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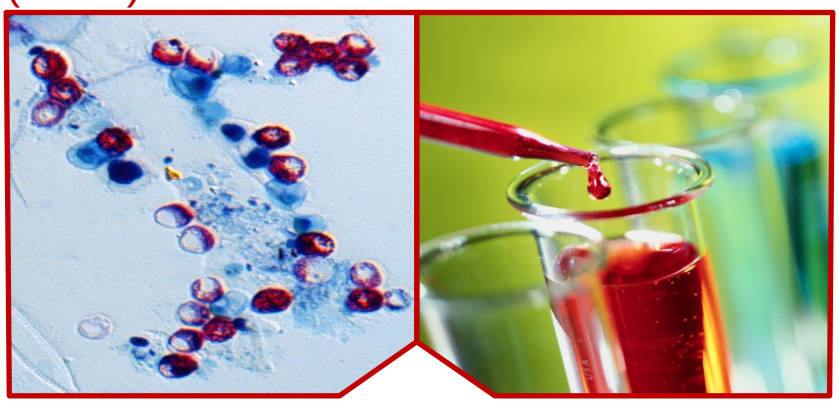
Selected Analytical Methods for Environmental Remediation and Recovery (SAM) – 2012



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Selected Analytical Methods for Environmental Remediation and Recovery (SAM) 2012

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
Cincinnati, OH 45268

Office of Research and Development
National Homeland Security Research Center

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Use of This Document

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

At this time, only some of the methods selected have been validated for the listed analyte and sample type. However, the methods are considered to contain the most appropriate currently available techniques based on expert judgment. Unless a published method listed in this document states specific applicability to the analyte/sample type for which it has been selected, it should be assumed that method evaluation is needed, and adjustments may be required to accurately account for variations in analyte/sample type characteristics, environmental samples, analytical interferences, and data quality objectives (DQOs).

EPA will strive to continue development and evaluation of analytical protocols, including optimization of procedures for measuring target analytes or agents in specific sample types, as appropriate. In those cases where method procedures are determined to be insufficient for a particular situation, NHSRC will continue to provide technical support regarding appropriate actions. NHSRC has also compiled information and published documents regarding field screening equipment, sample collection materials, rapid screening/ preliminary identification equipment, and disposal of samples corresponding to SAM analytes and sample types. These documents are available at www.epa.gov/sam/samcomp.htm.

Abbreviations and Acronyms

ACS	American Chemical Society
amp-ELISA	Amplified-enzyme-linked immunosorbent assay
APCI	Atmospheric Pressure Chemical Ionization
APHA	American Public Health Association
APHL	Association of Public Health Laboratories
AOAC	AOAC International (formerly the Association of Official Analytical Chemists)
API	Atmospheric pressure ionization
ASM	American Society for Microbiology
ASR	Analytical Service Requests
ASTM	ASTM International (formerly the American Society for Testing and Materials)
AWWA	American Water Works Association
BAM	Bacteriological Analytical Manual
BGMK	Buffalo green monkey kidney
BHT	Butylated hydroxytoluene
BMBL	*Biosafety in Microbiological and Biomedical Laboratories*
BSL	Biosafety level
BZ	Quinuclidinyl benzilate
°C	Degree Celsius
CAS RN	Chemical Abstracts Service Registry Number
CBR	Chemical, biological and/or radiological
CCID	Coordinating Center for Infectious Diseases
CDC	Centers for Disease Control and Prevention
CFR	Code of Federal Regulations
CFSAN	Center for Food Safety and Applied Nutrition
CIEIA	Competitive inhibition enzyme immunoassay
CLLE	Continuous liquid-liquid extraction
CLP	Contract Laboratory Program
CPE	Cytopathic effect
cps	Counts per second
CT	Cycle threshold
CVAA	Cold vapor atomic absorption
2-CVAA	2-Chlorovinylarsonous acid
CVAFS	Cold vapor atomic fluorescence spectrometry
CWA	Chemical Warfare Agent
2,4-D	2,4-Dichlorophenoxyacetic acid
DAPI	4',6-Diamidino-2-phenylindole
DAS	Diacetoxyscirpenol
DAS-HG-HSA	Diacetoxyscirpenol hemiglutarate human serum albumin
DAS-HS-HRP	Diacetoxyscirpenol hemisuccinate horseradish peroxidase conjugate
DB-1	100% Dimethylpolysiloxane
DBPR	Division of Bioterrorism Preparedness and Response
DHS	U.S. Department of Homeland Security
DIC	Differential interference contrast
DIG-ELISA	Digoxigenin labeled enzyme-linked immunosorbent assay
DIMP	Diisopropyl methylphosphonate
DL	Detection limit
DNA	Deoxyribonucleic acid
2,4-DNPH	2,4-Dinitrophenylhydrazine
DoD	U.S. Department of Defense
DOE	U.S. Department of Energy

DOT	U.S. Department of Transportation
DPD	N,N-Diethyl-*p*-phenylenediamine
DQO	Data quality objective
DTPA	Diethylenetriamine-pentaacetate
DVL	Detection verification level
EA2192	S-2-(Diisopropylamino)ethyl methylphosphonothioic acid
ECD	Electron capture detector
e-CFR	Electronic Code of Federal Regulations
ECL	Electrochemiluminescence
ED	Ethyldichloroarsine
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
EDEA	N-Ethyldiethanolamine
EDL	Estimated detection limit
EDTA	Ethylenediaminetetraacetic acid
EDXA	Energy dispersive X-ray analysis
EIA	Enzyme immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
EMC	Emission Measurement Center
EML	Environmental Measurements Laboratory
EMMI	Environmental Monitoring Methods Index
EMPA	Ethyl methylphosphonic acid
EMSL	Environmental Monitoring and Support Laboratory
EPA	U.S. Environmental Protection Agency
EQL	Estimated quantitation limit
ERLN	Environmental Response Laboratory Network
ESI	Electrospray ionization
ETV	Environmental Technology Verification
FA	Fluorescence assay
FBI	U.S. Federal Bureau of Investigation
FDA	U.S. Food and Drug Administration
FEMS	Federation of European Microbiological Societies
FGC-ECD	Fast gas chromatography with electron capture detection
FGI	Fluorescein derivative of *Conus geographus* α-conotoxin
FID	Flame ionization detector
FL	Fluorescence detector
FPD	Flame photometric detector
FRET	Fluorescence resonance energy transfer
FRhK-4	Fetal rhesus monkey kidney
FRMAC	Federal Radiological Monitoring and Assessment Center
FSIS	Food Safety and Inspection Service
GA	Tabun
GB	Sarin
GC	Gas chromatograph or Gas chromatography
GC-ECD	Gas chromatography-electron capture detector
GC-FID	Gas chromatography-flame ionization detector
GC-FPD	Gas chromatography-flame photometric detector
GC-MS	Gas chromatography-mass spectrometry
GC-NPD	Gas chromatography-nitrogen-phosphorus detector
GD	Soman
GE	1-Methylethyl ester ethylphosphonofluoridic acid
Ge	Germanium
Ge(Li)	Germanium (Lithium)

GESTIS	A German database (Gefahrstoffdatenbanken) containing data and information on hazardous substances and products
GF	Cyclohexyl sarin
GFAA	Graphite furnace atomic absorption spectrophotometer or Graphite furnace atomic absorption spectrophotometry
HASL	Health and Safety Laboratory, currently known as Environmental Measurements Laboratory (EML)
HAV	Hepatitis A Virus
HCoV	Human Coronavirus
HEV	Hepatitis E Virus
HD	Sulfur mustard / mustard gas; bis(2-chloroethyl) sulfide
HFBA	Heptafluorobutyric anhydride
HFBI	Heptafluorobutyrylimidazole
HHS	U.S. Health and Human Services
HLB	Hydrophilic-lipophilic-balanced
HMTD	Hexamethylenetriperoxidediamine
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
HN-1	Nitrogen mustard 1; bis(2-chloroethyl)ethylamine
HN-2	Nitrogen mustard 2; 2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine
HN-3	Nitrogen mustard 3; tris(2-chloroethyl)amine
HP(Ge)	High purity Germanium
HPLC	High performance liquid chromatography
HPLC-FL	High performance liquid chromatography-fluorescence
HPLC-MS	High performance liquid chromatography-mass spectrometry
HPLC-MS-MS	High performance liquid chromatography tandem mass spectrometry
HPLC-PDA	High performance liquid chromatography-photodiode array detector
HPLC-TSP-MS	High performance liquid chromatography-thermospray-mass spectrometry
HPLC-UV	High performance liquid chromatography-ultraviolet
HPLC-vis	High performance liquid chromatography-visible
HRP	Horseradish peroxidase
HTO	Tritiated water
HV	High volume
IBRD	Interagency Biological Restoration Demonstration
IC	Ion chromatograph or Ion chromatography
IC 20	Inhibitory concentration – Concentration to inhibit 20%
IC 50	Inhibitory concentration – Concentration to inhibit 50%
ICLN	Integrated Consortium of Laboratory Networks
ICP	Intestinal contents preparation (pathogens); Inductively coupled plasma (chemistry)
ICP-AES	Inductively coupled plasma – atomic emission spectrometry
ICP-MS	Inductively coupled plasma – mass spectrometry
ICR	Information Collection Requirements Rule
IDL	Instrument detection limit
IFA	Immunofluorescence
ILM	Inorganic Laboratory Method
IMPA	Isopropyl methylphosphonic acid
IMS	Immunomagnetic separation
INCHEM	INCHEM is a means of rapid access to internationally peer reviewed information on chemicals commonly used throughout the world, which may also occur as contaminants in the environment and food. It consolidates information from a number of intergovernmental organizations whose goal it is to assist in the sound management of chemicals. http://www.inchem.org/

IO	Inorganic
i.p.	Intraperitoneally
IRIS	Integrated Risk Information System (EPA)
ISE	Ion specific electrode
ISG	Impregnated silica gel
ISO	International Organization for Standardization
KHP	Potassium hydrogen phthalate
L-1	Lewisite 1; 2-Chlorovinyldichloroarsine
L-2	Lewisite 2; bis(2-Chlorovinyl)chloroarsine
L-3	Lewisite 3; tris(2-Chlorovinyl)arsine
LC	Liquid chromatograph or Liquid chromatography
LC/APCI-MS	Liquid chromatography / atmospheric pressure chemical ionization – mass spectrometry
LC/ESI-MS	Liquid chromatography / electrospray ionization – mass spectrometry
LCMRL	Lowest common minimum reporting level
LC-MS	Liquid chromatography-mass spectrometry
LC-MS-MS	Liquid chromatography tandem mass spectrometry
LC-TSP	Liquid chromatography-thermospray
LFD	Lateral flow device
LLD	Lower limit of detection
LOD	Limit of detection
LOQ	Limit of quantitation
LRN	Laboratory Response Network
LSC	Liquid scintillation counter
LSE	Liquid-solid extraction
Ltd.	A private company limited by shares
M	Molar
mAbs	Monoclonal antibodies
MAE	Microwave-assisted extraction
MALDI	Matrix-assisted laser-desorption ionization
MARLAP	*Multi-Agency Radiological Laboratory Analytical Protocols* (EPA/402/B-04/001A, B, C)
MDEA	N-Methyldiethanolamine
MDL	Method detection limit
MFA	Monofluoroacetate
MIC	Methyl isocyanate
MLD	Minimum lethal dose
MPA	Methylphosphonic acid
MRM	Multiple reaction monitoring
mRNA	Messenger ribonucleic acid
MS	Mass spectrometer or Mass spectrometry
MS-MS	Tandem mass spectrometry
MS/MSD	Matrix spike/Matrix spike duplicate
MSE	Microscale solvent extraction
MTBE	Methyl *tert*-butyl ether
MW	Molecular weight
NA	Not applicable
NaI(Tl)	Thallium-activated sodium iodide
NAREL	National Air and Radiation Environmental Laboratory
NBD chloride	7-Chloro-4-nitrobenzo-2-oxa-1,3-diazole
NBD-F	7-Fluoro-4-nitro-2,1,3-benzoxadiazole
NCPDCID	National Center for the Prevention, Detection, and Control of Infectious Diseases

NCRP	National Council on Radiation Protection and Measurements
NEMI	National Environmental Methods Index
NERL	National Exposure Research Laboratory (EPA)
NHSRC	National Homeland Security Research Center (EPA)
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
nM	Nanomolar
NMAM	NIOSH Manual of Analytical Methods
NNSA	National Nuclear Security Administration
NPD	Nitrogen-phosphorus detector
NRC	U.S. Nuclear Regulatory Commission
NRMRL	National Risk Management Research Laboratory (EPA)
nS	Nano siemens
NTIS	National Technical Information Service
NTU	Nephelometric turbidity units
OAQPS	Office of Air Quality Planning and Standards (EPA)
OAR	Office of Air and Radiation (EPA)
ORAU	Oak Ridge Associated Universities
ORD	Office of Research and Development (EPA)
ORIA	Office of Radiation and Indoor Air (EPA)
ORISE	Oak Ridge Institute for Science and Education
OSWER	Office of Solid Waste and Emergency Response (EPA)
OSHA	Occupational Safety and Health Administration
OVS	OSHA versatile sampler
OW	Office of Water (EPA)
PBS	Phosphate buffered saline
PCDDs	Polychlorinated dibenzo-*p*-dioxins
PCDFs	Polychlorinated dibenzofurans
PCR	Polymerase chain reaction
PDA	Photodiode array detector
PEL	Permissible exposure limit
PETN	Pentaerythritol tetranitrate
PFBHA	O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine
PFE	Pressurized fluid extraction
PMPA	Pinacolyl methyl phosphonic acid
1,2-PP	1-(2-Pyridyl)piperazine
ppbv	Parts per billion by volume
pptv	Parts per trillion by volume
PTFE	Polytetrafluoroethylene
PubMED	U.S. National Library of Medicine (http://www.pubmed.gov)
PUF	Polyurethane foam
PVC	Polyvinyl chloride
PVDF	Polyvinylidene fluoride
QA	Quality assurance
QAP	Quality assessment program
QC	Quality control
®	Registered trademark
R 33	Methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester (VR)
RCRA	Resource Conservation and Recovery Act
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
RESL	Radiological and Environmental Sciences Laboratory
RLAB	Regional laboratory method

RNA	Ribonucleic acid
RTECS	Registry of Toxic Effects of Chemical Substances
RV-PCR	Rapid Viability-Polymerase Chain Reaction
SAED	Select area electron diffraction
SAM	*Selected Analytical Methods for Environmental Remediation and Recovery*
SARS	Severe Acute Respiratory Syndrome
SEA	Staphylococcal enterotoxin type A
SEB	Staphylococcal enterotoxin type B
SEC	Staphylococcal enterotoxin type C
SIM	Selective ion monitoring
SM	*Standard Methods for the Examination of Water and Wastewater*
sMac	sorbitol-MacConkey's
SPE	Solid-phase extraction
SRC	Syracuse Research Corporation
SRM	Single reaction monitoring
SRS	Savannah River National Laboratory, Savannah River Site
STEC	Shiga-toxigenic *E. coli*
STEL	Short term exposure limit
STX	Saxitoxin
Stx-1	Shiga toxin Type 1
Stx-2	Shiga toxin Type 2
SW	Solid Waste
TBD	To be determined
TCLP	Toxicity Characteristic Leaching Procedure
TDG	Thiodiglycol
TEA	Triethanolamine
TEM	Transmission electron microscope or Transmission electron microscopy
TEPP	Tetraethylpyrophosphate
TETS	Tetramethylenedisulfotetramine or tetramine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
™	Unregistered trademark
1,3,5-TNB	1,3,5-Trinitrobenzene
2,4,6-TNT	2,4,6-Trinitrotoluene
T_2O	Tritium oxide
TO	Toxic Organic
TOF	Time-of-flight
TOXNET	Toxicology Data Network
TRU	Transuranic
TSP	Thermospray
TTN	Technical Transfer Network
TTX	Tetrodotoxin
U.S.	United States
USDA	U.S. Department of Agriculture
USGS	U.S. Geological Survey
UV	Ultraviolet
VBNC	Viable but non-culturable
VCSB	Voluntary Consensus Standard Body
VE	Phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester
VG	Phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester
vis	Visible detector
VM	Phosphonothioic acid, methyl-,S-(2-(diethylamino)ethyl) O-ethyl ester

VOC	Volatile organic compound
VR	Methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester (R 33)
VX	O-Ethyl-S-(2-diisopropylaminoethyl)methylphosphonothiolate
WCIT	Water Contaminant Information Tool
WEF	Water Environment Federation
WLA	Water Laboratory Alliance
WHO	World Health Organization
WSD	Water Security Division (EPA, Office of Water)

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Selected Analytical Methods for Environmental Remediation and Recovery (SAM)

SAM 2012

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Section 1.0: Introduction

After the terrorist attacks of September 11, 2001 and the anthrax attacks in the fall of 2001, federal and state personnel provided response, recovery and remediation under trying circumstances, including unprecedented demand on laboratory capabilities to analyze environmental samples. In reviewing these events, the Environmental Protection Agency (EPA) identified several areas to enhance the resiliency of the nation following homeland security events related to intentional and unintentional contamination. The need to improve the nation's laboratory capacity and capability to analyze environmental samples following such events (i.e., chemical, biological and/or radiological [CBR] contamination) was one of the most important areas identified.

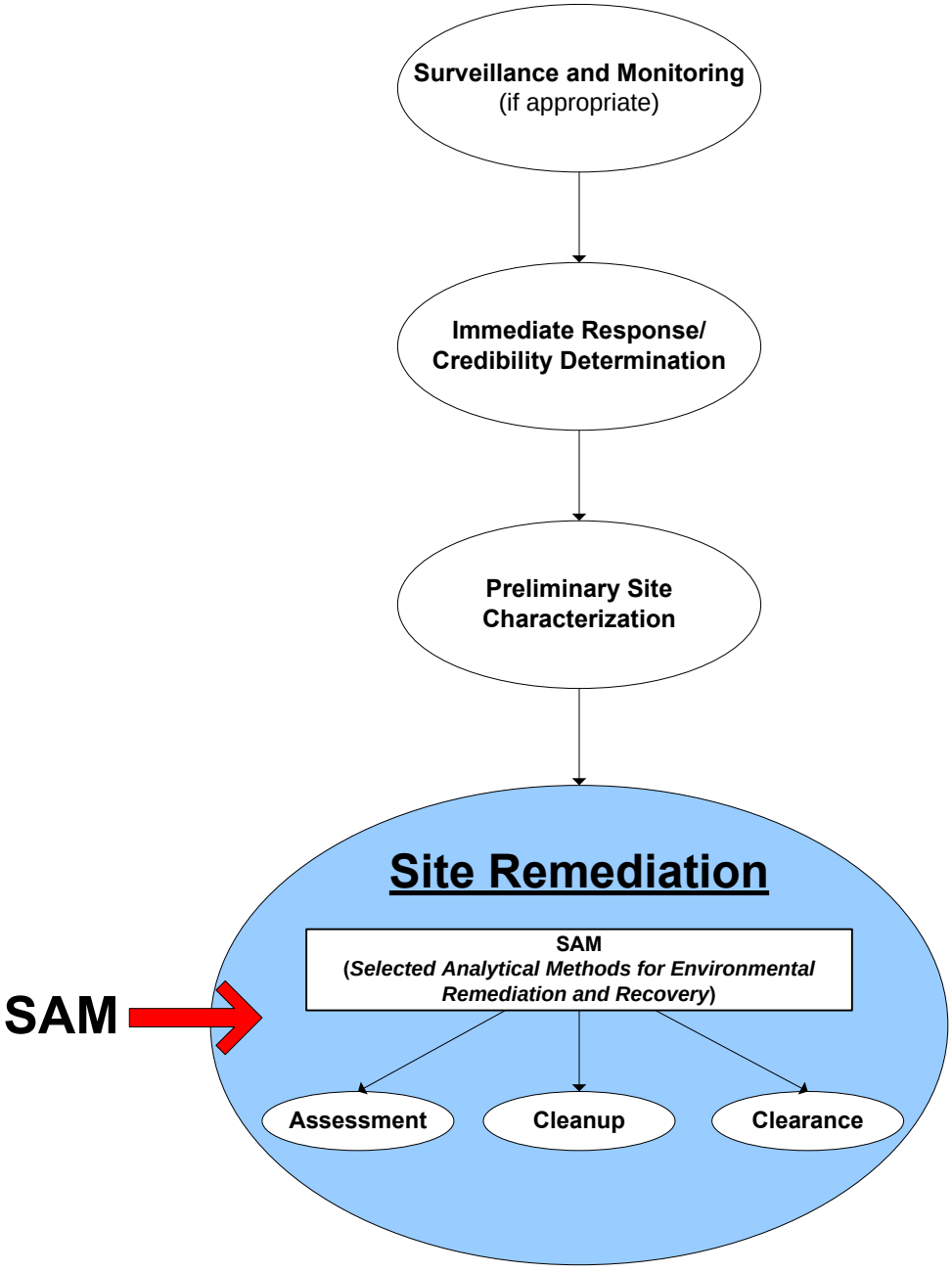
To address this need, EPA formed the Homeland Security Laboratory Capacity Work Group to identify and implement opportunities for near-term improvements and to develop recommendations for addressing longer-term laboratory issues. The EPA Homeland Security Laboratory Capacity Work Group consisted of representatives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

A critical area identified by the work group was the need for a list of selected analytical methods to be used (ideally) by all laboratories when analyzing contamination event samples and, in particular, when analysis of many samples is required over a short period of time. Using the same selected methods would reduce confusion, permit sharing of sample load between laboratories, improve data comparability, and simplify the task of outsourcing analytical support to the commercial laboratory sector. Use of such methods would also improve the follow-up activities of validating results, evaluating data and making decisions. To this end, work group members formed an Analytical Methods Subteam to address homeland security methods issues.

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

[1] Formerly EPA's *Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events* (SAM). SAM and its methods are available at: www.epa.gov/sam

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



Section 2.0: Background

In support of this document, EPA periodically assembles methods experts from within EPA, as well as other federal, state and local agencies; public utilities; national laboratories; and academia, to review methods and, if necessary, revise the methods listed. SAM analytes are included based on selection criteria that address the needs and priorities of EPA as well as other federal agencies (e.g., environmental persistence, half lives, availability, toxicity). The sample types listed in SAM are specific to each technical section and have been determined by the SAM technical work groups to be a concern during site remediation. SAM identifies a single method or method group per analyte/sample type to ensure a consistent analytical approach across multiple laboratories when analyzing environmental samples following an event. Method selection is based on consideration of specific criteria that emphasize method performance and include existing laboratory capabilities, laboratory capacity, method applicability to multiple environmental sample types, and method applicability to multiple SAM analytes. For some analytes, the preferred method is a clear choice; for others, competing criteria make the choice more difficult. Final method selections are based on technical recommendations from the SAM work groups under the direction of EPA's National Homeland Security Research Center (NHSRC). For analytes where limited laboratory capacity exists, such as chemical warfare agents (CWAs), methods were selected based on their applicability to similar chemicals (e.g., nerve agents and some pesticides). In these cases, laboratory studies to evaluate the ability of the selected method to measure the target analyte(s) are either underway or needed. **Figure 2-1** summarizes steps and provides the criteria used during the SAM method selection process. It is important to note that the method selection criteria included in this figure are listed in non-hierarchical order and, in some cases, only a subset of the criteria was considered.

Since 2004, NHSRC has brought together experts from across EPA and its sister agencies to develop this compendium of analytical methods to be used when analyzing environmental samples, and to address site characterization, remediation and clearance following homeland security events. Participants have included representatives from EPA program offices, EPA regions, EPA laboratories, Centers for Disease Control and Prevention (CDC), Food and Drug Administration (FDA), Department of Homeland Security (DHS), Federal Bureau of Investigation (FBI), Department of Defense (DoD), Department of Agriculture (USDA), U.S. Geological Survey (USGS), numerous state agencies and universities. Methodologies are considered for chemical, radiochemical, biological and biotoxin agents of concern in the types of environmental samples that would be anticipated. The primary objective of this effort is to support EPA's Environmental Response Laboratory Network (ERLN) and Water Laboratory Alliance (WLA) by identifying appropriate SAM methods that represent a balance between providing existing, documented techniques and providing consistent and valid analytical results.

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

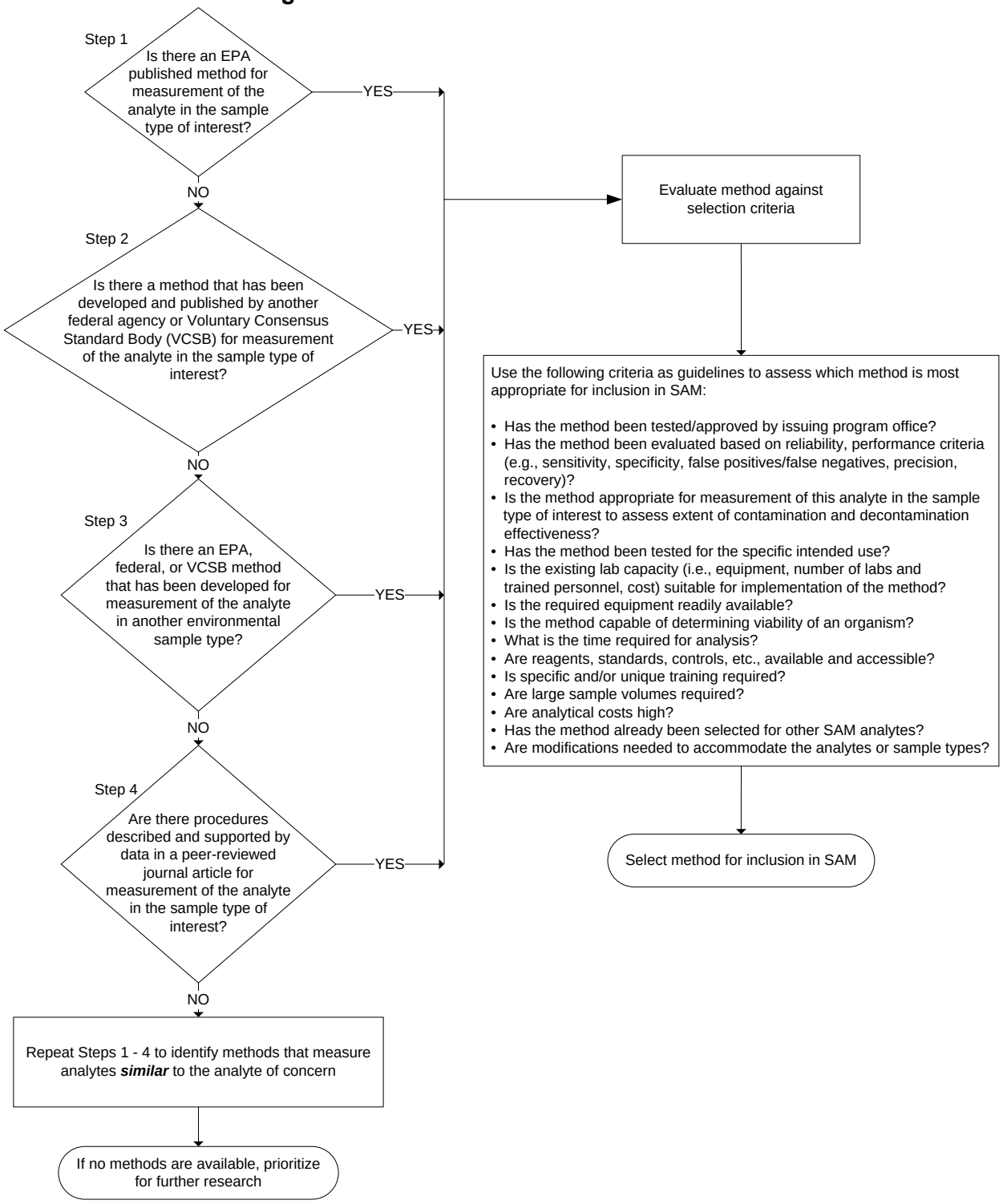
- National Environmental Methods Index (NEMI) and NEMI for Chemical, Biological and Radiological Methods (NEMI-CBR)
- Environmental Monitoring Method Index (EMMI)
- EPA Test Methods Index
- EPA Office of Water Methods
- EPA Office of Solid Waste SW-846 Methods
- EPA Microbiological Methods
- National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

- Scientific Literature

Since publication of SAM Revision 1.0 in September 2004, NHSRC has continued to convene technical work groups to evaluate and, if necessary, update the analytes and methods that are listed. Details regarding changes that have been incorporated into each revision of SAM are provided in Attachment 1. SAM 2012 also reflects a title change agreed to by stakeholders (i.e., EPA's NHSRC; Office of Groundwater and Drinking Water, Water Security Division (WSD) and WLA; Office of Emergency Management and ERLN; Office of Radiation and Indoor Air (ORIA); and Regional Offices) during a 2010 SAM Summit, to better reflect SAM's focus on providing selected analytical methods for use across multiple laboratories during environmental remediation and recovery. This current revision (SAM 2012) includes the addition of vegetation as a sample type under the radiochemistry sections, the addition of method applicability tiers to Appendix A (Selected Chemical Methods), several new methods added or replaced for currently listed chemical analytes, clarification of immunoassay methods listed for biotoxin analytes, and the addition of restructured pathogen sections to more clearly define scope and application.

