Validation of U.S. Environmental Protection Agency Environmental Sampling Techniques that Support the Detection and Recovery of Microorganisms

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Prepared by:

The FEM Method Validation Team

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Contributors:

Alan Lindquist, Ph.D.
National Homeland Security Research Center
Cincinnati, OH

Keya Sen, Ph.D.
Office of Water
Cincinnati, OH

Eric Rhodes, Ph.D.
National Exposure Research Laboratory
Cincinnati, OH

Ann Grimm, Ph.D.
National Exposure Research Laboratory
Cincinnati, OH

Sarah Perkins, Ph.D.
National Homeland Security Research Center
Cincinnati, OH

Sarah Taft, Ph.D.
National Homeland Security Research Center
Cincinnati, OH

Eunice Varughese, M.S.
National Exposure Research Laboratory
Cincinnati, OH

Margo Hunt, Ph.D.
Retired, U.S. EPA/ Consultant
Denver, CO

Mark Meckes, Ph.D.
National Risk Management Research Laboratory
Cincinnati, OH

Frank Schaefer, Ph.D.
National Homeland Security Research Center
Cincinnati, OH

Charlena Yoder Bowling
National Homeland Security Research Center
Cincinnati, OH

Matthew Arduino, Dr.P.H.
Centers for Disease Control and Prevention
Atlanta, GA

Sanjiv Shah, Ph.D.
National Homeland Security Research Center
Washington, D.C.

Stephen Morse, Ph.D.
Centers for Disease Control and Prevention
Atlanta, GA

Mano Sivaganesan, Ph.D.
National Risk Management Research Laboratory
Cincinnati, OH

Michele Burgess
Office of Emergency Management
Washington, D.C.

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Some text in this document is taken from the companion document U.S. EPA. 2009 *Method Validation of U.S. Environmental Protection Agency Microbiological Methods of Analysis,* FEM 2009-01 (U.S. EPA 2009).

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Foreword

The EPA's mission is to protect human health and the environment in which people live, learn and work. Microbes are ubiquitous in the environment and have the potential to both provide benefits and inflict harm on people and the environment. Because of the potential risk posed by microbes, EPA is involved extensively in the study, monitoring and environmental measurement of microorganisms in the environment. Sampling the air, water and soil for microbial flora is an integral part of environmental measurement. However, before a sampling project can begin, a sampling technique must be selected. Sampling techniques adopted by EPA require an extensive evaluation process to be validated; i.e., to be proven reliable, successful, replicable and well suited for the task.

The technique validation process is central to EPA's ability to monitor and measure microbial life. The EPA Science Policy Council established the Forum on Environmental Measurements (FEM) in April 2003. The FEM BioSampling Workgroup was formed in 2008 to address sampling issues related to microorganisms, and create Agency-wide, internal guidance for validation and peer review of associated methods prior to publication and general use. This comprehensive guidance document is the result of the Workgroup's multi-year effort and should prove useful not only to EPA personnel, but to EPA clients as well as contractors, researchers and other agencies that are interested in EPA's process for validation, approval and acceptance of EPA methods.

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FEM BioSampling Document Workgroup

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Gene Rice, EPA
Worth Calfee, EPA
Lara Phelps, EPA
Tonya Nichols, EPA
Phillip Campagna, EPA
Marissa Mullins, EPA
Nanci Hemberger, The Scientific Consulting Group, Inc.
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Denise Hoffman, The Scientific Consulting Group, Inc.

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Individual Reviewers

Noreen Adcock (EPA ORD)	Kristen Keteles (EPA Region 8)	Mark Rodgers (EPA ORD)
Jennifer Best (EPA OW)	Andrew Lincoff (EPA Region 9)	Laura Rose (CDC)
Zia Bukhari (American Water)	Carrie Miller (EPA OW)	Jim Seidel (EPA OCEFT)
Jennifer Cashdollar (EPA ORD)	Robin Oshiro (EPA OW)	Mike Ware (EPA ORD)
Mark Doolittle (Contractor R1)	Sandhya Parshionikar (EPA OW)	Elizabeth Hedrick (EPA OW)
Stephanie Harris (EPA Region 10)	Viola Reynolds (EPA Region 4)	

FEM Members and FEM Participants with Their EPA Office or Region (R)

Michael Shapiro (OW)	Tom Norris (NEIC)	Kim Kirkland (ORCR)
Lara Phelps (OSA)	Marilyn Livingood (OITA)	Pamela Barr (OW)
Deb Szaro (R2)	Marty Monell (OCSPP)	Gregory Carroll (OW)
Anand Mudambi (OSA)	Denise Rice (OCSPP)	Jan Matuszko (OW)
Bill Lamason (OAR)	Thomas Behymer (ORD)	Maria Gomez-Taylor (OW)
Robin Segall (OAR)	Denise MacMillan (ORD)	Louise Camalier (OEI)
Ron Fraass (OAR)	Jeff Heimerman (OSWER)	Dennis Wesolowski (R5)
John Griggs (OAR)	Dan Powell (OSWER)	Melanie Hoff (OSWER)
Michael Brody (OCFO)	James Michael (ORCR)	Mary Greene (OSA)
David Charters (OSWER)	Dennis Mikel (OAR)	Tim Hanley (OAR)
Elizabeth Flynt (OCSPP)	Marietta Echeverria (OPP)	Yaorong Qian (OCSPP)
Stephen Wente (OCSPP)	John McKernan (ORD)	Teresa Harten (ORD)
Tim Watkins (ORD)	Michael Johnson (OSWER)	Shen-Yi Yang (ORCR)
Susan Holdsworth (OW)	Marion Kelly (OW)	Darvene Adams (R2)
Kevin Kubik (R2)	Dale Bates (R7)	Brenda Bettencourt (R9)

Christopher Cagurangan (R9)	Robert Hall (R9)	Charles Sellers (ORCR)
Troy Strock (R5)	Patricia Mundy (OEI)	Christian Byrne (ORD)
Eric Nottingham (NEIC)	Gerry Sotolongo (R1)	Thuy Nguyen (OCSPP)
Eric Villegas (ORD)	Swinburne Augustine (ORD)	Charles Kovatch (OW)
Jeffery Robichaud (R7)	Laurie Trinca (OAR)	Michelle Henderson (ORD)
Judy Brisbin (OW)	Kevin Bolger (R5)	Patrick Churilla (R5)
Jeff Pritt (R7)	Linda Mauel (R2)	Kenna Yarbrough (NEIC)
Glynda Smith (OW)	Beth Mishalanie (NEIC)	Dale Bates (R7)
Carolyn Bernota (OECA)	Francisca Liem (OECA)	Carrie Middleton (NEIC)
Cary Secrest (OECA)	Carole Braverman (R5)	Motria Caudill (R5)
Barbara Sheedy (ORD)		

EPA Offices: NEIC, National Enforcement Investigations Center; OAR, Office of Air and Radiation; OCEFT, Office of Criminal Enforcement, Forensics and Training; OCFO, Office of Chief Financial Officer; OCSPP, Office of Chemical Safety and Pollution Prevention; OECA, Office of Enforcement and Compliance Assurance; OEI, Office of Environmental Information; OGC, Office of General Counsel; OITA, Office of International and Tribal Affairs; OPP, Office of Pesticide Programs; ORCR, Office of Resource Conservation and Recovery; ORD, Office of Research and Development; OSA, Office of the Science Advisor; OSW, Office of Solid Waste and Emergency Response; OW, Office of Water; R, Regional Office
Other Offices: CDC, Centers for Disease Control and Prevention

Executive Summary

The EPA Science Policy Council established the FEM, a standing committee of senior EPA managers, in April 2003, to provide EPA and the public with a focal point for addressing measurement and methods issues that have a multi-program impact; action teams are commissioned by the FEM to address specific issues. In October 2008, the BioSampling Workgroup was established to develop Agency-wide, internal guidance for validating and peer reviewing EPA methods prior to publication for general use.

This document provides Agency-wide guidance for EPA personnel who will evaluate the performance and suitability of new sampling techniques for microbiological parameters before publication by EPA. The validation principles in the document are based on current, international approaches and guidelines for intra-laboratory (single laboratory) and inter-laboratory (multiple laboratory) validation studies. Please note that this document relates to culture-based and molecular-based microbiological analytical methods. The Workgroup's goal was to collect information from the referenced documents into one guidance document; the document is not intended to supersede established practices.

