection personnel to directly contact sampling media

4.2.1 Swab Samples

Swabs are typically used for sampling small (4 inch^2 [26 cm^2]) non-porous surfaces such as crevices, corners, supply air diffusers, air return grills, irregular surfaces, 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). CDC Laboratory Response Network (LRN) laboratories are capable of processing samples collected in accordance with this sample collection technique. Sampling efficiency can be negatively impacted when samples are collected from surfaces with visible amounts of dust or other particulates.

4.2.2 Sponge Stick Samples

In addition to swabs (Section 4.2.1, the CDC also currently recommends cellulose sponge sticks for the collection of *B. anthracis* spores on smooth, non-porous surfaces (CDC 2012). Sponge

sticks are sponge wipes that are attached to a handle and are preferred for surface sampling of areas of 100 inch^2 (645 cm^2) per sponge stick. Multiple sponge sticks can be used to cover larger areas. Sponge stick sampling efficiency can be negatively impacted by visible amounts of dust or other substances present on the surfaces being samples.

4.2.3 Gauze Wipes

Gauze wipes are generally more durable than swabs or sponge sticks and are appropriate for sampling areas of 144 inch^2 (1 foot^2 [929 cm^2]) per wipe, of non-porous surfaces.

5.0 Waste Management

Waste generation and management begin as soon as the response to a contamination incident is initiated. Used PPE, materials from sampling activities, and liquids from decontamination associated with sample collection activities can be generated by sampling personnel. Generation of these waste streams will continue throughout the response and recovery phases. Planning for waste management is critical.

It is the responsibility of all sampling personnel to comply with the site- or incident-specific WMP (see Appendix B) and with federal, state and local regulations governing waste management, including biohazard and hazardous waste identification, tracking and reporting, accumulation documentation, and land disposal restrictions. It is also the responsibility of sampling personnel to minimize and control all releases. Sampling personnel can refer to the site- or incident-specific WMP for instructions on anticipated waste generation due to sampling, as well as waste management requirements and procedures.

Sampling personnel and planners also can refer to EPA's Waste Management Options for Homeland Security Incidents website and EPA's Incident Waste Decision Support Tool (I-WASTE DST) which provide information regarding regulations and guidance to support decision-making regarding waste treatment and disposal (websites last accessed 4/29/2021).[7] In general:

- Excess sample materials and supplies, reference materials, and accumulated waste that will not be reused are placed in appropriate waste container(s) separating solid waste from liquid waste, and stored separately from collected samples and sampling equipment prior to removal from a contaminated site. The site-specific WMP should be consulted regarding whether decontamination of these materials will be conducted prior to removal from a contaminated site or at a facility designated for decontamination or disposal.
- Unused and uncontaminated sample collection materials can be retained for additional sampling or shipped to the laboratory with each batch of samples. (These materials can serve as QC samples [field blanks] providing information to determine if analytical results might be impacted by interferences contained in the equipment used [Section 9.0]).

[7] I-WASTE is a decision support tool that organizes information related to waste management. The tool also provides access to technical information, regulations and guidance to work through waste management issues to facilitate safe and efficient removal, transport and management of waste materials. Pre-registration is needed to access EPA's I-WASTE Tool and Disposal Decision Tool at http://www2.ergweb.com/bdrtool/login.asp

6.0 Sample Documentation

Documentation collected associated with sample collection is necessary to determine how the contaminant was disseminated, determine the extent of contamination, inform the investigation and drive the need for sampling, and to help understand and evaluate the analytical results associated with each sample. It is also necessary for validating those results; documenting the protocols used, sampling conditions, sample location, and individual sampling personnel; and tracking the sample during transfer to ensure sample integrity. This section summarizes some of the key components of documentation that should be implemented and maintained by individuals involved in sample collection and documented in the data management plan (see Appendix B). Additional guidance is provided in EPA's Sampling, Laboratory and Data Considerations for Microbial Data Collected in the Field (Silvestri et al. 2018).

Electronic data recording devices are also available for use, and it is EPA Policy (Stanislaus 2016) to use Scribe (U.S. EPA 2018) wherever practical to collect, store and report sampling and analytical data. Scribe is a database management tool developed by EPA's Environmental Response Team for managing environmental data, and was designed to capture sampling data, observational information, monitoring field data and analytical data.[8]

> Documentation produced during collection and processing of samples should be considered a legal record by the sampling team. Training is required for sampling personnel in order to accurately generate/maintain legal records. Legibility and permanence should be maintained. If an error is made, it should either be struck out using a single line and initialed and dated, or re-written, checked for accuracy, initialed and dated, and attached to the original for record keeping.

6.1 Sample Identification

Each sample collected must include an identification (ID) label, including QC samples (Section 9.0). Each field and QC sample must have a unique ID, and the ID must be recorded on all field documentation, sample container labels, chain-of-custody (COC) forms, and any other documents pertaining to the sample. This recording ties all sample collection, handling and transport information directly to the sample, and is critical for sample tracking and data analysis. The ID is used to track information linked to the sample, including sample location and type, date and time of collection, sample collector and associated QC samples. Determination of sample IDs are site- or incident-specific; sampling personnel can consult the SAP to determine sample ID assignments.

6.2 Sample Labels

A unique sample label must be applied to each individual sample container, with information that identifies and describes the sample. Sample information is added in waterproof ink, and the label is covered with clear tape. Alternatively, pre-prepared labels that uniquely identify the sample, such as a bar or Quick Response code that tracks the sample information, can be affixed to each container. Sample container labels will be incident- and site-specific and must, at a minimum, include the sample ID. Additional information that can be included on these labels includes:

- Time and date sample collected
- Sample matrix (e.g., particulates)
- Sample area
- Preservation, if applicable

[8] For additional information regarding Scribe, see https://www.epa.gov/ert/environmental-response-team-information-management (last accessed 04/29/2021).

- Sample collection location (Global Positioning System [GPS] coordinates or brief description)
- Signature or initials of the sample collector

Sample labels are placed on the outside of each primary and secondary sample container (see Section 8.3).

6.3 Sample Documentation Information

During sample collection, information associated with each sampling event is recorded and maintained in logbooks, on sample tracking forms, or in other sample documentation designated in the incident SAP and data management plan. These field records are completed at the time each sample is collected, and the copies accompany samples during shipment. The information recorded on these forms is essential to data validation, is extremely useful to laboratories and data users, and includes, at a minimum:

- Unique sample ID
- Date and time of sample collection
- Sampling location (including GPS coordinates, if appropriate)
- Sample type and collection method used
- Sample collection start and stop times
- Names of sampling team members

Additional information that might be requested and recorded could include but is not limited to site conditions, field analyses and other pertinent observations. Electronic devices may also be used as a means of recording information in the field. If electronic recording devices are to be used, they should be selected based on durability, accuracy, backup capability and ease of decontamination. If photographs are included as part of the sampling documentation, the name of the photographer, corresponding sample ID, date, time, site location and site description are recorded sequentially in the logbook as each photograph is taken. Once photographs are transferred to hard copy, the associated information included in the sample documentation is electronically associated with the photograph or written on the back of the photograph.

6.4 Sample Control and Chain of Custody (COC)

Once samples are collected, they must be maintained under controlled and secured conditions until transport to the laboratory. This control is required to ensure that samples are not compromised, and that analytical data are representative of site conditions. COC forms create a written record that can be used to trace the creation, possession, and handling of the sample from the moment of its collection through analysis. A COC form accompanies each sample or group of samples as custody is transferred from one custodian to another.

Sample progress is tracked and recorded at each step of sample handling, from collection through processing, packaging, and shipment. Sampling teams are responsible for initiation, maintenance, and completion of COC forms. The individual(s) performing each step of sample transfer is required to record their initials or signature on the sample label, field records, COC form, and any other document associated with the sample to qualify the condition of the sample at that point of sample progression. For example, the sample collector will sign off (e.g., electronically or on documentation) to relinquish the samples after collection for packaging and shipment. One copy of the form is retained by the original sample collector. If multiple laboratories are receiving samples, the COCs provided to each individual laboratory only identifies the contents of the sample shipment being sent to the receiving laboratory. Although COC forms vary in style, format and detail, the forms should contain the same minimal

information required to identify the sample and document custody. In cases where multiple samples are transferred as a group, the COC should account for each individual sample.

EPA policy (Stanislaus 2016) is to use Scribe wherever practical to generate COC forms. At a minimum, sampling teams are responsible for providing the following information:

- General incident information (sample owners, contact information, site name)
- Detailed site map for locating sampling points
- Sample information (e.g., sample IDs, sample types, number and type of sample containers, and date/time samples were collected)
- Date and time the samples were relinquished
- Signature of persons transferring the samples

6.5 Custody Seals

Custody seals are part of the COC process and are attached over the sealed opening of sample containers to ensure that the samples have not been opened or tampered with after collection and packaging. A custody seal also can be placed over the shipping or transport container, making it impossible to open the container without ripping the seal. Typically, there is one seal per sample container and two seals are placed on opposite sides of the transport container. Custody seals contain the signature of the person responsible for packing the container and the date sealed. The seal must be sufficiently sturdy to resist incidental contact but able to break when the cap or lid is moved. Sample collectors will sign and date the sample custody seal (usually a 1 x 3-inch white paper label with adhesive backing) using waterproof ink.