In addition to updating SAM analytes and methods, SAM work groups have identified four areas for development of SAM companion documents to provide information regarding field screening equipment, sample collection, rapid screening and preliminary analysis equipment, and sample disposal to supplement the analytical methods included in SAM. The information listed in these documents generally corresponds to the analytes and methods in SAM and will be updated as resources allow to reflect revisions to SAM. Currently available SAM companion documents are listed in Attachment 1.

Figure 2-1. SAM Method Selection Process



Section 3.0: Scope and Application

The premise and purpose of this document is to select the analytical methods that will be used in cases when multiple laboratories are called on to analyze environmental samples following an intentional or unintentional incident such as a homeland security event (e.g., CBR contamination). The document is intended to support the ERLN and WLA, and also can be used as a tool to assist state and local laboratories in planning for and analyzing environmental samples following such events. The methods presented in this document should be used to:

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

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

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

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

At this time, only some of the methods selected have been validated for the listed analyte and sample type. However, the methods are considered to contain the most appropriate currently available techniques based on expert judgment. Unless a published method listed in this document states specific applicability to the analyte/sample type for which it has been selected, it should be assumed that method evaluation is needed, and adjustments may be required to accurately account for variations in analyte/sample type characteristics, environmental samples, analytical interferences, and data quality objectives (DQOs).

EPA will strive to continue development and evaluation of analytical protocols, including optimization of procedures for measuring target analytes or agents in specific sample types, as appropriate. In those cases where method procedures are determined to be insufficient for a particular situation, NHSRC will continue to provide technical support regarding appropriate actions. NHSRC has also compiled information and published documents regarding field screening equipment, sample collection materials, rapid screening/preliminary identification equipment, and disposal of samples corresponding to SAM analytes and sample types. These documents are available at www.epa.gov/sam/samcomp.htm.

EPA recognizes that specification of a single method may limit laboratory capacity and techniques that may be needed to evaluate difficult samples. In those cases where method procedures are determined to be insufficient for a particular situation, EPA will provide technical advice regarding appropriate actions (see list of contacts in Section 4). Additional information is also provided in the Agency Policy Directive Number FEM-2010-01.[2] Where further development and testing are necessary, EPA is continuing to develop and evaluate analytical protocols based on the methods that are listed in this document and on current EPA policies for validating analytical methods. Once validation is complete, data regarding the resulting method performance and data quality objectives (DQOs) will be available. EPA plans to continue to update the SAM document as appropriate to address the needs of homeland security, to reflect improvements in analytical methodology and new technologies, and to incorporate changes in analytes based on needs. EPA also anticipates that addenda may be generated to provide updates regarding information and issues that currently are not addressed by this document. Information regarding the use of deviations from the methods referenced in this document is provided in Section 4.

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

- Selecting technologies that may not be the most cost-effective technologies currently available for addressing the particular situation at hand;

[2] U.S. EPA, Forum of Environmental Measurements, July 21, 2010, *Ensuring the Validity of Agency Methods Validation and Peer Review Guidelines: Methods of Analysis Developed for Emergency Response Situations,* Agency Policy Directive Number FEM-2010-01.

- Selecting methodologies that may not be appropriate for use in responding to a particular event because EPA did not anticipate having to analyze for a particular analyte or analyte/sample type combination; and
- Discouraging development and adoption of new and better measurement technologies.

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

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

Public officials need to accurately assess and characterize site contamination following an emergency situation. This assessment includes initial characterization of potential site contamination for determination of immediate public and environmental risk, determination of the extent of contamination, and effective approaches for site remediation. EPA recognizes that having data of known and documented quality is critical in making proper decisions during each of these activities, and strives to establish DQOs for each response activity.[3] These DQOs are based upon needs for both quality and response time. During initial assessments, time is of utmost importance and DQOs must be established that weigh the need for rapid analytical response (e.g., using screening methods) against the need for very high quality data. Many of the methods listed in this document include quality control (QC) requirements for collecting and analyzing samples. EPA will assess these QC requirements to ensure analytical data quality supports decisions concerning site remediation and release. These QC requirements may be adjusted as necessary to maximize data and decision quality. Specific QC considerations and recommendations for analysis of samples for chemical, radiochemical, pathogen and biotoxin analytes are provided in each corresponding section of this document (i.e., Sections 5.1.2, 6.1.2, 7.1.2 and 8.1.2, respectively). EPA's ERLN, which is tasked with providing laboratory support following homeland security-related contamination events, also has established data reporting procedures. Requirements for receiving, tracking, storing, preparing, analyzing and reporting data are specified in the U.S. EPA (2011) *Environmental Response Laboratory Network Laboratory Requirements Document* at: http://epa.gov/erln/techsupport.html; project-specific requirements also are included in individual Analytical Service Requests (ASRs).

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

Section 4.0: Points of Contact

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

General	
Kathy Hall - *Primary* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 379-5260 hall.kathy@epa.gov	Romy Lee - *Alternate* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 569-7016 lee.romy@epa.gov
Chemical Methods	
Steve Reimer - *Primary* U.S. EPA Region 10 - Manchester Laboratory 7411 Beach Drive East Port Orchard, WA 98366 (360) 871-8718 reimer.steve@epa.gov	Matthew Magnuson - *Alternate* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 569-7321 magnuson.matthew@epa.gov
Radiochemical Methods	
John Griggs - *Primary* U.S. EPA Office of Radiation and Indoor Air Environmental Laboratory 540 South Morris Avenue Montgomery, AL 36115-2601 (334) 270-3450 griggs.john@epa.gov	Kathy Hall - *Alternate* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 379-5260 hall.kathy@epa.gov
Pathogen Methods	
Sanjiv Shah - *Primary* National Homeland Security Research Center U.S. EPA ORD - 8801RR 1300 Pennsylvania Avenue, NW Washington, DC 20460 (202) 564-9522 shah.sanjiv@epa.gov	Erin Silvestri - *Alternate* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 569-7619 silvestri.erin@epa.gov
Biotoxins Methods	
Matthew Magnuson - *Primary* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 569-7321 magnuson.matthew@epa.gov	Sanjiv Shah - *Alternate* National Homeland Security Research Center U.S. EPA ORD - 8801RR 1300 Pennsylvania Avenue, NW Washington, DC 20460 (202) 564-9522 shah.sanjiv@epa.gov

Section 5.0: Selected Chemical Methods

Appendix A provides a list of methods to be used in analyzing environmental samples for chemical contaminants during remediation activities that result from a homeland security event. Methods are listed for each analyte and for each sample type that potentially may need to be measured and analyzed when responding to an environmental contamination incident. Procedures from peer-reviewed journal articles are listed for those analyte-sample type combinations where methods are not available. Once standard procedures are available, the literature references will be replaced.

Please note: This section provides guidance for selecting chemical methods that have a high likelihood of assuring analytical consistency when laboratories are faced with a large scale environmental restoration crisis. Not all methods have been verified for the analyte/sample type combination listed in Appendix A. Please refer to the specified method to identify analyte/sample type combinations that have been verified. Any questions regarding information discussed in this section should be addressed to the appropriate contact(s) listed in Section 4.

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

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

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

5.1 General Guidelines

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

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

- Syracuse Research Corporation's (SRC) PHYSPROP (http://srcinc.com/what-we-do/product.aspx?id=133) and CHEMFATE (http://srcinc.com/what-we-do/product.aspx?id=132&terms=Environmental+Fate+and+Exposure) contain information pertaining to chemical structures, names, physical properties and persistence. PHYSPROP and CHEMFATE are sponsored by EPA.
- INCHEM (http://www.inchem.org/) contains both chemical and toxicity information.
- The Registry of Toxic Effects of Chemical Substances (RTECS) database can be accessed via the National Institute for Occupational Safety and Health (NIOSH) website (http://www.cdc.gov/niosh/rtecs/default.html) for toxicity information.
- EPA's Integrated Risk Information System (IRIS) (http://www.epa.gov/iris/) contains toxicity information (searchable on TOXNET).
- EPA's Water Contaminant Information Tool (WCIT) (http://www.epa.gov/wcit) can be accessed by registered users.
- *Forensic Science and Communications* (http://www.fbi.gov/about-us/lab/forensic-science-communications) is published by the Laboratory Division of the Federal Bureau of Investigation (FBI).
- Joint Research Centre / Institute for Health & Consumer Protection (http://ihcp.jrc.ec.europa.eu/) contains information regarding European Directive 67/548/EEC and Annex V.
- Agency of Toxic Substances & Disease Registry (ATSDR) Toxic Substances Portal (http://www.atsdr.cdc.gov/toxprofiles/index.asp) provides Toxicological Profiles.

Additional research on chemical contaminants is ongoing within EPA. Databases to manage this information are currently under development.

5.1.1 Standard Operating Procedures for Identifying Chemical Methods

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

The fitness of a method for an intended use is related to site-specific data quality objectives (DQOs) for a particular environmental remediation activity. These selected chemical methods have been assigned the tiers (below) to indicate a level of method usability for the specific analyte and sample type. The assigned tiers reflect the conservative view for DQOs involving timely implementation of methods for analysis of a

high number of samples (such that multiple laboratories are necessary), low limits of identification and quantification, and appropriate QC:

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

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

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

Once a method has been identified in Appendix A, **Table 5-1** can be used to locate the method summary. Sections 5.2.1 through 5.2.99 below provide summaries of the sample preparation and determinative methods listed in Appendix A.

Table 5-1. Chemical Methods and Corresponding Section Numbers

Analyte	CAS RN	Method	Section
Acephate	30560-19-1	538 (EPA OW)	5.2.10
		Chromatographia (2006) 63(5/6): 233 – 237	5.2.89
		J. Chromatogr. A (2007) 1154(1): 3 – 25	5.2.94
Acrylamide	79-06-1	3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8316 (EPA SW-846)	5.2.34
		PV2004 (OSHA)	5.2.71
Acrylonitrile	107-13-1	524.2 (EPA OW)	5.2.7
		3570 (EPA SW-846)	5.2.20
		5035A (EPA SW-846)	5.2.22
		8260C (EPA-SW846)	5.2.30
		8290A Appendix A (EPA SW-846)	5.2.32
		PV2004 (OSHA)	5.2.71
Aldicarb (Temik)	116-06-3	531.2 (EPA OW)	5.2.9
		3570 (EPA SW-846)	5.2.20
Aldicarb sulfone	1646-88-4	8290A Appendix A (EPA SW-846)	5.2.32
		8318A (EPA SW-846)	5.2.35
Aldicarb sulfoxide	1646-87-3	5601 (NIOSH)	5.2.54
		D7645-10 (ASTM)	5.2.79

Analyte	CAS RN	Method	Section
Allyl alcohol	107-18-6	5030C (EPA SW-846)	5.2.21
		5035A (EPA SW-846)	5.2.22
		8260C (EPA-SW846)	5.2.30
		TO-15 (EPA ORD)	5.2.45
4-Aminopyridine	504-24-5	3535A (EPA SW-846)	5.2.17
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8330B (EPA SW-846)	5.2.37
Ammonia	7664-41-7	350.1 (EPA OW)	5.2.6
		6015 (NIOSH)	5.2.59
		4500-NH3 B (SM)	5.2.84
		4500-NH3 G (SM)	5.2.85
Ammonium metavanadate (analyze as total vanadium) Arsenic, Total Arsenic trioxide (analyze as total arsenic)	7803-55-6 7440-38-2 1327-53-3	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3050B (EPA SW-846)	5.2.14
		6010C (EPA SW-846)	5.2.23
		6020A (EPA SW-846)	5.2.24
		IO-3.1 (EPA ORD)	5.2.40
		IO-3.4 (EPA ORD)	5.2.41
		IO-3.5 (EPA ORD)	5.2.42
		9102 (NIOSH)	5.2.64
Arsine (analyze as total arsenic in non-air samples)	7784-42-1	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3050B (EPA SW-846)	5.2.14
		6010C (EPA SW-846)	5.2.23
		6020A (EPA SW-846)	5.2.24
		6001 (NIOSH)	5.2.55
		9102 (NIOSH)	5.2.64
Asbestos	1332-21-4	D5755-03 (ASTM)	5.2.73
		D6480-05 (ASTM)	5.2.74
		10312:1995 (ISO)	5.2.83
Boron trifluoride	2095581	ID216SG (OSHA)	5.2.70
Brodifacoum Bromadiolone	56073-10-0 28772-56-7	3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8321B (EPA SW-846)	5.2.36
		D7644-10 (ASTM)	5.2.78
BZ [Quinuclidinyl benzilate]	1709855	3520C (EPA SW-846)	5.2.16
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8321B (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.44

Analyte	CAS RN	Method	Section
Calcium arsenate (analyze as total arsenic)	7778-44-1	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3050B (EPA SW-846)	5.2.14
		6010C (EPA SW-846)	5.2.23
		6020A (EPA SW-846)	5.2.24
		IO-3.1 (EPA ORD)	5.2.40
		IO-3.4 (EPA ORD)	5.2.41
		IO-3.5 (EPA ORD)	5.2.42
		9102 (NIOSH)	5.2.64
Carbofuran (Furadan)	1563-66-2	531.2 (EPA OW)	5.2.9
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8318A (EPA SW-846)	5.2.35
		5601 (NIOSH)	5.2.54
		D7645-10 (ASTM)	5.2.79
Carbon disulfide	75-15-0	524.2 (EPA OW)	5.2.7
		5030C (EPA SW-846)	5.2.21
		5035A (EPA SW-846)	5.2.22
		8260C (EPA-SW846)	5.2.30
		TO-15 (EPA ORD)	5.2.45
Carfentanil	59708-52-0	3520C (EPA SW-846)	5.2.16
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8321B (EPA SW-846)	5.2.36
Chlorfenvinphos	470-90-6	3520C (EPA SW-846)	5.2.16
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Chlorine	7782-50-5	4500-Cl G (SM)	5.2.86
		Analyst (1999) 124(12): 1853–1857	5.2.88
2-Chloroethanol	107-07-3	5030C (EPA SW-846)	5.2.21
		5035A (EPA SW-846)	5.2.22
		8260C (EPA-SW846)	5.2.30
		2513 (NIOSH)	5.2.51
3-Chloro-1,2-propanediol	96-24-2	TO-10A (EPA ORD)	5.2.44
		Eur. J. Lipid Sci. Technol. (2011) 113: 345 – 355	5.2.91
		J. Chromatogr. A (2000) 866: 65 – 77	5.2.96

Analyte	CAS RN	Method	Section
Chloropicrin	76-06-2	551.1 (EPA OW)	5.2.12
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		PV2103 (OSHA)	5.2.72
Chlorosarin Chlorosoman	1445-76-7 7040-57-5	3520C (EPA SW-846)	5.2.16
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
2-Chlorovinylarsonous acid (2-CVAA) (analyze as total arsenic)	85090-33-1	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3050B (EPA SW-846)	5.2.14
		6010C (EPA SW-846)	5.2.23
		6020A (EPA SW-846)	5.2.24
		IO-3.1 (EPA ORD)	5.2.40
		IO-3.4 (EPA ORD)	5.2.41
		IO-3.5 (EPA ORD)	5.2.42
		9102 (NIOSH)	5.2.64
Chlorpyrifos	2921-88-2	525.2 (EPA OW)	5.2.8
		3511 (EPA SW-846)	5.2.15
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Chlorpyrifos oxon	5598-15-2	525.2 (EPA OW)	5.2.8
		3520C (EPA SW-846)	5.2.16
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Crimidine	535-89-7	3511 (EPA SW-846)	5.2.15
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
Cyanide, Amenable to chlorination	NA	RLAB Method 3135.2I	5.2.39
Cyanide, Total	57-12-5	335.4 (EPA OW)	5.2.5
		ISM01.3 CN (EPA CLP)	5.2.38
		6010 (NIOSH)	5.2.57
Cyanogen chloride	506-77-4	TO-15 (EPA ORD)	5.2.45
		Encyclopedia of Anal. Chem. (2006) DOI:10.1002/9780470027318.a0809	5.2.90

Analyte	CAS RN	Method	Section
Cyclohexyl sarin (GF)	329-99-7	CWA Protocol (EPA NHSRC)	5.2.48
1,2-Dichloroethane	107-06-2	524.2 (EPA OW)	5.2.7
		5030C (EPA SW-846)	5.2.21
		5035A (EPA SW-846)	5.2.22
		8260C (EPA-SW846)	5.2.30
		TO-15 (EPA ORD)	5.2.45
Dichlorvos	62-73-7	525.2 (EPA OW)	5.2.8
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Dicrotophos	141-66-2	3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Diesel range organics	NA	3520C (EPA SW-846)	5.2.16
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8015C (EPA SW-846)	5.2.29
		8290A Appendix A (EPA SW-846)	5.2.32
Diisopropyl methylphosphonate (DIMP)	1445-75-6	538 (EPA OW)	5.2.10
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8321B (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.44
		D7597-09 (ASTM)	5.2.75
		E-2866-12 (ASTM)	5.2.82
Dimethylphosphite	868-85-9	3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Dimethylphosphoramidic acid	33876-51-6	3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		5035A (EPA SW-846)	5.2.22
		8290A Appendix A (EPA SW-846)	5.2.32
		8321B (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.44

Analyte	CAS RN	Method	Section
Diphacinone	82-66-6	3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8321B (EPA SW-846)	5.2.36
		D7644-10 (ASTM)	5.2.79
Disulfoton	298-04-4	525.2 (EPA OW)	5.2.8
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		5600 (NIOSH)	5.2.53
Disulfoton sulfone oxon Disulfoton sulfoxide Disulfoton sulfoxide oxon	2496-91-5 2497-07-6 2496-92-6	525.2 (EPA OW)	5.2.8
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		5600 (NIOSH)	5.2.53
1,4-Dithiane	505-29-3	3511 (EPA SW-846)	5.2.15
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4	3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8321B (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.44
Ethyl methylphosphonic acid (EMPA)	1832-53-7	8290A Appendix A (EPA SW-846)	5.2.32
		8321B (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.44
		D7597-09 (ASTM)	5.2.75
		E-2866-12 (ASTM)	5.2.82
Ethyldichloroarsine (ED)	598-14-1	3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		8270D (EPA SW-846)	5.2.31
		TO-15 (EPA ORD)	5.2.45
		9102 (NIOSH)	5.2.64
N-Ethyldiethanolamine (EDEA)	139-87-7	3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		8321B (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.44
		EPA 600/R-11/143 (EPA / NIOSH)	5.2.47
		D7599-09 (ASTM)	5.2.77

Analyte	CAS RN	Method	Section
Ethylene oxide	75-21-8	5030C (EPA SW-846)	5.2.21
		5035A (EPA SW-846)	5.2.22
		8260C (EPA SW-846)	5.2.30
		TO-15 (EPA ORD)	5.2.45
Fenamiphos	22224-92-6	525.2 (EPA OW)	5.2.8
		3511 (EPA SW-846)	5.2.15
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Fentanyl	437-38-7	3520C (EPA SW-846)	5.2.16
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8321B (EPA SW-846)	5.2.36
Fluoride	16984-48-8	300.1, Rev 1.0 (EPA OW)	5.2.4
Fluoroacetamide	640-19-7	J. Chromatogr. B (2008) 876(1): 103 – 108	5.2.98
Fluoroacetic acid and fluoroacetate salts	NA	S301-1 (NIOSH)	5.2.65
		J. Chromatogr. A (2007) 1139: 271 – 278	5.2.95
		J. Chromatogr. B. (2010) 878: 1045 –1050	5.2.97
2-Fluoroethanol	371-62-0	5030C (EPA SW-846)	5.2.21
		5035A (EPA SW-846)	5.2.22
		8260C (EPA-SW846)	5.2.30
		2513 (NIOSH)	5.2.51
Formaldehyde	50-00-0	556.1 (EPA OW)	5.2.13
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8315A (EPA SW-846)	5.2.33
		2016 (NIOSH)	5.2.50
Gasoline range organics	NA	3570 (EPA SW-846)	5.2.20
		5030C (EPA SW-846)	5.2.21
		5035A (EPA SW-846)	5.2.22
		8015C (EPA SW-846)	5.2.29
		8290A Appendix A (EPA SW-846)	5.2.32
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4	3535A (EPA SW-846)	5.2.17
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8330B (EPA SW-846)	5.2.37
Hexamethylenetriperoxidediamine (HMTD)	283-66-9	3535A (EPA SW-846)	5.2.17
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8330B (EPA SW-846)	5.2.37
		Analyst (2001) 126: 1689 – 1693	5.2.87

Analyte	CAS RN	Method	Section
Hydrogen bromide Hydrogen chloride	10035-10-6 7647-01-0	7903 (NIOSH)	5.2.61
Hydrogen cyanide	74-90-8	6010 (NIOSH)	5.2.57
Hydrogen fluoride	7664-39-3	7903 (NIOSH)	5.2.61
Hydrogen sulfide	2148878	6013 (NIOSH)	5.2.58
Isopropyl methylphosphonic acid (IMPA)	1832-54-8	3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8321B (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.44
		D7597-09 (ASTM)	5.2.75
		E-2866-12 (ASTM)	5.2.82
Kerosene	64742-81-0	3570 (EPA SW-846)	5.2.20
		5030C (EPA SW-846)	5.2.21
		5035A (EPA SW-846)	5.2.22
		8015C (EPA SW-846)	5.2.29
		8290A Appendix A (EPA SW-846)	5.2.32
Lead arsenate (analyze as total arsenic) Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic) Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic) Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic) Lewisite oxide (analyze as total arsenic)	7645-25-2 541-25-3 40334-69-8 40334-70-1 1306-02-1	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3050B (EPA SW-846)	5.2.14
		6010C (EPA SW-846)	5.2.23
		6020A (EPA SW-846)	5.2.24
		IO-3.1 (EPA ORD)	5.2.40
		IO-3.4 (EPA ORD)	5.2.41
		IO-3.5 (EPA ORD)	5.2.42
		9102 (NIOSH)	5.2.64
Mercuric chloride (analyze as total mercury)	7487-94-7	245.1 (EPA OW)	5.2.3
		7473 (EPA SW-846)	5.2.27
		9102 (NIOSH)	5.2.64
Mercury, Total	7439-97-6	245.1 (EPA OW)	5.2.3
		7473 (EPA SW-846)	5.2.27
		IO-5 (EPA ORD)	5.2.43
		9102 (NIOSH)	5.2.64
Methamidophos	10265-92-6	538 (EPA OW)	5.2.10
		Chromatographia (2006) 63(5/6): 233 – 237	5.2.89
		J. Chromatogr. A (2007) 1154(1): 3 – 25	5.2.94
Methomyl	16752-77-5	531.2 (EPA OW)	5.2.9
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8318A (EPA SW-846)	5.2.35
		5601 (NIOSH)	5.2.54
		D7645-10 (ASTM)	5.2.79

Analyte	CAS RN	Method	Section
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2	245.1 (EPA OW)	5.2.3
		7473 (EPA SW-846)	5.2.27
		IO-5 (EPA ORD)	5.2.43
		9102 (NIOSH)	5.2.64
Methyl acrylonitrile	126-98-7	524.2 (EPA OW)	5.2.7
		3570 (EPA SW-846)	5.2.20
		5035A (EPA SW-846)	5.2.22
		8260C (EPA-SW846)	5.2.30
		8290A Appendix A (EPA SW-846)	5.2.32
		PV2004 (OSHA)	5.2.71
Methyl fluoroacetate (analyze as fluoroacetate ion)	453-18-9	S301-1 (NIOSH)	5.2.65
		J. Chromatogr. A (2007) 1139: 271 – 278	5.2.95
		J. Chromatogr. B (2010) 878: 1045 –1050	5.2.97
Methyl hydrazine	60-34-4	3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		3510 (NIOSH)	5.2.52
		J. Chromatogr. (1993) 617: 157 – 162	5.2.92
Methyl isocyanate	624-83-9	OSHA 54	5.2.67
Methyl paraoxon Methyl parathion	950-35-6 298-00-0	3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Methylamine	74-89-5	OSHA 40	5.2.66
N-Methyldiethanolamine (MDEA)	105-59-9	3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		8321B (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.44
		EPA 600/R-11/143 (EPA / NIOSH)	5.2.47
		D7599-09 (ASTM)	5.2.77
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3	3520C (EPA SW-846)	5.2.16
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44

Analyte	CAS RN	Method	Section
Methylphosphonic acid (MPA)	993-13-5	3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8321B (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.44
		D7597-09 (ASTM)	5.2.75
		E-2866-12 (ASTM)	5.2.82
Mevinphos	7786-34-7	525.2 (EPA OW)	5.2.8
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Monocrotophos	6923-22-4	3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)-ethylamine]	538-07-8	3520C (EPA SW-846)	5.2.16
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)-methylamine] Mustard, nitrogen (HN-3) [tris(2-chloroethyl)-amine]	51-75-2 555-77-1	3520C (EPA SW-846)	5.2.16
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Mustard, sulfur / Mustard gas (HD)	505-60-2	CWA Protocol (EPA NHSRC)	5.2.48
Nicotine compounds	54-11-5	3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0	3535A (EPA SW-846)	5.2.17
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8330B (EPA SW-846)	5.2.37

Analyte	CAS RN	Method	Section
Osmium tetroxide (analyze as total osmium)	20816-12-0	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3050B (EPA SW-846)	5.2.14
		6010C (EPA SW-846)	5.2.23
		IO-3.1 (EPA ORD)	5.2.40
		IO-3.4 (EPA ORD)	5.2.41
		9102 (NIOSH)	5.2.64
Oxamyl	23135-22-0	531.2 (EPA OW)	5.2.9
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8318A (EPA SW-846)	5.2.35
		5601 (NIOSH)	5.2.54
		D7645-10 (ASTM)	5.2.79
Paraoxon	311-45-5	3520C (EPA SW-846)	5.2.16
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
Parathion	56-38-2	3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Paraquat	4685-14-7	549.2 (EPA OW)	5.2.11
		J. Chromatogr. A (2008) 1196-97: 110 – 116	5.2.93
Pentaerythritol tetranitrate (PETN)	78-11-5	3535A (EPA SW-846)	5.2.17
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8330B (EPA SW-846)	5.2.37
Phencyclidine	77-10-1	3511 (EPA SW-846)	5.2.15
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Phorate	298-02-2	3535A (EPA SW-846)	5.2.17
Phorate sulfone	2588-04-7	3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
Phorate sulfone oxon	2588-06-9	3570 (EPA SW-846)	5.2.20
Phorate sulfoxide	2588-03-6	8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
Phorate sulfoxide oxon	2588-05-8	TO-10A (EPA ORD)	5.2.44
Phosgene	75-44-5	OSHA 61	5.2.68

Analyte	CAS RN	Method	Section
Phosphamidon	13171-21-6	3520C (EPA SW-846)	5.2.16
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Phosphine	7803-51-2	6002 (NIOSH)	5.2.56
Phosphorus trichloride	2125683	6402 (NIOSH)	5.2.60
Pinacolyl methyl phosphonic acid (PMPA)	616-52-4	3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8321B (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.44
		D7597-09 (ASTM)	5.2.75
		E-2866-12 (ASTM)	5.2.82
Propylene oxide	75-56-9	5030C (EPA SW-846)	5.2.21
		5035A (EPA SW-846)	5.2.22
		8260C (EPA-SW846)	5.2.30
		1612 (NIOSH)	5.2.49
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4	3520C (EPA SW-846)	5.2.16
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Sarin (GB) Soman (GD)	107-44-8 96-64-0	CWA Protocol (EPA NHSRC)	5.2.48
Sodium arsenite (analyze as total arsenic)	7784-46-5	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3050B (EPA SW-846)	5.2.14
		6010C (EPA SW-846)	5.2.23
		6020A (EPA SW-846)	5.2.24
		IO-3.1 (EPA ORD)	5.2.40
		IO-3.4 (EPA ORD)	5.2.41
		IO-3.5 (EPA ORD)	5.2.42
		9102 (NIOSH)	5.2.64
Sodium azide (analyze as azide ion)	26628-22-8	300.1, Rev 1.0 (EPA OW)	5.2.4
		ID-211 (OSHA)	5.2.69
		J. Forensic Sci. (1998) 43(1): 200 – 202	5.2.99