EPA's recommended format for writing sampling techniques is the Environmental Monitoring Management Council (EMMC) Method Format, which includes the following components: scope and application, technique summary, definitions, interferences, health and safety, equipment and supplies, reagents and standards, sample collection, preservation and storage, quality control (QC), calibration and standardization, procedural steps, calculations and data analysis, technique performance, pollution prevention, and waste management. In particular, Section 17 of the EMMC addresses validation data. In addition, this guidance document recommends including the numerical and descriptive specifications of the techniques' operational limits and performance attributes that are determined from intra-laboratory testing results during primary validation.

Peer review is required for all EPA sampling techniques for microbiological parameters prior to publication. The EPA Science Policy Council's 2006 *Peer Review Handbook* (U.S. EPA 2006a) provides Agency-wide requirements and options for that process.

Acronyms

AOAC	AOAC International (formerly Association of Analytical Chemists)
ASTM	ASTM International (formerly American Society for Testing and Materials)
ATCC™	(formerly American Type Culture Collection)
CAS	Chemical Abstract Service
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
DNA	deoxyribonucleic acid
DOT	U.S. Department of Transportation
DQI	data quality indicator
DQO	data quality objective
EMMC	Environmental Monitoring Management Council
EPA	U.S. Environmental Protection Agency
FEM	Forum on Environmental Measurements
FN	number of false negatives
FP	number of false positives
GPS	global positioning system
HMR	hazardous materials regulations
IATA	International Air Transportation Association
ISO	International Organization for Standardization
LOD	limit of detection
LOQ	limit of quantitation
MDL	method detection limit
NIST	National Institute of Standards and Technology
OSHA	Occupational Safety and Health Administration
QA	quality assurance
QC	quality control
SHEM	Safety, Health, and Environmental Management
SPC	specimen processing control
SOP	standard operating procedure
SOW	statement of work
TN	number of true negatives
TP	number of true positives
UN/ICAO	United Nations International Civil Aviation Organization

Glossary

accuracy: The degree of agreement between an observed value and an accepted reference value. Accuracy includes a combination of random error (precision) and systematic error (bias) components, which are due to sampling and analytical operations; a data quality indicator

blank: A specimen that is intended to contain none of the analytes of interest and is subjected to the usual analytical or measurement process to establish a zero baseline or background value

bias: The constant or systematic distortion of a measurement process, different from random error, which manifests itself as a persistent positive or negative deviation from the known or true value. This can result from improper data collection, poorly calibrated analytical or sampling equipment, or limitations or errors in analytical methods and techniques

calibration: Set of operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by material measure or a reference material, and the corresponding values realized by standards

compatibility: The capability for one data set to be reconciled or integrated with another; often expressed as a statistical measure

data quality objectives (DQOs): Qualitative and quantitative statements derived from the DQO Planning Process that clarify the purpose of the study, define the most appropriate type of information to collect, determine the most appropriate conditions from which to collect that information, and specify tolerable levels of potential decision errors

holding time: (1) The maximum times that samples may be held, after the sample is taken, prior to analysis and still be considered valid or not compromised; (2) The maximum times that samples may be held, after the sample is taken, prior to preparation and/or analysis and still be considered valid or not compromised

Latin square: An $n \times n$ array filled with n^2 different Latin letters or numbers, each occurring exactly once in each row and exactly once in each column. Such an arrangement can be used as the basis of experimental procedures in which it is desired to control or allow for two sources of variability while investigating a third

limit of detection (LOD) and limit of quantitation (LOQ): The LOD and LOQ concentrations are calculated by applying the compound's calibration curve to the noise response of a sample to obtain a value which is then multiplied by a factor of 3 for LOD (3 times of noise) and 10 for LOQ (10 times of noise). The responses of the analytes are not considered in this approach. Only the noise level is included in the calculation. In some cases, the concentration of the lowest calibration standard is treated as the LOQ. The LOD is not defined in this case, although the LOD is often assumed to be 1/3 of the LOQ. The lowest possible LOD and LOQ values are not critical in these cases. The rationale of this approach is that the expected analyte concentrations in the samples are high and above the lowest calibration concentration and knowledge of the

actual LOD/LOA is not necessary

method detection limit (MDL): The minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte. The MDL is determined using the procedure provided in 40 CFR 136

method: (1) A body of procedures and techniques for performing an activity (e.g., sampling, analysis, quantification) systematically presented in the order in which they are to be executed. (2) Logical sequence of operations, described generically, used in the performance of measurements

precision: The consistency of measurement values quantified by measures of dispersion, such as the sample standard deviation. Precision must be defined in context—e.g., for a certain analyte, matrix, method, perhaps concentration, laboratory or group of laboratories

procedure: A specified way to carry out an activity or process

quality assurance (QA): An integrated system of management activities involving planning, implementation, documentation, assessment, reporting and quality improvement to ensure that a process, item or service is of the type and quality needed and expected by the client

quality control (QC): (1) The overall system of technical activities whose purpose is to measure and control the quality of a product or service so that it meets the needs of users; (2) The overall system of technical activities that measures the attributes and performance of a process, item or service against defined standards to verify that they meet the stated requirements established by the customer; operational techniques and activities that are used to fulfill requirements for quality

robustness: The ability to match a particular sample collection technique with multiple analytical assay techniques

ruggedness: The degree of reproducibility obtained by the analysis of the same samples under a variety of test conditions, such as pH, temperature, humidity, etc.

sample size: The number of items or the quantity (volume, mass or area) of material constituting a sample

sample stability: The capability of a sample material to retain the initial property of a measured constituent for a period of time within specified limits when the sample is stored under defined conditions

selectivity: The extent to which a method can determine particular analytes in mixtures or matrices without interferences from other components

sensitivity: (1) The capability of a method or instrument to discriminate between small differences in analyte concentration; (2) A qualitative description of an instrument's or analytical method's detection limit

specificity: The measure of the proportion of negatives that are correctly identified

standard operating procedure: (1) A written document outlining an analytical method that provides a level of detail intended to allow advanced analysts or analysts familiar with the method outlined in the SOP to perform that analytical method; (2) A written document that details the method of an operation, analysis or action whose techniques and procedures are thoroughly prescribed and which is accepted as the method for performing certain routine or repetitive tasks.

standardization: The process of adjusting instrument output to a previously established calibration; the experimental establishment of the concentration of a reagent solution; correlation of an instrument response to a standard of known accuracy

systematic error: Consistent biases in measurement which cause the mean "observed" value of many separate measurements to differ significantly from the "actual" value of the measured quantity or attribute; equal to total error minus random error

technique: The systematic procedure by which a complex or scientific task is accomplished

uncertainty: (1) The range of values that contains the true value of what is being evaluated at some level of confidence; (2) A measure of the total variability associated with sampling and measuring that includes the two error components: systematic error (bias) and random error.

validation: (1) Confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled; (2) In design and development, the process of examining a product or result to determine its conformance to user needs

1. Introduction

EPA program offices publish a wide variety of measurement methods and techniques for use by EPA personnel, other government agencies and the private sector. These methods and techniques originate from many sources, such as EPA laboratories and contractors, scientific organizations, other government laboratories and the private sector. Because these methods could be published as regulations, incorporated as references in regulations or published as guidance, they must be tested thoroughly and peer reviewed prior to publication as EPA methods.

The FEM is a standing committee of senior EPA managers who provide EPA and the public with a focal point for addressing measurement and methods issues that have a multi-program impact. The FEM commissions action teams to address specific issues to provide Agency-wide, internal guidance for validating and peer reviewing EPA methods and techniques prior to their publication for general use. The BioSampling Workgroup, a FEM action team, developed this document to assist EPA staff charged with developing or reviewing microbiological sample collection techniques.

Understanding the fate of microorganisms in the environment and their impact on the environment and human health are important aspects of EPA's mission. The Agency extensively studies and monitors microorganisms in a variety of environmental matrices, and sampling is an integral part of environmental measurements of microbiological contaminants. Effective sampling approaches must address the overwhelming complexities in environmental microbiology, including the different forms of microorganisms, types of samples and sampling devices, and interfering substances and organisms. Sampling technique validation is the process of demonstrating that a sampling technique is suitable for its intended use; multiple studies are required to evaluate a technique's performance under defined conditions. Properly designed and successful sampling-technique validation studies ensure the reliability of a sampling technique. EPA depends on proven and reliable microbial sampling methods to understand the impact of microbes on the environment and human health.

Analytical methods, used in conjunction with sampling techniques, depend on effective sampling and recovery of target microorganisms. Sampling is merely a portion of a method, as a typical method includes sample collection and processing, extraction or isolation procedures, analyte detection, data analysis and other essential information. Any determination of uncertainty or bias in an environmental measurement must include both the analytical procedures and the sampling technique. To fulfill accurate measurement objectives, each step of the process should be analyzed and validated. (Note that this document relates to culture-based and molecular-based microbiological analytical methods.)