7.0 Sampling Supplies and Reagents

Samples collected in response to a contamination incident involving microbiological agents should be collected using dedicated and sterile sampling devices (e.g., swabs, sponge sticks), materials (e.g., containers) and reagents (e.g., wetting agent, neutralizing buffer) to minimize interferences and cross-contamination. In most cases, pre-packaged sampling devices and materials are available and can be used. This section provides general information regarding requirements and considerations associated with the sampling devices and materials needed for sample collection. Sampling kits are prepared and provided to sample collection teams prior to field sampling (see Sections 8.3.1 – 8.3.4) and consist of the following components as described in Sections 7.1 – 7.6.

7.1 Sampling Supplies and Reagents: Swabs

- Sterile macrofoam swabs, 3/16-inch thick, medical-grade polyurethane foam head, 100 pores per inch, thermally bonded to a polypropylene handle (e.g., Fisher Scientific Catalog No. 22-029-573, Puritan Catalog No. 25-1607 1PF SC, or equivalent)
- Sterile scissors
- 2-mL vials (e.g., Fisher Scientific Catalog No.50-476-678 or equivalent)
- Sterile wetting agent or neutralizing solution (Section 7.5)
- Sterile, sealable, leak-proof containers (e.g., 15-mL, Fisher Scientific Catalog No. 12-565-269, or equivalent)
- Disposable 2″ × 2″ template (4-inch^2 [26-cm^2])
- 4″ × 6″ clean, sealable plastic bags
- 1-quart clean, sealable plastic bags

7.2 Sampling Supplies and Reagents: Sponge Sticks

- Sterile sponge sticks, 1.5″ × 3″ sterile cellulose sponge pre-moistened with neutralizing buffer (e.g., 3M™ Sponge-Stick [3M, St. Paul, MN; Catalog No. SSL-10NB], Hygiena™ Stick Sponge [Hygiena, Camarillo, California; Catalog No. SS100NB], or equivalent)
- Disposable 10″ × 10″ template (100 inch^2 [645 cm^2]) for non-porous surfaces
- Sterile individually wrapped, 4-ounce (118-mL) screw-cap specimen container (e.g., Kendall Healthcare, Mansfield, MA; Catalog No. 8889-207026, or equivalent)
- 1-quart sealable plastic bags
- 1-gallon sealable plastic bags

7.3 Sampling Supplies and Reagents: Gauze Wipes

- Sterile, non-cotton gauze 2″ × 2″ wipes (e.g., Curity Catalog No. 8042, or equivalent)
- Sterile wetting agent or neutralizing buffer, 10 mL (Section 7.5)
- Sterile powder free gloves (e.g., Ansell™ Catalog No. 6034153, or equivalent)
- Sterile, sealable, leak-proof containers (e.g., 15-mL, Fisher Scientific Catalog No. 12-565-269, or equivalent)
- Sterile transfer pipets, individually wrapped, 5-mL (e.g., Greenwood Products, Inc. Catalog No. GS137038, or equivalent)
- Sterile, sealable, leak-proof containers (e.g., 50-mL centrifuge tube, Fisher Scientific Catalog No. 06-443-20, or equivalent)
- Disposable 12″ × 12″ template, 144-inch^2 (1-foot^2 [929 cm^2])
- 1-quart sealable plastic bags
- 1-gallon sealable plastic bags

7.4 Wetting Solutions

The type and the amount of wetting solution that is used to moisten swabs, sponge sticks or wipes is based on several considerations, including the target microbiological agent(s) and the presence of disinfecting agent residual. Typical wetting solutions include wetting agents and neutralizing buffers (7.4.1). Note: Consult the SAP and analyzing laboratory to determine the appropriate type and amount wetting solution that will be used during a specific sampling event.

7.4.1 Wetting Agents and Neutralizing Buffers

- Phosphate buffered saline (PBS)
- Sterile water
- Butterfield's buffer with 0.02% Tween® 80 neutralizes phenolic compounds and acts as a surfactant
- PBS, pH 7.2 with 0.02% Tween® 80 neutralizes phenolic compounds and acts as a surfactant
- Neutralizing buffer (e.g., Hardy Diagnostics Catalog No. K105, or equivalent), neutralizes quaternary ammonium and chlorine compounds
- Dey Engley neutralizing broth (e.g., Hardy Diagnostics Catalog No. K108, or equivalent) neutralizes chlorine compounds and iodine, but may encourage growth during transport
- Letheen broth (e.g., Hardy Diagnostics Catalog No. K105, or equivalent) neutralizes quaternary ammonium compounds, but may encourage growth during transport

7.5 General Supplies

- New, clean powder-free nitrile gloves (Note: For sample collection, the sample collector uses two pairs of sterile gloves for each sample collected. Because the outer pair of gloves comes into direct contact with the sampling media, this pair is changed between each sample collected and, as needed, during collection.)
- PPE, as required by the SAP and HASP
- Pre-printed labels for sample containers (see Section 6.2)
- Permanent marker(s) and indelible ink pens
- Disinfectant wipes (e.g., Dispatch® wipes, Catalog No. 69150, or equivalent)
- Masking tape
- 1-gallon sealable plastic bag containing additional disposable templates specified for the collection device (approximately 10% more than needed for sampling event)
- Sample documentation materials (e.g., digital camera, electronic tablet, forms, and/or logbook [Section 6.0])
- Custody seals (see Section 6.5)
- 1-gallon sealable plastic bags (for contaminated equipment)
- Sealable waste containers (e.g., 5-gallon or 20-gallon buckets with lids)

7.6 Sample Transport Containers and Packing Materials

- Transport container – Rigid, insulated cooler able to withstand an internal pressure of 14 pounds per square inch (psi), with a secure, sealable lid. Capable of 1) surviving impacts without being compromised or damaged, and 2) containing and maintaining ice packs (See Section 11.2.2)
- Durable absorbent packing material (See Section 11.2)
- Self-contained ice or cold packs
- Sealing tape
- Custody seals (see Section 6.5)
- Shipping documentation (see Section 11.0)
- COC forms (see Section 6.4)

8.0 Preparation for Sample Collection

Adequate and appropriate preparation for sample collection is critical to ensuring that representative samples are collected properly and as needed to meet analytical requirements, as well as ensuring the safety of sampling personnel, transporters, and laboratory technicians. This section summarizes several of the activities that are completed prior to initiating the sampling steps described in Section 10.0, including sampling teams (Section 8.1), use of techniques to minimize cross contamination (Section 8.2), and preparation of sampling kits (Section 8.3). Sampling personnel should work closely with the incident commander or site/project managers to ensure that sampling activities are conducted in accordance with the SAP.

8.1 Sampling Teams

Any sampling effort requiring the collection of multiple samples, particularly those involving hazardous conditions and/or collection of samples containing pathogens, should involve a sampling team consisting of at least two personnel. Additional personnel may be required for large-scale sampling efforts or when site-specific hazards may be encountered. Individual team members are trained to assume specific activities or duties related to the sampling effort. Depending on the size of the sampling event and the number of samples required, a three-person sampling team consisting of a *collector*, a *supplier*, and a *support person*, is recommended. While not as optimal as a three-person team, a two-person sampling team could be acceptable in some situations with the supplier conducting the duties of the support person.

- **Collector** - handling the sample collection devices and materials and collecting the sample.
- **Supplier** - providing the collector with devices, materials and solutions needed to collect the sample and decontaminating sample containers. The supplier does not come into direct contact with any of the materials or solutions that will come into direct contact with the sample.
- **Support Person** - responsible for sample documentation and radio communication.

This team approach can reduce the time required for sample collection and adds an additional layer of quality assurance. Importantly, sampling teams also provide an additional level of safety. Each team member must be trained in the collection of samples using aseptic techniques (see Section 8.2). Prior to initiating sample collection activities, sampling personnel will:

- Review and understand all specifications and requirements that are included in the incident SAP and/or QAPP, HASP and WMP (see Appendix B).
- Communicate with all sampling team members to ensure roles and responsibilities have been established and are understood.
- Sampling teams should have access to a detailed site map to assist in locating sample points.
- Contain (e.g., in an overpack bag) pre-prepared materials (e.g., sampling kits) prior to site entry and until use, to protect from contamination.
- Assemble and don the appropriate PPE prior to site entry, as directed in the HASP. Summary information regarding PPE for use during sample collection following a pathogen contamination incident is provided in Section 3.0.
- Understand site egress procedures, which address decontamination and transfer of sample containers and contained waste in accordance with the SAP and HASP.

Sampling personnel are required to decontaminate prior to exiting the contaminated area (hot zone), as instructed in the HASP, to ensure contamination is not spread outside the area. A prescribed level of

personal monitoring might also be required. For additional personnel decontamination information, see EPA's Decontamination Line Protocol Evaluation for Biological Contamination Incidents Assessment and Evaluation Report (U.S. EPA 2015).

8.2 Techniques to Minimize Potential Cross Contamination

The use of clean and dedicated sampling devices, and appropriate PPE helps to prevent contamination during sample collection. As sources of contamination may or may not be obvious, inclusion of the appropriate field quality control (QC) samples, as described in Section 9.0, can help identify the presence and sources of contamination. Cross contamination between samples can usually be avoided by adherence to techniques to minimize potential cross contamination and changing gloves between samples, and by avoiding contact between sampling equipment and contaminated surfaces. Sampling personnel receive training in these techniques prior to collecting samples. To limit possible contamination, templates should only be taped to the surface if necessary and should remain in place after sampling (Calfee et al. 2016).