Analyte	CAS RN	Method	Section
Strychnine	57-24-9	3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
Tabun (GA)	77-81-6	3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Tetraethyl pyrophosphate (TEPP)	107-49-3	3511 (EPA SW-846)	5.2.15
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
Tetramethylenedisulfotetramine (TETS)	80-12-6	3511 (EPA SW-846)	5.2.15
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
		EPA 600/R-11/091 (EPA / CDC)	5.2.46
Thallium sulfate (analyze as total thallium)	10031-59-1	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3050B (EPA SW-846)	5.2.14
		6010C (EPA SW-846)	5.2.23
		6020A (EPA SW-846)	5.2.24
		IO-3.1 (EPA ORD)	5.2.40
		IO-3.4 (EPA ORD)	5.2.41
		IO-3.5 (EPA ORD)	5.2.42
		9102 (NIOSH)	5.2.64
Thiodiglycol (TDG)	111-48-8	TO-10A (EPA ORD)	5.2.44
		D7598-09 (ASTM)	5.2.76
		E2787-11 (ASTM)	5.2.80
		E2838-11 (ASTM)	5.2.81
Thiofanox	39196-18-4	538 (EPA OW)	5.2.10
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8321B (EPA SW-846)	5.2.36
		5601 (NIOSH)	5.2.54
		D7645-10 (ASTM)	5.2.79

Analyte	CAS RN	Method	Section
1,4-Thioxane	15980-15-1	3511 (EPA SW-846)	5.2.15
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
Titanium tetrachloride (analyze as total titanium)	7550-45-0	3050B (EPA SW-846)	5.2.14
		6010C (EPA SW-846)	5.2.24
		6020A (EPA SW-846)	5.2.25
		9102 (NIOSH)	5.2.64
Triethanolamine (TEA)	102-71-6	3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		8321B (EPA SW-846)	5.2.36
		TO-10A (EPA ORD)	5.2.44
		EPA 600/R-11/143 (EPA / NIOSH)	5.2.47
		D7599-09 (ASTM)	5.2.77
Trimethyl phosphite	121-45-9	3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
1,3,5-Trinitrobenzene (1,3,5-TNB) 2,4,6-Trinitrotoluene (2,4,6-TNT)	99-35-4 118-96-7	3535A (EPA SW-846)	5.2.17
		3570 (EPA SW-846)	5.2.20
		8290A Appendix A (EPA SW-846)	5.2.32
		8330B (EPA SW-846)	5.2.37
Vanadium pentoxide (analyze as total vanadium)	1314-62-1	200.7 (EPA OW)	5.2.1
		200.8 (EPA OW)	5.2.2
		3050B (EPA SW-846)	5.2.14
		6010C (EPA SW-846)	5.2.23
		6020A (EPA SW-846)	5.2.24
		IO-3.1 (EPA ORD)	5.2.40
		IO-3.4 (EPA ORD)	5.2.41
		IO-3.5 (EPA ORD)	5.2.42
		9102 (NIOSH)	5.2.64
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester] VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester] VM [phosphonothioic acid, methyl-,S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0 78-53-5 21770-86-5	3520C (EPA SW-846)	5.2.16
		3535A (EPA SW-846)	5.2.17
		3541 (EPA SW-846)	5.2.18
		3545A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.20
		8270D (EPA SW-846)	5.2.31
		8290A Appendix A (EPA SW-846)	5.2.32
		TO-10A (EPA ORD)	5.2.44
VX [O-ethyl-S-(2-diisopropylaminoethyl)methyl-phosphonothiolate]	50782-69-9	CWA Protocol (EPA NHSRC)	5.2.48
White phosphorus	12185-10-3	3570 (EPA SW-846)	5.2.20
		7580 (EPA SW-846)	5.2.28
		8290A Appendix A (EPA SW-846)	5.2.32
		7905 (NIOSH)	5.2.62

Analyte	CAS RN	Method	Section
The following analytes should be prepared and/or analyzed by the following methods only if problems (e.g., insufficient recovery, interferences) occur when using the sample preparation / determinative techniques identified for these analytes in Appendix A.			
Allyl alcohol	107-18-6	TO-10A (EPA ORD)	5.2.44
3-Chloro-1,2-propanediol	96-24-2	TO-15 (EPA ORD)	5.2.45
Chlorosarin Chlorosoman	1445-76-7 7040-57-5	TO-15 (EPA ORD)	5.2.45
Crimidine	535-89-7	8321B (EPA SW-846)	5.2.36
Diisopropyl methylphosphonate (DIMP)	1445-75-6	TO-15 (EPA ORD)	5.2.45
Dimethylphosphoramidic acid	33876-51-6	8270D (EPA SW-846)	5.2.31
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4	8270D (EPA SW-846)	5.2.31
Hydrogen fluoride	7664-39-3	7906 (NIOSH)	5.2.63
Mercuric chloride (analyze as total mercury) Mercury, Total	7487-94-7 7439-97-6	7470A (EPA SW-846)	5.2.25
		7471B (EPA SW-846)	5.2.26
Methamidophos	10265-92-6	5600 (NIOSH)	5.2.53
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2	7470A (EPA SW-846)	5.2.25
		7471B (EPA SW-846)	5.2.26
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3	TO-15 (EPA ORD)	5.2.45
Sarin (GB) Soman (GD)	107-44-8 96-64-0	TO-15 (EPA ORD)	5.2.45
1,4-Thioxane	15980-15-1	5030C (EPA SW-846)	5.2.21
		5035A (EPA SW-846)	5.2.22
		8260C (EPA SW-846)	5.2.30

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

Table 5-2. Sources of Chemical Methods

Name	Publisher	Reference
National Environmental Methods Index (NEMI)	EPA, U.S. Geological Survey (USGS)	http://www.nemi.gov
EPA Contract Laboratory Program (CLP) Methods	EPA, CLP	http://www.epa.gov/superfund/programs/clp/
EPA Office of Water (OW) Methods	EPA OW	http://www.epa.gov/safewater/methods/sourcalt.html
EPA Solid Waste (SW)-846 Methods	EPA Office of Solid Waste and Emergency Response (OSWER)	http://www.epa.gov/epaoswer/hazwaste/test/main.htm
EPA Office of Research and Development (ORD) Methods	EPA ORD	http://www.epa.gov/ttnamti1/
EPA Air Toxics Methods	EPA Office of Air and Radiation (OAR)	http://www.epa.gov/ttn/amtic/airtox.html

Name	Publisher	Reference
EPA / Centers for Disease Control and Prevention (CDC) / NIOSH Reports	EPA ORD, CDC	http://www.epa.gov/nhsrc/news/news042407.html and http://cfpub.epa.gov/si/si_public_record_Report.cfm?dirEntryId=238103
EPA Analytical Protocols	EPA NHSRC	http://www.epa.gov/sam/contact_us.htm
Occupational Safety and Health Administration (OSHA) Methods	OSHA	http://www.osha.gov/dts/sltc/methods/index.html
NIOSH Methods	NIOSH	http://www.cdc.gov/niosh/nmam/
Standard Methods for the Examination of Water and Wastewater (SM), 21st Edition, 2005*	American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF)	http://www.standardmethods.org
*Annual Book of ASTM Standards**	ASTM International	http://www.astm.org
GESTIS Substance Database	Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung (IFA)	http://www.dguv.de/ifa/en/gestis/stoffdb/index.jsp
International Organization for Standardization (ISO) Methods*	ISO	http://www.iso.org
Official Methods of Analysis of AOAC International*	AOAC International	http://www.aoac.org
Analyst*	Royal Society of Chemistry	http://www.rsc.org/Publishing/Journals/AN/
Analytical Letters*	Taylor & Francis	http://www.informaworld.com/smpp/title~content=t713597227
Journal of Chromatography A and B*	Elsevier Science Publishers	http://www.journals.elsevier.com/journal-of-chromatography-a/
Journal of Forensic Sciences*	ASTM International	http://www.astm.org/DIGITAL_LIBRARY/JOURNALS/FORENSIC/PAGES/JFS16113J.htm
Chromatographia*	Vieweg+Teubner	http://www.springer.com/chemistry/analytical+chemistry/journal/10337
Encyclopedia of Analytical Chemistry*	Wiley	http://www.wiley.com/WileyCDA/WileyTitle/productCd-0471976709.html
European Journal of Lipid Science and Technology*	Wiley	http://www.wiley-vch.de/publish/en/journals/alphabeticIndex/2114/
EPA Water Contamination Information Tool (WCIT)	EPA OW Water Security Division (WSD)	http://www.epa.gov/wcit

Name	Publisher	Reference
Analytical Chemistry*	American Chemical Society(ACS)	http://pubs.acs.org/journal/ancham
Journal of Agricultural and Food Chemistry*	ACS	http://pubs.acs.org/journal/jafcau

* Subscription and/or purchase required.

5.1.2 General QC Guidelines for Chemical Methods

Having analytical data of appropriate quality requires that laboratories: (1) conduct the necessary QC activities to ensure that measurement systems are in control and operating correctly; (2) properly document results of the analyses; and (3) properly document measurement system evaluation of the analysis-specific QC, including corrective actions.[4] In addition to the laboratories being capable of generating accurate and precise data during site remediation, they must be able to deliver results in a timely and efficient manner. Therefore, laboratories must be prepared with calibrated instruments, the proper standards, standard analytical procedures, standard operating procedures, and qualified and trained staff. Moreover, laboratories also must be capable of providing rapid turnaround of sample analyses and data reporting.

The level or amount of QC needed during sample analysis and reporting depends on the intended purpose of the data that are generated (e.g., the decision(s) to be made). The specific needs for data generation should be identified. QC requirements and DQOs should be derived based on those needs, and should be applied consistently across laboratories when multiple laboratories are used. For almost all of the chemical warfare agents (CWAs), most laboratories will not have access to analytical standards for calibration and QC. Use of these agents is strictly controlled by the Department of Defense (DoD) and access is limited. For information regarding laboratory analysis of samples containing CWAs or laboratory requirements to possess and use ultra-dilute agent standards, please use the contact information provided on the Environmental Response Laboratory Network (ERLN) website at: http://www.epa.gov/oemerln1/contact.html.

A minimum set of analytical QC procedures should be planned, documented and conducted for all chemical testing. Some method-specific QC requirements are described in many of the individual methods that are cited in this document and will be referenced in any analytical protocols developed to address specific analytes and sample types of concern. Individual methods, sampling and analysis protocols or contractual statements of work should also be consulted to determine if any additional QC might be needed. Analytical QC requirements generally consist of analysis of laboratory control samples to document whether the analytical system is in control; matrix spikes to identify and quantify measurement system accuracy for the media of concern and, at the levels of concern, various blanks as a measure of freedom from contamination; as well as matrix spike duplicates or sample replicates to assess data precision.

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

- Demonstration that the measurement system is operating properly
  - Initial calibration
  - Method blanks

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

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

QC tests should be consistent with EPA's Good Laboratory Practice Standards (http://www2.epa.gov/compliance/good-laboratory-practices-standards-compliance-monitoring-program) and be run as frequently as necessary to ensure the reliability of analytical results. Additional guidance can be found at: www.epa.gov/quality/qatools.html; in Chapter 1 of EPA SW-846 "Test Methods for Evaluating Solid Waste, Physical/Chemical Methods," (http://www.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/chap1.pdf); and in EPA's 2005 "Manual for the Certification of Laboratories Analyzing Drinking Water," (EPA 815-R-05-004) (http://www.epa.gov/ogwdw000/methods/pdfs/manual_labcertification.pdf). As with the identification of needed QC samples, the frequency of QC sampling should be established based on an evaluation of DQOs. The type and frequency of QC tests can be refined over time.

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

Please note: The appropriate point of contact identified in Section 4 should be consulted regarding appropriate quality assurance (QA) and QC procedures prior to sample analysis. These contacts will consult with the EPA ERLN or Water Laboratory Alliance (WLA) coordinator responsible for laboratory activities during the specific event to ensure QA/QC procedures are performed consistently across laboratories. EPA program offices will be responsible for ensuring that the QA/QC practices are implemented.

5.1.3 Safety and Waste Management

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

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

- Centers for Disease Control and Prevention (CDC) – Title 42 of the Code of Federal Regulations part 72 (42 CFR 72). Interstate Shipment of Etiologic Agents
- CDC – 42 CFR part 73. Select Agents and Toxins
- Department of Transportation (DOT) – 49 CFR part 172. Hazardous Materials Table, Special Provisions, Hazardous Materials Communications, Emergency Response Information, and Training Requirements
- EPA – 40 CFR part 260. Hazardous Waste Management System: General. Available at: http://www.access.gpo.gov/nara/cfr/waisidx_07/40cfr260_07.html
- EPA – 40 CFR part 270. EPA Administered Permit Programs: The Hazardous Waste Permit Program. Available at: http://www.access.gpo.gov/nara/cfr/waisidx_07/40cfr270_07.html
- OSHA – 29 CFR part 1910.1450. Occupational Exposure to Hazardous Chemicals in Laboratories. Available at: http://www.access.gpo.gov/nara/cfr/waisidx_06/29cfr1910a_06.html
- OSHA – 29 CFR part 1910.120. Hazardous Waste Operations and Emergency Response.

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

5.2 Method Summaries

Summaries for the analytical methods listed in Appendix A are provided in Sections 5.2.1 through 5.2.99. These sections contain summary information extracted from the selected methods. Each method summary contains a table identifying the contaminants in Appendix A to which the method applies, a brief description of the analytical method, and a link to, or source for, obtaining a full version of the method. Summaries are provided for informational use. Tiers that have been assigned to each method/analyte pair (see Section 5.1.1) also are provided in Appendix A. The full version of the method should be consulted prior to sample analysis.

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

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

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

Method Developed for: Determination of metals in solution. This method is a consolidation of existing methods for water, wastewater and solid wastes.
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid and drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Method detection limits (MDLs) in aqueous samples have been found to be 0.008 mg/L for arsenic, 0.003 mg/L for vanadium, and 0.001 mg/L for thallium.

Description of Method: This method will determine metal-containing compounds only as the total metal (e.g., total arsenic) in aqueous samples. An aliquot of a well-mixed, homogeneous sample is accurately weighed or measured for sample processing. For total recoverable analysis of a sample containing undissolved material, analytes are first solubilized by gentle refluxing with nitric and hydrochloric acids. After cooling, the sample is made up to volume, mixed, and centrifuged or allowed to settle overnight prior to analysis. For determination of dissolved analytes in a filtered aqueous sample aliquot, or for the "direct analysis" total recoverable determination of analytes in drinking water where sample turbidity is < 1 nephelometric turbidity units (NTU), the sample is made ready for analysis by the addition of nitric acid, and then diluted to a predetermined volume and mixed before analysis. The prepared sample is analyzed using ICP-AES. Specific analytes targeted by Method 200.7 are listed in Section 1.1 of the method.

Special Considerations: Laboratory testing is currently underway for speciation of lewisite 1 using GC-MS techniques. Users should consult with the appropriate point of contact listed in Section 4.0 regarding use of graphite furnace atomic absorption spectrophotometry (GFAA) as a back-up or for additional confirmatory analyses.

Source: EPA. 1994. "Method 200.7: Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry," Revision 4.4.
http://www2.epa.gov/sites/production/files/2015-06/documents/epa-200.7.pdf

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

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

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

Method Developed for: Dissolved and total elements in ground water, surface water, drinking water, wastewater, sludges and soils.
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid and drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: MDLs for arsenic in aqueous samples have been found to be 1.4 µg/L in scanning mode, and 0.4 µg/L in selected ion monitoring mode. The recommended calibration range is 10 to 200 µg/L.

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

Special Considerations: Laboratory testing is currently underway for speciation of lewisite 1 using GC-MS techniques. Users should consult with the appropriate point of contact listed in Section 4.0 regarding use of GFAA as a back-up or for additional confirmatory analyses.

Source: EPA. 1994. "Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry," Revision 5.4.
http://www2.epa.gov/sites/production/files/2015-06/documents/epa-200.8.pdf

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

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

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

Method Developed for: Mercury in surface waters. It may be applicable to saline waters, wastewaters, effluents, and domestic sewages providing potential interferences are not present.
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid and drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Applicable concentration range is 0.2 to 10.0 µg Hg/L. The detection limit for this method is 0.2 µg Hg/L.

Description of Method: This method will determine mercuric chloride and methoxyethylmercuric acetate as total mercury. If dissolved mercury is targeted, the sample is filtered prior to acidification. To

detect total mercury (inorganic and organic mercury), the sample is treated with potassium permanganate and potassium persulfate to oxidize organic mercury compounds prior to analysis. Inorganic mercury is reduced to the elemental state (using stannous chloride) and aerated from solution. The mercury vapor passes through a cell positioned in the light path of a CVAA spectrophotometer. The concentration of mercury is measured using the CVAA spectrophotometer.

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

Source: EPA. 1994. "Method 245.1: Determination of Mercury in Water by Cold Vapor Atomic Absorption Spectrometry CVAA)." http://www2.epa.gov/sites/production/files/2015-06/documents/epa-245.1.pdf

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

Analyte(s)	CAS RN
Fluoride	16984-48-8
Sodium azide (analyze as azide ion)	26628-22-8

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

Method Developed for: Inorganic anions in reagent water, surface water, ground water and finished drinking water
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid and drinking water samples for fluoride. It also should be used for analysis of solid, air and/or wipe samples for sodium azide when appropriate sample preparation techniques have been applied. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit for fluoride in reagent water is 0.009 mg/L. The MDL varies depending upon the nature of the sample and the specific instrumentation employed. The estimated calibration range should not extend over more than 2 orders of magnitude in concentration over the expected concentration range of the samples.

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

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

Source: EPA. 1997. "Method 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography," Revision 1.0. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-300.1.pdf

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

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

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

Method Developed for: Cyanide in drinking, ground, surface and saline waters, and domestic and industrial wastes
Method Selected for: SAM lists this method for preparation and analysis of drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The applicable range is 5 to 500 µg/L.

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

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

Source: EPA. 1993. "Method 335.4: Determination of Total Cyanide by Semi-automated Colorimetry," Revision 1.0. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-335.4.pdf

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

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

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

Method Developed for: Ammonia in drinking, ground, surface and saline waters, and domestic and industrial wastes
Method Selected for: SAM lists this method for preparation and analysis of drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range for ammonia is 0.01 to 2.0 mg/L.

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

to the ammonia concentration. The blue color formed is intensified with sodium nitroprusside and measured spectrophotometrically.

Special Considerations: Reduced volume distillation techniques, such as midi-distillation or micro-distillation, can be used in place of traditional macro-distillation techniques.

Source: EPA. 1993. "Method 350.1: Nitrogen, Ammonia (Colorimetric, Automated Phenate)," Revision 2.0. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-350.1.pdf

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

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

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Purge-and-trap
Determinative Technique: Gas chromatography–mass spectrometry (GC-MS)

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

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

Special Considerations: The most recent version of this method (Method 524.3) requires instrumentation, such as cryogenic auto samplers, which are not currently in common use. If laboratory use of this equipment increases, Method 524.3 may be considered for SAM applications.

Source: EPA. 1992. "Method 524.2: Measurement of Purgeable Organic Compounds in Water by Capillary Column Gas Chromatography/Mass Spectrometry," Revision 4.0.
http://www2.epa.gov/sites/production/files/2015-06/documents/epa-524.2.pdf

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

Analyte(s)	CAS RN
Chlorpyrifos	2921-88-2
Chlorpyrifos oxon[1]	5598-15-2
Dichlorvos	62-73-7
Disulfoton	298-04-4
Disulfoton sulfone oxon[1]	2496-91-5
Disulfoton sulfoxide	2497-07-6
Disulfoton sulfoxide oxon[1]	2496-92-6
Fenamiphos	22224-92-6
Mevinphos	7786-34-7

[1] If problems occur when using this method for measurement of oxon compounds, analysts should consider use of procedures included in "Oxidation of Selected Organophosphate Pesticides During Chlorination of Simulated Drinking Water." Water Research. 2009. 43(2): 522–534. http://www.sciencedirect.com/science/journal/00431354

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

Method Developed for: Organic compounds in finished drinking water, source water or drinking water in any treatment stage
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid and/or drinking water samples. For chlorpyrifos in aqueous liquids, use Method 3511 for sample preparation and Method 8270D for analysis. For chlorpyrifos oxon in aqueous liquids, use Methods 3520C/3535A for sample preparation and Method 8270D for analysis. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The applicable concentration range for most analytes is 0.1 to 10 µg/L.

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

Special Considerations: Refer to footnote provided in analyte table above for special considerations that should be applied when measuring specific analytes. SPE using C_{18} resin may not work for certain compounds having high water solubility. In these cases, other sample preparation techniques or different SPE resins may be required.

Source: EPA. 1995. "Method 525.2: Determination of Organic Compounds in Drinking Water by Liquid-Solid Extraction and Capillary Column Gas Chromatography/Mass Spectrometry," Revision 2.0. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-525.2.pdf

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

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

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Direct injection
Determinative Technique: HPLC

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

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

Source: EPA. 2001. "Method 531.2: Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC With Postcolumn Derivatization," Revision 1.0. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-531.2.pdf

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

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

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Direct injection
Determinative Technique: Liquid Chromatography Tandem Mass Spectrometry (LC-MS-MS)

Method Developed for: Acephate, DIMP, methamidophos and thiofanox in drinking water samples
Method Selected for: SAM lists this method for preparation and analysis of drinking water samples. See Appendix A for corresponding method usability tiers.

Detection and Quantitation: The MDLs for acephate, DIMP, methamidophos and thiofanox in reagent water were calculated to be 0.019, 0.014, 0.017 and 0.090 µg/L, respectively. The Lowest Common Minimum Reporting Levels (LCMRLs) in reagent water were calculated to be 0.044, 0.022, 0.032 and 0.18 µg/L, respectively

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

Source: EPA. 2009. "Method 538: Determination of Selected Organic Contaminants in Drinking Water by Direct Aqueous Injection-Liquid Chromatography/Tandem Mass Spectrometry (DAI-LC/MS/MS)," Revision 1.0. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-538.pdf

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

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

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

Method Developed for: Diquat and paraquat in drinking water sources and finished drinking water
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid and drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: MDL for paraquat is 0.68 µg/L. The analytical range depends on the sample matrix and the instrumentation used.

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

Source: EPA. 1997. "Method 549.2: Determination of Diquat and Paraquat in Drinking Water by Liquid-Solid Extraction and High Performance Liquid Chromatography With Ultraviolet Detection," Revision 1.0. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-549.2.pdf

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

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

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction
Determinative Technique: Gas chromatography-electron capture detector (GC-ECD)

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

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

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

Source: EPA. 1995. "Method 551.1: Determination of Chlorination Disinfection Byproducts, Chlorinated Solvents, and Halogenated Pesticides/Herbicides in Drinking Water by Liquid-Liquid Extraction and Gas Chromatography With Electron-Capture Detection," Revision 1.0.
http://www2.epa.gov/sites/production/files/2015-06/documents/epa-551.1.pdf

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

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

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

Method Developed for: Formaldehyde in drinking water samples
Method Selected for: SAM lists this method for preparation and analysis of drinking water samples. See Appendix A for corresponding method usability tiers.

Detection and Quantitation: MDLs for formaldehyde in reagent water were calculated as 0.09 and 0.08 µg/L for primary and secondary columns, respectively. The applicable concentration range is approximately 5 to 40 µg/L.

Description of Method: A 20-mL volume of water sample is adjusted to pH 4 with potassium hydrogen phthalate (KHP) and the analytes are derivatized at 35 ºC for 2 hours with 15 mg of O-(2,3,4,5,6-pentafluorobenzyl)-hydroxylamine (PFBHA) reagent. The oxime derivatives are extracted from the water with 4 mL of hexane. The extract is processed through an acidic wash step, and analyzed by FGC-ECD. The target analytes are identified and quantified by comparison to a procedural standard. Two chromatographic peaks will be observed for many of the target analytes. Both (E) and (Z) isomers are formed for carbonyl compounds that are asymmetrical, and that are not sterically hindered. The (E) and (Z) isomers may not be chromatographically resolved in a few cases. Compounds with two carbonyl groups, such as glyoxal and methyl glyoxal, can produce even more isomers. Chromatographic peaks used for analyte identification are provided in Section 17, Table 1 and Figure 1 of the method.

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

Source: EPA. 1999. "Method 556.1: Determination of Carbonyl Compounds in Drinking Water by Fast Gas Chromatography," Revision 1.0. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-556.1.pdf

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

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

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

Method Developed for: Metals in sediments, sludges, and soil samples
Method Selected for: SAM lists this method for preparation of solid samples. See Appendix A for corresponding method usability tiers.

Description of Method: This method is used to prepare samples for the determination of arsenic trioxide, arsine, lewisite, lewisite degradation products, calcium and lead arsenate and sodium arsenite as total arsenic; thallium sulfate as total thallium; titanium tetrachloride as titanium; osmium tetroxide as osmium; and ammonium metavanadate and vanadium pentoxide as total vanadium. A 1-g to 2-g sample is digested with nitric acid and hydrogen peroxide. Sample volumes are reduced, then brought up to a final volume of 100 mL. Samples are analyzed for total arsenic, total thallium, total titanium or total vanadium by Method 6010C or 6020A (SW-846); use Method 6010C (SW-846) for total osmium; use Method 7010 (SW-846) for arsine.

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

Source: EPA. 1996. "Method 3050B (SW-846): Acid Digestion of Sediments, Sludges, and Soils," Revision 2. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf

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

Analyte(s)	CAS RN
Chlorpyrifos	2921-88-2
Crimidine	535-89-7
1,4-Dithiane	505-29-3
Fenamiphos	22224-92-6
Phencyclidine	77-10-1
Tetraethyl pyrophosphate (TEPP)	107-49-3
Tetramethylenedisulfotetramine (TETS)	80-12-6
1,4-Thioxane	15980-15-1

Analysis Purpose: Sample preparation
Sample Preparation Technique: Microextraction
Determinative Technique: GC-MS
Determinative Method: EPA SW-846 Method 8270D

Method Developed for: Volatile, semivolatile and nonvolatile organic compounds in water
Method Selected for: SAM lists this method for preparation of aqueous liquid and drinking water samples. Drinking water samples for TETS should be prepared and analyzed using EPA 600/R-11/091. Drinking water samples for chlorpyrifos and fenamiphos should be prepared and analyzed using EPA Method 525.2. See Appendix A for corresponding method usability tiers.

Description of Method: Samples are prepared by shake extraction with an organic solvent in sealed extraction tubes. Careful manipulation of the sample, solvent, drying agent and spiking solutions during the procedure minimizes loss of volatile compounds while maximizing extraction of volatile, semivolatile and nonvolatile compounds. Sample extracts are collected, dried, and concentrated using a modification of the Kuderna-Danish concentration method or other appropriate concentration technique. By increasing the number of theoretical plates and reducing the distillation temperature, extracts are concentrated without loss of volatile constituents. Samples should be prepared one at a time to the point of solvent addition (i.e., do not pre-weigh a number of samples then add the solvent). Samples should be extracted as soon after collection as possible, but no longer than 14 days from the date of collection for acid preserved samples. If samples are not acidified, the extraction should be performed within 7 days from the date of collection. Exposure to air before sample extraction should be minimized as much as possible.