All sampling techniques must be peer reviewed before publication. The authors refer readers to the current version of the EPA Science Policy Council's *Peer Review Handbook* (U.S. EPA 2006a), an excellent source of information for both internal and external peer review processes.

1.1 Purpose

This guidance document describes the process for validating environmental sampling techniques that support the detection and recovery of microorganisms. It describes the scientific principles that should be addressed during sampling technique validation studies for microbiological parameters and provides guidelines concerning the minimum levels of required validation and peer review before EPA recommends specific methods or techniques. This document provides general guidance for the validation of microbiological sampling techniques likely to be used in EPA methods.

This document also provides information on selecting an appropriate sampling technique for specific applications (e.g., clearance, characterization), as well as general background material on the wide range of approaches to microorganism sampling in environmental media. Testing should be conducted to ensure that sampling techniques written for a specific organism are effective for additional organisms prior to use, even when the microbes are closely related. Similarly, validation is needed to ensure that techniques tested for collecting samples for one type of assay will perform with equal proficiency with the proposed assays.

The EPA is involved in collecting microorganism samples from numerous matrices, such as water, biofilms, biosolids, soil, sediment, air, and other matrices. The sampling literature includes several EPA methods for microorganism detection (http://www.epa.gov/microbes).

1.2 Intended Audience

This guidance was written for EPA personnel who are responsible for sampling techniques for microbiological parameters that will be: (a) published as serially numbered EPA methods, (b) published as regulations or (c) incorporated by reference in regulations. In some cases, the validated methods may be needed for research by EPA and other organizations. This document also may be used by clients, contractors or other interested parties who, upon reviewing an EPA method or technique for potential use, are interested in EPA's process for method validation, approval and acceptance.

1.3 Scope of Guidance

In exploring requirements to validate a specific technique, the FEM BioSampling Workgroup concluded that specific guidance on how to conduct validation studies for every sampling technique was beyond the scope of this document; therefore, detailed validation protocols for specific techniques are not covered. Instead, guidelines for developing validation protocols are presented. This document also references validation protocols developed by EPA and other standards setting organizations, such as the International Organization for Standardization (ISO), ASTM International (formerly American Society for Testing and Materials) and AOAC International (formerly Association of Analytical Chemists). The intent of the document is not to supersede established practices but rather to collect information from the documents mentioned above and incorporate that information into one document.

Each step of a method, from the sampling technique through processing and assays, influences its selectivity, sensitivity and specificity. Performance measures of sampling techniques cannot be measured in the absence of appropriate analytical assays or set of assays. The performance measures cannot be interpreted in the absence of the specific combination of matrix analyte, sampling technique, analytical assay and other processing or analytical procedures used to develop the measure. This guidance was developed for EPA microbiological sampling techniques not validated prior to publishing as EPA methods or adapted as Agency-accepted regulatory standards, or for existing techniques not yet validated with a desired assay. The document avoids the term "method" with regard to sampling because stand-alone sampling methods for microorganisms are not typical. In addition, sampling techniques are not independent of the assay used; assay results depend on how the sample is delivered to the assay. If either the sampling technique or the assay is changed, the results also are likely to change. A separate guidance document was prepared to address validation issues concerning extraction procedures and the analytical detection of microbes (see U.S. EPA. 2009. *Method Validation of U.S. Environmental Protection Agency Microbiological Methods of Analysis*, FEM 2009-01).

Within the context of this document, microbiology includes viruses, bacteria, fungi, yeasts, toxins, algae, protozoa, metazoan parasites and DNA or other materials associated with these microorganisms collected in environmental matrices such as air, water bio-solids and soil. In addition, this information is designed to apply to all sampling techniques associated with antimicrobial agent testing. The Agency may need additional documents to address sampling needs for other macroscopic living organisms such as fish, shellfish, annelid worms, insects or birds. Although the document may be broad enough to encompass validation of sampling methods used in other areas of biology, it is not designed for that purpose.

The precise procedures of a sampling technique must be addressed when developing or validating sampling tools and techniques. All physical sampling techniques or collection tools that are used must be indicated, as well as whether the procedure is a field measurement process such as screening or a direct reading. This document does not address site characterization issues, nor the number of samples required for a representative study.

1.4 Terminology

Scientific terms and meanings change with time. For many years, national and international standards organizations have sought to harmonize terminology within scientific disciplines. For the purpose of this guidance, a glossary of terms and definitions is included on page x. Where possible, definitions were obtained from the FEM Environmental Measurement, ISO, and National Institute of Standards and Technology (NIST) glossaries.

2. Planning and Initiating Validation Studies

Proper planning is critical for successful validation studies. A sampling validation plan should encompass all aspects of validation activities and follow a standardized format similar to that shown in Appendix A. Sampling techniques are closely associated with the development of analytical techniques, and care must be taken to ensure that new collection techniques are consistent with the planned analytical techniques.

Sampling technique validation depends heavily on the environment in which the sample is collected. Temperature variations, variations in media type and the technique used to collect samples (e.g., pressure placed on a surface wipe) can cause sample collection variability. Care must be taken to document these variables and either limit the variables tested or ensure that a range of conditions are included in the test. In addition, the sample collection process should be well documented. For example, soil samples, typically collected via mechanical means, can be either grab or composite samples. Sampling techniques that are simple deviations from existing processes (e.g., new size of a split spoon sampler) require less rigorous validation than a novel sampling technology with little technical basis (e.g., extraction of microorganisms from drinking water samples using traveling wave electrostatic charge separation). The key to effective validation activity is control and documentation of technique variability. Through control and documentation, the developer validation body, or end user, may determine if the range of conditions is appropriate for a particular sampling technique or if conditions indicate that use of another technique may be more appropriate. Further, these factors allow a user to determine if the technique is performing as designed. Finally, well-controlled and documented procedures permit the data generated from the application of the procedure to be used in decision-making.

Normally, samples collected from multiple locations and under differing conditions should be tested in conjunction with matrix spikes and proficiency evaluation testing so a variety of performance characteristics such as interferences, reproducibility, robustness and ruggedness can be determined. This practice of performance verification provides a range of operating criteria and a better understanding of the consistency of the sampling technique. However, this practice should be balanced against the costs of sampling technique validation studies. Unfortunately, sampling technique difficulties may be revealed only after performing multiple tests, and it may be necessary to troubleshoot and optimize a technique after conducting additional validation studies that address those difficulties. If procedural changes are required, the changes may affect the technique performance characteristics, and repeating some or all aspects of the study may be necessary. Although site characterization is beyond the scope of this document, the process for selecting a location representative of the analyte(s) distribution in the local environment should be documented.

Safety is a prime consideration in any sampling event and should be addressed in both the sampling technique and the validation plan, including personal protective equipment and first aid. Additional safety concerns include physical and biological hazards such as harmful microorganisms.

3. General Guidelines for Sampling Procedure Validation

This section addresses elements to consider when developing a validation protocol. Including these components in a sampling technique validation plan should add value to the data generated. Omitting multiple items of this information may limit the applicability of a sampling technique or protocol.

This section is not intended to provide specific guidance for developing protocols for particular sampling techniques, as the possible range of variables to be considered is too great to cover in a general document. Information such as the definitions of verification and validation levels; development and contents of quality assurance (QA) project plans; and health and safety plans for the testing plan, data treatment and manipulation, and development of acceptance criteria for particular techniques are generally available elsewhere. (A selection of citations is provided in the "Further Reading and Additional Guidance" section of this document.) Depending on the proposed use of the data generated, this information may be specified by regulation or policy.

The specific activities of a validation process depend on the type of sampling technique or protocol being developed and tested, and on the potential application of the data. If a sampling technique is tested to improve an aspect of an existing method used for regulatory compliance, a different criterion would be expected than for a method being developed for research. These differences may be in the number and type of conditions tested, the level of QA, the treatment of the data and in the acceptance criteria, or in other areas of validation.

3.1 Sampling Technique Selection

Sampling technique options often are mandated by regulatory constraints. Several sampling techniques may be available if the regulatory framework is flexible or if the sample collection is not in direct response to a regulatory mandate.

Choosing an appropriate sampling technique should be based on an evaluation of various sampling techniques according to the project goals and requirements; therefore, the first step is to list and understand the mission requirements. The decision tree in Figure 1 will assist users with choosing the appropriate sampling technique to validate for a given purpose.