Techniques should be used to collect samples to reduce exposure risk to sampling personnel, contaminating the samples, or spreading contaminants in the environment. The use of these techniques is the first and most important step in ensuring consistent and accurate sampling results. The following practices can be used as a guideline:

- Minimize the amount of time sample containers are open.
- Hold open containers away from sources of contamination (e.g., blowing air, other possibly contaminated objects).
- Do not touch the inside of sample containers or caps.
- Once a container is filled, do not touch the contents.
- Work as quickly as possible, without compromising technique.
- Change gloves as prescribed by the SAP and/or HASP, using appropriate doffing/donning procedures.
- Avoid touching areas of the collection device that come into contact with surfaces or that concentrate contaminants.
- Avoid contact between the sampling equipment and contaminated surfaces.

8.3 Sampling Kits

It is essential that sampling personnel use pre-prepared sampling kits for sample collection, and that these kits be properly equipped, maintained, and organized before deployment of sampling teams. Information regarding the equipment and materials included in these kits is provided in Sections 7.2 – 7.4. However, sampling personnel can consult with the incident commander or site/project managers and the SAP to determine what equipment and materials will be required.

8.3.1 Swab Sampling Kit Assembly

Sampling kit assembly is conducted in a controlled clean area, preferably in a laboratory or similar controlled location, using new powder-free disposable nitrile gloves. Once prepared, the kits are stored in a clean and dry location prior to use. A unique sampling kit is required for each field and QC sample. Prior to sample collection, sampling personnel will check sampling kits to confirm they are complete.

- Don new gloves prior to kit assembly.
- Dispense wetting agent or neutralization buffer into a sterile 2-mL vial.
- Label a sterile, sealable, leak-proof 15-mL centrifuge tube unique sample ID.

- Label sealable 4″× 6″, 1-quart, and 1-gallon plastic bags with the same unique sample ID.
- Place the following into a sealable, pre-labeled 1-quart bag: sterile macrofoam swab, pre-labeled 15-mL centrifuge tube, and 4″× 6″ and 1-quart pre-labeled sealable plastic bags.
- Place the 1-quart bag, 2″ × 2″ template, 2-mL vial of wetting agent or neutralizing buffer, and sterile scissors in a pre-labeled 1-gallon plastic overpack bag; and seal the overpack bag.

Swab Sampling Kit Check List

- Sterile macrofoam swabs
- 2 mL vial of wetting agent or neutralizing buffer
- 2″ × 2″ template
- Labeled sterile 15-mL centrifuge tube
- Labeled sealable 1-quart sealable plastic bag
- Labeled sealable 1-gallon plastic overpack bag
- Sterile scissors

8.3.2 Sponge Stick Sampling Kit Assembly

Sampling kit assembly be conducted in a controlled clean area, preferably in a laboratory or similar controlled location, using new powder-free disposable nitrile gloves. Once prepared, the kits are stored in a clean and dry location prior to use. A unique sampling kit is required for each field and QC sample. Prior to sample collection, sampling personnel will check sampling kits to confirm they are complete.

- Don new gloves prior to kit assembly.
- Label sealable 1-quart and 1-gallon plastic bags with the same unique sample ID.
- Label a sterile 4-ounce screw-cap specimen cup.
- Place the following into a sealable, pre-labeled 1-gallon bag: pre-packaged sterile pre-moistened sponge stick, folded 10″ × 10″ template, pre-labeled sterile 4-ounce specimen cup, a 1-quart pre-labeled sealable plastic bag, and 1-gallon bags.

Sponge Stick Sampling Kit Check List (see Figure 8.1)

- Sterile sponge stick
- Disposable 10″ × 10″ template for non-porous surfaces
- 5-mL individually wrapped sterile pipettes
- Labeled 4-ounce sterile screw-cap specimen container (tapered-side specimen cups are preferred over straight-side specimen cups to ease in breaking of sponge-stick head from handle if 3M™ sponge-sticks are used [Calfee et al., 2016])
- Labeled sealable 1-quart plastic bag
- Labeled sealable 1-gallon plastic overpack bag
- 1-gallon plastic bags

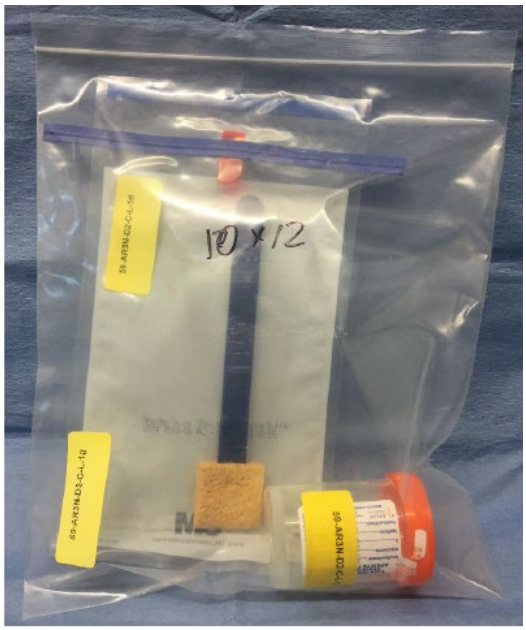

Figure 8.1. Sponge Stick Sampling Kit
[Note: Sampling template not shown]

8.3.3 Gauze Wipe Sampling Kit Assembly

Sampling kit assembly is conducted in a controlled, clean area, preferably in a laboratory or similar controlled location, using new powder-free disposable nitrile gloves. Once prepared, the kits are stored in a clean and dry location prior to use. A unique sampling kit is required for each field and QC sample. Sample kits containing wipes, will be prepared with either wipes that are pre-moistened during sample kit preparation or dry wipes with a wetting solution that can be applied in the field. **Note**: It is recommended that pre-moistened wipes are provided in the sampling kit(s) and building of pre-wetted wipes will require additional time for building the kit. Prior to sample collection, sampling personnel check sampling kits to confirm they are complete.

- Don new gloves prior to kit assembly.
- Pre-moistened Wipes: Gauze often comes two to a pack. Either apply 5 mL of wetting agent or neutralizing buffer to both wipes in its original packaging and discard one gauze OR discard one gauze and add 2.5 mL wetting agent or neutralizing buffer to the remaining gauze (preferred). Place the moistened wipe in a sterile 50-mL screw-cap centrifuge tube and place the tube in a 4″× 6″ plastic sealable bag.
- Dry Wipes (not preferred as it is easier for the samplers to handle pre-wetted wipes than preparing them in the field): Dispense 10 mL of wetting agent or neutralizing buffer into a sterile 15-mL screw-cap tube. Note: Wetting agent or neutralization buffer will be added to the wipe onsite.
- Label a sterile, sealable, leak-proof 50-mL centrifuge tube with the unique sample ID.
- Label sealable a 4″× 6″, 1-quart and 1-gallon plastic bag with the same unique sample ID.
- Place the 50-mL centrifuge tube into a sealable, pre-labeled 4″× 6″ bag.
- Place the following into a sealable, pre-labeled 1-quart bag: gauze wipe (dry or pre-moistened), 10 mL of wetting agent or neutralization buffer if wetting onsite, bagged pre-labeled 50-mL centrifuge tube and a 1-quart pre-labeled sealable plastic bag.

- Place the wipe kit bag, folded 10″ × 10″ inch template, in a pre-labeled 1-gallon plastic overpack bag and seal the overpack bag.

Gauze Wipe Sampling Kit Check List

• Sterile gauze wipe • 10-mL of sterile wetting or neutralization buffer (if wipes are not pre-moistened) • Sterile 5-mL pipettes • 12” x 12” template • Labeled sterile 50-mL centrifuge tube • Labeled sealable 1-quart sealable plastic bag • Labeled sealable 1-gallon plastic overpack bag

8.3.4 Supplemental Supplies and Materials

In addition to the sampling kits, sampling personnel will ensure they have all the additional materials, supplies and equipment needed for sample collection, decontamination, documentation, and packaging activities. Materials needed for documentation of activities, sample tracking and sample packaging accompany the sampling kits. At a minimum, the following should be made available to sampling teams:

- Pre-assembled sampling kit (one per sample, see Sections 8.3.1- 8.3.3)
- PPE (e.g., protective clothing, gloves) as required by the SAP and HASP
- Site maps
- Sample preservation (e.g., ice packs)
- Sample documentation (see Section 6.0)
- Sample packaging supplies (e.g., containers, clear sealing tape, custody seals)
- Decontamination materials (see Section 7.4)
- Extra gloves and template(s).

8.4 Decontamination

Decontamination of the primary sample receptacle using wipes should be completed prior to removal from the exclusion zone to minimize contaminant transfer (Calfee 2016; CDC 2012). While it is outside the scope of this document to provide full instructions on decontamination of sampling equipment, general information is provided. Unless determined to be free of contamination, materials used for decontamination must be collected as waste and removed from the sampling site for proper disposal. 5- or 20-gallon buckets with lids can be used for waste containment. Drums or large garbage cans can be used to contain contaminated PPE, accumulated wastes, containers or equipment. The sample outer packaging should be decontaminated upon arrival at the receiving laboratory. Specific procedures for waste management are included in the WMP.