Source: EPA. 2002. "Method 3511 (SW-846): Organic Compounds in Water by Microextraction," Revision 0. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-3511.pdf

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

Analyte(s)	CAS RN
BZ [Quinuclidinyl benzilate]	6581-06-2
Carfentanil	59708-52-0
Chlorfenvinphos	470-90-6
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos oxon	5598-15-2
Diesel range organics	NA
Fentanyl	437-38-7
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl) methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
Paraoxon	311-45-5
Parathion	56-38-2
Phosphamidon	13171-21-6
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5

Analysis Purpose: Sample preparation
Sample Preparation Technique: Continuous liquid-liquid extraction (CLLE)
Determinative Technique: Gas chromatography-flame ionization detector (GC-FID) / GC-MS / HPLC
Determinative Method: EPA SW-846 Method 8015C, Method 8270D or Method 8321B. Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Organic compounds in aqueous samples
Method Selected for: SAM lists this method for preparation of aqueous liquid and/or drinking water samples. *Please note*: Drinking water samples for chlorpyrifos oxon should be prepared and analyzed using EPA Method 525.2. See Appendix A for corresponding method usability tiers.

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

Special Considerations: Some of the target compounds will hydrolyze in water, with hydrolysis rates dependant on various factors such as sample pH and temperature.
Source: EPA. 1996. "Method 3520C (SW-846): Continuous Liquid-Liquid Extraction," Revision 3. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-3520c.pdf

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

Analyte(s)	CAS RN
4-Aminopyridine	504-24-5
BZ [Quinuclidinyl benzilate]	6581-06-2
Carfentanil	59708-52-0
Chlorfenvinphos	470-90-6
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos oxon	5598-15-2
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diesel range organics	NA
Dimethylphosphoramidic acid	33876-51-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4
Ethyldichloroarsine (ED)	598-14-1
Fentanyl	437-38-7
Hexamethylenetriperoxidediamine (HMTD)	283-66-9
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4
Methyl paraoxon	950-35-6
Methyl parathion	298-00-0
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Mevinphos	7786-34-7
Monocrotophos	6923-22-4
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
Nicotine compounds	54-11-5
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0
Paraoxon	311-45-5
Parathion	56-38-2
Pentaerythritol tetranitrate (PETN)	78-11-5
Phorate	298-02-2
Phorate sulfone	2588-04-7
Phorate sulfone oxon[1]	2588-06-9
Phorate sulfoxide	2588-03-6
Phorate sulfoxide oxon[1]	2588-05-8
Phosphamidon	13171-21-6
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
Strychnine	57-24-9
Tabun (GA)	77-81-6
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5

[1] If problems occur when using this method for measurement of oxon compounds, analysts should consider use of procedures included in "Oxidation of Selected Organophosphate Pesticides During Chlorination of Simulated Drinking Water." Water Research. 2009. 43(2): 522–534. http://www.sciencedirect.com/science/journal/00431354

Analysis Purpose: Sample preparation
Sample Preparation Technique: SPE

Determinative Technique: GC-FID / GC-MS / HPLC
Determinative Method: EPA SW-846 Method 8015C, Method 8270D, Method 8321B or Method 8330B. Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Organic compounds in ground water, wastewater and Toxicity Characteristic Leaching Procedure (TCLP, Method 1311) leachates
Method Selected for: SAM lists this method for preparation of aqueous liquid and/or drinking water samples. *Please note*: Drinking water samples for chlorpyrifos oxon, dichlorvos and mevinphos should be prepared and analyzed by EPA Method 525.2. All other drinking water samples and all aqueous liquid samples should be prepared using this method (SW-846 Method 3535A). See Appendix A for corresponding method usability tiers.

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

Special Considerations: Refer to footnote provided in analyte table above for special considerations that should be applied when measuring specific analytes. Some of the target compounds will hydrolyze in water, with hydrolysis rates dependant on various factors such as sample pH and temperature.

Source: EPA. 1998. "Method 3535A (SW-846): Solid-Phase Extraction (SPE)," Revision 1. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-3535a.pdf

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

Analyte(s)	CAS RN
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
BZ [Quinuclidinyl benzilate]	6581-06-2
Carfentanil	59708-52-0
Chlorfenvinphos	470-90-6
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos oxon	5598-15-2
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diesel range organics	NA
Dimethylphosphoramidic acid	33876-51-6
Diphacinone	82-66-6
Disulfoton sulfone oxon[1]	2496-91-5
Disulfoton sulfoxide	2497-07-6
Disulfoton sulfoxide oxon[1]	2496-92-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4
Ethyldichloroarsine (ED)	598-14-1
N-Ethyldiethanolamine (EDEA)	139-87-7
Fentanyl	437-38-7
Methyl hydrazine	60-34-4

Analyte(s)	CAS RN
Methyl paraoxon	950-35-6
Methyl parathion	298-00-0
N-Methyldiethanolamine (MDEA)	105-59-9
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Mevinphos	7786-34-7
Monocrotophos	6923-22-4
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
Nicotine compounds	54-11-5
Paraoxon	311-45-5
Parathion	56-38-2
Phorate	298-02-2
Phorate sulfone	2588-04-7
Phorate sulfone oxon[1]	2588-06-9
Phorate sulfoxide	2588-03-6
Phorate sulfoxide oxon[1]	2588-05-8
Phosphamidon	13171-21-6
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
Strychnine	57-24-9
Tabun (GA)	77-81-6
Thiofanox	39196-18-4
Triethanolamine (TEA)	102-71-6
Trimethyl phosphite	121-45-9
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5

[1] If problems occur when using this method for measurement of oxon compounds, analysts should consider use of procedures included in "Oxidation of Selected Organophosphate Pesticides During Chlorination of Simulated Drinking Water." Water Research. 2009. 43(2): 522–534. http://www.sciencedirect.com/science/journal/00431354

Analysis Purpose: Sample preparation
Sample Preparation Technique: Automated Soxhlet extraction
Determinative Technique: GC-FID / GC-MS / HPLC
Determinative Method: EPA SW-846 Method 8015C, Method 8270D or Method 8321B. Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Organic compounds in soil, sediment, sludges and waste solids
Method Selected for: SAM lists this method for preparation of solid samples. See Appendix A for corresponding method usability tiers.

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

Special Considerations: Refer to footnote provided in analyte table above for special considerations that should be applied when measuring specific analytes. Some of the target compounds will hydrolyze in water, with hydrolysis rates dependant on various factors such as sample pH and temperature.

Source: EPA. 1994. "Method 3541 (SW-846): Automated Soxhlet Extraction," Revision 0. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-3541.pdf

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

Analyte(s)	CAS RN
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
BZ [Quinuclidinyl benzilate]	6581-06-2
Carfentanil	59708-52-0
Chlorfenvinphos	470-90-6
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos oxon	5598-15-2
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diesel range organics	NA
Dimethylphosphoramidic acid	33876-51-6
Diphacinone	82-66-6
Disulfoton sulfone oxon[1]	2496-91-5
Disulfoton sulfoxide	2497-07-6
Disulfoton sulfoxide oxon[1]	2496-92-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4
Ethyldichloroarsine (ED)	598-14-1
N-Ethyldiethanolamine (EDEA)	139-87-7
Fentanyl	437-38-7
Methyl hydrazine	60-34-4
Methyl paraoxon	950-35-6
Methyl parathion	298-00-0
N-Methyldiethanolamine (MDEA)	105-59-9
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Mevinphos	7786-34-7
Monocrotophos	6923-22-4
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
Nicotine compounds	54-11-5
Paraoxon	311-45-5
Parathion	56-38-2
Phorate	298-02-2
Phorate sulfone	2588-04-7
Phorate sulfone oxon[1]	2588-06-9
Phorate sulfoxide	2588-03-6
Phorate sulfoxide oxon[1]	2588-05-8
Phosphamidon	13171-21-6
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
Strychnine	57-24-9
Tabun (GA)	77-81-6
Thiofanox	39196-18-4

Analyte(s)	CAS RN
Triethanolamine (TEA)	102-71-6
Trimethyl phosphite	121-45-9
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5

[1] If problems occur when using this method for measurement of oxon compounds, analysts should consider use of procedures included in "Oxidation of Selected Organophosphate Pesticides During Chlorination of Simulated Drinking Water." Water Research. 2009. 43(2): 522–534. http://www.sciencedirect.com/science/journal/00431354

Analysis Purpose: Sample preparation
Sample Preparation Technique: PFE
Determinative Technique: GC-FID / GC-MS / HPLC
Determinative Method: EPA SW-846 Method 8015C, Method 8270D or Method 8321B. Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Organic compounds in soils, clays, sediments, sludges and waste solids
Method Selected for: SAM lists this method for preparation of solid samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: This method has been validated for solid matrices containing 250 to 12,500 µg/kg of semivolatile organic compounds, 250 to 2500 µg/kg of organophosphorus pesticides, 5 to 250 µg/kg of organochlorine pesticides, 50 to 5000 µg/kg of chlorinated herbicides, and 1 to 2500 ng/kg of polychlorinated dibenzo-*p*-dioxins (PCDDs) / polychlorinated dibenzofurans (PCDFs).

Description of Method: Approximately 10 to 30 g of soil sample is prepared for extraction either by air drying the sample, or by mixing the sample with anhydrous sodium sulfate or pelletized diatomaceous earth. The sample is then ground and loaded into the extraction cell. The extraction cell containing the sample is heated to the extraction temperature, pressurized with the appropriate solvent system, and extracted for 5 minutes (or as recommended by the instrument manufacturer). The extract may be concentrated, if necessary, and exchanged into a solvent compatible with the cleanup or determinative step being employed.

Special Considerations: Refer to footnote provided in analyte table above for special considerations that should be applied when measuring specific analytes. Sodium sulfate can cause clogging, and air-drying or pelletized diatomaceous earth may be preferred. Some of the target compounds will hydrolyze in water, with hydrolysis rates dependant on various factors such as sample pH and temperature.

Source: EPA. 1998. "Method 3545A (SW-846): Pressurized Fluid Extraction (PFE)," Revision 1. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-3545a.pdf

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

Analyte(s)	CAS RN
Acrylamide	79-06-1
Acrylonitrile	107-13-1
Aldicarb (Temik)	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
4-Aminopyridine	504-24-5
BZ [Quinuclidinyl benzilate]	6581-06-2
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
Carfentanil	59708-52-0

Analyte(s)	CAS RN
Carbofuran (Furadan)	1563-66-2
Chlorfenvinphos	470-90-6
Chloropicrin	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos	2921-88-2
Chlorpyrifos oxon	5598-15-2
Crimidine	535-89-7
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diesel range organics	NA
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Dimethylphosphite	868-85-9
Dimethylphosphoramidic acid	33876-51-6
Diphacinone	82-66-6
Disulfoton	298-04-4
Disulfoton sulfone oxon[1]	2496-91-5
Disulfoton sulfoxide	2497-07-6
Disulfoton sulfoxide oxon[1]	2496-92-6
1,4-Dithiane	505-29-3
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4
Ethyl methylphosphonic acid (EMPA)	1832-53-7
Fenamiphos	22224-92-6
Fentanyl	437-38-7
Formaldehyde	50-00-0
Gasoline range organics	NA
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4
Hexamethylenetriperoxidediamine (HMTD)	283-66-9
Isopropyl methylphosphonic acid (IMPA)	1832-54-8
Kerosene	64742-81-0
Methomyl	16752-77-5
Methyl acrylonitrile	126-98-7
Methyl hydrazine	60-34-4
Methyl paraoxon	950-35-6
Methyl parathion	298-00-0
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Methylphosphonic acid (MPA)	993-13-5
Mevinphos	7786-34-7
Monocrotophos	6923-22-4
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
Nicotine compounds	54-11-5
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0
Oxamyl	23135-22-0
Paraoxon	311-45-5
Parathion	56-38-2
Pentaerythritol tetranitrate (PETN)	78-11-5
Phencyclidine	77-10-1
Phorate	298-02-2

Analyte(s)	CAS RN
Phorate sulfone	2588-04-7
Phorate sulfone oxon[1]	2588-06-9
Phorate sulfoxide	2588-03-6
Phorate sulfoxide oxon[1]	2588-05-8
Phosphamidon	13171-21-6
Pinacolyl methyl phosphonic acid (PMPA)	616-52-4
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
Strychnine	57-24-9
Tabun (GA)	77-81-6
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Thiofanox	39196-18-4
1,4-Thioxane	15980-15-1
Trimethyl phosphite	121-45-9
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5
White phosphorus	12185-10-3

[1] If problems occur when using this method for measurement of oxon compounds, analysts should consider use of procedures included in "Oxidation of Selected Organophosphate Pesticides During Chlorination of Simulated Drinking Water." Water Research. 2009. 43(2): 522–534. http://www.sciencedirect.com/science/journal/00431354

Analysis Purpose: Sample preparation
Sample Preparation Technique: MSE
Determinative Technique: Gas chromatography – nitrogen-phosphorus detector (GC-NPD) / GC-FID / GC-MS / HPLC
Determinative Method: EPA SW-846 Methods 7580, 8015C, 8270D, 8315A, 8316, 8318A, 8321B and 8330B. Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Extracting volatile, semivolatile and nonvolatile organic compounds from solids such as soils, sludges and wastes
Method Selected for: SAM lists this method for preparation of solid and wipe samples. Some of the analytes listed in the table above have been assigned a different method for preparation of soil samples. See Appendix A for appropriate soil sample preparation methods and for corresponding method usability tiers.

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

Special Considerations: Refer to footnote provided in analyte table above for special considerations that should be applied when measuring specific analytes.

Source: EPA. 2002. "Method 3570 (SW-846): Microscale Solvent Extraction (MSE)," Revision 0. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-3570.pdf

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

Analyte(s)	CAS RN
Allyl alcohol	107-18-6
Carbon disulfide	75-15-0
2-Chloroethanol	107-07-3
1,2-Dichloroethane	107-06-2
Ethylene oxide	75-21-8
2-Fluoroethanol	371-62-0
Gasoline range organics	NA
Kerosene	64742-81-0
Propylene oxide	75-56-9
The following analytes should be prepared by this method (and determined by the corresponding SW-846 Method 8260C) **only** if problems (e.g., insufficient recovery, interferences) occur when using the sample preparation/determinative techniques identified for these analytes in Appendix A.	
1,4-Thioxane	15980-15-1

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

Method Developed for: VOCs in aqueous and water miscible liquid samples
Method Selected for: SAM lists this method for preparation of aqueous liquid and/or drinking water samples. For carbon disulfide and 1,2-dichloroethane, EPA Method 524.2 (rather than Method 5030C) should be used for preparation of drinking water samples. See Appendix A for corresponding method usability tiers.

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

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

Source: EPA. 2003. "Method 5030C (SW-846): Purge-and-Trap for Aqueous Samples, Revision 3. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-5030c.pdf

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

Analyte(s)	CAS RN
Acrylonitrile	107-13-1
Allyl alcohol	107-18-6
Carbon disulfide	75-15-0
2-Chloroethanol	107-07-3
1,2-Dichloroethane	107-06-2
Ethylene oxide	75-21-8
2-Fluoroethanol	371-62-0
Gasoline range organics	NA
Kerosene	64742-81-0
Methyl acrylonitrile	126-98-7
Propylene oxide	75-56-9
The following analytes should be prepared by this method (and determined by the corresponding SW-846 Method 8260C) **only** if problems (e.g., insufficient recovery, interferences) occur when using the sample preparation/determinative techniques identified for these analytes in Appendix A.	
1,4-Thioxane	15980-15-1

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

Method Developed for: VOCs in solid materials (e.g., soils, sediments and solid waste) and oily wastes
Method Selected for: SAM lists this method for preparation of solid samples. See Appendix A for corresponding method usability tiers.

Description of Method: This method describes a closed-system purge-and-trap process for analysis of VOCs in solid samples containing low levels (0.5 to 200 µg/kg) of VOCs. The method also provides specific procedures for preparation of samples containing high levels (>200 µg/kg) of VOCs. For low-level VOCs, a 5-g sample is collected into a vial that is placed into an autosampler device. Reagent water, surrogates and internal standards are added automatically, and the vial is heated to 40 degrees Celsius (°C). The volatiles are purged into an appropriate trap using an inert gas combined with sample agitation. When purging is complete, the trap is heated and backflushed with helium to desorb the trapped sample components into a GC for analysis. For high-level VOCs, samples are either collected into a vial that contains a water-miscible organic solvent or a portion of sample is removed from the vial and dispersed in a water-miscible solvent. An aliquot of the solvent is added to reagent water, along with surrogates and internal standards, then purged and analyzed using an appropriate determinative method [e.g., Method 8015C or 8260C (SW-846)].

Source: EPA. 2002. "Method 5035A (SW-846): Closed-System Purge-and-Trap and Extraction for Volatile Organics in Soil and Waste Samples," Draft Revision 1.
http://www2.epa.gov/sites/production/files/2015-07/documents/epa-5035a.pdf

5.2.23 EPA Method 6010C (SW-846): Inductively Coupled Plasma - Atomic Emission Spectrometry

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2

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

Analysis Purpose: Analyte determination and measurement
Determinative Technique: ICP-AES
Sample Preparation Method: EPA SW-846 Method 3050B (solid samples) and NIOSH Method 9102 (wipe samples)
Sample Preparation Technique: Acid digestion

Method Developed for: Trace elements in solution
Method Selected for: SAM lists this method for analysis of solid and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Detection limits vary with each analyte. Estimated instrument detection limits (IDLs) for arsenic and titanium are 30 µg/L and 5.0 µg/L, respectively. The upper end of the analytical range may be extended by sample dilution.

Description of Method: This method determines arsenic trioxide, lewisite, lewisite degradation products, calcium and lead arsenate and sodium arsenite as total arsenic; osmium tetroxide as osmium; thallium sulfate as thallium; titanium tetrachloride as titanium; and ammonium metavanadate and vanadium pentoxide as total vanadium. Soil samples (prepared using SW-846 Method 3050B) and wipe samples (prepared using NIOSH Method 9102) are analyzed by ICP-AES.

Special Considerations: Laboratory testing is currently underway for speciation of lewisite 1 using GC-MS techniques. Users should consult with the appropriate point of contact listed in Section 4.0 regarding use of GFAA as a back-up or for additional confirmatory analyses.

Source: EPA. 2007. "Method 6010C (SW-846): Inductively Coupled Plasma-Atomic Emission Spectrometry," Revision 3. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-6010c.pdf

5.2.24 EPA Method 6020A (SW-846): Inductively Coupled Plasma - Mass Spectrometry

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonous acid (2-CVAA) (analyze as total arsenic)	85090-33-1

Analyte(s)	CAS RN
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)	40334-70-1
Lewisite oxide (analyze as total arsenic)	1306-02-1
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Titanium tetrachloride (analyze as total titanium)	7550-45-0
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

Analysis Purpose: Analyte determination and measurement
Determinative Technique: ICP-MS
Sample Preparation Method: EPA SW-846 Method 3050B (solid samples) and NIOSH Method 9102 (wipe samples)
Sample Preparation Technique: Acid digestion

Method Developed for: Elements in water samples and in waste extracts or digests
Method Selected for: SAM lists this method for analysis of solid and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: In relatively simple sample types, detection limits will generally be below 0.1 µg/L. Less sensitive elements, such as arsenic, may have detection limits of 1.0 µg/L or higher. The upper end of the analytical range may be extended by sample dilution.

Description of Method: This method will determine arsenic trioxide, lewisite, lewisite degradation products, calcium and lead arsenate and sodium arsenite as total arsenic. The method also will determine thallium sulfate as total thallium, titanium tetrachloride as titanium, and ammonium metavanadate and vanadium pentoxide as total vanadium. Soil samples (prepared using SW-846 Method 3050B) and wipe samples (prepared using NIOSH Method 9102) are analyzed by ICP-MS. IDLs, sensitivities and linear ranges vary with sample type, instrumentation and operation conditions.

Special Considerations: Laboratory testing is currently underway for speciation of lewisite 1 using GC-MS techniques. Users should consult with the appropriate point of contact listed in Section 4.0 regarding use of GFAA as a back-up or for additional confirmatory analyses.

Source: EPA. 1998. "Method 6020A (SW-846): Inductively Coupled Plasma-Mass Spectrometry," Revision 1. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-6020a.pdf

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

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

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Acid digestion (solid and aqueous liquid samples) and acid digestion by NIOSH Method 9102 (wipe samples)
Determinative Technique: CVAA

Method Developed for: Mercury in mobility-procedure extracts, aqueous wastes and ground waters
Method Selected for: SAM lists this method for reference if problems occur when using EPA Method 245.1 for these analytes during preparation and analysis of aqueous liquid samples. (See Footnote 9 of Appendix A.)
Detection and Quantitation: The detection limit for the method is 0.2 µg/L.

Description of Method: A 100-mL aqueous sample is digested with acids, permanganate solution, persulfate solution and heat. The sample is cooled and reduced with hydroxylamine-sodium chloride solution. Just prior to analysis, the sample is treated with Sn(II), reducing the mercury to Hg(0). The reduced sample is sparged and the mercury vapor is analyzed by CVAA.

Special Considerations: Chloride and copper are potential interferences.

Source: EPA. 1994. "Method 7470A (SW-846): Mercury in Liquid Waste (Manual Cold-Vapor Technique)," Revision 1. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-7470a.pdf

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

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

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Acid digestion (solid and aqueous liquid samples) and acid digestion by NIOSH Method 9102 (wipe samples)
Determinative Technique: CVAA

Method Developed for: Total mercury in soils, sediments, bottom deposits and sludge-type materials
Method Selected for: SAM lists this method for use if problems occur when using EPA SW-846 Method 7473 for these analytes during preparation and analysis of solid and wipe samples. (See Footnote 8 of Appendix A)

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

Special Considerations: Chloride and copper are potential interferences.

Source: EPA. 1998. "Method 7471B (SW-846): Mercury in Solid or Semisolid Waste (Manual Cold-Vapor Technique)," Revision 2. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-7471b.pdf

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

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

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Thermal decomposition (solid and aqueous liquid samples) and acid digestion by NIOSH Method 9102 (wipe samples)
Determinative Technique: Visible spectrophotometry

Method Developed for: Total mercury in solids, aqueous samples and digested solutions
Method Selected for: SAM lists this method for preparation and analysis of solid and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The IDL is 0.01 ng total mercury. The typical working range for this method is 0.05 to 600 ng.

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

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

Source: EPA. 1998. "Method 7473 (SW-846): Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry," Revision 0.
http://www2.epa.gov/sites/production/files/2015-07/documents/epa-7473.pdf

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

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

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

Method Developed for: White phosphorus in soil, sediment and water
Method Selected for: SAM lists this method for preparation and analysis of solid, aqueous liquid, drinking water and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: MDLs for reagent water, well water and pond water were calculated to be 0.008, 0.009, 0.008 µg/L, respectively. MDLs for sand, a sandy loam soil (Lebanon soil), and soil from the Rocky Mountain Arsenal (U.S. Army Environmental Center soil) were calculated to be 0.02, 0.43, 0.07 µg/kg, respectively. This procedure provides sensitivity on the order of 0.01 µg/L for water samples and 1 µg/kg for soil samples.

Description of Method: Method 7580 may be used to determine the concentration of white phosphorus in soil, sediment and water samples using solvent extraction and GC. Water samples are extracted by one

of two procedures, depending on the sensitivity required. For the more sensitive procedure, a 500-mL water sample is extracted with 50 mL of diethyl ether. The extract is concentrated by back extraction with reagent water, yielding a final extract volume of approximately 1.0 mL. A 1.0 µL aliquot of this extract is injected into a GC equipped with a nitrogen-phosphorus detector (NPD). Wet soil or sediment samples are analyzed by extracting a 40 g wet-weight aliquot of the sample with a mixture of 10.0 mL degassed reagent water and 10.0 mL isooctane. The extraction is performed in a glass jar on a platform shaker for 18 hours. A 1.0 µL aliquot of the extract is analyzed by GC-NPD.

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

Source: EPA. 1996. "Method 7580 (SW-846): White Phosphorus (P_4) by Solvent Extraction and Gas Chromatography," Revision 0. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-7580.pdf

5.2.29 EPA Method 8015C (SW-846): Nonhalogenated Organics Using GC/FID

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

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

Method Developed for: Various nonhalogenated VOCs and semivolatile organic compounds in water samples
Method Selected for: SAM lists this method for analysis of solid, aqueous liquid, drinking water and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The estimated MDLs vary with each analyte and range between 2 and 48 µg/L for aqueous liquid samples. The MDLs in other matrices have not been evaluated. The analytical range depends on the target analyte(s) and the instrument used.

Description of Method: This method provides GC conditions for the detection of certain nonhalogenated volatile and semivolatile organic compounds. Depending on the analytes of interest, samples may be introduced into the GC by a variety of techniques including purge-and-trap, direct injection of aqueous liquid samples, and solvent extraction. An appropriate column and temperature program are used in the GC to separate the organic compounds. Detection is achieved by a flame ionization detector (FID). The method allows the use of packed or capillary columns for the analysis and confirmation of the non-halogenated individual analytes.

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

Source: EPA. 2000. "Method 8015C (SW-846): Nonhalogenated Organics Using GC/FID," Revision 3. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-8015c.pdf

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

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

Analysis Purpose: Analyte determination and measurement
Determinative Technique: GC-MS
Sample Preparation Method: EPA SW-846 Method 5035A (solid samples), Method 5030C (aqueous liquid and drinking water samples), and Method 3570/8290A Appendix A (wipe samples).
Sample Preparation Technique: Purge-and-trap (solid samples, aqueous liquid and drinking water samples) and MSE / solvent extraction (wipe samples).

Method Developed for: Applicable to nearly all types of samples, regardless of water content, including various air sampling trapping media, ground and surface water, aqueous sludges, caustic liquors, acid liquors, waste solvents, oily wastes, mousses (emulsified oil), tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils and sediments.
Method Selected for: SAM lists this method for analysis of solid, aqueous liquid, drinking water and/or wipe samples. For acrylonitrile, carbon disulfide, 1,2-dichloroethane and methyl acrylonitrile only, EPA Method 524.2 (rather than 8260C) should be used for analysis of drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Using standard quadrupole instrumentation and the purge-and-trap, estimated quantitation limits (EQLs) are 5 µg/kg (wet weight) for soil/sediment samples and 5 µg/L for ground water. Somewhat lower limits may be achieved using an ion trap MS or other instrumentation of improved design. No matter which instrument is used, EQLs will be proportionately higher for sample extracts and samples that require dilution or when a reduced sample size is used to avoid saturation of the detector. The EQL for an individual analyte is dependent on the instrument as well as the choice of sample preparation/introduction method.

Description of Method: Volatile compounds are introduced into a GC by purge-and-trap or other procedures (see Section 1.2 in Method 8260C). The analytes can be introduced directly to a wide-bore capillary column or cryofocused on a capillary pre-column before being flash evaporated to a narrow-bore capillary for analysis. Alternatively, the effluent from the trap is sent to an injection port operating in the split mode for injection to a narrow-bore capillary column. The column is temperature-programmed to separate the analytes, which are then detected with a MS interfaced to the GC. Analytes eluted from the capillary column are introduced into the MS via a jet separator or a direct connection.

Source: EPA. 2006. "Method 8260C (SW-846): Volatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)," Revision 3. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-8260c.pdf

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

Analyte(s)	CAS RN
Chlorfenvinphos	470-90-6
Chloropicrin[1]	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos	2921-88-2
Chlorpyrifos oxon	5598-15-2
Crimidine[2]	535-89-7
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Dimethylphosphite	868-85-9
Disulfoton	298-04-4
Disulfoton sulfone oxon[3]	2496-91-5
Disulfoton sulfoxide	2497-07-6
Disulfoton sulfoxide oxon[3]	2496-92-6
1,4-Dithiane	505-29-3
Ethyldichloroarsine (ED)	598-14-1
Fenamiphos	22224-92-6
Methyl paraoxon	950-35-6
Methyl parathion	298-00-0
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Mevinphos	7786-34-7
Monocrotophos	6923-22-4
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl) methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
Nicotine compounds	54-11-5
Paraoxon	311-45-5
Parathion	56-38-2
Phencyclidine	77-10-1
Phorate	298-02-2
Phorate sulfone	2588-04-7
Phorate sulfone oxon[3]	2588-06-9
Phorate sulfoxide	2588-03-6
Phorate sulfoxide oxon[3]	2588-05-8
Phosphamidon	13171-21-6
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
Strychnine	57-24-9
Tabun (GA)	77-81-6
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine[1]	80-12-6
1,4-Thioxane[4]	15980-15-1
Trimethyl phosphite[1]	121-45-9

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

[1] If problems occur with analyses, lower the injection temperature.
[2] If problems occur when using this method, it is recommended that SW-846 Method 8321B be used. Sample preparation methods should remain the same.
[3] If problems occur when using this method for measurement of oxon compounds, analysts should consider use of procedures included in "Oxidation of Selected Organophosphate Pesticides During Chlorination of Simulated Drinking Water." Water Research. 2009. 43(2): 522–534. http://www.sciencedirect.com/science/journal/00431354
[4] If problems occur when using this method, it is recommended that SW-846 Method 8260C and appropriate corresponding sample preparation procedures (i.e., Method 5035A for solid samples and Method 5030C for aqueous liquid and drinking water samples) be used.