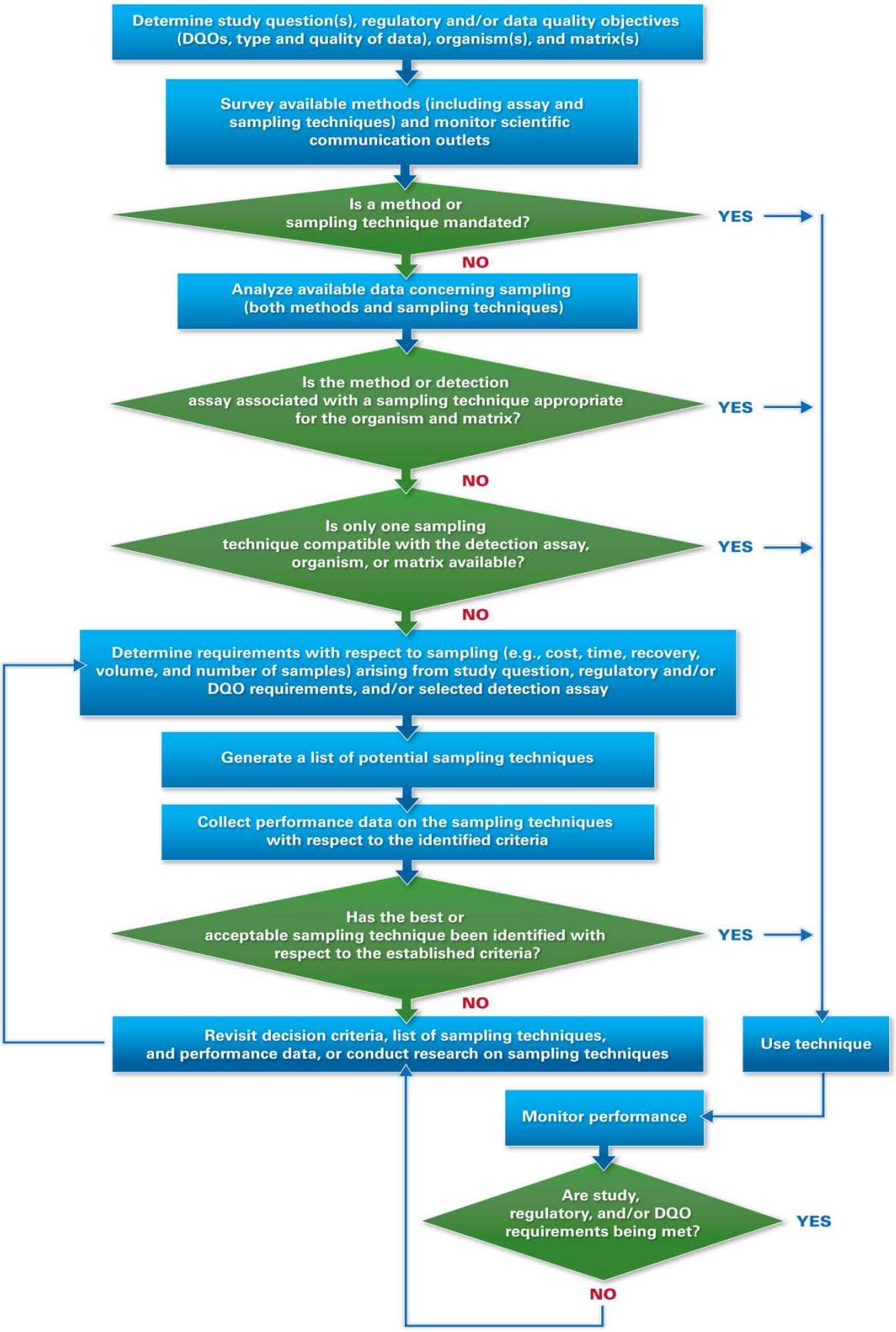

Figure 1. Sampling Technique Selection Decision Tree

Additional questions that may help clarify sampling technique requirements include:

- What size is the sample, or how much material should be collected?
- What characteristics of the underlying population to be sampled (e.g., initial and final volume, temperature, collection location, other characteristics) are required to provide adequate information to address the underlying question(s)? What question(s) is the sampling and analysis effort attempting to address?
- Is there a mandatory maximum sample holding time between sample collection and sample analysis?
- If no single sampling technique is mandated, is there a subset of sampling techniques from which a technique should be selected so that the data are acceptable?
- Are there requirements that will allow data from a particular sampling technique to be considered in future decisionmaking? If there are multiple requirements, how are they prioritized? What are the requirements for collecting a representative sample, and will multiple samples be required? Is there a requirement to sample the same location multiple times, or must samples be collected from diverse locations? Will multiple samples be used to make a composite sample or is each sample to be analyzed individually?
- What are the logistical constraints, such as cost restrictions, on the sampling effort? Are the logistical requirements for potential sample collection techniques within these constraints?

Additional characteristics to consider when selecting sampling equipment and techniques include:

- Material compatibility (gloves, sampling implements, sampling containers, etc.);
- Chemical compatibility;
- Sample volume capabilities;
- Physical requirements;
- Ease of operation;
- Convenience;
- Decontamination effort needed for sampling equipment;
- Field processing requirements;
- Existence of techniques optimized for this sample type, assay and purpose.

It might be useful to evaluate possible required performance characteristics for the sampling technique. These characteristics may include:

- Selectivity;
- Microbial viability;
- Sample storage and preservation;
- Area or volume sampled;
- Detection and quantitation limits;
- Robustness;
- Ruggedness;
- Resource requirements;
- Safety;

- Waste minimization;
- Operational limits;
- QA/quality control (QC); and
- Chain of custody.

3.2 Sampling Technique Optimization

Techniques are developed to perform under a defined set of conditions and may be adjusted to provide higher quality data. Optimization is the process of altering a technique, measuring the effect on the data and retaining changes that improve technique outcomes. Optimization implies an improved data parameter as measured by some predefined criterion, and optimization of a technique for one parameter frequently results in changes to other parameters. Secondary changes may be beneficial or detrimental to the overall criteria by which the technique is judged. Optimization of a sampling technique is possible when the analytical method is constant.

Some parameters are difficult to quantify and optimize, and parameters that are easier to measure often are optimized first. Technique developers should consider the prioritization of these parameters, and optimization should be weighted toward parameters that represent significant programmatic concerns rather than those that are easiest to measure.

Most techniques require optimization of several parameters; the simplest optimization technique for multiple parameters is to optimize each significant parameter serially. After a parameter is optimized, previously optimized parameters must be tested to ensure that they remain optimal, and necessary adjustments made. Multi-parameter optimization is a cost-effective technique when optimizing several parameters. This technique combines multiple parameters to form a Latin square-type design and arrives at a simultaneous optimization without testing each square. There are numerous algorithms for minimizing testing with this strategy, but selection of an appropriate algorithm is beyond the scope of this document as it is situationally dependent.

A general technique description could be developed to provide suboptimal data for a wide range of conditions but should address optimization levels. Alternately, a description could be written for a narrow application and be optimized for specific parameters within a narrow range of conditions. The technique description should detail the conditions and parameters considered in optimization—how to ensure that the conditions exist in a given sample, how to address samples that are not within the conditions described and how to treat data collected outside the optimal range.

There are potential discontinuities in optimization parameters. A parameter may have a relatively continuous distribution throughout a range of conditions, but may change radically in other conditions; the technique description should note these discontinuities. Often, these conditions are revealed only after a technique is applied over a period of time. In these cases, the technique must be modified, amended or otherwise annotated so users are aware of the changes.

The interplay of programmatic objectives and optimization parameters cannot be understated. For instance, optimization for a low cost per sample may result in a decrease in percent recovery and an increase in variability. To compensate for these deficits, the number of samples would

have to increase, eliminating the overall cost savings. To select the applicable optimization state, therefore, factors other than cost/sample must be considered. Although cost/sample is higher, single-sample techniques might prove useful when obtaining multiple samples is impractical, costly or impossible because of restricted site access. Alternatively, the option with a lower cost/sample may suffice when the area or medium being sampled requires flexibility, adaptability or multiple sampling sites. If cost or statistical performance of different optimization states is equivalent, other operational concerns must be considered.

To optimize a technique effectively, it is necessary to define and reconcile parameters, their performance criteria and the conditions under which they may be tested based on their programmatic components. This should provide an acceptable range of response variables prior to technique optimization. Because multiple parameter optimization will require decisions that value one parameter over another, acceptable trade-off conditions should be considered in advance.

The optimization parameters, data ranges, test conditions and trade-offs should be developed in consultation with, and accepted by, the user community, and decisions should be documented. The user community should understand that it is not possible to guarantee that all recognized performance measures and ranges will be met. The user community also should be provided with information that occurs during the optimization process, as it is EPA policy to consult with stakeholders. Developers should not promise delivery on performance criteria during the parameter-formulation period, as this might influence parameter selection and bias outcomes.