8.5 Media Blanks

In addition to field blanks and trip blanks (which accompany sampling teams in the field and are used to evaluate potential contamination introduced during sample collection), media blanks are unexposed (e.g., unopened) sampling media to confirm sterility (see Section 9.3). These blanks are tested prior to preparing sampling kits to ensure they are sterile. The blanks consist of two unopened sampling devices (e.g., swab, sponge stick, or gauze wipe) per lot used and two unopened, unused samples of the wetting solution (if not using pre-moistened media). The media blanks are provided to the processing laboratory to confirm sterility.

9.0 Quality Control Samples

Additional samples are collected to assess the validity of the analytical results, as well as possible analytical interferences, contamination, and sample integrity (QC). For samples collected using swabs, sponge sticks or wipes, QC samples typically consist of field blanks, trip blanks and media blanks. The sampling task leader or on scene coordinator should consult with the laboratory or refer to the SAP to determine the type and number (or frequency) of QC samples that will be collected.

Results of QC samples can be used to provide information regarding the accuracy of both the sampling and analytical procedures. For this reason, QC samples are often submitted blind to the laboratory to increase objectivity (i.e., sample documentation received by the laboratory does not identify which QC samples correspond to which field sample). Sampling personnel should refer to the SAP to determine how all samples (field and QC) are to be identified and labeled for transport to the laboratory.

9.1 Field Blanks

Field blanks are used to monitor contamination that may be introduced into samples during sample collection. If required, field blanks are prepared at the sample collection site prior to sample collection, then transported to the laboratory along with the field samples for analysis. Field blanks for surface sampling are prepared by placing an unused swab, sponge stick or wipe, of the same material and wetting agent or neutralizing buffer solution used to collect the sample, into a primary sample container. The field blank is exposed to the on-site field conditions but is not used for sample collection.

9.2 Trip Blanks

Trip blanks are used to monitor contamination that might be introduced into samples during handling and transport. Trip blanks are prepared prior to going into the field, taken to the sampling site, and shipped back to the laboratory, unused and unopened, with the samples. Unlike field blanks, the trip blanks are not exposed to field conditions. At no time after their preparation are the sample containers (e.g., plastic bags, sterile containers) opened before they reach the laboratory. **Note**: In some cases, a trip blank might also be used as a media blank (Section 9.3) by the laboratory.

9.3 Media Blanks

Media blanks are used to assess the sterility of a batch of swabs, sponge sticks and/or wipes that will be used for sample collection. These blanks also include samples of the wetting agent or neutralizing buffer that will be used. If required, these blanks are provided to the laboratory, unopened as provided by the manufacturer (see Section 8.5). The blanks are shipped directly to the laboratory and not taken into the field.

10.0 Sample Collection

This section outlines instructions specific to the collection of representative environmental samples from non-porous surfaces to be analyzed for pathogens during site characterization and post-decontamination sampling following a contamination incident involving a pathogen. The sampling techniques provided should not be considered all-inclusive of the techniques that exist but might be more commonly used for remediation during a contamination incident in which EPA is responsible for sample collection. Prior to field sampling, all sampling personnel should be familiar with the incident-specific SAP, HASP and WMP, in addition to the following information:

- Health and safety (Section 3.0)
- Waste management (Section 5.0)
- Sample documentation (Section 6.0)
- Equipment and supplies (Section 7.0)
- Sample collection preparation (Section 8.0)
- QC requirements (Section 9.0)
- Packaging and transport (Section 11.0)

Sampling personnel also must understand their role(s) and responsibilities regarding sample collection and practice the sampling activities as a team to ensure consistent and efficient collection of representative samples while taking into account the pattern and duration of sampling. As noted in Section 8.1, in most cases, a three-person sampling team is recommended, consisting of a collector, a supplier and a support person. The number of individuals needed, however, will depend on the size of the sampling event and number of samples required.

In addition, sampling personnel should be aware of any specific laboratory sample acceptance requirements (e.g., appropriate primary and secondary containers, preservation, holding time, integrity) and ensure that samples are collected and handled in accordance with these requirements. All requirements should be detailed in the incident-specific SAP. However, if there is any uncertainty regarding sampling activities, sampling personnel should contact the incident commander or site/project managers for clarification.

Table 10-1 provides a summary of the sample collection approaches described in this section.

Table 10-1. Summary of Sampling Approaches for Collection of Pathogens from Non-Porous Surfaces

Sampling Approach	Pathogen Type	Approach	Section Reference
Macrofoam Swabs	All	Swab 4-inch^2 (26-cm^2) non-porous surface area with sterile, moistened macrofoam swab	10.2
Cellulose Sponge Sticks	All	Wipe 100-inch^2 (645-cm^2) non-porous surface area with sterile, moistened cellulose sponge stick	10.3
Gauze Wipes	All	Wipe 144-inch^2 (1-foot^2 [929-cm^2]) non-porous surface area with sterile, moistened gauze wipe	10.4

10.1 Materials, Supplies, and Equipment

In addition to being familiar with the documentation and information noted above, sampling teams must have access to all necessary sampling equipment prior to entering the area of

contamination and initiating sample collection activities. Required equipment and supplies include, but are not limited to:

- Appropriate PPE (Section 3.0)
- Sampling kits (Section 8.3.1)
- Sample documentation (Section 6.3)
- Decontamination supplies (Section 7.5)
- Sample transport containers and packing materials (Section 7.6)

Sampling personnel must ensure that the sampling kits and documentation are complete and specific for the sample(s) that are to be collected. Note: New gloves and a sampling kit are required for each field and QC sample collected. If multiple samples are to be collected, avoid cross-contamination by changing gloves between samples. Multiple swabs, sponge-sticks or wipes can be used to cover larger or more complex surface areas as necessary. In addition, composite samples also can be collected by applying the instructions below, using the same swab, sponge- stick, or wipe for collection from multiple areas.

10.2 Swab Sampling

Swabs are typically used to collect particulates from relatively small non-porous surface areas, as well as from complex surfaces such as crevices, corners, and other hard-to-reach areas. The sampling approach described in this section is adapted from CDC protocols for swab sampling for *B. anthracis* spores (CDC 2012). See also the CDC/NIOSH video "Anthrax surface sampling: how to sample with macrofoam swabs on nonporous surfaces" (CDC, NIOSH 2015a; last accessed 02/26/2021).

Macrofoam swabs	Used to collect samples from relatively small (4-inch^2 [26-cm^2]) and complex or irregular non-porous surface areas

I. Preparation and Setup

Supplier and Collector:	Don or put on new, clean sampling gloves (Note: the support person also dons or put on new, clean gloves if they accidently touch the sample or contaminated area).
Supplier:	Open the sample collection bin and remove swab sampling kit.
Supplier:	Hold the sampling kit label out for support person to document the sample ID and related information.
Support Person:	Scan the bar code, record the sample ID, or radio the ID information to a data recorder in the support zone.
Supplier:	Open the sample kit outer bag and remove the 2″× 2″ sampling template. Hand the template to the collector.
Collector:	Gently place the sampling template on the area to be sampled, being careful to minimize disruption of any particulates present (Figure 10.2-1).

Figure 10.2-1. Placing a Template Prior to Swab Sampling

Support Person: If required, photograph the sampled area, and/or draw a map of the location in the logbook.

II. Collection

Supplier: Remove the bag containing the prepackaged swab. Open the bag and move the handle of the swab toward the opening of the bag without touching the swab. Hold the bag open so that the collector can remove the swab.

Collector: Grasp the swab at the top of its handle and carefully remove the swab from the bag without touching the bag or the tip of swab.

Supplier: Discard the swab packaging as waste once the collector has removed the swab.

Supplier: Move the 2-mL vial containing the wetting solution to the top of its containment bag and open the vial for the collector to moisten the swab.

Collector: Moisten the swab by gently dipping it in the wetting solution. Remove excess liquid by pressing the head of the swab on the inside surface of the vial.

Supplier: Close the vial and discard the remaining solution and vial as waste.

Collector: Use the moistened swab to collect a sample from the area inside the template.

- Swab the surface area horizontally, using overlapping S-strokes rolling motion to cover the entire area within the template (Figure 10.2-2) with a consistent amount of gentle but firm pressure. The strokes should overlap by 50% with each previous pass, and therefore covering the entire surface area of the template at least twice with the swab.

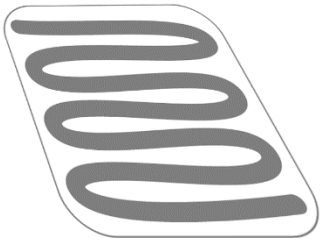

Figure 10.2-2. Horizontal Sampling

- Turn the swab over and swab the entire surface area within the template, using vertical S-strokes (Figure 10.2-3) and a consistent amount of gentle but firm pressure.

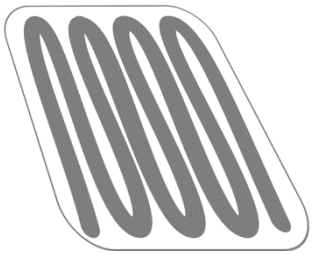

Figure 10.2-3. Vertical Sampling

- Turn the swab on its side and swab the entire surface area within the template, using diagonal S-strokes (Figure 10.2-4) and a consistent amount of gentle but firm pressure.