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

Method Developed for: Semivolatile organic compounds in extracts prepared from many types of solid waste matrices, soils, air sampling media and water samples
Method Selected for: SAM lists this method for analysis of solid, aqueous liquid, drinking water and/or wipe samples. *Please note*: Drinking water samples for chlorpyrifos, chlorpyrifos oxon, dichlorvos, disulfoton, disulfoton sulfoxide, fenamiphos and mevinphos should be prepared and analyzed by EPA Method 525.2; aqueous liquid and drinking water samples for chloropicrin should be prepared and analyzed by EPA Method 551.1; drinking water samples for TETS should be analyzed using EPA 600/R-11/091; all other analyte/sample type combinations should be analyzed by this method (SW-846 8270D). See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The EDLs vary with each analyte and range between 10 and 1000 µg/L for aqueous liquid samples and 660 and 3300 µg/kg for soil samples. The analytical range depends on the target analyte(s) and the instrument used.

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

Special Considerations: Refer to footnotes provided in analyte table above for special considerations that should be applied when measuring specific analytes.

Source: EPA. 1998. "Method 8270D (SW-846): Semivolatile Organic Compounds by Gas Chromatography/Mass Spectrometry (GC/MS)," Revision 4.
http://www2.epa.gov/sites/production/files/2015-07/documents/epa-8270d.pdf

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

Analyte(s)	CAS RN
Acrylamide	79-06-1
Acrylonitrile	107-13-1
Aldicarb (Temik)	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
4-Aminopyridine	504-24-5
BZ [Quinuclidinyl benzilate]	6581-06-2
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
Carfentanil	59708-52-0
Carbofuran (Furadan)	1563-66-2
Chlorfenvinphos	470-90-6
Chloropicrin	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos	2921-88-2
Chlorpyrifos oxon	5598-15-2
Crimidine	535-89-7
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diesel range organics	NA
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Dimethylphosphite	868-85-9
Dimethylphosphoramidic acid	33876-51-6
Diphacinone	82-66-6
Disulfoton	298-04-4
Disulfoton sulfone oxon[1]	2496-91-5
Disulfoton sulfoxide	2497-07-6
Disulfoton sulfoxide oxon[1]	2496-92-6
1,4-Dithiane	505-29-3
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4
Ethyl methylphosphonic acid (EMPA)	1832-53-7
Fenamiphos	22224-92-6
Fentanyl	437-38-7
Formaldehyde	50-00-0
Gasoline range organics	NA
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4
Hexamethylenetriperoxidediamine (HMTD)	283-66-9
Isopropyl methylphosphonic acid (IMPA)	1832-54-8
Kerosene	64742-81-0
Methomyl	16752-77-5
Methyl acrylonitrile	126-98-7
Methyl hydrazine	60-34-4
Methyl paraoxon	950-35-6
Methyl parathion	298-00-0
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Methylphosphonic acid (MPA)	993-13-5
Mevinphos	7786-34-7

Analyte(s)	CAS RN
Monocrotophos	6923-22-4
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl) methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
Nicotine compounds	54-11-5
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0
Oxamyl	23135-22-0
Paraoxon	311-45-5
Parathion	56-38-2
Pentaerythritol tetranitrate (PETN)	78-11-5
Phencyclidine	77-10-1
Phorate	298-02-2
Phorate sulfone	2588-04-7
Phorate sulfone oxon[1]	2588-06-9
Phorate sulfoxide	2588-03-6
Phorate sulfoxide oxon[1]	2588-05-8
Phosphamidon	13171-21-6
Pinacolyl methyl phosphonic acid (PMPA)	616-52-4
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
Strychnine	57-24-9
Tabun (GA)	77-81-6
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Thiofanox	39196-18-4
1,4-Thioxane	15980-15-1
Trimethyl phosphite	121-45-9
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5
White phosphorus	12185-10-3

[1] If problems occur when using this method for measurement of oxon compounds, analysts should consider use of procedures included in "Oxidation of Selected Organophosphate Pesticides During Chlorination of Simulated Drinking Water." Water Research. 2009. 43(2): 522–534. http://www.sciencedirect.com/science/journal/00431354

Analysis Purpose: Sample preparation
Sample Preparation Technique: Solvent extraction
Determinative Technique: GC-NPD / GC-FID / GC-MS / HPLC
Determinative Method: EPA OW Method 300.1 Revision 1.0; EPA SW-846 Methods 7580, 8015C, 8270D, 8315A, 8316, 8318A, 8321B and 8330B. Refer to Appendix A for which of these determinative methods should be used for a particular analyte.

Method Developed for: Evaluation of surface contamination by 2,3,7,8-substituted PCDD and PCDF congeners
Method Selected for: SAM lists this method for preparation of wipe samples. See Appendix A for corresponding method usability tiers.

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

Special Considerations: Refer to footnote provided in analyte table above for special considerations that should be applied when measuring specific analytes. The solvent systems described in this method extraction have been evaluated for PCDD and PCDF congeners only. Other analytes may require different solvent systems for optimal sample extraction.

Source: EPA. 2007. "Method 8290A, Appendix A (SW-846): Procedure for the Collection, Handling, Analysis, and Reporting of Wipe Tests Performed Within the Laboratory," Revision 1.
http://www2.epa.gov/sites/production/files/2015-07/documents/epa-8290a.pdf

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

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

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

Method Developed for: Free carbonyl compounds in aqueous, soil, waste and stack samples
Method Selected for: SAM lists this method for preparation and analysis of solid, aqueous liquid and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDL for formaldehyde varies depending on sample conditions and instrumentation, but is approximately 6.2 µg/L for aqueous liquid samples.

Description of Method: A measured volume of aqueous liquid sample (approximately 100 mL), or an appropriate amount of solids extract (approximately 25 g), is buffered to pH 3 and derivatized with 2,4-dinitrophenylhydrazine (2,4-DNPH). Using the appropriate extraction technique, the derivatives are extracted using methylene chloride and the extracts are exchanged with acetonitrile prior to HPLC analysis. HPLC conditions are described permitting the separation and measurement of various carbonyl compounds in the extract by absorbance detection at 360 nm. If formaldehyde is the only analyte of interest, the aqueous liquid sample and/or solid sample extract should be buffered to pH 5.0 to minimize the formation of artifact formaldehyde.

Source: EPA. 1996. "Method 8315A (SW-846): Determination of Carbonyl Compounds by High Performance Liquid Chromatography (HPLC)," Revision 1.
http://www2.epa.gov/sites/production/files/2015-07/documents/epa-8315a.pdf

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

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

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

Sample Preparation Technique: Direct injection (aqueous liquid and drinking water samples), water extraction (solid), and MSE / solvent extraction by EPA SW-846 Method 3570/8290A Appendix A (wipe samples)
Determinative Technique: HPLC

Method Developed for: Acrylamide, acrylonitrile and acrolein in water samples
Method Selected for: SAM lists this method for preparation and/or analysis of solid, aqueous liquid, drinking water and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Acrylamide has an MDL of 10 µg/L; acrylonitrile has an MDL of 20 µg/L.

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

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

Source: EPA. 1994. "Method 8316 (SW-846): Acrylamide, Acrylonitrile and Acrolein by High Performance Liquid Chromatography (HPLC)," Revision 0.
http://www2.epa.gov/sites/production/files/2015-07/documents/epa-8316.pdf

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

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

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

Method Developed for: *N*-methylcarbamates in soil, water and waste matrices
Method Selected for: SAM lists this method for preparation and/or analysis of solid and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The estimated MDLs vary with each analyte and range from 1.7 to 9.4 µg/L for aqueous samples and 10 to 50 µg/kg for soil samples.

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

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

Source: EPA. 2000. "Method 8318A (SW-846): N-Methylcarbamates by High Performance Liquid Chromatography (HPLC)," Revision 1. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-8318a.pdf

5.2.36 EPA Method 8321B (SW-846): Solvent-Extractable Nonvolatile Compounds by High Performance Liquid Chromatography-Thermospray-Mass Spectrometry (HPLC-TS-MS) or Ultraviolet (UV) Detection

Analyte(s)	CAS RN
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
BZ [Quinuclidinyl benzilate]	6581-06-2
Carfentanil	59708-52-0
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Dimethylphosphoramidic acid[1]	33876-51-6
Diphacinone	82-66-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid]	73207-98-4
Ethyl methylphosphonic acid (EMPA)	1832-53-7
N-Ethyldiethanolamine (EDEA)	139-87-7
Fentanyl	437-38-7
Isopropyl methylphosphonic acid (IMPA)	1832-54-8
N-Methyldiethanolamine (MDEA)	105-59-9
Methylphosphonic acid (MPA)	993-13-5
Pinacolyl methyl phosphonic acid (PMPA)	616-52-4
Thiofanox	39196-18-4
Triethanolamine (TEA)	102-71-6
The following analyte should be determined by this method **only** if problems (e.g., insufficient recovery, interferences) occur when using SW-846 Method 8270D. Sample preparation methods should remain the same as those listed in Appendix A.	
Crimidine[1]	535-89-7

[1] This analyte is determined using a wavelength of 230 nm.

Analysis Purpose: Analyte determination and measurement
Determinative Technique: High performance liquid chromatography-mass spectrometry (HPLC-MS); HPLC (using electrospray or other atmospheric pressure ionization interface)
Sample Preparation Method: EPA SW-846 Method 3541/3545A (solid samples), 3520C/3535A (aqueous liquid and drinking water samples), and Method 3570/8290A Appendix A (wipe samples). For thiofanox, EPA Method 538 (rather than Method 3520C/3535A) should be used for preparation of drinking water samples. Refer to Appendix A for which of these preparation methods should be used for a particular analyte/sample type combination.
Sample Preparation Technique: Varies with analyte/sample type (see Appendix A).

Method Developed for: Solvent-extractable nonvolatile compounds, including dyes, organophosphorus compounds, phenoxyacid herbicides and carbamates in solid and water samples
Method Selected for: SAM lists this method for analysis of solid, aqueous liquid, drinking water and wipe samples. It is intended to serve as a general-purpose HPLC method; SAM users should refer to other LC methods listed in Appendix A for these analytes for specific instrument conditions. See Appendix A for corresponding method usability tiers.

Description of Method: As published, Method 8321B provides reversed-phase HPLC, thermospray (TSP) MS and UV conditions for detection of the target analytes. TSP instrumentation is obsolete, however, and currently available electrospray or atmospheric pressure ionization (API) techniques are recommended. SAM users should refer to other LC methods listed in Appendix A for these analytes for specific instrument conditions. Quantitative analysis may be performed by MS detection, using either an external or internal standard approach. Primary analysis of some analytes (e.g., carbamates) may be performed by UV detection; however, results should be confirmed using MS. The instrument conditions, analytical range and detection limits vary depending on the target analyte, sample type and instrument used.

Special Considerations: Refer to footnote provided in analyte table above for special considerations that should be applied when measuring specific analytes.

Source: EPA. 1998. "Method 8321B (SW-846): Solvent-Extractable Nonvolatile Compounds by High Performance Liquid Chromatography-Thermospray-Mass Spectrometry (HPLC-TSP-MS) or Ultraviolet (UV) Detection," Revision 2. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-8321b.pdf

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

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

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

Method Developed for: Trace analysis of explosives and propellant residues in water, soil or sediment
Method Selected for: SAM lists this method for preparation and/or analysis of solid, aqueous liquid, drinking water and wipe samples. Aqueous liquid and drinking water samples are prepared using Methods 3535A or 8330B prior to analysis. For HMTD, procedures adapted from Analyst (2001) 126:1689 – 1693 are used for sample analysis. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limits, ranges and interferences depend on the target compound

Description of Method: This method is intended for the trace analysis of explosives and propellant residues by HPLC using a dual wavelength UV detector in a water, soil or sediment matrix. All of the compounds listed in this method are either used in the manufacture of explosives or propellants, or they are the degradation products of compounds used for that purpose. Samples are prepared for analysis by high performance liquid chromatography-ultraviolet (HPLC-UV) detection using the appropriate sample preparation technique (SPE by Method 3535A or solvent extraction by Method 8330B) and, if necessary, sample cleanup procedures. Direct injection of diluted and filtered water samples can be used for water

samples of higher concentration. Soil and sediment samples are extracted using acetonitrile in an ultrasonic bath, filtered and chromatographed.

Source: EPA. 2006. "Method 8330B (SW-846): Nitroaromatics, Nitramines, and Nitrate Esters by High Performance Liquid Chromatography (HPLC)," Revision 2.
http://www2.epa.gov/sites/production/files/2015-07/documents/epa-8330b.pdf

5.2.38 EPA CLP ISM01.3 Cyanide: Analytical Methods for Total Cyanide Analysis

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

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

Method Developed for: Metals in water, sediment, sludge and soil
Method Selected for: SAM lists this method for preparation and analysis of solid, aqueous liquid and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The method quantitation limits are 10 µg/L for aqueous samples and 0.5 mg/kg for solid samples.

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

Source: EPA Contract Laboratory Program (CLP). "ISM01.2: Exhibit D – Part D: Analytical Methods for Total Cyanide Analysis." http://www2.epa.gov/sites/production/files/2015-07/documents/epa-ism01.3.pdf

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

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

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

Method Developed for: Cyanide in drinking, ground and surface waters, domestic and industrial wastewaters, sediments and solid waste
Method Selected for: SAM lists this method for preparation and analysis of solid, aqueous liquid, drinking water and wipe samples. See Appendix A for corresponding method usability tiers.

Detection and Quantitation: The applicable range is 0.003 to 0.500 mg/L cyanide in the distillate. This range can be expanded by sample dilution, either by using less sample for distillation or diluting the distillate.

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

Special Considerations: Alternate cyanide analyzer equipment may be used, provided it is used according to the procedures described and the laboratory can demonstrate equivalent performance.

Source: EPA. 2008. "RLAB Method 3135.2I: Cyanide, Total and Amenable in Aqueous and Soil Samples Automated Colorimetric With Manual Digestion."
http://www2.epa.gov/sites/production/files/2015-07/documents/epa-3135.2i.pdf

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

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

Analysis Purpose: Sample preparation
Sample Preparation Technique: Acid extraction
Determinative Technique: ICP-AES / ICP-MS
Determinative Method: EPA Method IO-3.4 or Method IO-3.5. Osmium tetroxide should be analyzed by Method IO-3.4.

Method Developed for: Particulate metals in air.
Method Selected for: SAM lists this method for preparation of air samples. See Appendix A for corresponding method usability tiers.

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

Source: EPA. 1999. "IO Compendium Method IO-3.1: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Selection, Preparation and Extraction of Filter Material." http://www2.epa.gov/sites/production/files/2015-07/documents/epa-io-3.1.pdf

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

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

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

Method Developed for: Metals in ambient particulate matter
Method Selected for: SAM lists this method for analysis of air samples. See Appendix A for corresponding method usability tiers.

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

Special Considerations: Laboratory testing is currently underway for speciation of lewisite 1 using GC-MS techniques. Concerns have been raised regarding the use of nitric acid when analyzing samples for osmium tetroxide; hydrochloric acid should be considered and evaluated as a possible alternative.

Source: EPA. 1999. "IO Compendium Method IO-3.4: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma (ICP) Spectroscopy." EPA/625/R-96/010a.
http://www2.epa.gov/sites/production/files/2015-07/documents/epa-io-3.4.pdf

EPA. 1999. "IO Compendium Method IO-2.1: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Sampling of Ambient Air for Total Suspended Particulate Matter (SPM) and PM_{10} Using High Volume (HV) Sampler." EPA/625/R-96/010a.
http://www2.epa.gov/sites/production/files/2015-07/documents/epa-io-2.1.pdf

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

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

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

Method Developed for: Metals in ambient particulate matter
Method Selected for: SAM lists this method for analysis of air samples. See Appendix A for corresponding method usability tiers.

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

Special Considerations: Laboratory testing is currently underway for speciation of lewisite 1 using GC-MS techniques.

Source: EPA. 1999. "IO Compendium Method IO-3.5: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Determination of Metals in Ambient Particulate Matter Using Inductively Coupled Plasma/Mass Spectrometry (ICP/MS)." EPA/625/R-96/010a.
http://www2.epa.gov/sites/production/files/2015-07/documents/epa-io-3.5.pdf

EPA. 1999. "IO Compendium Method IO-2.1: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Sampling of Ambient Air for Total Suspended Particulate Matter (SPM) and PM_{10} Using High Volume (HV) Sampler." EPA/625/R-96/010a. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-io-2.1.pdf

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

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

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

Method Developed for: Vapor and particle phase mercury in ambient air
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limits are 30 pg/m^3 for particulate mercury and 45 pg/m^3 for vapor phase mercury. Detection limits, analytical range and interferences are dependent on the instrument used.

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

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

Source: EPA. 1999. "IO Compendium Method IO-5: Compendium of Methods for the Determination of Inorganic Compounds in Ambient Air: Sampling and Analysis for Vapor and Particle Phase Mercury in Ambient Air Utilizing Cold Vapor Atomic Fluorescence Spectrometry (CVAFS)." EPA/625/R-96/010a. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-io-5.pdf

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

Analyte(s)	CAS RN
BZ [Quinuclidinyl benzilate][1]	6581-06-2
Chlorfenvinphos	470-90-6
3-Chloro-1,2-propanediol[2]	96-24-2
Chlorosarin[2]	1445-76-7

Analyte(s)	CAS RN
Chlorosoman[2]	7040-57-5
Chlorpyrifos	2921-88-2
Chlorpyrifos oxon	5598-15-2
Dichlorvos	62-73-7
Dicrotophos	141-66-2
Diisopropyl methylphosphonate (DIMP)[2]	1445-75-6
Dimethylphosphite	868-85-9
Dimethylphosphoramidic acid[1]	33876-51-6
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid][1]	73207-98-4
Ethyl methylphosphonic acid (EMPA)[1]	1832-53-7
N-Ethyldiethanolamine (EDEA)	139-87-7
Fenamiphos	22224-92-6
Isopropyl methylphosphonic acid (IMPA)[1]	1832-54-8
Methyl paraoxon	950-35-6
Methyl parathion	298-00-0
N-Methyldiethanolamine (MDEA)	105-59-9
1-Methylethyl ester ethylphosphonofluoridic acid (GE)[2]	1189-87-3
Methylphosphonic acid (MPA)[1]	993-13-5
Mevinphos	7786-34-7
Monocrotophos	6923-22-4
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl) methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
Paraoxon	311-45-5
Parathion	56-38-2
Phencyclidine	77-10-1
Phorate	298-02-2
Phorate sulfone	2588-04-7
Phorate sulfone oxon[3]	2588-06-9
Phorate sulfoxide	2588-03-6
Phorate sulfoxide oxon[3]	2588-05-8
Phosphamidon	13171-21-6
Pinacolyl methyl phosphonic acid (PMPA)[1]	616-52-4
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
Tabun (GA)	77-81-6
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Thiodiglycol (TDG)	111-48-8
Triethanolamine (TEA)	102-71-6
Trimethyl phosphite	121-45-9
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5
The following analyte should be determined by this method **only** if problems (e.g., insufficient recovery, interferences) occur when using Method TO-15.	
Allyl alcohol	107-18-6

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

[2] If problems occur when using this method, it is recommended that the canister Method TO-15 be used.

[3] If problems occur when using this method for measurement of oxon compounds, analysts should consider use of procedures included in "Oxidation of Selected Organophosphate Pesticides During Chlorination of Simulated Drinking Water." Water Research. 2009. 43(2): 522–534. http://www.sciencedirect.com/science/journal/00431354

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

Method Developed for: Pesticides and polychlorinated biphenyls in ambient air
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The limit of detection (LOD) will depend on the specific compounds measured, the concentration level, and the degree of specificity required. This method is applicable to multicomponent atmospheres, 0.001 to 50 µg/m^3 concentrations, and 4 to 24-hour sampling periods.

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

Special Considerations: Refer to footnotes provided in analyte table above for special considerations that should be applied when measuring specific analytes. See Special Considerations for EPA SW-846 8270D in Section 5.2.31 for information regarding derivatization of compounds.

Source: EPA. 1999. "Air Method, Toxic Organics-10A (TO-10A): Compendium of Methods for the Determination of Toxic Organic Compounds in Ambient Air: Determination of Pesticides and Polychlorinated Biphenyls in Ambient Air Using Low Volume Polyurethane Foam (PUF) Sampling Followed by Gas Chromatographic/Multi-Detector Detection (GC/MD)." EPA 625/R-96/010b. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-to-10a.pdf

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

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

Analysis Purpose: Sample preparation, and analyte determination and measurement

Sample Preparation Technique: Samples are collected using canisters.
Determinative Technique: GC-MS

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

Description of Method: The atmosphere is sampled by introduction of air into a specially prepared stainless steel canister (electropolished or silica-coated). A sample of air is drawn through a sampling train comprising components that regulate the rate and duration of sampling into the pre-evacuated and passivated canister. Grab samples also may be collected. After the air sample is collected, the canister valve is closed, an identification tag is attached to the canister, and the canister is transported to the laboratory for analysis. To analyze the sample, a known volume of sample is directed from the canister through a solid multisorbent concentrator. Recovery of less volatile compounds may require heating the canister.

After the concentration and drying steps are completed, VOCs are thermally desorbed, entrained in a carrier gas stream, and then focused in a small volume by trapping on a cryo-focusing (ultra-low temperature) trap or small volume multisorbent trap. The sample is then released by thermal desorption and analyzed by GC-MS.

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

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

5.2.46 EPA 600/R-11/091: High Throughput Determination of Tetramine in Drinking Water by Solid Phase Extraction and Isotope Dilution Gas Chromatography/Mass Spectrometry (GC/MS)

Analyte(s)	CAS RN
Tetramethylenedisulfotetramine (TETS)	80-12-6

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: SPE using 96 well-plates
Determinative Technique: GC-MS

Method Developed for: TETS in drinking water
Method Selected for: SAM lists this method for preparation and analysis of drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDL for TETS is 0.15 µg/L. The reporting range is 0.5 − 250 µg/L.

Description of Method: A 50-mL water sample is collected, and a preservative and/or dechlorinating agent is added as required by site-specific conditions. An aliquot is pipetted into a well of a preconditioned 96-well solid phase extraction plate, and isotopically labeled TETS is added. Following a wash step with 5% methanol/95%water, tetramine is eluted in acetonitrile. The extract is concentrated to dryness under nitrogen and heat, and then adjusted to a 100-µL volume in acetonitrile. TETS is separated from the sample matrix and identified by GC-MS analysis, operated in selective ion monitoring (SIM) mode or equivalent. Analyte identification is accomplished by comparing the acquired mass spectra, including ion ratios, and retention times to reference spectra and retention times for calibration standards acquired under identical GC-MS conditions. Quantitation is performed using the internal standard technique. Utilization of an isotopically-labeled internal standard provides a high degree of accuracy and precision for sample quantitation by accounting for analyte recovery and analytical efficiency.

Source: EPA and CDC. 2011. "High Throughput Determination of Tetramine in Drinking Water by Solid Phase Extraction and Isotope Dilution Gas Chromatography/Mass Spectrometry (GC/MS)." EPA 600/R-11/091. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-600-r-11-091.pdf

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

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

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

Method Developed for: TEA, EDEA and MDEA in wipe surfaces
Method Selected for: SAM lists this method for preparation and analysis of wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Detection limits (DL) for EDEA, MDEA and TEA are 0.06, 0.07 and 0.12 ng/cm^2, respectively. The limit of quantitation (LOQs) for EDEA, MDEA and TEA are 6.25, 6.85 and 12.3 ng/cm^2,respectively. The reporting range for all three target compounds is 0.1 – 5.0 ng/cm^2.

Description of Method: Samples are collected from surfaces with wipes and stored at 4 °C (± 2 °C) if not analyzed within 24-hours. Samples are brought to ambient temperature, then spiked with a surrogate compound and solvent. Samples are then sonicated, extracted with a syringe filter unit, concentrated, and analyzed directly by LC-MS-MS operated in the positive electrospray ionization (ESI+) mode. Each target compound is separated and identified by retention time and by comparing the sample primary multiple reaction monitoring (MRM) transition to the known standard MRM transition from reference spectra under identical LC-MS-MS conditions. The retention time for the analytes in the sample must fall within ± 5% of the retention time of the analytes in standard solution. The concentration of each analyte is determined by the instrumentation software using external calibration.

Source: EPA and CDC. 2011. "Surface Analysis Using Wipes for the Determination of Nitrogen Mustard Degradation Products by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)." EPA/600/R-11/143. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-600-r-11-143.pdf

5.2.48 Analytical Protocol for Chemical Warfare Agents in Water, Soil, and Wipes

Analyte(s)	CAS RN
Cyclohexyl sarin (GF)	329-99-7
Mustard, sulfur / Mustard gas (HD)	505-60-2
Sarin (GB)	107-44-8
Soman (GD)	96-64-0
VX	50782-69-9

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

Method Developed for: Determination of GF, GB, GD, HD and VX in water, soil and wipes
Method Selected for: SAM lists this procedure for preparation and analysis of aqueous liquid, drinking water, solid, wipe and air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The calibration ranges in full scan mode are 11.4 – 114 µg/L (GB, GF and VX) and 5.7 – 57 µg/L (GD and HD) for water samples; 20 – 200 µg/kg (GB, GF and VX) and 10 – 100 µg/kg (GD and HD) for soil samples; 0.02 – 0.2 µg/cm^2 (GB, GF and VX) and 0.01 – 0.1 µg/cm^2 (GD and HD) for wipes.

Description of Method: The method involves solvent extraction of the sample followed by GC/MS analysis to determine semivolatile CWAs. Prior to analysis, samples must be prepared using sample preparation techniques appropriate for each matrix type. Aqueous, solid and wipe samples are spiked with surrogates and extracted by microscale extraction. All target compounds in aqueous and wipe samples and all target compounds except for VX in solid samples are extracted with methylene chloride. VX in solid samples is extracted first using a tris buffer solution, followed by extraction with methylene chloride. Extracts are dried, concentrated (solids and wipe extracts only) by nitrogen evaporation, then analyzed by GC-MS, using either a mass selective detector in full scan mode or time-of-flight (TOF).

Special Considerations: The method has been extensively tested in reagent water, sand, wipes, drinking water and groundwater, and is currently undergoing multi-laboratory testing and validation in additional environmental matrices. The procedures are specifically for use by laboratories with EPA approval for handling and analysis of samples containing CWAs, and may require modifications for application to environmental sample types.

Source: EPA. March 2011. "DRAFT Analytical Protocol for Chemical Warfare Agents in Water, Soil and Wipes." Copies of this analytical protocol may be requested from NHSRC at http://www.epa.gov/sam/contact_us.htm

5.2.49 NIOSH Method 1612: Propylene Oxide

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

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

Method Developed for: Propylene oxide in air
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range is between 8 and 295 ppm for air samples of 5 L.

Description of Method: A sample tube containing coconut shell charcoal is used for sample collection with a flow rate of 0.01 to 0.2 L/minute. A 1-mL volume of carbon disulfide is added to the vial and allowed to sit for 30 minutes prior to analysis with occasional agitation. Analysis is performed on a GC-FID. No interferences have been found.

Special Considerations: The presence of propylene oxide should be confirmed by either a secondary GC column or by an MS.

Source: NIOSH. 1994. "Method 1612: Propylene Oxide," Issue 2.
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-1612.pdf

5.2.50 NIOSH Method 2016: Formaldehyde

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

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

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

Description of Method: This method can be used for the determination of formaldehyde using HPLC with a UV detector. Air is sampled onto a cartridge containing silica gel coated with 2,4-DNPH, at a rate of 0.03 to 1.5 L/minute. The cartridge is extracted with 10 mL of acetonitrile and analyzed by HPLC-UV at a wavelength of 360 nm. Ozone has been observed to consume the 2,4-DNPH reagent and to degrade the formaldehyde derivative. Ketones and other aldehydes can react with 2,4-DNPH; the derivatives produced, however, are separated chromatographically from the formaldehyde derivative.