3.3 Sampling Operational Limits

The operational limits of a sampling technique represent constraints on the use or operation of the technique. Sampling technique descriptions should list these limits because they may affect decisionmaking regarding technique selection for a particular application. For example, a wetted-wall cyclonic sampler that uses water to entrain particulates from a stream of air may malfunction at temperatures below freezing. Another example is the operational limits of cascade impactor samplers that collect airborne microorganisms from an air stream onto a semisolid agar medium. Semisolid media are subject to dehydration, depending on atmospheric conditions, which may limit the duration of sampling time. The duration also may be related to the sample flow rate.

Other sampling technique constraints may be related to meteorological conditions, altitude and distance from the sample or other known factors. Holding time and temperature can affect microbiological samples, potentially causing either an artificial increase or decrease in the number and viability of target organisms. Increased logistical support can overcome or moderate some operational limits, however. A temperature-controlled enclosure, for example, can overcome the operational limits of a temperature-sensitive sampling device.

Operational limits often are not tested by or obvious to developers, who frequently develop techniques in laboratory settings. Field conditions such as meteorological conditions, low light levels, the absence of horizontal surfaces or the requirement to wear protective gear may limit the performance of a technique, and laboratory tests might not reveal these limits. Technique

documentation should include known operational limits. In addition, testing conditions should be described so that potential users can assess whether test conditions accurately replicate potential difficulties.

3.4 Critical Sampling Technique Performance Characteristics

Each step of a method, from sampling technique through processing and assays, influences its selectivity, sensitivity and specificity. Performance measures of sampling techniques cannot be measured in the absence of an appropriate analytical assay or set of assays. The performance measures cannot be interpreted in the absence of specific combinations of matrix analyte, sampling technique, analytical assay and other processing or analytical procedures used to develop the measure. However, method steps can be investigated and verified separately, as was done for Methods 1622 and 1615.

The sampling technique development team also must incorporate all technique performance components. These factors may include personnel, laboratory facilities, reagents, supplies, equipment, calibrations, reporting standards, record keeping, data analysis, safety and quality. These decisions should be based on the impact that these factors will have on the technique used, the data quality and whether or not the data are acceptable for use.

3.4.1 Sampling Technique Selectivity

Sampling technique selectivity determines whether the procedure can be used to collect the target biological material appropriately, with or without its non-target surroundings/interferences—the more selective the technique, the narrower the range of targets. For example, the non-selective grab sample technique would have substantial drawbacks for a narrow range of targets. This may result in a false negative if the sampling technique is not specific enough to the assay. The selected sampling technique should be evaluated carefully based on project goals, matrix, location of microbes, heterogeneity, sampling device, the target itself, exposure to sunlight and introduction of dissolved oxygen.

An internal control functions like the target in its ability to be collected, concentrated and detected. A sample process control, or internal control, may be required or needed when the target organism is low in prevalence and highly pathogenic (i.e., low infectious dose), or if the surrogate is an indicator for the sanitary condition of the water rather than a specific pathogen or set of pathogens. Care should be given when selecting surrogates to ensure that the properties of the control(s) are similar to those of the target organisms especially with regard to sampling (i.e., is the interaction of the surrogate with the matrix and sampling device the same as that of the target?). Although a control organism or compound may occur naturally in the environment, often it is added ("over-spiked") into a sample in precise numbers as a specimen-processing control (SPC) or to understand the effects of treatment, antibiotics, etc. Thus, nonpathogenic surrogate organisms, such as other strains of *Bacillus spp*., have been shown to be acceptable for use in place of *B. anthracis; Hafnia alvei* is used in place of *E. coli* O157:H7 and *Salmonella spp.* in other matrices. The ability to capture the surrogate in a manner similar to the target should be considered when selecting a sampling technique. Conversely, a surrogate can be selected if it is adequate for the sampling technique chosen for the target. Standards such as

EasySeed™ for protozoa and ColorSeed™ (both BTF Pty Ltd) for *Giardia* spp. and *Cryptosporidium* spp.; BioBall™ (BTF Pty Ltd, BIOMÉRIEUX Inc., Pittsburgh, PA) for bacteria; and Armored RNA® (Ambion, Inc., Applied Biosystems, Foster City, CA) for viruses, can be spiked directly into water to determine the efficacy of the test technique to monitor respective organisms.

3.4.2 Sampling Technique Sensitivity

Sensitivity measures the proportion of actual positives that are identified correctly. For example, sensitivity would be the proportion of target organisms that can be detected and is expressed mathematically as:

Sensitivity = TP / (TP + FN) * 100%

Where:
TP = Number of true positives
FN = Number of false negatives

Data to calculate sensitivity typically are generated by repeated testing of serial dilutions of a known spike standard.

Limit of Detection (LOD)

The LOD is the minimum amount of analyte that can be detected reliably and distinguished from a known and characterized background with a given level of confidence; LODs establish a baseline detection value for optimal conditions. If no organisms are detected in a sample, results should be reported as less than the LOD per sample area or volume. The method detection limit (MDL) is defined as the minimum amount of an analyte that can be measured and for which it can be reported with 99 percent confidence that the analyte concentration in an interference-free matrix is greater than zero. The MDL is determined by analyzing a matrix sample containing the analyte. (Refer to U.S. EPA *Method Validation of U.S. Environmental Protection Agency Microbiological Methods of Analysis*, FEM 2009-01 for more information on the MDL).

Limit of Quantitation (LOQ)

The LOQ is the lowest amount of analyte that can be measured with acceptable precision and accuracy as required by data quality objectives. What is considered “acceptable” is determined by the method, if it requires it, or by the user. Both the LOQ and the range of quantitation are established from a standard curve of reference sample measurements. The standard curve defines the relationship between the detector or instrument response and the analyte amount. Methods designed to obtain a quantitative analysis may have several required operational limits and performance attributes, one of which is a standard curve.

3.4.3 Sampling Technique Specificity

Specificity measures the proportion of negatives that are correctly identified. Specificity is expressed mathematically as:

Specificity = TN / (TN + FP) * 100%

Where:
TN = Number of true negatives
FP = Number of false positives

Microbiology methods and media specificity traditionally are demonstrated using pure positive and negative control cultures. For example, appropriate ATCC™ strains for several groups of enteric control culture bacteria are provided in Section 5.1.6.4 of EPA's *Manual for the Certification of Laboratories Analyzing Drinking Water*, 5th Edition (U.S. EPA, 2005). Positive cultures listed for Enterococci include *Enterococcus faecalis* ATCC 11700 and *Enterococcus faecium* ATCC 6057. Appropriate negative controls include *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739 or 25922, and *Serratia marcescens* ATCC 14756. Appropriate target and nontarget control culture definitions, or other standards for both validation and routine QC use, are expected when developing new microbial methods. In an effective method, a single target organism should be discernible in complex matrices that may contain millions of nontarget organisms.

3.4.4 Sampling Technique Viability

The most important aspect of effective sampling is maintaining the integrity of target microorganism(s) until samples are analyzed. Although simply detecting organisms in an environmental matrix can be informative, risk assessment may require information on viability. Thus, if a study's purpose is to determine human health risk, including a viability assay within a detection method could be essential. Certain bacteria strains can enter a viable but nonculturable state, for example, thereby preventing detection of potentially viable organisms. In such cases, holding or incubating samples under specific conditions may be necessary to allow cells to resuscitate.

For methods that include a viability assay, the way in which samples are collected and processed is important. If viability measurements are included or if viable organisms are to be detected, then collection and storage techniques may or may not differ from those that are used to detect nonviable organisms, or DNA or toxins from the organism. For most organisms to be detected in a viable condition, sampling and further downstream processing should be conducted at lower temperatures (4°C) within hours to days. Additionally, other conditions such as incubation, growth media or anaerobic conditions may be required, depending on the microorganism and its condition. Sampling technique standard operating procedures (SOPs) should specify each of these conditions so that technique selection is based on project goals.

3.4.5 Required Sampling Measurements

Documentation during sampling may be required or suggested for some data parameters or sample-matrix parameters. These may be specified in the sampling plan or may be integral to the sample collection process and should be included in the sampling process description. For some data parameters (e.g., alkalinity, turbidity and hardness), data may be collected in the field, or a portion of the collected sample may be sent to an analytical laboratory. If a split or replicate of a sample is to be sent to a laboratory for analysis, the potential impact that sample collection and transportation might have on the parameter of interest must be considered. For example, air incorporated into a water sample during collection may change the pH of the sample from an anoxic environment. Some parameters are required by the method (e.g., to ensure viability of the targeted microorganisms); others are collected for research purposes (e.g., comparing the presence of a microorganism to pH or turbidity).