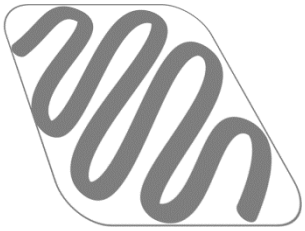

Figure 10.2-4. Diagonal Sampling

III. After Collection of Each Sample

Supplier: Open the 4″ x 6″ bag containing the sterile 15-mL centrifuge tube. Without touching the tube, move the tube to the opening of the bag and unscrew the cap. Hold the tube so that the collector can place the sample into the tube.

Collector: Carefully place the head of the swab directly into the tube (Figure 10.2-5). Bend the handle to break off the head of the swab into the tube. The end of the handle should not touch the inside of the tube. **(Note**: The head of the swab should break off easily with bending. Sterile scissors also are included with the general supplies and can be used if needed to facilitate removal.)

Collector: Leave the sampling template in place and dispose of the swab handle as waste.

Figure 10.2-5. Placing the Swab into the Centrifuge Tube

Supplier: Securely tighten the lid of the tube and place the tube in a clean 4″ x 6″ sealable plastic bag. Remove excess air, seal the bag, and place it in a larger, clean 1-quart sealable bag.

Supplier: Decontaminate the outer surface of the outer bag.

Supplier: Apply a custody seal over the sealed opening of the outer bag.

Supplier: Place the double-bagged sample into the sample collection bin for transfer to sample packaging and transport (see Section 11.0).

Support Person: Record the size of the surface area sampled, and complete sample documentation (see Section 6.0).

Collector: Ensure that all solid and liquid waste is contained (e.g., solid wastes in a plastic bag and liquid wastes in a durable sealable container) and removed from the sampling site for proper decontamination and disposal.

All Personnel: Doff sampling gloves prior to moving to the next location.

10.2.1 QC Samples

QC samples that might be requested for swab sampling include field blanks and trip blanks. The number, type and locations of QC samples required will be specified in the SAP.

- Field blanks (Section 9.1) are prepared and handled as described above with the exception that the swab does not contact any potentially contaminated surface.
- Trip blanks (Section 9.2) are supplied as unopened/unused sampling kit assemblies and are transferred and transported along with the field samples.

10.3 Sponge Sticks

Instructions for collecting particulate samples using cellulose sponge sticks are provided below. The approach described is adapted from CDC protocols for sampling for *B. anthracis* spores (CDC 2012). See also the CDC/NIOSH video "Anthrax surface sampling: how to sample with cellulose sponges on nonporous surfaces" (CDC, NIOSH 2015b).

Cellulose sponge sticks	Used to collect samples from relatively large (100 inch^2 [645-cm^2]) smooth non-porous surface areas

I. Equipment Preparation and Setup

Supplier and Collector: Don or put on new, clean sampling gloves (Note: the support person also dons or put on new, clean gloves if they accidently touch the sample or contaminated area).

Supplier: Open sample collection bin and remove the 1-gallon bag containing the sampling kit.

Supplier: Hold the kit barcode label out for Support Person to document sample ID and related information.

Support Person: Scan the bar code, record the sample ID, or radio the ID information to a data recorder in the support zone.

Supplier: Open the sample kit outer bag and remove the 10″× 10″ sampling template. Hand the template to the Collector.

Collector: Gently place sampling template on the area to be sampled, being careful to minimize disruption of any settled particulates.

Support Person: If required, photograph the sampled area, and/or draw a map of the location in the logbook.

II. Collection

Supplier: Open the 1-gallon bag and remove the inner bag containing the sterile, pre-moistened sponge-stick.

Supplier: Open the bag containing the sponge stick and position the sponge stick so that the Collector can grasp its handle to remove it from the bag.

Collector: Grasp the top of the handle of the sponge stick to remove it from the bag. Do not touch the sponge stick below the thumb stop.

Collector: Wipe the surface to be sampled using the moistened sponge stick by laying the widest part of the sponge on the surface, leaving the leading edge slightly lifted.

- Wipe the surface area horizontally, using overlapping S-strokes to cover the entire area within the template (Figure 10.3-1) with a consistent amount of gentle but firm pressure.

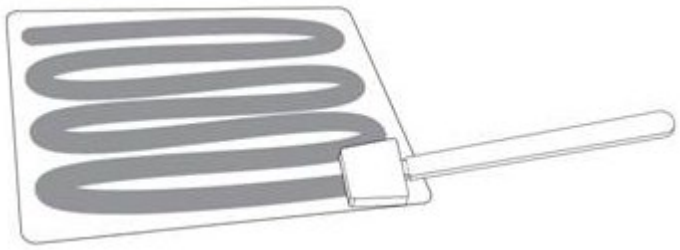

Figure 10.3-1. Horizontal Sampling

- Turn the sponge over, and wipe the same area again using vertical "S" strokes (Figure 10.3-2).

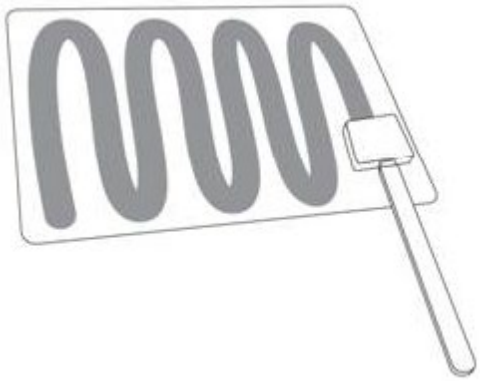

Figure 10.3-2. Vertical Sampling

- Use the edges of the sponge (narrow sides) to wipe the same area using diagonal "S" strokes (Figure 10.3-3).

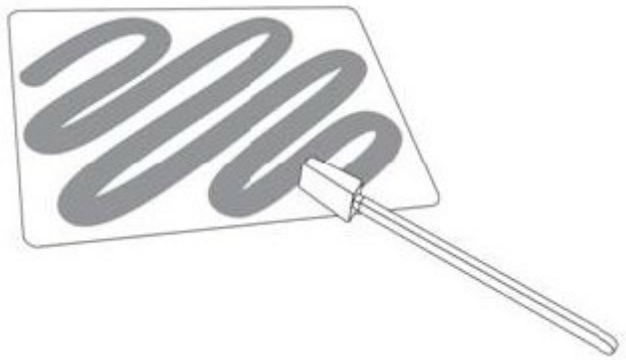

Figure 10.3-3. Diagonal Sampling

- Use the tip of the sponge to wipe the perimeter of the sampling area (Figure 10.3-4).

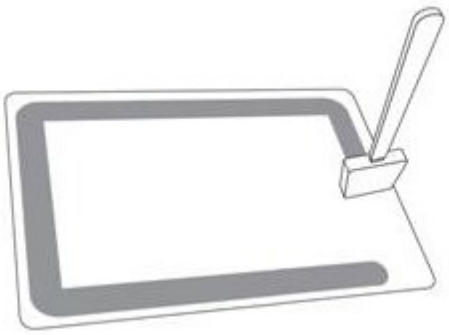

Figure 10.3-4. Perimeter Sampling

III. After Collection of Each Sample

Supplier:	Remove the 4-ounce sterile specimen cup from the 1-gallon bag. Open and hold out the open, sterile 4-ounce specimen cup for collector.
Collector:	Place the head of the sponge directly into the sterile, 4-ounce sample container.
Collector:	Depending on the type of sponge-stick used, either the handle may need to be bent by rocking it back and forth until the handle breaks off the head of the sponge or the head of the sponge is detached from the stick. The end of the sponge handle should not touch the inside of the sample container.
Supplier:	Securely seal the container and place it in the pre-labeled 1-quart resealable plastic bag. Seal the bag. Note: If needed, excess air can be removed from resealable plastic bags to increase the number of samples that can be packaged in one secondary sample container.
Supplier:	Decontaminate the outer surface of the bag.
Supplier:	Open the pre-labeled 1-gallon bag and place the 1-quart bag into it.
Supplier:	Seal the 1-gallon bag and decontaminate the outer surface.
Supplier:	Apply a custody seal over the opening of the sealed 1-gallon bag.
Supplier:	Place the double-bagged sample into the sample collection bin for transfer to sample packaging and transport (see Section 11.0).
Support Person:	Record the size of the surface area sampled, and complete sample documentation (see Section 6.3).

Collector: Leave the sampling template in place. Ensure that all solid and liquid waste is contained (e.g., solid wastes in a plastic bag and liquid wastes in a durable sealable container) and removed from sampling site for proper decontamination and disposal.

All Personnel: Doff sampling gloves prior to moving to the next location.

10.3.1 QC Samples

QC samples that might be requested for surface sampling include field blanks and trip blanks. The number, type and locations of QC samples required will be specified in the SAP.

- Field blanks (Section 9.1) are prepared and handled as described above for field samples.
- Trip blanks (Section 9.2) are supplied as unopened/unused sampling kit assemblies and are transferred and transported along with the field samples.

10.4 Gauze Wipes

Instructions for collection of particulate samples using gauze wipes are provided in this section. The sampling approach described is adapted from CDC protocols for wipe sampling for *B. anthracis* spores (CDC 2012).