Source: NIOSH. 2003. "Method 2016: Formaldehyde," Issue 2.
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-2016.pdf

5.2.51 NIOSH Method 2513: Ethylene Chlorohydrin

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

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

Method Developed for: Ethylene chlorohydrin (2-chloroethanol) in air
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range of the method is 0.5 to 15 ppm for a 20-L air sample.

Description of Method: Samples are drawn into a tube containing petroleum charcoal at a rate of 0.01 to 0.2 L/minute and transferred into vials containing eluent (carbon disulfide, 2-propanol and *n*-pentadiene as an internal standard). Vials must sit for 30 minutes prior to analysis by GC-FID. No interferences have been identified. Humidity may decrease the breakthrough volume during sample collection.

Special Considerations: The presence of 2-chloroethanol should be confirmed by either a secondary GC column or by an MS.

Source: NIOSH. 1994. "Method 2513: Ethylene Chlorohydrin," Issue 2.
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-2513.pdf

5.2.52 NIOSH Method 3510: Monomethylhydrazine

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

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

Method Developed for: Monomethylhydrazine in air
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range of the method is 0.027 to 2.7 ppm for a 20-L air sample.

Description of Method: Samples are collected into a bubbler containing hydrochloric acid using a flow rate of 0.5 to 1.5 L/minute. Samples are transferred to a 25-mL flask, mixed with phosphomolybdic acid solution, diluted to the mark with 0.1 M hydrochloric acid, and then transferred to a large test tube for spectrophotometric analysis. Positive interferences include other hydrazines, as well as stannous ion, ferrous ion, zinc, sulfur dioxide and hydrogen sulfide. Negative interferences may occur by oxidation of the monomethylhydrazine by halogens, oxygen (especially in the presence of copper (I) ions) and hydrogen dioxide.

Source: NIOSH. 1994. "Method 3510: Monomethylhydrazine," Issue 1.
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-3510.pdf

5.2.53 NIOSH Method 5600: Organophosphorus Pesticides

Analyte(s)	CAS RN
Disulfoton	298-04-4
Disulfoton sulfone oxon[1]	2496-91-5
Disulfoton sulfoxide	2497-07-6
Disulfoton sulfoxide oxon[1]	2496-92-6

[1] If problems occur when using this method for measurement of oxon compounds, analysts should consider use of procedures included in "Oxidation of Selected Organophosphate Pesticides During Chlorination of Simulated Drinking Water." Water Research. 2009. 43(2): 522–534. http://www.sciencedirect.com/science/journal/00431354

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

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

Description of Method: This method is used for the detection of organophosphorus pesticides using GC with a flame photometric detector (FPD). Samples are prepared by desorbing the XAD-2 resin with 2 mL of toluene/acetone (90/10 v/v) solution. The method also may be applicable to the determination of other organophosphorus compounds after evaluation for desorption efficiency, sample capacity, sample stability, and precision and accuracy. The working range for each analyte is provided in Table 5 of the method. These ranges cover from 0.1 to 2 times the OSHA PELs (see Table 5 of the method). The method also is applicable to Short Term Exposure Limit (STEL) measurements using 12-L samples.

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

Source: NIOSH. 1994. "Method 5600: Organophosphorus Pesticides," Issue 1.
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-5600.pdf

5.2.54 NIOSH Method 5601: Organonitrogen Pesticides

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

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

Method Developed for: Organonitrogen pesticides in air
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit for aldicarb is 1.2 µg per sample and 0.6 µg per sample for carbofuran, methomyl and oxamyl. The working ranges for aldicarb, carbofuran and oxamyl are listed in Table 2 of the method, and range from 0.5 to 10 times the OSHA PEL.

Description of Method: This method can be used for the determination of organonitrogen pesticides using HPLC with a UV detector. Samples are prepared by desorbing the XAD-2 resin with 2 mL of triethylamine-phosphate solution, rotating end-over-end for 45 minutes, and filtering. The method also may be applicable to the determination of other organonitrogen compounds and to a broad range of pesticides having UV chromophores, e.g., acetanilides, acid herbicides, organophosphates, phenols, pyrethroids, sulfonyl ureas, sulfonamides, triazines and uracil pesticides. Because of the broad response of the UV detector at shorter wavelengths, there are many potential interferences. Those tested include solvents (chloroform and toluene), antioxidants (butylated hydroxytoluene [BHT]), plasticizers (dialkyl phthalates), nitrogen compounds (nicotine and caffeine), impurities in HPLC reagents (e.g., in triethylamine), other pesticides (2,4-Dichlorophenoxyacetic acid [2,4-D], atrazine, parathion, etc.), and pesticide hydrolysis products (1-naphthol). Confirmation techniques are recommended when analyte identity is uncertain.

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

Source: NIOSH. 1998. "Method 5601: Organonitrogen Pesticides," Issue 1.
http://www.cdc.gov/niosh/docs/2003-154/pdfs/5601.pdf

5.2.55 NIOSH Method 6001: Arsine

Analyte(s)	CAS RN
Arsine (analyze as total arsenic in non-air samples)	7784-42-1

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

Method Developed for: Arsine in air
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range of the method is 0.001 to 0.2 mg/m^3 for a 10-L sample.

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

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

Source: NIOSH. 1994. "Method 6001: Arsine," Issue 2.
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-6001.pdf

5.2.56 NIOSH Method 6002: Phosphine

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

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

Method Developed for: Phosphine in air
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range of the method is 0.02 to 0.9 mg/m^3 for a 16-L sample.

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

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

Source: NIOSH. 1994. "Method 6002: Phosphine," Issue 2.
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-6002.pdf

5.2.57 NIOSH Method 6010: Hydrogen Cyanide

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

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

Method Developed for: Hydrogen cyanide in air
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range of the method is 3 to 260 mg/m^3 for a 3-L sample.

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

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

Source: NIOSH. 1994. "Method 6010: Hydrogen Cyanide," Issue 2.
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-6010.pdf

5.2.58 NIOSH Method 6013: Hydrogen Sulfide

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

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction

Determinative Technique: IC with conductivity detection
Method Developed for: Hydrogen sulfide in air
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range of the method is 0.9 to 20 mg/m^3 for a 20-L sample.

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

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

Source: NIOSH. 1994. "Method 6013: Hydrogen Sulfide," Issue 1.
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-6013.pdf

5.2.59 NIOSH Method 6015: Ammonia

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

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

Method Developed for: Ammonia in air
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range of the method is 0.15 to 300 mg/m^3 for a 10-L sample. Twice the recommended sample volume should be collected in order to achieve an action level of 70 µg/m^3.

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

Source: NIOSH. 1994. "Method 6015: Ammonia," Issue 2.
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-6015.pdf

5.2.60 NIOSH Method 6402: Phosphorus Trichloride

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

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

Method Developed for: Phosphorus trichloride in air

Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range of the method is 1.2 to 80 mg/m^3 for a 25-L sample.

Description of Method: In this method, phosphorus trichloride is determined as phosphate. A volume of 11 to 100 L of air is drawn through a bubbler containing reagent water. The resulting phosphorus acid solution is oxidized with bromine to phosphoric acid and color agent (sodium molybdate) and reducing agent (hydrazine sulfate) are added. The solution is analyzed for the resulting molybdenum blue complex by visible spectrophotometry. Phosphorus (V) compounds do not interfere. Sample solutions are stable to oxidation by air during sampling.

Source: NIOSH. 1994. "Method 6402: Phosphorus Trichloride," Issue 2.
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-6402.pdf

5.2.61 NIOSH Method 7903: Acids, Inorganic

Analyte(s)	CAS RN
Hydrogen bromide	10035-10-6
Hydrogen chloride	7647-01-0
Hydrogen fluoride	7664-39-3

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

Method Developed for: Inorganic acids in air
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The working range of this method is 0.01 to 5 mg/m^3 for a 50-L sample.

Description of Method: Acids are analyzed as bromide, chloride and fluoride. A volume of 3 to 100 L of air is drawn through a silica gel sorbent. The sorbent portions are extracted with a buffered carbonate/bicarbonate solution and the extract is analyzed by IC.

Special Considerations: Particulate salts of the acids are an interference (trapped on the glass wool filter plug in the sorbent tube). Chlorine and bromine are also interferences. Acetate, formate and propionate interferences may be reduced by use of a weaker eluent. If problems occur when using this method for analysis of hydrogen fluoride, it is recommended that NIOSH Method 7906 be used.

Source: NIOSH. 1994. "Method 7903: Acids, Inorganic," Issue 2.
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-7903.pdf

5.2.62 NIOSH Method 7905: Phosphorus

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

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

Method Developed for: Phosphorus in air

Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The LOD for samples analyzed by GC-FPD is 0.005 µg per sample. The working range for samples analyzed by GC-FPD is 0.056 to 0.24 mg/m^3 for a 12-L sample.

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

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

Source: NIOSH. 1994. "Method 7905: Phosphorus," Issue 2.
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-7905.pdf

5.2.63 NIOSH Method 7906: Fluorides, Aerosol and Gas, by IC

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

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

Method Developed for: Fluorides in aerosol and gas
Method Selected for: SAM lists this method for use if problems occur when using NIOSH Method 7903 for the analysis of hydrogen fluoride during preparation and analysis of air samples. (See Footnote 6 of Appendix A.)
Detection and Quantitation: The working range of the method is 0.04 to 8 mg/m^3 for 250-L samples.

Description of Method: Hydrogen fluoride is determined as fluoride ion by this method. A volume of 1 to 800 L of air is drawn through a 0.8-µm cellulose ester membrane (to trap particulate fluorides) and a cellulose pad treated with sodium carbonate (to trap gaseous fluoride). The pad is extracted with reagent water and the extract is analyzed for fluoride by IC.

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

Source: NIOSH. 1994. "Method 7906: Fluorides, Aerosol and Gas by IC," Issue 1.
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-7906.pdf

5.2.64 NIOSH Method 9102: Elements on Wipes

Analyte(s)	CAS RN
Ammonium metavanadate (analyze as total vanadium)	7803-55-6
Arsenic, Total	7440-38-2
Arsenic trioxide (analyze as total arsenic)	1327-53-3
Arsine (analyze as total arsenic in non-air samples)	7784-42-1
Calcium arsenate (analyze as total arsenic)	7778-44-1
2-Chlorovinylarsonous acid (2-CVAA) (analyze as total arsenic)	85090-33-1

Analyte(s)	CAS RN
Ethyldichloroarsine (ED)	598-14-1
Lead arsenate (analyze as total arsenic)	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine] (analyze as total arsenic)	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)	40334-70-1
Lewisite oxide (analyze as total arsenic)	1306-02-1
Mercuric chloride (analyze as total mercury)	7487-94-7
Mercury, Total	7439-97-6
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2
Osmium tetroxide (analyze as total osmium)	20816-12-0
Sodium arsenite (analyze as total arsenic)	7784-46-5
Thallium sulfate (analyze as total thallium)	10031-59-1
Titanium tetrachloride (analyze as total titanium)	7550-45-0
Vanadium pentoxide (analyze as total vanadium)	1314-62-1

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

Method Developed for: Measurement of metals on wipe surfaces using ICP-AES
Method Selected for: SAM lists this method for preparation of wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The range for arsenic is 0.261 to105 µg/wipe; for thallium 0.136 to 50.0 µg/wipe; for vanadium 0.0333 to 25.0 µg/wipe.

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

Special Considerations: ICP-MS may also be used for the analysis of wipe samples; however, at this time, this technique has not been evaluated for wipes. Nitric and perchloric acids are strong oxidizers and extremely corrosive. Perform all perchloric acid digestions in a perchloric acid hood. When working with acids, use gloves and avoid inhalation or contact with skin or clothing.

Source: NIOSH. 2003. "Method 9102, Issue 1: Elements on Wipes."
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-9102.pdf

5.2.65 NIOSH Method S301-1: Fluoroacetate Anion

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

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

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

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

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

Source: NIOSH. 1977. "Method S301-1: Sodium Fluoroacetate."
http://www2.epa.gov/sites/production/files/2015-07/documents/niosh-s301-1.pdf

5.2.66 OSHA Method 40: Methylamine

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

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

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

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

Source: OSHA. 1982. "Method 40: Methylamine." Method originally obtained from www.osha.gov, but is provided here for reference.
http://www2.epa.gov/sites/production/files/2015-07/documents/osha-method40.pdf

5.2.67 OSHA Method 54: Methyl Isocyanate (MIC)

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

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

Method Developed for: Methyl isocyanate in air
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.

Description of Method: This method determines the concentration of methyl isocyanate in air by using HPLC with a FL or UV detector. Samples are collected by drawing a known volume of air through XAD-7 tubes coated with 0.3 mg of 1-(2-pyridyl)piperazine (1-2PP). Samples are desorbed with acetonitrile and analyzed by HPLC using a FL or UV detector.

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

5.2.68 OSHA Method 61: Phosgene

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

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

Method Developed for: Phosgene in air samples
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.

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

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

Source: OSHA. 1986. "Method 61: Phosgene." Method originally obtained from www.osha.gov, but is provided here for reference.
http://www2.epa.gov/sites/production/files/2015-07/documents/osha-method61.pdf

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

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

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

Method Developed for: Sodium azide and hydrazoic acid in workplace atmospheres
Method Selected for: SAM lists this method for preparation and analysis of air and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit was found to be 0.001 ppm as hydrazoic acid (HN_3) or 0.003 mg/m^3 as sodium azide (NaN_3) for a 5-L air sample. The quantitation limit was found to be 0.004 ppm as HN_3 or 0.011 mg/m^3 as NaN_3 for a 5-L air sample.

Description of Method: This method describes sample collection and analysis of airborne azides [as NaN_3 and hydrazoic acid HN_3]. Particulate NaN_3 is collected on a polyvinyl chloride (PVC) filter or in the glass wool plug of the sampling tube. Gaseous HN_3 is collected and converted to NaN_3 by the impregnated silica gel (ISG) sorbent within the sampling tube. The collected azide on either media is desorbed in a weak buffer solution, and the resultant anion (N_3^-) is analyzed by IC using a variable wavelength UV detector at 210 nm. A gravimetric conversion is used to calculate the amount of NaN_3 or HN_3 collected.

Source: OSHA. 1992. "Method ID-211: Sodium Azide and Hydrazoic Acid in Workplace Atmospheres." http://www2.epa.gov/sites/production/files/2015-07/documents/osha-id-211.pdf

5.2.70 OSHA Method ID216SG: Boron Trifluoride (BF_3)

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

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

Method Developed for: Boron trifluoride in air samples
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit is 10 µg in a 30-L sample.

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

Source: OSHA. 1989. "Method ID216SG: Boron Trifluoride (BF_3)." Method originally obtained from www.osha.gov, but is provided here for reference.
http://www2.epa.gov/sites/production/files/2015-07/documents/osha-id216sg.pdf

5.2.71 OSHA Method PV2004: Acrylamide

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

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

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

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

Source: OSHA. 1991. "Method PV2004: Acrylamide."
http://www2.epa.gov/sites/production/files/2015-07/documents/osha-pv2004.pdf

5.2.72 OSHA Method PV2103: Chloropicrin

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

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

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

Description of Method: This method determines the concentration of chloropicrin in air by GC-ECD. Samples are collected by drawing a known volume of air through two XAD-4 tubes in series. Samples are desorbed with ethyl acetate and analyzed by GC-ECD.
Special Considerations: The presence of chloropicrin should be confirmed by either a secondary GC column or by an MS. Chloropicrin is light sensitive, and samples should be protected from light.

Source: OSHA. 1991. "Method PV2103: Chloropicrin."
http://www2.epa.gov/sites/production/files/2015-07/documents/osha-pv2103.pdf

5.2.73 ASTM Method D5755-03: Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Surface Loading

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

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

Method Developed for: Asbestos in dust
Method Selected for: SAM lists this method for preparation and analysis of solid (e.g., soft surfaces-microvac) samples. See Appendix A for corresponding method usability tiers.

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

Source: ASTM. 2003. "Method D5755-03: Standard Test Method for Microvacuum Sampling and Indirect Analysis of Dust by Transmission Electron Microscopy for Asbestos Structure Number Surface Loading." http://www.astm.org/Standards/D5755.htm

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

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

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

Method Developed for: Asbestos in samples wiped from surfaces
Method Selected for: SAM lists this method for preparation and analysis of wipe (e.g., hard surfaces-wipes) samples. See Appendix A for corresponding method usability tiers.

Description of Method: This method describes a procedure to identify asbestos in samples wiped from surfaces and to provide an estimate of the concentration of asbestos reported as the number of asbestos structures per unit area of sampled surface. A sample is collected by wiping a surface of known area with a wipe material. The sample is transferred from the wipe material to an aqueous suspension of known volume. Aliquots of the suspension are then filtered through a membrane filter, and a section of the membrane filter is prepared and transferred to a TEM grid, using the direct transfer method. The

asbestiform structures are identified, sized, and counted by TEM, using electron diffraction and EDXA at a magnification from 15,000 to 20,000X.

Source: ASTM. 2005. "Method D6480-05: Standard Test Method for Wipe Sampling of Surfaces, Indirect Preparation, and Analysis for Asbestos Structure Number Concentration by Transmission Electron Microscopy." http://www.astm.org/Standards/D6480.htm

5.2.75 ASTM Method D7597-09: Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Hydrogen Dimethylamidophosphate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Water by Liquid Chromatography/Tandem Mass Spectrometry

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

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

Method Developed for: Diisopropyl methylphosphonate, ethyl hydrogen dimethylamidophosphate, isopropyl methylphosphonic acid, methylphosphonic acid and pinacolyl methylphosphonic acid in surface water
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid and drinking water samples. For DIMP in drinking water samples, use EPA Method 538 for sample preparation and analysis. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection verification levels (DVLs) and reporting range for this method vary for each analyte and range from 0.25 to 20 µg/L and 5 to 1500 µg/L.

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

Source: ASTM. 2009. "Method D7597-09: Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Hydrogen Dimethylamidophosphate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Water by Liquid Chromatography/Tandem Mass Spectrometry." http://www.astm.org/Standards/D7597.htm

5.2.76 ASTM Method D7598-09: Standard Test Method for Determination of Thiodiglycol in Water by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry

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

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

Method Developed for: Thiodiglycol in surface water samples
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid and drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The DVL for thiodiglycol is 20 µg/L; the reporting range is 100 to 10000 µg/L.

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

Source: ASTM. 2009. "Method D7598-09: Standard Test Method for Determination of Thiodiglycol in Water by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry." http://www.astm.org/Standards/D7598.htm

5.2.77 ASTM Method D7599-09: Standard Test Method for Determination of Diethanolamine, Triethanolamine, *N*-Methyldiethanolamine and *N*-Ethyldiethanolamine in Water by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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

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

Method Developed for: Diethanolamine, triethanolamine, *n*-methyldiethanolamine and *n*-ethyldiethanolamine in surface water samples
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid and drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The DVL and reporting range for EDEA and TEA are 5 µg/L and 25 to 500 µg/L, respectively. The DVL and reporting range for MDEA are 10 µg/L and 50 to 500 µg/L, respectively.

Description of Method: Target compounds are analyzed by direct injection without derivatization by LC-MS-MS. Samples are shipped to the laboratory at 0 to 6 °C, spiked with surrogates, filtered using a

syringe-driven filter unit and analyzed directly by LC-MS-MS within 7 days. Target compounds are identified by comparing sample SRM transitions to the known standard SRM transitions. The retention time for the analytes of interest must also fall within the retention time of the standard by ± 5%. Target compounds are quantitated using the SRM transition and external standard calibration.

Source: ASTM. 2009. "Method D7599-09: Standard Test Method for Determination of Diethanolamine, Triethanolamine, *N*-Methyldiethanolamine and *N*-Ethyldiethanolamine in Water by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)." http://www.astm.org/Standards/D7599.htm

5.2.78 ASTM Method D7644-10: Standard Test Method for Determination of Bromadiolone, Brodifacoum, Diphacinone and Warfarin in Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

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

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

Method Developed for: Bromadiolone, brodifacoum and diphacinone in water
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid and drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The DVLs and reporting range for each analyte are 0.020 µg/L and 0.125 – 2.5 µg/L, respectively.

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

Source: ASTM. 2009. "Method D7644-10: Standard Test Method for Determination of Bromadiolone, Brodifacoum, Diphacinone and Warfarin in Water by Liquid Chromatography/Tandem Mass Spectrometry." http://www.astm.org/Standards/D7644.htm. Note: If this method is no longer available through ASTM, please refer to the appropriate point of contact listed in Section 4 for information on obtaining the method.

5.2.79 ASTM Method D7645-10: Standard Test Method for Determination of Aldicarb, Aldicarb Sulfone, Aldicarb Sulfoxide, Carbofuran, Methomyl, Oxamyl and Thiofanox in Water by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

Analyte(s)	CAS RN
Aldicarb	116-06-3

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

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

Method Developed for: Aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbofuran, oxamyl, methomyl and thiofanox in water
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid samples for aldicarb, aldicarb sulfone, aldicarb sulfoxide, carbofuran, methomyl, oxamyl and thiofanox. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The DVL for aldicarb sulfone, aldicarb sulfoxide and thiofanox is 250 ng/L. The reporting range is 1 – 100 µg/L.

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

Source: ASTM. 2010. "Method D7645: Standard Test Method for Determination of Aldicarb, Aldicarb Sulfone, Aldicarb Sulfoxide, Carbofuran, Methomyl, Oxamyl and Thiofanox in Water by Liquid Chromatography/Tandem Mass Spectrometry." http://www.astm.org/Standards/D7645.htm. Note: If this method is no longer available through ASTM, please refer to the appropriate point of contact listed in Section 4 for information on obtaining the method.

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

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

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

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

Description of Method: Samples are shipped to the laboratory at 0 to 6 °C and must be extracted, concentrated, and analyzed by LC-MS-MS within 7 days. Approximately 5 – 30 g of soil is mixed with an appropriate amount (depending on the wetness of the soil) of drying agent (diatomaceous earth), spiked with a surrogate, and extracted in a PFE system using methanol. Extracts are filtered using a 0.2-micron filter and concentrated to a final volume of 0.4 mL using a nitrogen evaporation device. The volume of the extract is brought up to 2 mL with HPLC-grade water and analyzed by LC-MS-MS. The target analytes are identified by comparing the sample SRM transitions to the known standard SRM transitions. The retention time for the analytes in the sample must fall within ± 5% of the retention time of the analytes in standard solution. Target analytes are measured using the SRM transition and external standard calibration.

Source: ASTM. 2011. "Method E2787: Standard Test Method for Determination of Thiodiglycol in Soil Using Pressurized Fluid Extraction Followed by Single Reaction Monitoring Liquid Chromatography/ Tandem Mass Spectrometry." http://www.astm.org/Standards/E2787.htm. Note: If this method is no longer available through ASTM, please refer to the appropriate point of contact listed in Section 4 for information on obtaining the method.

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

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

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

Method Developed for: Thiodiglycol in wipes
Method Selected for: SAM lists this method for preparation and analysis of wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDL for thiodiglycol is 0.085 µg/wipe. The reporting range is 1 – 80 µg/wipe.

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

Source: ASTM. 2011. "Method E2383: Standard Test Method for Determination of Thiodiglycol on Wipes Using Pressurized Fluid Extraction Followed by Single Reaction Monitoring Liquid Chromatography/Tandem Mass Spectrometry." http://www.astm.org/Standards/E2838.htm. Note: If this method is no longer available through ASTM, please refer to the appropriate point of contact listed in Section 4 for information on obtaining the method.

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

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

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

Method Developed for: Diisopropyl methylphosphonate, ethyl hydrogen dimethylamidophosphate, isopropyl methylphosphonic acid, methylphosphonic acid and pinacolyl methylphosphonic acid in soil
Method Selected for: SAM lists this method for preparation and analysis of solid samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDLs range from 1.3 to 8.7 µg/kg. The reporting range for all analytes is 40 to 2000 µg/kg.

Description of Method: Target compounds are analyzed by direct injection without derivatization by LC-MS-MS. Samples are shipped to the laboratory at 0 to 6 °C and must be extracted, concentrated, and analyzed by LC-MS-MS within 7 days. Approximately 5 – 30 g of soil are mixed with an appropriate amount (depending on the wetness of the soil) of drying agent (diatomaceous earth), spiked with a surrogate, and extracted in a PFE system using water. Extracts are filtered using a 0.2-micron filter and analyzed by LC-MS-MS. The target compounds are identified by comparing the sample SRM transitions to the known standard SRM transitions. The retention time for the analytes of interest must also fall within the retention time of the standard by ± 5%. Target compounds are quantitated using the SRM transition of the target compounds and external standard calibration.

Source: ASTM. 2012. "Method E2866-12: Standard Test Method for Determination of Diisopropyl Methylphosphonate, Ethyl Hydrogen Dimethylamidophosphate, Ethyl Methylphosphonic Acid, Isopropyl Methylphosphonic Acid, Methylphosphonic Acid and Pinacolyl Methylphosphonic Acid in Water by Liquid Chromatography/Tandem Mass Spectrometry." http://www.astm.org/Standards/E2866.htm.
Note: If this method is no longer available through ASTM, please refer to the appropriate point of contact listed in Section 4 for information on obtaining the method.

5.2.83 ISO Method 10312:1995: Ambient Air - Determination of Asbestos Fibres - Direct-Transfer Transmission Electron Microscopy Method

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

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

Method Developed for: Asbestos in ambient air
Method Selected for: SAM lists this method for preparation and analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: In a 4000-L air sample with approximately 10 pg/m^3 (typical of clean or rural atmospheres), an analytical sensitivity of 0.5 structure/L can be obtained. This is equivalent to a detection limit of 1.8 structure/L when an area of 0.195 mm of the TEM specimen is examined. The range of concentrations that can be determined is 50 to 7,000 structures/mm^2 on the filter.

Description of Method: This method determines the type(s) of asbestos fibers present, but cannot discriminate between individual fibers of the asbestos and non-asbestos analogues of the same amphibole mineral. The method is defined for polycarbonate capillan/pore filters or cellulose ester (either mixed esters of cellulose or cellulose nitrate) filters through which a known volume of air has been drawn. The method is suitable for determination of asbestos in both exterior and building atmospheres.

Source: ISO. 2005. "Method 10312: 1995: Ambient Air - Determination of Asbestos Fibres - Direct Transfer Transmission Electron Microscopy Method."
http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=18358

5.2.84 Standard Method 4500-NH_3 B: Nitrogen (Ammonia) Preliminary Distillation Step

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

Analysis Purpose: Sample preparation
Sample Preparation Technique: Distillation
Determinative Technique: Visible spectrophotometry
Determinative Method: Standard Method 4500-NH_3 G

Method Developed for: Nitrogen (ammonia) in drinking waters, clean surface or groundwater, and good-quality nitrified wastewater effluent
Method Selected for: SAM lists this method for preparation of aqueous liquid samples. See Appendix A for corresponding method usability tiers.

Description of Method: A 0.5- to 1-L sample is dechlorinated, buffered, adjusted to pH 9.5, and distilled into a sulfuric acid solution. The distillate is brought up to volume, neutralized with sodium hydroxide, and analyzed by Method 4500-NH_3 G.

Source: APHA, AWWA, and WEF. 2005. "Method 4500-NH3 B: Nitrogen (Ammonia) Preliminary Distillation Step." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition.
http://www.standardmethods.org/

5.2.85 Standard Method 4500-NH_3 G: Nitrogen (Ammonia) Automated Phenate Method

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

Analysis Purpose: Analyte determination and measurement
Determinative Technique: Visible spectrophotometry
Sample Preparation Method: Standard Method 4500-NH_3 B
Sample Preparation Technique: Distillation

Method Developed for: Nitrogen (ammonia) in drinking waters, clean surface or groundwater, and good-quality nitrified wastewater effluent
Method Selected for: SAM lists this method for analysis of aqueous liquid samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The range of the method is 0.02 to 2.0 mg/L.

Description of Method: Ammonia is determined as indophenol blue by this method. A portion of the neutralized sample distillate (from procedure 4500-NH_3 B) is run through a manifold. The ammonium in the distillate reacts with solutions of disodium ethylenediaminetetraacetic acid (EDTA), sodium phenate, sodium hypochlorite and sodium nitroprusside. The resulting indophenol blue is detected by colorimetry in a flow cell. Photometric measurement is made between the wavelengths of 630 and 660 nm.