Examples of sampling site and matrix parameters include but are not limited to:

- Date of sample collection;
- Location (possible global positioning system [GPS] coordinates);
- Time of day;
- Current and past weather conditions;
- Percent of canopy cover at sample location area;
- Description of vegetation;
- Depth of water at which sample was collected;
- Presence of animals or other materials (e.g., feces) at collection site;
- Dissolved oxygen levels;
- Turbidity;
- Temperature;
- Salinity;
- Volume;
- pH;
- Hardness;
- Alkalinity;
- Depth;
- Biological oxygen demand;
- Total organic carbon;
- Ammonia-nitrogen levels;
- Oxidation-reduction potential;
- Percent solid or amount of suspended solids;
- Indicator microorganisms (fecal coliforms, *E. coli*, enterococci, bacteriophages) and/or total heterotrophic bacteria; and
- Disinfectant residuals.

3.4.6 Mass, Area or Volume Sampled

Sample collection areas must be specified within the collection parameters to determine the final quantity (e.g., spores/cm^2). If samples are not collected from a surface area, the volume or mass

must be recorded to establish the basis of the found quantity (e.g., spores/m^3 air or viable cells/mL liquid). Without a known mass, area or volume, concentration of the analyte cannot be calculated. In addition, the sampling amounts must conform to the sampling plan. Volume or area sampled also should consider spatial heterogeneity in the matrix.

3.4.7 Sampling Technique Robustness

Robustness is the ability to match the performance of a sample collection technique with multiple microorganisms and analytical assay techniques; each collection and assay combination *must* be verified.

3.4.8 Sampling Technique Ruggedness

Ruggedness is the degree of reproducibility that is obtained by analyzing the same samples under a variety of test conditions; conditions may include different laboratories, sample collectors, temperatures, pH and relative humidity.

3.4.9 Resource Requirements

Personnel are a primary resource that should be addressed in all techniques. Technique descriptions should include the number of people required to complete the technique, training and skill requirements, and the initial evaluation and continuing assurance of personnel performance. Methods to monitor personnel and corrective actions to take if personnel fail to meet specified requirements also must be included.

Personnel descriptions help ensure that sample collectors are trained and experienced (whether in the laboratory or in the field), so that costly operator errors are prevented and public health risks are minimized. Inadequate or unnecessary requirements for training, performance evaluation and experience may limit a technique's usefulness and the availability of useful data. Other resources include time, funding, supplies, equipment, transportation, laboratory space, etc.

3.5 Safety and Security

Live organisms such as pathogenic microorganisms create potentially high biohazard and infection risks. It is the user's responsibility to establish appropriate safety and health practices prior to adopting a sampling technique. In particular, users must develop a safety plan and observe all safety procedures within the plan. Sampling technique safety considerations should include but are not limited to:

- Toxicity, carcinogenicity or other potential health hazards of reagents and organisms;
- Controlling and limiting exposure to health hazards through protective measures (e.g., gloves and glove boxes, particle masks, protective eyewear);
- Occupational Safety and Health Administration (OSHA) regulations for safe handling of chemicals and organisms;
- Material Safety Data Sheets on file and readily available;

- Biosafety in Microbiological and Biomedical Laboratories (BMBL) for biosafety;
- All hazards associated with collecting a sample, including physical and environmental dangers (e.g., inclement weather, slip hazards, unsafe atmospheric conditions, shock hazards);
- All hazards associated with packing and shipping samples and wastes, including ensuring that shipping containers are free from contamination;
- Medical clearances and appropriate vaccinations;
- Disinfection and clearance procedures required for specific microbes;
- Emergency procedures, incident reporting and recordkeeping;
- Department of Transportation (DOT), Centers for Disease Control and Prevention (CDC), International Air Transport Association (IATA), state and other hazardous material shipping regulations;
- Locations and contact information for local hospitals, and emergency contacts for personnel;
- Locations and availability of emergency showers, eyewash stations and other first aid; and
- Chain of custody requirements (see Section 3.8.2 of this document).

In addition, the corresponding health and safety plan must be approved by the local Safety, Health and Environmental Management (SHEM) office or the chemical hygiene officer before starting a project. A security plan also should be developed that includes the site, staff, storage and transport of potentially hazardous materials.

3.6 Waste Minimization and Waste Management

Waste management considerations, including treatment and ultimate disposal of both the sample and the sampling materials, should be factored into the sampling technique. State or local regulatory agencies should be contacted early to determine state or local requirements and available treatment and disposal options. Please refer to Section 3.8.3 of this document for information about shipping hazardous wastes.

3.7 QA and QC

QA and QC programs should provide scientifically sound and legally defensible documented data. As risk assessment often drives biological sampling, sampling and analytical procedures should correspond to the precision necessary to understand the nature and extent of contamination and enable proper assessment of potential human health or ecological risks. This section provides general descriptions of quality practices and goals for sampling procedures for various biological environmental matrices. The guidelines provide basic QA and QC considerations. It is the project managers'/coordinators' responsibility to incorporate additional requirements to ensure adequate and proper data quality.

3.7.1 Management System

Before sampling or analytical work begins, a management system should be in place that accurately reflects the operating and QA/QC programs in the laboratory. The management system should be documented in the laboratory's quality manual and other referenced quality

documents. The QA manual should address but is not limited to the following elements:

- Quality manual maintenance and update procedures;
- QA objectives and policies;
- QA project plan specific to the project (see http://www.epa.gov/quality/qapps.html for more information on QA project plans);
- Personnel qualifications and training;
- Control of records and documents;
- Data management;
- Analytical methods;
- Equipment calibration and maintenance procedures;
- Reagents and standards;
- Sampling materials and procedures;
- Handling and transportation of samples;
- Sample retention and disposal;
- Internal QC procedures, including who is responsible for QC but not involved in routine work;
- Data validation and reporting;
- QA reports.

The quality manual should be updated as needed, and reviewed and approved by appropriate personnel at least annually.

3.7.2 Training

Persons responsible for overseeing or coordinating sampling are required to ensure that sample collectors are trained properly in procedures and techniques. Development of proficiency tests for sampling assays is recommended. Training should be documented in laboratory records, including a description of the training program content and duration; training records and performance evaluation records should be maintained and readily available.

3.7.3 SOPs

SOPs describe all sampling procedures including sample collection, transportation, analysis, storage and disposal as well as SOPs for equipment use, QA/QC, calibration, and production of reports. SOPs should include all relevant steps in a procedure and be written so that appropriately trained personnel can apply the procedures. Any required operator training or apparatus for a procedure (including all required reagents and materials) must be stated. SOPs can cross-reference other SOPs or documents if necessary, but referenced documents should be cited properly and available for review. Personnel should review SOPs annually and make modifications as necessary. Initial sampling technique SOPs may be refined when compared to another sampling SOP that changes a parameter (e.g., pressure when applying a wipe to a surface, filtration volume, filter membrane type, etc.).

3.7.4 Records

Staff must maintain proper and adequate records and files. Records include but are not limited to:

- Sample numbering and tracking system;
- Analytical data and results for all samples;
- Reports generated from analysis;
- QC data.

Hard copy records (e.g., sampler forms, original hand written data) should be electronically scanned or otherwise converted to an electronic format. Electronic records should be backed up regularly, and each laboratory is responsible for ensuring that records are stored securely and can be retrieved easily.

3.7.5 Equipment Maintenance and Calibration

Sampling and sample analysis equipment must be maintained as documented in the appropriate SOPs or manufacturer's guidelines to ensure analytical quality. Laboratories should apply standards that use the established limits for all equipment; this applies to general equipment such as pH meters as well as to sophisticated analytical instruments and vehicles. Field equipment such as balances, pipetters and pH meters in particular should have regular maintenance and/or calibration schedules. Frequency of calibration checks should be based on established practices and the stability of the equipment; form and frequency of these checks should be documented properly. Calibration and maintenance records should be kept for all equipment, which will aid in assessing repair status.

3.7.6 Sampling Plan

Variations in sampling procedures can have a significant effect on analyses. All sampling procedures, therefore, should be well documented, with clear details provided for sampling precautions and sampling strategies.

Recommendations for sampling QA include:

- Strictly adhere to sampling SOPs.
- Ensure that all equipment is clean and in working order.
- Record all applicable conditions during sampling.
- Take strict precautions to avoid sample contamination.