Gauze wipes	Used to collect samples from relatively large (144 inch^2 [1 foot^2 or 929-cm^2]) flat, non-porous surface areas

I. Equipment Preparation and Setup

As with collection of samples using swabs and sponge sticks, multiple wipes can be used to cover larger surface areas as needed. Composite samples also can be collected by applying the instructions below, using the same wipe for collection from multiple areas.

Supplier and Collector: Don or put on new, clean sampling gloves (Note: the support person also dons or put on new, clean gloves if they accidently touch the sample or contaminated area).

Supplier: Open the sample collection bin and remove the wipe sampling kit.

Supplier: Hold the kit barcode label out for Support Person to document sample ID and related information.

Support Person: Scan the bar code, record the sample ID, or radio the ID information to a data recorder in the support zone.

Supplier: Open the sample kit outer bag and remove the 12″ × 12″ sampling template. Hand the template to the Collector.

Collector: Gently place the sampling template on the area to be sampled, being careful to minimize disruption of any settled particulates. Note: If the surface to be sampled is touched while placing the template, replace outer gloves with a new pair of sterile gloves before initiating sample collection.

Support Person: If required, photograph the sampling area, and/or draw a map of the location in the logbook.

Supplier: Remove the gauze wipe package from the sample kit.

If wipes have not been pre-moistened:

Supplier: Partially peel open a sterile gauze wipe package, carefully exposing but not touching the wipe.

Supplier: Measure 5 mL of wetting solution from the 10-mL container using a disposable pipette and apply it to the wipe in its original packaging. [Note: Moistened wipes should not be dripping. Unused wetting agent or neutralizing solution and the pipette used should be discarded.]

Collector: Remove outer gloves and don new pair of sterile gloves.

Collector: Remove one of the moistened wipes (if two per package), squeeze wipe to remove excess liquid and dispose of or retain the other wipe as a field blank (Section 10.4.1).

Collector: Completely unfold the moistened wipe, and then fold it in half.

If wipes have been pre-moistened:

Supplier: Open the outer sample kit bag and move the 50-mL tube containing the pre-moistened wipe to the top of the bag.

Supplier: Holding the tube in the bag, flick downward so wipe slides to cap.

Supplier: Carefully open cap (wipe should be stuck to cap). Present the cap to the Collector, being careful not to drop the wipe.

Collector: Carefully remove the wipe, taking care not to touch the cap or the tube.

Supplier: Place the cap back on the tube and place the tube back into the sample kit bag.

II. Collection

Collector: Wipe the surface to be sampled, holding fingertips together and applying gentle but firm pressure.

- Wipe the surface area horizontally, using overlapping S-strokes to cover the entire area within the template (Figure 10.4-1) with a consistent amount of gentle but firm pressure.

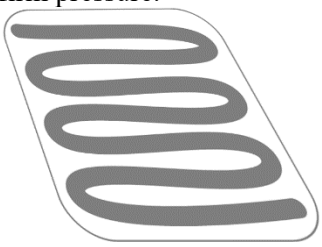

Figure 10.4-1. Horizontal Surface Wipe Sampling

- Fold the wipe in half (exposed side of the gauze wipe in) and wipe the same area again using vertical 'S' strokes as shown in Figure 10.4-2 and 10.4-3.

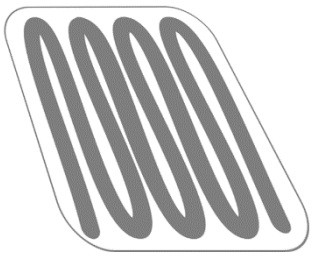

Figure 10.4-2. Vertical Surface Wipe Sampling

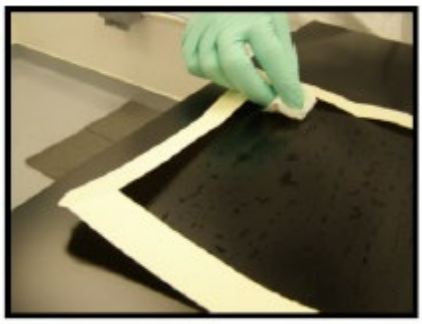

Figure 10.4-3. Wipe Sampling Using a Template

- Fold the exposed side of the gauze in half once more (exposed side of the gauze wipe in), and wipe the same area using diagonal 'S' strokes as shown in Figure 10.4-4.

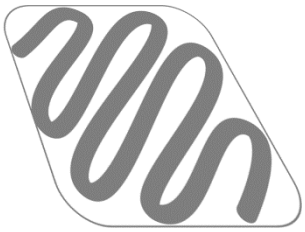

Figure 10.4-4. Diagnol Surface Wipe Sampling

III. After Collection of Each Sample

Supplier: After the sample has been collected, move the 50-mL centrifuge tube to the end of the sample kit bag and unscrew its cap.

Collector: Fold the wipe in half again (exposed side in) and place it into the tube, taking care not to touch the inside of the tube with anything other than the wipe.

Supplier: Securely tighten the cap of the tube and place it back into 1-quart sample kit bag. Removed excess air, seal the bag and decontaminate its outer surface.

Supplier: Place the decontaminated bag containing the sample into a clean 1-gallon bag and seal the 1-gallon bag.

Supplier: Decontaminate the outer surface of the outer bag.

Supplier: Apply a custody seal over the sealed opening of the outer bag.

Supplier: Place the double-bagged sample into the sample collection bin for transfer to sample packaging and transport (see Section 11.0).

Support Person: Record the size of the surface area sampled and complete sample documentation (see Section 6.3).

Collector: Leave the sampling template in place and gather any additional contaminated materials or supplies for proper disposal.

Collector: Ensure that all solid and liquid waste is contained (e.g., solid wastes in a plastic bag and liquid wastes in a durable sealable container) and removed from sampling site for proper decontamination and disposal.

All Personnel: Doff sampling gloves prior to moving to the next location.

10.4.1 QC Sampling

QC samples that might be requested for surface sampling include field blanks and trip blanks. The number, type and locations of QC samples required will be specified in the SAP.

- Field blanks (Section 9.1) are prepared and handled as described above for field samples.
- Trip blanks (Section 9.2) are supplied as unopened/unused sampling kit assemblies and are transferred and transported along with the field samples.

11.0 Sample Packaging and Transport

This section provides general information on packaging and preparing samples for transport and to help ensure that sample integrity is maintained during these processes. Information regarding packaging for Select Agents is covered in section 11.3. Since pathogenic samples often degrade quickly over time, it is imperative to have procedures for sample storage (short and long term), packaging, and transport that are efficient and preserve sample integrity. Laboratories should receive samples that are properly preserved, packaged, and received within the holding time requirements needed to support analysis, and receiving laboratories can and will reject samples if sample packaging and transport requirements are not met. Samples also must be accompanied by the appropriate documentation. Information regarding packaging for select agents is not covered in this document and can be found in CDC's Federal Select Agent Program Guidance on the Shipment and Receipt of Packages with Select Agents and Toxins (CDC 2014).

11.1 Sample Holding Time and Temperature

Many variables go into making decisions regarding sample storage temperatures and holding time. Caution should be taken when applying requirements that were developed for other sampling media or organisms. The best storage temperature for a given sample often varies depending on the type of biological organism, the sample matrix, the sample's intended use, and how long the sample will be stored. When storing samples, it is also important to consider the target microbiological agent's molecular structure such as nucleic acids, proteins, etc. (Holland *et al*., 2003; Budowle *et al*., 2006; NRC, 2014; Shabihkhani *et al*., 2014) and the degree of integrity required for analysis. Some general considerations for holding times and temperatures are included in **Table 11-1**, and Sections 11.1.1 (Sample Holding Times) and 11.1.2 (Temperature) below:

Table 11-1. Transport Conditions and Holding Times

Microbiological Agent	Storage Temperature	Recommended Holding Time	Other Considerations
Bacteria	2-8 ºC; do not freeze	Vegetative bacterial samples should be analyzed as soon as possible. (Maximum holding time of 24 – 48 hours.) Samples containing bacterial spores should be analyzed within 48 hours (CDC, 2012).	• Samples should not come in direct contact with ice or ice packs • Ice packs should be placed outside the secondary receptable • Consult the SAP for temperature monitoring or other transport requirements designated by the receiving laboratory
Viruses	2-8 ºC; do not freeze	Samples should be analyzed as soon as possible. (Maximum holding time of 24 – 72 hours.)	• Samples should not come in direct contact with ice or ice packs • Ice packs should be placed outside the secondary receptable. • Consult the SAP for temperature monitoring or other transport requirements designated by the receiving laboratory
Protozoa/ Helminths	2-8 ºC; do not freeze	Samples should be analyzed as soon as possible. (Maximum holding time of 96 hours.)	• Samples should not come in direct contact with ice or ice packs • Ice packs should be placed outside the secondary receptable • Consult the SAP for temperature monitoring or other transport requirements designated by the receiving laboratory
*Vibrio cholera**	Room temperature	Samples should be analyzed as soon as possible.	• Consult the SAP for temperature monitoring or other transport requirements designated by the receiving laboratory

*****Note: Samples to be analyzed for *Vibrio cholerae* are kept at room temperature and must not be cooled.

11.1.1 Sample Holding Time

Maximum sample holding time, a critical aspect to consider when making decisions regarding sample packaging and transport, is the sum of the time between sample collection and receipt at the laboratory and the time between sample receipt at the laboratory and sample analysis. In all cases, samples should be transported to the laboratory and analyzed as quickly as possible following collection, in a manner that stabilizes the sample and minimizes the loss of viability.