Source: APHA, AWWA, and WEF. 2005. "Method 4500-NH_3 G: Nitrogen (Ammonia) Automated Phenate Method." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

5.2.86 Standard Method 4500-Cl G: Chlorine (Residual) DPD Colorimetric Method

Analyte(s)	CAS RN
Chlorine	7782-50-5

Analysis Purpose: Sample preparation, and/or analyte determination and measurement
Sample Preparation Technique: Water samples are buffered and colorimetric agent is added. Buffered water extraction by Analyst, 1999. 124: 1853–1857 are used for preparation of air samples.
Determinative Technique: Visible spectrophotometry

Method Developed for: Chlorine in water and wastewater
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid and drinking water samples. It also should be used for analysis of air samples when appropriate sample preparation techniques have been applied. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The method can detect 10 µg/L chlorine.

Description of Method: A portion of aqueous liquid sample is buffered and reacted with N,N-diethyl-*p*-phenylenediamine (DPD) color agent. The resulting free chlorine is determined by colorimetry. If total chlorine (including chloroamines and nitrogen trichloride) is to be determined, potassium iodide crystals are added. Results for chromate and manganese are blank corrected using thioacetamide solution.

Special Considerations: Organic contaminants and strong oxidizers may cause interference.

Source: APHA, AWWA, and WEF. 2005. "Method 4500-Cl G: DPD Colorimetric Method." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

5.2.87 Literature Reference for Hexamethylenetriperoxidediamine (HMTD) (Analyst, 2001. 126:1689–1693)

Analyte(s)	CAS RN
Hexamethylenetriperoxidediamine (HMTD)	283-66-9

Analysis Purpose: Analyte determination and measurement
Sample Preparation Technique: SW-846 Methods 8330B/3535A (solid samples, aqueous liquid and drinking water samples), and 3570/8290A Appendix A (wipe samples). Refer to Appendix A for which of these preparation methods should be used for a particular analyte/sample type combination.

Determinative Technique: LC-MS-MS

Method Developed for: Trace quantities of HMTD in explosives or explosive mixtures
Method Selected for: SAM lists this procedure for analysis of solid, aqueous liquid, drinking water and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The LOD is 20 µg/L.

Description of Method: Prepared samples are analyzed by positive mode atmospheric pressure chemical ionization (APCI) LC-MS-MS using a C_{18} analytical column (150 mm x 2.0 mm I.D., 5µm particle size) coupled with a C_{18} guard cartridge system (10 mm x 2.0 mm I.D.). Elution using a 95/5 water/methanol solution detects HMTD at m/z = 209 and a retention time of ~ 15.5 minutes.

Special Considerations: The procedure has been developed for the determination of HMTD in explosives or explosive mixtures; modifications will be needed for application to environmental samples such as soils, wipes and water samples. Until modifications can be developed and tested, it is recommended that the sample preparation procedures described in SW-846 Methods 8330B and 3535A (solid samples, aqueous liquid and drinking water samples) and SW-846 Methods 3570 and 8290A Appendix A (wipe samples) be used.

Source: Crowson, A. and Berardah, M.S. 2001. "Development of an LC/MS Method for the Trace Analysis of Hexamethylenetriperoxidediamine (HMTD)." Analyst 126(10): 1689-1693. http://pubs.rsc.org/en/Content/ArticleLanding/2001/AN/b107354k

5.2.88 Literature Reference for Chlorine in Air (Analyst, 1999. 124(12): 1853–1857)

Analyte(s)	CAS RN
Chlorine	7782-50-5

Analysis Purpose: Sample preparation
Sample Preparation Technique: Buffered water extraction
Determinative Technique: Visible spectrophotometry
Determinative Method: Standard Method 4500-Cl G

Method Developed for: Active chlorine in air
Method Selected for: SAM lists this procedure for preparation of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Detection limit of 0.1 µg of chlorine; the collection efficiency was >90%; recovery of chlorine spikes from 0.05-g aliquots of the sorbent was not quantitative (~60%) but was reproducible.

Description of Method: A procedure is described for determination of total combined gas-phase active chlorine (i.e., Cl_2, hypochlorous acid [HOCl], and chloramines) and is based on a sulfonamide-functionalized silica gel sorbent. For determination of the collected chlorine, a modified version of the DPD colorimetric procedure is used, which yielded a detection limit of 0.1 µg of chlorine. At flow rates ranging from 31 to 294 mL/minute, the collection efficiency was >90% based on breakthrough analysis. Recovery of chlorine spikes from 0.05-g aliquots of the sorbent was not quantitative (~60%) but was reproducible; the recovery is accounted for in samples by adding weighed amounts of sorbent to the standards.

Source: Johnson, B.J., Emerson, D.W., Song, L., Floyd, J. and Tadepalli, B. 1999. "Determination of Active Chlorine in Air by Bonded Phase Sorbent Collection and Spectrophotometric Analysis." Analyst. 124(12): 1853–1857. http://pubs.rsc.org/en/content/articlelanding/1999/an/a906305f

5.2.89 Literature Reference for Methamidophos (Chromatographia. 2006. 63(5/6): 233–237)

Analyte(s)	CAS RN
Acephate	30560-19-1
Methamidophos	10265-92-6

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

Method Developed for: Pesticides (methamidophos) in water samples
Method Selected for: SAM lists this procedure for preparation and analysis of aqueous liquid samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The LOD is 30 µg/L.

Description of Method: A multi-residue analytical method is described for monitoring polar pesticides, such as acephate and methamidophos, in water with SPE and LC-MS-MS. Samples are analyzed using a C_{18} analytical column (150 mm x 3.2 mm I.D., 5µm particle size) coupled with a C_{18} guard cartridge system (4 mm x 3.0 mm I.D.).

Source: Liu, F., Bischoff, G., Pestemer, W., Xu, W. and Kofoet, A. 2006. "Multi-residue Analysis of Some Polar Pesticides in Water Samples With SPE and LC/MS/MS." Chromatographia. 63(5/6): 233–237. http://www.springerlink.com/content/gg87150ll26390x6/

5.2.90 Literature Reference for Cyanogen Chloride (Encyclopedia of Anal. Chem. 2006 DOI: 10.1002/9780470027318.a0809)

Analyte(s)	CAS RN
Cyanogen chloride	506-77-4

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

Method Developed for: Determination of cyanogen chloride in drinking water
Method Selected for: SAM lists this procedure for preparation and analysis of aqueous liquid, drinking water and solid samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: In drinking water, the MDL is 0.13 µg/L when using purge-and-trap GC-MS or liquid-liquid microextraction GC-ECD, and 0.04 µg/L when using headspace GC-ECD.

Description of Method: The method describes three different sample preparation techniques (purge-and-trap, headspace and micro liquid-liquid extraction) and two different determinative techniques (GC-MS and GC-ECD). Using the purge-and-trap technique, cyanogen chloride and an internal standard are extracted (purged) from the sample matrix by bubbling an inert gas through the sample. Purged sample components are trapped in a tube containing suitable sorbent materials. When purging is complete, the sorbent tube is heated. Simultaneously, a short piece of deactivated fused silica precolumn is cooled with liquid nitrogen to refocus the analytes. The cryotrap is heated to inject the sample onto a GC-MS.

For headspace GC-ECD analyses, a 40-mL vial is filled with sample without headspace. With the vial upside down, a volume of nitrogen is forced into the sample using a syringe, and an equivalent sample

volume is dispelled through a second syringe. The sample is shaken by hand and, after settling, a volume of the headspace is sampled by syringe and injected into a split-mode GC-ECD. For liquid-liquid microextraction GC-ECD analyses, 30 mL of water sample is extracted in a 40-mL vial, with 10 g of Na_2SO_4, 4 mL of MTBE and an internal standard. The sample is shaken by mechanical shaker or by hand. After allowing the phases to separate, the MTBE layer is transferred to another vial and injected into a GC-ECD.

Special Considerations: This procedure has been developed for water samples; modifications may be needed for application to environmental samples such as solid samples.

Source: Xie, Y. 2006. "Cyanogen Chloride and Cyanogen Bromide Analysis in Drinking Water." Encyclopedia of Analytical Chemistry, 1 – 11. http://onlinelibrary.wiley.com/doi/10.1002/9780470027318.a0809/abstract

5.2.91 Literature Reference for 3-Chloro-1,2-propanediol (Eur. J. Lipid Sci. Technol. 2011, 113: 345–355)

Analyte(s)	CAS RN
3-Chloro-1,2-propanediol	96-24-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction, followed by solid phase extraction cleanup and derivatization
Determinative Technique: GC-MS

Method Developed for: Trace quantities of 3-chloro-1,2-propanediol in foodstuffs
Method Selected for: SAM lists this procedure for preparation and analysis of solid and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The low calibration standard is 5 µg/L. The MDL in food ranges from 4 to 16 µg/kg. The working range is 4 – 4,000 µg/kg.

Description of Method: Foodstuffs (olive oil, cereal and potato products) are solvent extracted with hexane/diethyl ether and centrifuged. The resulting organic layer is washed several times (by adding water, vortexing and then centrifuging), then dried with sodium sulfate. The extract is concentrated to dryness, and redissolved in tetrahydrofuran (THF) to which acidified methanol is added. The reaction mixture is neutralized with sodium bicarbonate and washed with 3 aliquots of hexane, and the residue is quantitatively transferred to a sodium chloride solution. This solution is mixed with the contents of a highly pure diatomaceous earth based solid phase refill sachet, transferred to a chromatography column, and then eluted with diethyl ether. The collected eluent is concentrated by rotary evaporation and derivatized with heptaflurobutyrylimidazole (HFBI) at 70 °C for 15 – 20 minutes. After washing with water, the extracts are analyzed using a GC-MS.

Special Considerations: The procedure has been developed for the determination of 3-chloro-1,2-propanediol in foodstuffs only; modifications may be needed for application to environmental samples.

Source: Hamlet, C. G. and Asuncion, L. 2011. "Single-Laboratory Validation of a Method to Quantify Bound 2-Chloropropane-1,3-diol and 3-Chloropropane-1,2-diol in Foodstuffs Using Acid Catalysed Transesterification, HFBI Derivatisation and GC/MS Detection." Eur. J. Lipid Sci. Technol., 113(3): 345–355. http://onlinelibrary.wiley.com/doi/10.1002/ejlt.v113.3/issuetoc

5.2.92 Literature Reference for Methyl Hydrazine (Journal of Chromatography 1993 (617), 157-162)

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

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: SW-846 Method 3541/3545 (for solids), SW-846 Methods 3570/8290 Appendix A (for wipes), filtration for water samples, followed by derivatization for all sample types
Determinative Technique: HPLC-UV

Method Developed for: Determination of hydrazine in human plasma
Method Selected for: SAM lists this procedure for preparation and analysis of aqueous liquid and drinking water samples, and for the analysis of solid and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Detection limit in pooled plasma is 1 µg/L. The reporting range is 5 – 1000 µg/L.

Description of Method: Samples are prepared in a single-step reaction by protein denaturation with trichloroacetic acid, and derivatization to a stable azine with 4-hydroxybenzaldehyde. Chromatographic separation is carried out on a reversed-phase (octadecylsilane) column with methanol:water (60:40) as the mobile phase and UV detection at 340 nm. Retention time of the azine derivative of methyl hydrazine is 3.5 minutes.

Special Considerations: This procedure has been developed for human plasma; modifications may be needed for application to environmental samples such as aqueous liquid, drinking water, solid and wipes samples.

Source: Kircherr, H. 1993. "Determination of Hydrazine in Human Plasma by High Performance Liquid Chromatography." Journal of Chromatography B, 617(1): 157-162.
http://www.sciencedirect.com/science/article/pii/0378434793804368

5.2.93 Literature Reference for Paraquat (Journal of Chromatography A, 2008, 1196–1197, 110–116)

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

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Extraction by digestion, shaking or microwave-assisted extraction (MAE) followed by SPE cleanup
Determinative Technique: LC-UV or LC-MS-MS

Method Developed for: Determination of quaternary ammonium herbicides in soil
Method Selected for: SAM lists this procedure for preparation and analysis of solid and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: Limits of detection are 10 µg/kg by digestion and 50 µg/kg by MAE when using LC-UV and 1.0 µg/kg by digestion and 3.0 µg/kg by MAE when using LC-MS-MS. Estimated quantification limits are 20 µg/kg and 100 µg/kg when using LC-UV and 2.0 µg/kg by digestion and 7.5µg/kg by MAE when using LC-MS-MS.

Description of Method: Soil matrices can be extracted using one of the following three procedures: (1) digestion with an acidic methanol/ EDTA solution, (2) shaking in an EDTA/ammonium formate solution, or (3) using a microwave assisted extraction system in a benzalkonium chloride/acid solution. Cleanup of extracts is performed by SPE using silica cartridges for all three extraction procedures. Detection of these herbicides is carried out by either LC-UV or LC-MS-MS.

Special Considerations: This procedure has been developed for soil samples; modifications may be needed for application to environmental samples such as wipes samples.

Source: Pateiro-Moure, M., Martínez-Carballo, E., Arias-Estévez, M. and Simal-Gándara, J. 2008. "Determination of Quaternary Ammonium Herbicides in Soils. Comparison of Digestion, Shaking and Microwave-Assisted Extractions." Journal of Chromatography A, 1196−1197, 110−116. http://www.elsevier.com/locate/chroma

5.2.94 Literature Reference for Methamidophos (Journal of Chromatography A, 2007. 1154: 3–25)

Analyte(s)	CAS RN
Acephate	30560-19-1
Methamidophos	10265-92-6

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

Method Developed for: Pesticides (methamidophos) in crops
Method Selected for: SAM lists this procedure for preparation and analysis of solid, air and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The LOD for this method is 0.01 mg/kg.

Description of Method: A liquid chromatography–tandem quadrupole mass spectrometry (LC-MS-MS) multi-residue method for the simultaneous target analysis of a wide range of pesticides and metabolites in fruit, vegetables and cereals is described. Gradient elution has been used in conjunction with ESI+ tandem mass spectrometry to detect up to 171 pesticides and/or metabolites in different crop matrices using a single chromatographic run. Pesticide residues are extracted/partitioned from the samples with acetone/dichloromethane/light petroleum. Samples are analyzed by LC-MS-MS using a C_{18} analytical column (150 mm x 3.2 mm I.D., 5µm particle size) coupled with a C_{18} guard cartridge system (4 mm x 3.0 mm I.D.).

Special Considerations: The procedure has been developed for the analysis of various pesticides (methamidophos) in crops using LC-MS-MS; modifications will be needed for application to environmental samples such as soils, wipes and air samples collected on sorbent/filters.

Source: Hiemstra, M. and de Kok, A. 2007. "Comprehensive Multi-residue Method for the Target Analysis of Pesticides in Crops Using Liquid Chromatography-Tandem Mass Spectrometry." Journal of Chromatography A. 1154(1): 3–25. http://www.sciencedirect.com/science/journal/00219673

5.2.95 Literature Reference for Fluoroacetic Acid/Fluoroacetate Salts/Methyl Fluoroacetate (Journal of Chromatography A, 1139 (2007) 271–278)

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

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Water extraction followed by SPE cleanup and derivatization for solid and wipe samples. Use NIOSH Method S301-1 for air samples.
Determinative Technique: LC-MS

Method Developed for: Determination of fluoroacetate in food
Method Selected for: SAM lists this procedure for preparation and analysis of solids and wipes and for the analysis of air samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The LOD is 0.8 µg/L. The calibration range is 20 – 10,000 µg/L.

Description of Method: The method utilizes a water extraction, SPE cleanup, and LC-MS for determination of fluoroacetate as monofluoroacetate (MFA). SPE is performed using C_{18} cartridges. The LC-MS system utilizes a C_{18} column and the MS is operated in atmospheric pressurized chemical ionization (APCI) negative mode. If significant interferences are observed, the method describes a qualitative procedure that can be used to confirm the presence of fluoroacetate. The sample is first prepared as described in the quantitative method. Then an aliquot is derivatized by adding 2-nitrophenylhydrazine, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and pyridine buffer, and heating at 65 °C for 15 minutes. The extract is then cleaned by putting it through a C_{18} cartridge. The extracts are then blown to dryness, reconstituted in 2 mL of water/methanol (20/80), and filtered through a 0.2 µm filter. Analysis of the cleaned extract is performed on an LC-MS using a C8 column and gradient elution, beginning with 25% methanol for the first 3 minutes, followed by 80% methanol over the next 10 minutes. A post run equilibration (7 minutes) is used prior to the next injection.

Special Considerations: This procedure has been developed for food; modifications may be needed for application to environmental samples such as solid and wipe samples. In addition, the air filter extraction procedure (described in NIOSH Method S301-1) was not developed for the LC-MS-MS detector, and it may be necessary to alter the extraction method if interferences arising from the extraction are observed.

Source: Noonan, G.O., Begley, T.H. and Diachenko, G.W. 2007. "Rapid Quantitative and Qualitative Confirmatory Method for the Determination of Monofluoroacetic Acid in Foods by Liquid Chromatography–Mass Spectrometry." Journal of Chromatography A, 1139: 271–278. http://www.elsevier.com/locate/chroma

5.2.96 Literature Reference for 3-Chloro-1,2-propanediol (Journal of Chromatography A, 2000. 866: 65–77)

Analyte(s)	CAS RN
3-Chloro-1,2-propanediol	96-24-2

Analysis Purpose: Sample preparation, and analyte determination and measurement
Sample Preparation Technique: Solvent extraction followed by derivatization
Determinative Technique: GC-ECD

Method Developed for: Determination of 3-chloro-1,2-propanediol in water
Method Selected for: SAM lists this procedure for preparation and analysis of aqueous liquid and drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The MDL is 0.73 µg/L. The reporting range is 11 – 169 µg/L.

Description of Method: Sodium sulfate, sodium bisulfate and a surrogate are added to a 5-mL sample and extracted twice with 5 mL of ethyl acetate. The two ethyl acetate extracts are combined and concentrated to 50 µL under nitrogen evaporation. Then 100 µL of acetonitrile is added, and the solution is mixed and transferred to a drying column containing sodium sulfate. An additional 100 µL of acetonitrile is used to rinse the sample vial and the rinse is transferred to the drying column. After letting the sample sit on the column for 10 minutes, it is eluted with 2 mL of acetonitrile. The dried extract is derivatized by adding 50 µL of heptafluorobutyric anhydride (HFBA) and heating at 75 °C for 30 minutes. The derivatized sample is extracted with water, then hexane, followed by a saturated sodium bicarbonate solution. The aqueous layer is discarded, and the hexane layer is washed twice with sodium bicarbonate solution and shaking for 30 seconds. The hexane extract is then transferred to a GC vial and analyzed by GC-ECD with a DB5-MS column.

Special Considerations: The procedure has been tested for reagent grade water and seawater; modifications may be needed for application to environmental samples.

Source: Matthew, B.M. and Anastasio, C. 2000. "Determination of Halogenated Mono-alcohols and Diols in Water by Gas Chromatography With Electron-Capture Detection." Journal of Chromatography A, 866(1): 65–77. http://www.elsevier.com/locate/chroma

5.2.97 Literature Reference for Fluoroacetic Acid/Fluoroacetate Salts/Methyl Fluoroacetate (Journal of Chromatography B, 2010, 878: 1045–1050)

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

Analysis Purpose: Sample preparation (aqueous liquid and drinking water only), and analyte determination and measurement
Sample Preparation Technique: SPE using 96-well plates (see Special Considerations)
Determinative Technique: LC-MS-MS

Method Developed for: Determination of fluoroacetate in urine
Method Selected for: SAM lists this procedure for preparation and analysis of drinking water and aqueous liquid samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit for fluoroacetate in human urine is 0.9 µg/L. The reporting range is 50 to 5000 µg/L.

Description of Method: Fluoroacetate, in the form of monofluoroacetate, is extracted from urine by placing aliquots onto a 96-well plate and performing SPE using hydrophilic-lipophilic-balanced (HLB) reverse-phase sorbent plates (or equivalent). The extracts are then separated with isocratic high-performance liquid chromatography with a reverse-phase analytical column with embedded basic ion-pairing groups. Target compounds are identified using ESI tandem mass spectrometry. The retention time, when using the conditions described in this journal article, are expected to be ~1.4 minutes.

Special Considerations: This procedure has been developed for urine samples; modifications may be needed for application to environmental samples. For drinking water samples and relatively clean

aqueous liquid samples, direct injection may be suitable. If the laboratory has the capability of performing IC-MS, they may consider using the following method, which has been developed specifically for water samples: http://www.dionex.com/en-us/webdocs/110767-AN276-IC-MS-Flouroacetate-Water-AN70478_E.pdf

Source: Hamelin, E., Mawhinney, D.B., Parry R. and Kobelski, R.J. 2010. "Quantification of Monofluoroacetate and Monochloroacetate in Human Urine by Isotope Dilution Liquid Chromatography Tandem Mass Spectrometry." Journal of Chromatography B, 878(15-16): 1045–1050. http://www.elsevier.com/locate/chromb

5.2.98 Literature Reference for Fluoroacetamide (Journal of Chromatography B, 2008. 876(1): 103–108)

Analyte(s)	CAS RN
Fluoroacetamide	640-19-7

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

Method Developed for: Fluoroacetamide and tetramine in blood, urine and stomach contents
Method Selected for: SAM lists this procedure for preparation and analysis of solid, aqueous liquid, drinking water, air and wipe samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: The detection limit of this method for fluoroacetamide is 0.01 µg/g.

Description of Method: Samples are extracted by microscale liquid-liquid extraction using acetonitrile, ENVI-Carb™ and sodium chloride. Samples are analyzed by GC-MS using a 30-m DB-5MS capillary column (or equivalent) coupled with a 1.5 m Innowax capillary column (or equivalent) by a quartz capillary column connector. If analyzing for fluoroacetamide alone, only the Innowax capillary column is needed.

Special Considerations: The procedure has been developed for the analysis of fluoroacetamide and tetramine in blood, urine and stomach fluid samples; modifications will be needed for application to environmental samples.

Source: Xu, X., Song, G., Zhu, Y., Zhang, J., Zhao, Y., Shen, H., Cai, Z., Han, J. and Ren, Y. 2008. "Simultaneous Determination of Two Acute Poisoning Rodenticides Tetramine and Fluoroacetamide With a Coupled Column in Poisoning Cases." Journal of Chromatography B. 876(1): 103–108. http://www.sciencedirect.com/science/article/pii/S1570023208007757

5.2.99 Literature Reference for Sodium Azide (Journal of Forensic Sciences, 1998. 43(1): 200–202)

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

Analysis Purpose: Sample preparation
Sample Preparation Technique: Water extraction, filtration and/or acidification
Determinative Technique: IC with conductivity detection
Determinative Method: EPA Method 300.1, Revision 1.0

Method Developed for: Sodium azide in blood
Method Selected for: SAM lists this procedure for preparation of solid, aqueous liquid and drinking water samples. See Appendix A for corresponding method usability tiers.
Detection and Quantitation: This method can routinely quantify to at least 100 µg/L, and the detection limit is estimated to be 30 µg/L.

Description of Method: Samples are analyzed by IC using suppressed conductivity detection. Water extraction and filtration steps should be used for the preparation of solid samples. Filtration steps should be used for preparation of aqueous liquid and drinking water samples.

Special Considerations: The procedure described above has been developed for the analysis of sodium azide in blood samples.

Source: Kruszyna, R., Smith, R.P. and Kruszyna, H. 1998. "Determining Sodium Azide Concentration in the Blood by Ion Chromatography." Journal of Forensic Sciences. 43(1): 200–202.
http://www.astm.org/DIGITAL_LIBRARY/JOURNALS/FORENSIC/PAGES/JFS16113J.htm

Section 6.0: Selected Radiochemical Methods

A list of analytical methods to be used in analyzing environmental samples for radiochemical contaminants following a contamination incident is provided in Appendix B. Methods are listed for each isotope and for each sample type that potentially may need to be measured and analyzed when responding to an environmental emergency.

Please note: This section provides guidance for selecting radiochemical methods that have a high likelihood of assuring analytical consistency when laboratories are faced with a large scale environmental restoration crisis. Not all methods have been verified for the analyte/sample type combination listed in Appendix B. Please refer to the specified method to identify analyte/sample type combinations that have been verified. Any questions regarding information discussed in this section should be addressed to the appropriate contact(s) listed in Section 4.

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

- **Analyte(s).** The radionuclide(s) or contaminant(s) of interest.
- **Chemical Abstracts Service Registry Number (CAS RN).** A unique identifier for chemical substances that provides an unambiguous way to identify a chemical or molecular structure when there are many possible systematic, generic or trivial names. In this section (Section 6.0) and Appendix B, the CAS RNs correspond to the specific radionuclide identified.
- **Determinative technique.** An analytical instrument or technique used for qualitative and confirmatory determination of compounds or components in a sample.
- **Drinking water sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in drinking water samples. Methods have been identified for qualitative and confirmatory determination.
- **Aqueous and liquid phase sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in aqueous and/or non-aqueous liquid phase samples. Methods have been identified for qualitative and confirmatory determination.
- **Soil and sediment phase sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in soil and sediment samples. Methods have been identified for qualitative and confirmatory determination.
- **Surface wipe sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in surface wipe samples. Methods have been identified for qualitative and confirmatory determination.
- **Air filter sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in air filter samples. Methods have been identified for qualitative and confirmatory determination.
- **Vegetation sample methods.** The recommended methods/procedures for sample preparation and analysis to measure the analyte of interest in vegetation (i.e., grasses, leaves, trees, etc.) not intended for human consumption. Methods have been identified for qualitative and confirmatory determination.
- **Qualitative determination method identifier.** A unique identifier or number assigned to an analytical method by the method publisher. The identified method is intended to determine the presence of a radionuclide. These methods are less precise than confirmatory methods, and are used when greater sample throughput and more rapid reporting of results is required.
- **Confirmatory method identifier.** A unique identifier or number assigned to an analytical method by the method publisher. The identified method is for measurement of the activity from a particular radionuclide per unit of mass, volume or area sampled.

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

6.1 General Guidelines

The guidelines summarized in this section provide a general overview of how to identify the appropriate radiochemical method(s) for a given analyte-sample type combination, as well as recommendations for quality control (QC) procedures.

For additional information on the properties of the radionuclides listed in Appendix B, Toxicology Data Network (TOXNET) (http://toxnet.nlm.nih.gov/index.html), a cluster of databases on toxicology, hazardous chemicals, and related areas maintained by the National Library of Medicine, is an excellent resource. EPA's Radiation Protection Program (http://www.epa.gov/radiation/radionuclides/index.html) and the *Multi-Agency Radiological Laboratory Analytical Protocols Manual* (MARLAP) (http://www.epa.gov/radiation/marlap/manual.html) websites provide some additional information pertaining to radionuclides of interest and selection of radiochemical methods. Documents for emergency response operations for laboratories, recently developed by EPA's Office of Radiation and Indoor Air (ORIA), describe the likely analytical decision paths that would be required by personnel at a radioanalytical laboratory following a radiological or nuclear contamination incident. These documents may be found at http://www.epa.gov/narel/incident_guides.html.

6.1.1 Standard Operating Procedures for Identifying Radiochemical Methods

To determine the appropriate method to be used on an environmental sample, locate the analyte of concern in Appendix B: Selected Radiochemical Methods under the "Analyte Class" or "Analyte(s)" column. After locating the analyte of concern, continue across the table to identify the appropriate determinative technique (e.g., alpha spectrometry), then identify the appropriate qualitative and/or confirmatory method for the sample type of interest (drinking water, aqueous and liquid phase, soil and sediment, surface wipes and air filters) for the particular analyte.

Once a method has been identified in Appendix B, **Table 6-1** can be used to locate the method summary. Sections 6.2.1 through 6.2.36, below, provide summaries of the qualitative and confirmatory methods listed in Appendix B.

Table 6-1. Radiochemical Methods and Corresponding Section Numbers

Analyte / Analyte Class	CAS RN	Method	Section
Gross Alpha	NA	900.0 (EPA)	6.2.2
		FRMAC, Vol 2, pg. 33 (DOE)	6.2.30
Gross Beta	NA	AP1 (ORISE)	6.2.34
		7110 B (SM)	6.2.43
Gamma Select Mixed Fission Products*	NA	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.22
Total Activity Screening	NA	Y-12 Preparation of Samples for Total Activity Screening (DoD)	6.2.49

* Please note that this category does not cover all fission products.