A sampling plan must be prepared in advance for all sampling programs. Carefully considered plans ensure that changes between two sampling rounds are attributable to changes in the environment and not to procedural changes. Sampling plans should include the sampling objective (e.g., to test the prevalence of a certain microorganism in soil), site selection (location, type of environmental matrix), the time and date that samples are collected, the number and amount of samples collected, as well as methods for holding samples prior to analysis (e.g., temperature and maximum time). When designing the sampling plan, researchers must

ensure that collected samples properly represent the specific environment that is being sampled.

3.7.6.1 Data Quality Objectives (DQOs)

DQOs establish the sampling performance or acceptance criteria that are the basis for the sampling plan design and ensure that samples of sufficient quality and quantity are collected (U.S. EPA 2004). Detailed guidelines on the DQO process are available in the EPA's *Guidance on Systemic Planning Using the Data Quality Objectives Process* (U.S. EPA 2006b) or online at http://www.epa.gov/quality/dqos.html

Data quality indicators (DQIs) provide quantitative measures of identified DQOs by assessing completeness, comparability, representativeness, precision and accuracy. Completeness measures the amount of useable data from a data collection activity; comparability expresses the confidence with which data are considered equivalent; representativeness measures the extent to which the sampling data reflect the sample site; precision refers to the amount of agreement between independent test results; and accuracy is the closeness of agreement between a test result and the accepted reference value (U.S. EPA 2002).

3.7.7 QC

Ensuring QC in the field and laboratory includes using blanks and duplicate, replicate or spiked samples. In addition to normal variability in microorganism concentrations among samples, contamination is possible at all phases of the procedure. QC is specific to the purpose of sampling and how the samples will be analyzed. The following considerations will help ensure that reliable data are obtained:

1. A matrix blank is unspiked so that background levels of the microbe of interest can be measured, which is then subtracted from the recovery calculations.
2. Unspiked blank samples (using a sterile form of the environmental matrix sampled) should be run each time a sampling procedure is completed. If the blanks are positive for the selected microorganism, the procedure is contaminated, and data from that run may need to be discarded or repeated.
3. Duplicate or replicate samples should be acquired whenever feasible. Variability in the microbiological concentration between one sample volume and another is normal. Replicates provide additional QA and allow for averaging two or more samples to ensure the most accurate results. The required number of replicates should be determined statistically.
4. Spiked samples or positive controls should be run for each sampling procedure to establish that the technique was performed correctly. If positive controls are not positive for the microorganism of interest, there may be some concern about the technique used—for example, the media is not good or the incubation temperature is not correct. Spiked positive controls should be run to check for any matrix interference issues that may cause false negative results. Spiked negative control also can be run for the opposite reason. If additional sample is available, the technique may need to be repeated or the results discarded/invalidated.

5. Trip or travel blanks are samples of analyte-free media taken from the laboratory to the sampling site and returned to the laboratory unopened. A trip blank is used to document contamination attributable to shipping and field handling procedures.

Sufficient QA/QC procedures (blank samples, duplications and positive controls) can increase the cost of a sampling program substantially but is less costly than the costs associated with discarding sampling results because of questionable outcomes or inadequate QA.

3.7.8 Sampling Documentation

Careful documentation is required during sampling so that all relevant sample information is recorded clearly at the time of sampling. Field sampling forms (paper or electronic) should be included in the sampling plan and be completed by the person conducting the sampling. Forms should include sampling location, time and date of sample collection (and receipt of the sample by the analytical laboratory) and conditions; field-measured variables; equipment used (including inventory numbers); necessary sample preparation; and the sampler's name. A bound field log book generally is acceptable for record keeping. Electronic recording also may be acceptable when consistent with an organization's data requirements.

Field measurements such as pH and temperature must be performed on a separate subsample that subsequently is discarded to avoid contaminating samples for laboratory analysis; for example, a conductivity measurement should not be prepared with a sample that previously was used to measure pH, as potassium chloride from the pH probe may affect the conductivity reading.

3.7.9 Data and Reporting

A primary goal of QA is to ensure that data are suitable for their intended use, including results and interpretations. Data should be checked comprehensively and analyzed by experienced specialists, and results should be reported accurately and allow for individual interpretation. Reports should include information that affects interpretation, such as sampling conditions or the method of analysis; calibration and data QC should be referenced and readily available.

3.7.10 QA Checks

Regular quality compliance checks are necessary to maintain a QA system. Such procedures involve annual reviews as well as QA system audits, which should be independent, thorough and preferably unannounced. Reviews and audits should be documented formally and made available to persons responsible for the work. Deviations from required standards must be corrected as soon as possible.

(See EPA's *Guidance for Quality Assurance Project Plans* [U.S. EPA 2002]).

3.8 Sample Integrity and Tracking

3.8.1 Sample Receipt, Preservation, Storage and Disposal

Recommended preservation methods are used for all collected samples. If samples involve chemical preservatives, the chemicals must be checked first for efficacy. Sample preservation stabilizes parameters of interest by retarding chemical or biological changes. Samples containing microorganisms in particular deteriorate with time, and proper storage and timely transport can minimize deterioration and preserve sample integrity. Sample integrity is the unimpaired chemical and biological composition of a test sample upon the extraction of an aliquot for analysis. Preserving sample integrity ensures that samples arrive at laboratories in the same condition in which they were collected in the field. Field and transportation measures protect samples from physical contamination, loss of volume, volatilization, light exposure or damaging temperature change. Requirements and conditions for preserving and storing samples depend primarily upon the intended method of analysis. For example, if samples are collected from chlorinated water sources and the target analyte is a viable organism, as for culture-based microbiology methods, sample bottles must contain sufficient sodium thiosulfate to neutralize the residual chlorine present. Samplers and analysts must be vigilant in preventing contamination of samples and reagents.

Sample integrity elements that must be evaluated include but are not limited to:

- Temperature control;
- Storage times and conditions;
- Preservation chemicals;
- Container compatibility;
- Labeling/seal integrity;
- Volume; and
- Contamination control.

For direct field measurements, sample integrity involves no gain or loss of analyte when acquiring samples for the detector. In addition, appropriate QC steps must verify sample integrity.

Samples should be protected from contamination and deterioration before their arrival to a laboratory. To facilitate sample protection, equipment should be clean—sterile whenever possible—and in good working condition. Sample containers should be sterile and kept in a clean environment and away from dust, dirt and fumes. The sample container's inner portion should not be touched or handled by the operator. Reusable containers must be cleaned properly, sterilized and proven to be free of quantifiable target analyte before use. Sample collectors should use sterile gloves and other necessary techniques, such as washing hands and wearing facemasks, to reduce sample contamination. After collection, samples should be placed in sealed containers to prevent contamination during storage and/or transport. Storage (i.e., storage conditions), holding time (i.e., maximum time before analysis for unstable parameters) and transport procedures also must be considered.

Laboratory staff should record when samples arrive to ensure that samples are documented as they pass through the laboratory's analytical systems and that relevant SOPs are followed. Samples should be logged in and stored so that deterioration is minimized, and sample conditions and storage locations should be recorded. Subsampling, sample splitting to allow different storage conditions or sample pretreatment to increase stability also must be recorded. The source and identity of samples should be marked clearly and uniquely to avoid confusion.

Arrangements for disposal or appropriate disposition of samples and sampling materials should be made when samples exceed stable storage times and after the samples are no longer needed. BMBL should be followed for biosafety.

3.8.2 Chain of Custody Considerations

The primary objective of chain of custody is to create an accurate written record that traces the sample from the moment of collection through its destruction or disposal. Chain of custody helps avoid indefensible evidence in court by documenting samples as they pass from one person to the next. An agency must demonstrate the reliability of its evidence by proving the chain of custody of its samples. A chronological record must be maintained that records who has possessed the sample(s) and all analyses that were performed on the samples. Following chain-of-custody procedures when handling samples and data helps provide assurance that no tampering has occurred. A sampling technique must include chain-of-custody considerations and instructions for the lifespan of the sample. In general, the following chain-of-custody guidelines should be followed:

1. A minimum number of people should collect and handle samples and data.
2. Only people associated with the project should handle samples and data.
3. The transfer of samples and data from one person to another must be documented on chain-of-custody forms and site security for these samples should be maintained.
4. Chain-of-custody forms must accompany samples and data.
5. Samples and data must include identification that is legible and written with permanent ink.

Chain of custody is a progression of steps, each of which has its own chain-of-custody form. These may include, but are not limited to:

Chain-of-Custody Steps	Necessary Forms
1. Sampling preparations	Reagents and Supplies Form
2. Taking the sample	Field Sampling Data Sheet
3. Transporting the sample to the laboratory	Shipping and Receiving Form
4. Receipt, storage and transfer of the sample	Sample Receipt and Record Log
5. Sample analysis	Analytical Data Sheet
6. Data record keeping	Archive Contents Record

These forms are available at http://www.epa.gov/apti/coc/.