11.1.2 Sample Temperature

In all cases, sample collectors should consult the SAP for information regarding requirements for sample preservation (temperature) and transport conditions. A receiving laboratory may require temperature blanks or temperature monitoring devices to be placed in transport coolers to evaluate whether an appropriate temperature has been maintained throughout transport. The procedure to be followed for sample preservation and transport conditions should be resolved with the laboratory prior to initiation of sample collection and included as part of the SAP. If the SAP does not provide the requirements for sample temperature and transport conditions, the sampling team leader should consult with the project team leader or designee who in turn should consult with the laboratory to resolve any questions. In general:

- Samples containing microbiological agents should be stored at 2°C–8°C without freezing prior to processing (CDC, 2012), and analyzed as soon as possible (see Table **11-1**). An exception is made for samples that will be analyzed for *Vibrio cholerae*; these samples should be maintained at room temperature and should not be cooled.

- Samples containing microbiological agents often degrade over time when stored at room temperature, but some samples can lose integrity even at low temperatures if subjected to multiple freeze-thaw cycles. Samples that require low temperature preservation shall be considered acceptable if the arrival temperature of a representative sample container meets the temperature requirement.

11.2 Sample Container Transport and Labeling

Prior to shipping biological samples, the IATA Dangerous Goods Regulations (DGRs) for shipment by air, DOT requirements in 49 CFR Parts 171 through 180 (IATA 2015; U.S. DOT, 2012), and/or the Hazardous Materials Regulations for movement in public right-of-ways within the U.S. (U.S. DOT 2012; U.S. DOT 2020) should be consulted to determine whether the biological samples should be classified as hazardous materials (HAZMATs) and transported as a dangerous goods shipment. IATA and DOT publications are revised frequently, and individuals should consult the most current publications, the Federal Register, and publications of other governing agencies for complete instructions. It is the sample originator's responsibility to ensure adherence to all regulations. Only trained and certified HAZMAT shippers may ship biological agents that have been designated for dangerous goods shipments. Sampling personnel can refer to the following websites for information regarding the shipping of infectious substances and biological agents:

- International Air Transport Association (last accessed 04/29/2021)
- U.S. Department of Transportation (last accessed 04/29/2021)
- American Society for Microbiology (ASM 2018; last accessed 04/29/2021)
- American Biological Safety Association (last accessed 04/29/2021)
- WHO "Guidance on Regulations for the Transport of Infectious Substances 2019–2020" (WHO 2019; last accessed 04/29/2021)

11.3 Sample Packaging

This section provides general guidance on sample packaging; however, level of packaging is dependent on whether samples are considered a dangerous goods shipment or not and the most recent IATA and DOT publications should be consulted. Samples should be shipped with appropriate chain of custody forms and labels. Packaging kits should include inner and outer packaging, coolers, labels, and absorbent material. Battery packs for sampling pumps should be removed from the pump prior to shipment.

> CAUTION: Samples must not be frozen (see Table 11-1). To avoid freezing all or portions of the sample(s), samples should not be packed in direct contact with ice or ice packs.

11.3.1 Primary and Secondary Sample Containment

Following sample collection and prior to leaving the contaminated area, all field and QC samples are packaged in primary and secondary sample containers, as described in Section 10.2. For samples collected into filter cassettes using the procedures described:

- Primary sample containers are clean, leak-proof, and sealable 15- mL polypropylene conical tubes for containment of the filter nozzle (if needed, see Section 8.3.1), and/or 4″× 6″ sealable plastic bags for containment of the filter cassette
- Secondary sample containers are clean sealable plastic bags

Each primary and secondary sample container is sealed and labeled with the Sample ID (see Sections 6.3 and 10.2). Prior to leaving the area of contamination, the outer surface of the outer most container is decontaminated and placed into a clean bag or box for transfer to the transport container packaging area.

11.3.2 Packing Sample Transport Containers

Transport containers must be sufficiently durable and constructed of material that will ensure sample integrity, including maintaining appropriate temperatures. If the proper containers, packing materials, and labels are used incorrectly, damage to the samples can occur and sample integrity could be compromised. If the samples will be shipped by air, the container must be able to withstand an internal pressure of 14 pounds per square inch (psi). Rigid, insulated coolers with secure, sealable lids, that are capable of 1) surviving impacts without being compromised or damaged, and 2) containing and maintaining self-contained ice or cold packs are recommended. Inner containers should be cushioned with enough absorbent material to absorb any fluids (e.g., melted ice).

- Remove the contained sample(s) from the sample transfer bag or box and decontaminate the outside of the secondary sample container(s) using decontamination wipes (Section 8.4).
- Pack secondary sample containers into a transport container with sufficient absorbent packing material to absorb any fluids (e.g., condensation from ice packs) and to ensure they are protected and will not shift during transport.
- Self-contained ice or cold packs are recommended for cooling samples during transport.
- When multiple sample containers are packaged within a single transport container, absorbent packing material absorbent material (such as paper towels or absorbent gel sheets) should be used to separate containers and to ensure there is no contact between the containers.
- Complete a COC form, seal the form in a plastic bag along with other pertinent sample documentation, and adhere the bag to the inside of the transport container lid. Retain a copy of all documentation, including the COC.
- Seal the transport container, and place at least two custody seals on the transport container lid, in a manner such that the container cannot be opened without breaking the seals.

11.3.3 Transport Documents

All sample transport containers should be accompanied by sample documentation, including COC forms and field records (see Section 6.0). To maintain COC, the COC form must be readily accessible when transferring samples from one individual to another. Therefore, COC forms should be placed inside a waterproof self-sealing bag, which is adhered to the inside of the transport container lid. One copy of these forms should be retained by the sampling personnel. If the transport container is being shipped, the shipping receipt should be retained by the sampling personnel for the permanent record. Common carrier documents should be included with each shipment and completed as required by the individual carrier. All packages must securely display the following:

- Sampling contact information, mailing address, and phone number
- Laboratory name(s), mailing address, and phone number
- Quantity and description of contents
- Date of shipment

11.4 Transfer of Custody

An unbroken COC must be maintained for all samples from collection through analysis and archiving. Information that is included in a COC form is discussed in Section 6.4, and an example COC form is provided in Appendix C. Laboratories should be notified in advance of any shipments, and an accurate

description of the contents of the shipment and the expected date of arrival should be included. Once received at the laboratory, each individual releasing and receiving the samples must sign the COC form to provide evidence of the custody transfer. Upon receipt of samples, the laboratory will document the condition of each sample container received and report this information to the analytical services requestor to confirm that the package was not damaged or tampered with during transport.

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Appendix A: Applicable Microbiological Agents

This document is to be used in conjunction with laboratory analysis methods listed in the Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2017 (SAM) (U.S. EPA 2017) which provides a compendium of analytical methods that have been selected specifically for use during environmental response activities. SAM identifies a single selected method or suite of methods for each analyte/sample type. Example SAM bacterial agents can be found in Table A-1 and viruses, protozoa, and helminths can be found in Table A-2.

Table A-1. Example Subset of Bacterial Microbiological Agents

Bacteria	Disease	Typical Exposure Reservoir in United States [1]
Bacillus anthracis	Anthrax	Mammals, humans and soil
Brucella spp.	Brucellosis, Undulant Fever	Animals and by-products (e.g., contaminated milk)
Burkholderia mallei	Glanders	Not known to occur in U.S. Disease primarily affects animals, although exposure in humans can occur.
Burkholderia pseudomallei	Melioidosis	Water and soil
Campylobacter jejuni	Campylobacteriosis	Food and water
Chlamydophila psittaci (formerly known as *Chlamydia psittaci*)	Parrot Fever	Pet birds and by-products (e.g., cage debris)
Coxiella burnetii	Q-Fever	Animals and by-products (e.g., contaminated milk)
Escherichia coli O157:H7	Enterohemorrhagic *E. coli* or EHEC	Animals, humans, soil, water and food
Francisella tularensis	Tularemia, Rabbit Fever	Animals, insects, soil, water and vegetation
Legionella pneumophila	Legionellosis	Water
Leptospira interrogans	Leptospirosis	Animals, soil and water
Listeria monocytogenes	Listeriosis	Food
Non-typhoidal Salmonella (Not applicable to *S. Typhi*)	Salmonellosis	Animals, humans and food
Salmonella enterica serovar Typhi *(S.* Typhi*)*	Typhoid Fever	Humans, food and water
Shigella spp.	Shigellosis	Humans, water and food
Staphylococcus aureus	Staphylococcal Food Poisoning	Animals, humans, soil, water and food
Vibrio cholerae	Cholera	Shellfish, humans, water and food
Yersinia pestis	Plague	Animals and insects

[1] The occurrence and reservoirs listed for the bacteria are those described by CDC, 2009.