3.8.3 Shipping

Samples and wastes may be subject to DOT hazardous material regulations (HMR) (see HMR, 49 CFR Parts 171-180) and the CDC's Select Agent Program requirements (http://www.asm.org/index.php/policy/select-agent-background-information-and-web-sites.html). In addition, air shipments also must comply with IATA Dangerous Goods Regulations. Samples collected and wastes generated during field investigations or in response to hazardous material incidents must be classified by certified DOT/IATA personnel prior to shipment as either environmental or hazardous materials (dangerous goods) samples. Most uncharacterized environmental samples (including drinking water) and most groundwater and ambient surface water, soil, sediment and treated municipal and industrial wastewater effluent may not require a permit to ship, but organizational policy should be followed with regard to sample shipments. Suspected contaminated samples or wastes must be shipped as dangerous goods or possibly as select agents.

All hazardous goods shipments must comply with the regulations and guidance described above. Personnel with approved DOT/IATA training must perform all shipments of potentially hazardous materials, and such shipments should be packaged, labeled and shipped according to the appropriate ground or air regulations. Sample and packaging integrity must be maintained to ensure safe shipment. In addition, shipments may be subject to the Select Agent Rules (42 CFR 72.6), which are part of the Select Agent Program that is administered by the CDC and U.S. Department of Agriculture. Samples or wastes should be stored in a secure area that is protected from vermin and adverse weather conditions, and hazardous and nonhazardous samples and wastes must be separated.

4. Writing the Technique

The validation process includes preparing a written description of the technique. Historically, EPA techniques use the EMMC format that includes the following components: scope and application; technique summary; definitions; interferences; health and safety; equipment and supplies; reagents and standards; sample collection, preservation, and storage; QC; calibration and standardization; procedural steps; calculations and data analysis; technique performance; pollution prevention; and waste management. In particular, Section 17 of the EMMC addresses validation data. The EMMC can be accessed online at http://www.epa.gov/ttn/emc/guidlnd/gd-045.pdf. Table 1 in this report describes the components. Note that this is a *recommended* format and not a requirement. It is recommended that the numerical and descriptive specifications of the technique's operational limits be included as well as the performance attributes determined during validation. The validation process should be sufficient for meeting the technique's intended use.

5. Sampling Technique Validation Reports

SOPs and good recordkeeping are essential elements of validated sampling techniques. A specific sampling technique should document that the technique was validated appropriately and verified by field studies. Successful completion of such studies should be documented in the sampling technique validation report.

A suitable report should be prepared for placing in the public docket. The report should address the sampling technique validation topics outlined in this guidance document and summarized in Table 1 and provide: (a) background information on sampling technique development; (b) a description of the sampling technique; (c) a description of the sampling technique validation practices; (d) changes made to the sampling technique as a result of the validation studies; and (e) recommendations for future work. At a minimum, the sampling technique validation report must address the information contained in Table 1.

Table 1. Minimum Sampling Technique Validation Report Topics

Topic	Explanation
Sampling Technique	Provide the SOP or a thorough description of the sampling technique that is being validated.
Summary	Provide an overall summary of the validation report.
Introduction	1. Provide background information on the sampling technique development. 2. State the purpose of the technique, including the measurement objectives and the intended use of the data.
Methodology	1. Describe the experimental design for validating the sampling technique, including: • The test method/procedure; • Details of equipment/locations used, with calibration status. 2. Describe the technique's scope and applicability, including: • How the scope and applicability define the range of technique performance; • Sampling or field measurement process components to be validated; • Matrix to be sampled and unique properties of the sampling matrix (e.g., soil heterogeneity); • Nature of the analytes; • Range of analyte levels for which the technique is suited; • Advantages and limitations of the sampling technique; • Sampling equipment and supplies; • How the technique and analytical parameters meet the DQOs for the specific application.

Table 1: Minimum Technique Validation Report Topics, cont'd.

Sampling Technique Characteristics	1. *Selectivity:* Describe how selectivity was evaluated and how the sampling plan identified and addressed interferences. 2. *Sensitivity:* Describe the sensitivity of the technique and how the sampling plan addressed it. 3. *Specificity:* Describe how specificity was evaluated and how the sampling plan addressed it. 4. *Viability/Sample Integrity:* Describe how the sampling plan addresses viability assays, if required/applicable, and what the requirements are for maintaining/ preserving viability. Describe sample security from the collection site to the analytical laboratory. 5. *Sample Size/Area or Volume Sampled:* Discuss the number of items or the quantity that constitutes an adequate sample for the technique, and whether the samples are composites or grab. 6. *Robustness:* Describe how the sampling technique matches the performance of the applicable analytical assay technique(s). 7. *Ruggedness:* Describe sampling technique performance after experiencing minor changes in operating or environmental conditions. 8. *Resource Requirements and Required Measurements:* Describe the applicable resource requirements, such as personnel, skills, and equipment, as well as the required measurements to be taken, such as site parameters and matrix parameters.
Safety Considerations	Describe safety concerns and procedures that should be addressed, including personal protective equipment and first aid as well as physical and chemical hazards.
QC/QA	Describe the QC/QA checks used.
Discussion	1. Discuss technique development. 2. Discuss validation testing results. Evaluate the testing, including comparison with reference materials and preparations, acceptance criteria and recommendations. 3. Discuss sampling technique changes that were made as a result of the validation studies.
Conclusions	1. Discuss formal acceptance/rejection of work. 2. Provide recommendations for future work.

6. Multi-laboratory Validation Studies

Generally, EPA has recommended using inter-laboratory collaborative studies when validating techniques that are expected to be used widely or support regulatory activity. EPA typically uses a tiered approach that was developed under the streamlining initiative for validating microbiological techniques; this approach takes into consideration the level of intended use for a technique. This approach also minimizes the validation requirements of limited-use techniques (single-laboratory and single-industry use) and instead focuses resources on validating techniques that are intended for nationwide use. Because QC acceptance criteria are developed from validation studies and validation requirements vary with each tier, appropriate statistical procedures to develop the criteria will vary by tier as well.

For an in-depth discussion of multi-laboratory validation studies, refer to *Method Validation of U.S. Environmental Protection Agency Microbiological Methods of Analysis*, FEM 2009-01 (U.S. EPA 2009).

7. Peer Review

Prior to publication, EPA sampling techniques are peer reviewed according to the information provided in the current version of the EPA Science Policy Council's *Peer Review Handbook* (U.S. EPA, 2006a). The *Handbook* provides Agency-wide guidance for consistent implementation of peer review, and program offices have the flexibility to design peer reviews for their specific needs. The *Handbook* also provides detailed information about the products that are subject to peer review. In addition, information is available on selecting peer review mechanisms (internal and external), planning a peer review process, conducting a peer review and preparing peer-review records.

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Further Reading and Additional Guidance

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Appendix A: Sampling Technique Validation Plan

The sampling validation process is an integral element of the sampling technique, and planning documents should be included within the plan as a section or as a stand-alone document attached as an appendix. It should integrate the contributions and requirements of all stakeholders and present this information in a clear, concise format. To achieve this goal, validation planning should be part of the initial planning (e.g., directed planning process). The information and documentation identified in the plans should be communicated to the laboratory as part of the statement of work, project or study plan, standard operating procedure (SOP), or quality assurance (QA) project plan.

The sampling validation plan must address, but is not limited to, the following information:

1. Purpose of validation
2. Sampling procedure, including:
    a. Description of the main principle of the test method;
    b. Description of test procedures and test conditions (including precautions, reagents, reference and preparation substances);
    c. Details of equipment and facilities to be used (including measuring/recording equipment), with calibration status;
    d. Variables to be monitored.
3. Performance characteristics, as listed in this document:
    a. Selectivity;
    b. Sensitivity;
    c. Specificity;
    d. Viability;
    e. Required measurements;
    f. Area, volume or mass sampled;
    g. Robustness;
    h. Ruggedness; and
    i. Resource requirements.
4. Safety and security
5. Waste minimization and waste management
6. QA/quality control (QC), including:
    a. Blanks, equipment rinsate samples and field duplicates;
    b. QA/QC management system;
    c. Laboratory QC sample;
    d. Documentation and records management;
    e. Training;
    f. SOPs;
    g. Equipment maintenance and calibration.
7. Sample integrity
    a. Sample receipt, labels, logs, preservation, holding times, sample containers, transportation, etc.; and
    b. Chain-of-Custody forms.
8. Shipping considerations
9. Details of methods for recording and evaluating results, including statistical analysis