Table A-2. Example Subset of Viruses, Protozoa and Helminths

Viruses	
Adenoviruses: Enteric and Non-enteric (A-F)	
Astroviruses	
Caliciviruses: Noroviruses	
Caliciviruses: Sapovirus	
Coronaviruses: Severe Acute Respiratory Syndrome (SARS)-associated Human Coronavirus (HCoV)	
Hepatitis E Virus (HEV)	
Influenza H5N1 virus	
Picornaviruses: Enteroviruses	
Picornaviruses: Hepatitis A Virus (HAV)	
Reoviruses: Rotavirus (Group A)	
Protozoa	**Disease**
Cryptosporidium spp.	Cryptosporidiosis
Entamoeba histolytica	Amebiasis
Giardia spp.	Giardiasis
Naegleria fowleri	Naegleriasis
Toxoplasma gondii	Toxoplasmosis
Helminths	**Disease**
Baylisascaris procyonis	Raccoon roundworm infection

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Appendix B. Supplemental Plans

The sampling strategy developed in support of sample collection activities following a contamination incident needs several site- and incident-specific supplemental documented plans. These documents are consulted to determine which sample collection techniques to use. These supplemental items are necessary to support the sampling strategy and are addressed below.

B.1 Quality Assurance Project Plan (QAPP)

The Quality Assurance Project Plan (QAPP) is a comprehensive document describing in detail the activities that must be implemented to ensure that the results of the data and information collected satisfy the project performance criteria (U.S. EPA 2000). For a specific incident, it is possible that a QAPP will be developed for the overall incident, and then a more detailed SAP could be developed for each specific sampling activity to be conducted. The elements of a QAPP address aspects of project management; quality assurance (QA) and quality control (QC); and data collection, production and use (U.S. EPA 2001). Guidance on the technical requirements of a QAPP is provided in Requirements for Quality Assurance Project Plans EPA QA/R-5 (U.S. EPA 2001) and Guidance on Quality Assurance Project Plans, EPA QA/G-5 (U.S. EPA 2002a), which present advice intended to help prepare a QAPP. At a minimum, QAPPs address the following elements:

- Project Management – key personnel and their roles; organization chart; project description and background; data quality objectives and criteria for measurement data; documentation and records
- Data Generation and Acquisition – sample design, methods, and handling; analytical methods; quality control; instrument and equipment inspection, maintenance, and calibration; data management
- Assessment and Oversight – assessments and response actions; reports to management
- Data Validation and Usability – data review, verification, and validation; verification and validation methods

B.2 Sampling and Analysis Plan (SAP)

For collection of samples, a well-defined and thorough sampling and analysis plan (SAP) needs to be developed and implemented. The SAP is specific for the site being evaluated and outlines the sampling and analysis strategies that should be in place prior to initiating the sample collection. The information included in the SAP provides detailed site-specific instructions and requirements that are used in conjunction with sample collection and analysis. The SAP is important because analytical results can be used by the Incident Command, local health departments, decontamination teams, decision makers and attorneys. For this reason, laboratories performing the analyses should be consulted regarding sample sizes, containers, and shipment when developing the SAP and prior to sampling. The SAP also includes consideration of data quality objectives (DQO), which are used to ensure that collected data are of known and documented quality for their intended use. Information of specific importance to sampling teams includes:

- Types of samples to be collected or measurements to be performed (check with analyzing laboratory to see what can be accepted)
- Target agents and sample types
- Potential interferences, including environmental conditions and weather impacts
- Number of field samples to be collected
- Amount of material to be collected for each sample

- Required sample container size and type
- Sample locations and frequencies
- Sample collection techniques and procedures
- Data quality objectives (DQO's) of the sampling and analysis activities
- Type and frequency of field QC samples to be collected
- QC requirements and measurement quality objectives
- Sample preservation and holding time requirements
- Sample packaging and shipping requirements
- Documentation requirements

Guidance for information to be included when developing a SAP is provided in EPA's Sampling, Laboratory and Data Considerations for Microbial Data Collected in the Field (Silvestri et al. 2018). This document summarizes elements that should be considered when planning, developing and implementing a SAP for microbiological contamination incidents in which the EPA would be responsible for supporting sampling and analysis. The SAP template provided in EPA's Interim Draft Outline: Sampling and Analysis Plan for Environmental Sample Potentially Containing Pathogens, EPA/600/R-17/129 (U.S. EPA 2017a) can be used as a "ready to go" outline for creating a SAP and associated DQOs.

Additional information on sample collection strategies and designs can be found in: Guidance on Choosing a Sampling Design for Environmental Data Collection for use in Developing a Quality Assurance Project Plan, EPA QA/G-5S (EPA, 2002b). Additional information on sample preservation, holding times, and packaging and shipping requirements, are included in EPA's Sample Collection Information Document for Pathogens (Chattopadhyay 2017).

B.3 Health and Safety Plan (HASP)

Safety is a primary consideration for any sampling event. The Health and Safety Plan (HASP) is developed to be specific to a site and incident. Each microbiological agent and contamination incident pose specific health hazards, and an incident-specific HASP must be available to sampling personnel. HASPs will vary depending on the site, the sampling phase (site assessment, remediation or post decontamination), 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. HASPs follow guidelines provided by U.S. Department of Labor Occupational Safety and Health Administration (OSHA) (U.S. DOL 2008). At a minimum, HASPs include instructions and guidelines regarding:

- Names, positions, and contact information of key personnel and health and safety personnel
- Site- or incident-specific risk assessment or job hazard analysis addressing sample collection activities
- Training requirements
- Personal protective equipment (PPE) on site and usage requirements
- Medical screening requirements (maintain confidential documents properly and securely)
- Site or incident control
- Emergency response plan, containing off-site emergency contact information such as local hazardous materials response teams or additional trained rescue personnel (U.S. DOL 2002)
- Entry and egress procedures
- Spill containment
- Personnel decontamination procedures

Personnel safety requirements and considerations for a particular site might extend beyond concerns related to exposure to microbiological agents and can include exposures to physical hazards and chemicals that are toxic, corrosive, emit harmful or explosive vapors, or are incompatible when mixed. General health and safety considerations that should be considered when implementing sample collection described in this document are provided in Section 4.0.

B.4 Analytical Protocols and Laboratories

Analytical protocols describe the methods that will be used in the laboratory to analyze the collected samples. These protocols often include information that to be considered by individuals collecting samples (e.g., the types of QC samples required, sample holding times and conditions, use of dechlorinating or neutralizing agent, and sample sizes). Analytical protocols also often include procedures that might be required to prepare various sample types prior to implementing procedures for microbiological agent detection and measurement. Sample collection described in this document are intended to be used in conjunction with the analytical methods that are included in EPA's Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2017 (U.S. EPA 2017b; last accessed 04/29/2021). Sampling personnel should consult the SAP for specifications on the following sampling requirements which might affect subsequent sample analysis:

- Allowable sample holding times
- Required sample volumes and containers
- Preferred sampling device and collection reagents (e.g., wetting agents, selective media)
- Sample packaging and shipping/delivery requirements
- QC samples
- Sample decontamination procedures
- Sample throughput (number of samples a lab can process per unit time)

B.5 Waste Management Plan (WMP)

A Waste Management Plan (WMP) that outlines waste management requirements, procedures, strategies, and processes from the point of generation to final deposition. Ideally, the WMP should be in place prior to an incident. Ideally, a general WMP will be in place that can be used to prepare an incident-specific WMP. This incident-specific plan addresses federal, state and local waste management requirements for the different waste streams, waste characterization and waste acceptance sampling and analysis, identification of waste management facilities, on-site decontamination of waste, on-site waste management and minimization strategies and tactics,, off-site waste management, waste transportation, health and safety, as well as tracking and reporting of waste sampling results. State and local waste management officials should be contacted as early in the development process as possible. For more information on WMPs, see EPA's Waste Management Benefits, Planning and Mitigation Activities for Homeland Security Incidents website (available at: https://www.epa.gov/homeland-security-waste/waste-management-benefits-planning-and-mitigation-activities-homeland, last accessed 04/29/2021).

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U.S. EPA. (2017b) Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2017. U.S. Environmental Protection Agency: Washington, DC. EPA/600/R-17/356

Appendix C. Example Chain of Custody Form

	EPA	**USEPA** **Sample Traffic Report & Chain of Custody Record**	**Case No.:** **DAS No.:** **SDG No.:** **Page of**

Date Shipped	**Chain of Custody Record:**	**Sample Collector Signature:**	**For Lab Use Only**
Carrier Name	**Relinquished By: (Date/Time)**	**Received By: (Date / Time)**	**Lab Contract No.:**
Airbill:	**1)**		**Unit Price:**
Shipped To:	**2)**		**Transfer To:**
	3)		**Lab Contract No.:**
	4)		**Unit Price:**

Sample Identification Code		**Sample Collector**	**Matrix / Type**	**Volume / Mass**	**Analysis Required**	**Sampling Location / Sample Depth**	**Date / Time**	**Laboratory Sample No.**	**FOR LAB USE ONLY Sample Condition on Receipt**
1									
2									
3									
4									

Additional Sample Collector Signature(s):		**Sample(s) to be used for laboratory QC?**	**Temperature Upon Receipt:**	**Chain of Custody Seal Number:**
			Shipment Iced? (Yes/No)	**Custody Seal Intact? (Yes/No)**

Matrix codes: SO – Soil; DW – Drinking Water; AF – Air Filter; AI – Air Impinger; P – Particulate; WI – Wipe; SW – Swab; ST – Sponge stick; DCW – Decontamination Wastewater; VF – Vacuum Filter

DAS: Delivery as Analytical Services

SDG: Sample Delivery Group

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