Analyte / Analyte Class	CAS RN	Method	Section
Americium-241	14596-10-2	Rapid Radiochemical Method for Am-241 (EPA)	6.2.12
		Am-01-RC (HASL-300)	6.2.19
		Am-04-RC (HASL-300)	6.2.20
		Am-06-RC (HASL-300)	6.2.21
		Pu-12-RC (HASL-300)	6.2.24
		Actinides and Sr-89/90 in Soil Samples (SRS)	6.2. 32
		Actinides and Sr-89/90 in Vegetation (SRS)	6.2. 33
		AP11 (ORISE)	6.2.37
		D3084-05 (ASTM)	6.2.39
		7120 (SM)	6.2.44
		Rapid methods for acid or fusion digestion (EPA)	6.2.17 and 6.2.18
Californium-252	13981-17-4	Am-01-RC (HASL-300)	6.2.19
		Am-04-RC (HASL-300)	6.2.20
		Pu-12-RC (HASL-300)	6.2.24
		AP11 (ORISE)	6.2.37
		D3084-05 (ASTM)	6.2.39
Cesium-137	10045-97-3	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.22
Cobalt-60	10198-40-0	7120 (SM)	6.2.44
Curium-244	13981-15-2	Am-01-RC (HASL-300)	6.2.19
		Am-04-RC (HASL-300)	6.2.20
		Pu-12-RC (HASL-300)	6.2.24
		AP11 (ORISE)	6.2.37
		D3084-05 (ASTM)	6.2.39
Europium-154	15585-10-1	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.22
		7120 (SM)	6.2.44
Iodine-125	14158-31-7	Procedure #9 (ORISE)	6.2.38
Iodine-131	10043-66-0	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.22
Iridium-192	14694-69-0	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.22
		7120 (SM)	6.2.44
Molybdenum-99	14119-15-4	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.22
Phosphorus-32	14596-37-3	Rapid Radiochemical Method for P-32 (EPA)	6.2.9
		R4-73-014 (EPA)	6.2.10
		RESL P-2 (DOE)	6.2.31

Analyte / Analyte Class	CAS RN	Method	Section
Plutonium-238 Plutonium-239	13981-16-3 15117-48-3	EMSL-33 (EPA)	6.2.8
		Rapid Radiochemical Method for Pu-238 and -239/240 (EPA)	6.2. 13
		Actinides and Sr-89/90 in Soil Samples (SRS)	6.2.29
		Actinides and Sr-89/90 in Vegetation (SRS)	6.2. 33
		AP11 (ORISE)	6.2.37
		D3084-05 (ASTM)	6.2.39
		Rapid methods for acid or fusion digestion (EPA)	6.2.17 and 6.2.18
Polonium-210	13981-52-7	Method 111 (EPA)	6.2.1
		Po-02-RC (HASL-300)	6.2.23
Radium-226	13982-63-3	903.1 (EPA)	6.2.4
		EMSL-19 (EPA)	6.2.7
		Rapid Radiochemical Method for Ra-226 (EPA)	6.2. 14
		Ra-03-RC (HASL-300)	6.2.25
		D3084-05 (ASTM)	6.2. 39
		7500-Ra B (SM)	6.2.45
		7500-Ra C (SM)	6.2.46
		Rapid methods for acid or fusion digestion (EPA)	6.2.17 and 6.2.18
Ruthenium-103 Ruthenium-106 Selenium-75	13968-53-1 13967-48-1 14265-71-5	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.22
		7120 (SM)	6.2.44
Strontium-89	14158-27-1	905.0 (EPA)	6.2.5
		Strontium in Food and Bioenvironmental Samples	6.2.11
		Actinides and Sr-89/90 in Soil Samples (SRS)	6.2.32
		Actinides and Sr-89/90 in Vegetation (SRS)	6.2.33
Strontium-90	10098-97-2	905.0 (EPA)	6.2.5
		Rapid Radiochemical Method for Radiostrontium (EPA)	6.2.15
		Sr-03-RC (HASL-300)	6.2.26
		Actinides and Sr-89/90 in Soil Samples (SRS)	6.2. 32
		Actinides and Sr-89/90 in Vegetation (SRS)	6.2. 33
		D5811-08 (ASTM)	6.2.41
		Rapid methods for acid or fusion digestion (EPA)	6.2.17 and 6.2.18
Technetium-99	14133-76-7	Tc-01-RC (HASL-300)	6.2.27
		Tc-02-RC (HASL-300)	6.2.28
		AP5 (ORISE)	6.2.36
		D7168-05 (ASTM	6.2.42
Tritium (Hydrogen-3)	10028-17-8	906.0 (EPA)	6.2.6
		AP2 (ORISE)	6.2.35

Analyte / Analyte Class	CAS RN	Method	Section
Uranium-234 Uranium-235 Uranium-238	13966-29-5 15117-96-1 7440-61-1	EMSL-33 (EPA)	6.2.8
		Rapid Radiochemical Method for Isotopic Uranium (EPA)	6.2. 16
		U-02-RC (HASL-300)	6.2. 29
		Actinides and Sr-89/90 in Soil Samples (SRS)	6.2. 32
		Actinides and Sr-89/90 in Vegetation(SRS)	6.2. 33
		AP11 (ORISE)	6.2.37
		D3972-02 (ASTM)	6.2.40
		7500-U B (SM)	6.2.47
		7500-U C (SM)	6.2.48
		Rapid methods for acid or fusion digestion (EPA)	6.2.17 and 6.2.18

The method summaries are listed in order of method selection hierarchy (see Figure 2-1), starting with EPA methods, followed by methods from other federal agencies and voluntary consensus standard bodies (VCSBs). Methods are listed in numerical order under each publisher. Where available, a direct link to the full text of the selected analytical method is provided in the method summary. For additional information regarding sample preparation and analysis procedures and on methods available through consensus standards organizations, please use the contact information provided in **Table 6-2**.

Table 6-2 Sources of Radiochemical Methods

Name	Publisher	Reference
National Environmental Methods Index (NEMI)	EPA, U.S. Geological Survey (USGS)	http://www.nemi.gov
Code of Federal Regulations (CFR) Promulgated Test Methods	EPA, Technical Transfer Network (TTN) Emission Measurement Center (EMC)	http://www.epa.gov/ttn/emc/promgate.html
Prescribed Procedures for Measurement of Radioactivity in Drinking Water (EPA-600 4-80-032, August 1980)	EPA, ORD, Environmental Monitoring and Support Laboratory (EMSL)	http://www.sld.state.nm.us/Documents/forewd.pdf Also available from National Technical Information Service (NTIS)*, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, (703) 605-6000.
Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events (EPA 402-R-10-001)	EPA, ORIA, National Air and Radiation Environmental Laboratory (NAREL)	http://www.epa.gov/narel/
Rapid Radiochemical Method for Phosphorus-32 in Water	EPA, ORIA, NAREL	When published, method will be available at: http://www.epa.gov/narel/new_docs.html
Rapid Radiochemical Methods for acid digestion and sodium carbonate fusion of filters and swipes	EPA, ORIA, NAREL	When published, methods will be available at: http://www.epa.gov/narel/new_docs.html

Name	Publisher	Reference
Radiochemical Analytical Procedures for Analysis of Environmental Samples, March 1979. EMSL-LV-0539-17	EPA, EMSL	Available NTIS*, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, (703) 605-6000.
EML Procedures Manual, Health and Safety Laboratory (HASL-300), 28th Edition, February, 1997	Department of Energy (DOE), Environmental Measurements Laboratory (EML) / Now DHS	http://www.wipp.energy.gov/NAMP/EMLLegacy/ Also available from NTIS*, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, (703) 605-6000.
Federal Radiological Monitoring and Assessment Center (FRMAC) Laboratory Manual	DOE, National Nuclear Security Administration (NNSA)	http://www.nv.doe.gov/nationalsecurity/homelandsecurity/frmac/manuals.aspx
Y-12 National Security Complex (Y-12)	DOE, NNSA	http://www.y12.doe.gov/
Radiological and Environmental Sciences Laboratory (RESL) Analytical Chemistry Branch Procedures Manual	DOE, RESL	Available from NTIS, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161, (703) 605-6000.
Savannah River Site (SRS) Methods	DOE, SRS	Savannah River National Laboratory Savannah River Site Aiken, SC 29808, (803) 725-6211
Oak Ridge Institute for Science and Education (ORISE) Laboratory Procedures Manual	ORISE, Independent Environmental Assessment and Verification	http://orise.orau.gov/environmental-assessments-health-physics/capabilities/independent-verification.aspx
Annual Book of ASTM Standards, Vol. 11.02*	ASTM International	http://www.astm.org
Standard Methods for the Examination of Water and Wastewater, 21st Edition, 2005*	American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF)	http://www.standardmethods.org

* Subscription and/or purchase required.

6.1.2 General QC Guidelines for Radiochemical Methods

Having data of known and documented quality is critical so that public officials can accurately assess the activities that may be needed in remediating a site and determine effectiveness following remediation.[5] Having such data requires that laboratories: (1) conduct the necessary QC to ensure that measurement systems are in control and operating correctly; (2) properly document results of the analyses; and (3) properly document measurement system evaluation of the analysis-specific QC. Ensuring data quality also requires that laboratory results are properly evaluated and the results of the data quality evaluation are included within the data report when transmitted to decision makers.

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

The level or amount of QC needed often depends on the intended purpose of the data that are generated. Various levels of QC may be required if the data are generated during contaminant presence/absence qualitative determinations versus confirmatory analyses. The specific needs for data generation should be identified. QC requirements and data quality objectives (DQOs) should be derived based on those needs, and should be applied consistently across laboratories when multiple laboratories are used. For example, during rapid sample screening analyses, minimal QC samples (e.g., blanks, duplicates) and documentation might be needed to ensure data quality. Implementation of the analytical methods for evaluation of environmental samples during site assessment through site clearance, such as those identified in this document, might require increased QC frequency.

Some method-specific QC requirements are described in many of the individual methods that are cited in this manual. QC requirements will be referenced in analytical protocols developed to address specific analytes and sample types of concern. Additional information regarding QC requirements specific to radiochemical methods is included in the MARLAP manual at: http://www.epa.gov/radiation/marlap/manual.html. Individual methods, sampling and analysis protocols or contractual statements of work should also be consulted to determine any additional QC that may be needed.

QC samples are required to assess the precision, bias and reliability of sample results. All QC results are tracked on control charts and reviewed for acceptability and trends in analysis or instrument operation. QC parameters are measured as required per method at the prescribed frequency. QC of laboratory analyses using radiochemical methods includes ongoing analysis of QC samples and tracking QC parameters including, but not limited to the following:

- Method blanks
- Calibration checks
- Sample and sample duplicates
- Laboratory control sample recoveries
- Matrix spike/matrix spike duplicate (MS/MSD) recoveries and precision
- Tracer and/or carrier yield

Please note: The appropriate point of contact identified in Section 4 should be consulted regarding appropriate QA/QC procedures prior to sample analysis. These contacts will consult with the EPA Environmental Response Laboratory Network (ERLN) or Water Laboratory Alliance (WLA) coordinator responsible for laboratory activities during the specific event to ensure QA/QC procedures are performed consistently across laboratories. EPA program offices will be responsible for ensuring that the QA/QC practices are implemented.

6.1.3 Safety and Waste Management

It is imperative that safety precautions be used during collection, processing and analysis of environmental samples. Laboratories should have a documented radiation safety plan or manual in addition to a health and safety plan for handling samples that may contain target chemical, biological and/or radiological (CBR) contaminants, and laboratory staff should be trained in and implement the safety procedures in the plan or manual. In addition, many of the methods summarized or cited in Section 6.2 contain specific requirements, guidelines or information regarding safety precautions that should be followed when handling or processing environmental samples and reagents. These methods may also provide information regarding waste management. Laboratories should consult with the responsible government agencies prior to disposal of waste materials. Other resources that can be consulted for additional information include the following:

- Occupational Safety and Health Administration (OSHA) - 29 CFR part 1910.1450. Occupational Exposure to Hazardous Chemicals in Laboratories. Available at: http://www.access.gpo.gov/nara/cfr/waisidx_06/29cfr1910a_06.html
- EPA - 40 CFR part 260. Hazardous Waste Management System: General. Available at: http://www.access.gpo.gov/nara/cfr/waisidx_07/40cfr260_07.html
- EPA - 40 CFR part 270. EPA Administered Permit Programs: The Hazardous Waste Permit Program. Available at: http://www.access.gpo.gov/nara/cfr/waisidx_07/40cfr270_07.html
- U.S. Nuclear Regulatory Commission (NRC) - 10 CFR part 20. Standards for Protection Against Radiation. Available at: http://www.access.gpo.gov/nara/cfr/waisidx_00/10cfr20_00.html
- DOE. Order O 435.1: Radioactive Waste Management. July 1, 1999. Available at: www.directives.doe.gov/pdfs/doe/doetext/neword/435/o4351.html
- DOE. M 435.1-1. *Radioactive Waste Management Manual*. Office of Environmental Management. July 9, 1999. Available at: http://www.directives.doe.gov/pdfs/doe/doetext/neword/435/m4351-1.html
- DOE. *Compendium of EPA-Approved Analytical Methods for Measuring Radionuclides in Drinking Water*. Prepared by the Office of Environmental Policy and Assistance Air, Water and Radiation Division (EH-412). June 1998. Available at: http://www.orau.org/ptp/PTP%20Library/library/DOE/Misc/radmeth3.pdf
- EPA. 1996. *Profile and Management Options for EPA Laboratory Generated Mixed Waste*. ORIA, Washington, DC. EPA 402-R-96-015. Available at: http://www.epa.gov/rpdweb00/docs/mixed-waste/402-r-96-015.pdf
- EPA. 2001. Changes to 40 CFR 266 (Storage, Treatment, Transportation, and Disposal of Mixed Waste), *Federal Register* 66:27217-27266, May 16. Available at: http://frwebgate.access.gpo.gov/cgi-bin/getdoc.cgi?dbname=2001_register&docid=01-11408-filed.pdf
- EPA. 2008. *Resource Conservation and Recovery Act (RCRA) Orientation Manual.* Office of Solid Waste and Emergency Response (OSWER), Washington, DC. EPA530-R-02-016. 259 pp. Available at: http://www.epa.gov/osw/inforesources/pubs/orientat/
- MARLAP Manual. 2004. Chapter 17. Waste Management in a Radioanalytical Laboratory. EPA 402-B-04-001B. Available at: http://www.epa.gov/rpdweb00/docs/marlap/402-b-04-001b-17-final.pdf
- National Research Council. 1995. *Prudent Practices in the Laboratory; Handling and Disposal of Chemicals,* National Academy Press, Washington, DC. Available at: http://books.nap.edu/openbook.php?isbn=0309052297
- National Council on Radiation Protection and Measurements (NCRP). 2002. *Risk-Based Classification of Radioactive and Hazardous Chemical Wastes*, Report Number 139. 7910 Woodmont Avenue, Suite 400, Bethesda, MD 20814–3095
- NRC / EPA. 1995. Joint Nuclear Regulatory Commission/Environmental Protection Agency Guidance on the Storage of Mixed Radioactive and Hazardous Waste. *Federal Register* 60:40204-40211

6.2 Method Summaries

Summaries for the analytical methods listed in Appendix B are provided in Sections 6.2.1 through 6.2.49. These summaries contain information that has been extracted from the selected methods. Each method summary contains a table identifying the contaminants in Appendix B to which the method applies, a brief description of the analytical method, and a link to the full version of the method or a source for obtaining a full version of the method. Summaries are provided for informational use. The full version of the method should be consulted prior to sample analysis.

6.2.1 EPA Method 111: Determination of Polonium-210 Emissions from Stationary Sources

Analyte(s)	CAS RN
Polonium-210	13981-52-7

Analysis Purpose: Qualitative and confirmatory determination
Technique: Alpha spectrometry

Method Developed for: Polonium-210 in particulate matter samples collected from stationary source exhaust stacks
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of surface wipes and air filters.

Description of Method: This method covers the determination of polonium-210 in particulate matter samples collected from stationary sources such as exhaust stacks. Polonium-210 in the sample is put in solution, deposited on a metal disc, and the radioactive disintegration rate measured. Polonium in acid solution spontaneously deposits on surface metals that are more electropositive than polonium. Polonium-209 tracers should be added to determine the chemical yield.

Source: EPA EMC, prepared by the Office of Air Quality Planning and Standards (OAQPS). 2000. "Method 111: Determination of Polonium-210 Emissions from Stationary Sources." http://www2.epa.gov/sites/production/files/2015-06/documents/epa-111.pdf

6.2.2 EPA Method 900.0: Gross Alpha and Gross Beta Radioactivity in Drinking Water

Analysis Purpose: Gross alpha and gross beta determination
Technique: Alpha/Beta counting

Method Developed for: Gross alpha and gross beta particle activities in drinking water
Method Selected for: SAM lists this method for gross alpha and gross beta determination in drinking water samples.

Description of Method: The method provides an indication of the presence of alpha and beta emitters, including the following SAM analytes:

- Americium-241 (CAS RN 14596-10-2) Alpha emitter
- Californium-252 (CAS RN 13981-17-4) Alpha emitter
- Cesium-137 (CAS RN 10045-97-3) Beta emitter
- Cobalt-60 (CAS RN 10198-40-0) Beta emitter
- Curium-244 (CAS RN 13981-15-2) Alpha emitter
- Europium-154 (CAS RN 15585-10-1) Beta emitter
- Iridium-192 (CAS RN 14694-69-0) Beta emitter

- Plutonium-238 (CAS RN 13981-16-3) Alpha emitter
- Plutonium-239 (CAS RN 15117-48-3) Alpha emitter
- Polonium-210 (CAS RN 13981-52-7) Alpha emitter
- Radium-226 (CAS RN 13982-63-3) Alpha emitter
- Ruthenium-103 (CAS RN 13968-53-1) Beta emitter
- Ruthenium-106 (CAS RN 13967-48-1) Beta emitter
- Strontium-90 (CAS RN 10098-97-2) Beta emitter
- Uranium-234 (CAS RN 13966-29-5) Alpha emitter
- Uranium-235 (CAS RN 15117-96-1) Alpha emitter
- Uranium-238 (CAS RN 7440-16-1) Alpha emitter

An aliquot of a preserved drinking water sample is evaporated to a small volume (3 to 5 mL) and transferred quantitatively to a tarred 2-inch planchet. The aliquot volume is determined based on a maximum total solids content of 100 mg. The sample aliquot is evaporated to dryness in the planchet to a constant weight, cooled, and counted using a gas proportional or scintillation counting system. The counting system is calibrated with thorium-230 for gross alpha, and with strontium-90 for gross beta analysis.[6] A traceable standards-based efficiency curve must be developed for each calibration nuclide (thorium-230 and strontium-90) based on a range of total solids content in the 2-inch planchet from 0 to 100 mg (see method for specific recommendations and requirements for the use of cesium-137).

Special Considerations: Long counting time and increased sample size may be required to meet detection limits. Sensitivity is limited by the concentration of solids in the sample. The method provides an overall measure of alpha and beta activity, including activity for the radionuclides listed above, but does not permit the specific identification of any alpha or beta emitting radionuclides.

Source: EPA, EMSL. 1980. "Method 900.0: Gross Alpha and Gross Beta Radioactivity in Drinking Water." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*, EPA/600/4/80/032. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-900.0.pdf

6.2.3 EPA Method 901.1: Gamma Emitting Radionuclides in Drinking Water

Analyte(s)	CAS RN
Americium-241	14596-10-2
Cesium-137	10045-97-3
Cobalt-60	10198-40-0
Europium-154	15585-10-1
Iodine-131	10043-66-0
Iridium-192	14694-69-0
Molybdenum-99	14119-15-4
Ruthenium-103	13968-53-1
Ruthenium-106	13967-48-1
Selenium-75	14265-71-5
Select Mixed Fission Products	NA

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Gamma spectrometry

Method Developed for: Gamma emitting radionuclides in drinking water
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of select gamma emitters in drinking water samples.

[6] EPA lists standards for use when analyzing drinking water in the table at 40 CFR 141.25 (Footnote 11).

Description of Method: This method is applicable for analysis of water samples that contain radionuclides that emit gamma photons with energies ranging from approximately 60 to 2000 keV. The method uses gamma spectroscopy for measurement of gamma photons emitted from radionuclides without separating them from the sample matrix. A homogeneous aliquot of water is placed into a standard geometry (normally a Marinelli beaker) for gamma counting, typically using a high purity germanium detector. Detectors such as Germanium (Lithium) or thallium-activated sodium iodide also can be used. Sample aliquots are counted long enough to meet the required sensitivity of measurement. To reduce adsorbance of radionuclides on the walls of the counting container, the sample is acidified at collection time. Due to its lower resolution, significant interference can occur using the thallium-activated sodium iodide detector when counting a sample containing radionuclides that emit gamma photons of similar energies. When using this method, shielding is needed to reduce background interference. Detection limits are, in general, dependent on analyte radionuclide gamma-ray abundance, sample volume, geometry (physical shape) and counting time.

Source: EPA, EMSL. 1980. "Method 901.1: Gamma Emitting Radionuclides in Drinking Water." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*, EPA/600/4/80/032. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-901.1.pdf

6.2.4 EPA Method 903.1: Radium-226 in Drinking Water – Radon Emanation Technique

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha counting

Method Developed for: Radium-226 in drinking water
Method Selected for: SAM lists this method for confirmatory analysis of drinking water samples.

Description of Method: This method is specific for radium-226, and is based on the emanation and scintillation counting of radon-222, the immediate decay product of radium-226. Radium-226 is concentrated and separated from the water sample by coprecipitation on barium sulfate. The precipitate is dissolved in ethylenediamine tetraacetic acid (EDTA) reagent, placed in a sealed bubbler and stored for ingrowth of radon-222. After ingrowth, the radon-222 gas is purged into a scintillation cell. When the short-lived radon-222 daughters are in equilibrium with the parent (after ~4h), the scintillation cell is counted for activity. The absolute measurement of radium-226 is effected by calibrating the scintillation cell system with a standard solution of the nuclide. There are no radioactive interferences in this method. Based on a 1000-mL sample and 100-minute counting time, the minimum detectable level for this method is 0.5 pCi/L.

Source: EPA, EMSL. 1980. "Method 903.1: Radium-226 in Drinking Water – Radon Emanation Technique." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*, EPA/600/4/80/032. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-903.1.pdf

6.2.5 EPA Method 905.0: Radioactive Strontium in Drinking Water

Analyte(s)	CAS RN
Strontium-89	14158-27-1
Strontium-90	10098-97-2

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Beta counting

Method Developed for: Strontium-89, strontium-90 and total strontium in drinking water
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of aqueous/liquid and drinking water samples for strontium-89 and confirmatory analysis of drinking water samples for strontium-90.

Description of Method: Stable strontium carrier is added to the water sample. Both strontium-89 and strontium-90 are precipitated from the solution as insoluble carbonates. Interferences from calcium and from some radionuclides are removed by one or more precipitations of the strontium carrier as strontium nitrate. Barium and radium are removed by precipitation as chromates. The yttrium-90 decay product of strontium-90 is removed by a hydroxide precipitation step. The separated strontium-89 and strontium-90 are precipitated as carbonates, weighed for determination of the chemical recovery, and counted for beta particle activity. The counting result, ascertained immediately after separation, represents the total strontium activity (strontium-89 and strontium-90) plus an insignificant fraction of the yttrium-90 that has grown into the separated strontium-90. The yttrium-90 decay product is allowed to in-grow for approximately two weeks and then is separated with stable yttrium carrier as hydroxide and finally precipitated as the oxalate, weighed for chemical recovery, and mounted for beta counting. The strontium-90 concentration is determined from the yttrium-90 activity; strontium-89 concentration is determined from the difference.

Source: EPA, EMSL. 1980. "Method 905.0: Radioactive Strontium in Drinking Water, *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*, EPA/600/4/80/032.
http://www2.epa.gov/sites/production/files/2015-06/documents/epa-905.0.pdf

6.2.6 EPA Method 906.0: Tritium in Drinking Water

Analyte(s)	CAS RN
Tritium (Hydrogen-3)	10028-17-8

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Tritium (as T_2O or HTO) in drinking water
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of drinking water and aqueous/liquid phase samples.

Description of Method: An unpreserved 100-mL aliquot of a drinking water sample is distilled after adjusting pH with a small amount of sodium hydroxide and adding potassium permanganate. The alkaline treatment prevents other radionuclides, such as radioiodine and radiocarbon, from distilling with the tritium. The permanganate treatment oxidizes trace organics that may be present in the sample and prevents their appearance in the distillate. To determine the concentration of tritium, the middle fraction of the distillate is used, because the early and late fractions are more apt to contain materials interfering with the liquid scintillation counting process. A portion of this collected fraction is added to a liquid scintillator cocktail, and the solution is mixed, dark adapted and counted for beta particle activity. The efficiency of the system can be determined by the use of prepared tritiated water (HTO) standards having the same density and color as the sample.

Source: EPA, EMSL. 1980. "Method 906.0: Tritium in Drinking Water." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*, EPA/600/4/80/032.
http://www2.epa.gov/sites/production/files/2015-06/documents/epa-906.0.pdf

6.2.7 EPA Method EMSL-19: Determination of Radium-226 and Radium-228 in Water, Soil, Air and Biological Tissue

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha counting

Method Developed for: Radium-226 and radium-228 in water, soil, air, biological tissues and biological fluids
Method Selected for: SAM lists this method for confirmatory analysis of soil/sediment, surface wipe and air filter samples.

Description of Method: Following acid digestion and filtration of soil, sediment, surface wipe or air filter samples, radium is precipitated with barium sulfate. Barium-radium-sulfate is dissolved in a pentasodium diethylenetriamine-pentaacetate (DTPA) solution and transferred to an emanation tube. The radon is allowed to come to equilibrium for approximately 30 days. Radium-226 decays by alpha emission to radon-222. Radon-222 is separated and collected from the liquid by a de-emanation technique. The radon is counted by alpha scintillation 4.5 hours after de-emanation, at which time the short-lived progeny have reached 97% of equilibrium. An applicable measurement range has not been determined; however, samples that contain 0.1 pCi of Radium-226 have been analyzed.

Source: EPA, EMSL. 1979. "EMSL-19: Determination of Radium-226 and Radium-228 in Water, Soil, Air and Biological Tissue." *Radiochemical Analytical Procedures for Analysis of Environmental Samples*. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-emsl-19.pdf

6.2.8 EPA Method EMSL-33: Isotopic Determination of Plutonium, Uranium, and Thorium in Water, Soil, Air, and Biological Tissue

Analyte(s)	CAS RN
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Isotopic plutonium, uranium and thorium, together or individually, in soil, water, air filters, urine or ashed residues of vegetation, animal tissues and bone
Method Selected for: SAM lists this method for confirmatory analysis of drinking water, aqueous/liquid, soil/sediment, surface wipe and/or air filter samples.

Description of Method: This method is appropriate for the analysis of isotopic plutonium, uranium and thorium, together or individually, by alpha spectrometry. Plutonium-236, uranium-232 and thorium-234 tracer standards are added for the determination of chemical yields. Samples are decomposed by nitric-hydrofluoric acid digestion or ignition to assure that all of the plutonium is dissolved and chemically separated from the sample by coprecipitation with sodium and ammonium hydroxide, anion exchange and electrodeposition. The residues are dissolved in dilute nitric acid and successive sodium and ammonium hydroxide precipitations are performed in the presence of boric acid to remove fluoride and soluble salts.

The hydroxide precipitate is dissolved, the solution is pH-adjusted with hydrochloric acid, and plutonium and uranium are adsorbed on an anion exchange column, separating them from thorium. Plutonium is eluted with hydrobromic acid. The actinides are electrodeposited on stainless steel discs from an ammonium sulfate solution and subsequently counted by alpha spectrometry. This method is designed to detect environmental levels of activity as low as 0.02 pCi per sample. To avoid possible cross-contamination, sample aliquot activities should be limited to 25 pCi or less.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11.

Source: EPA, EMSL. 1979. "EMSL-33: Isotopic Determination of Plutonium, Uranium, and Thorium in Water, Soil, Air, and Biological Tissue." *Radiochemical Analytical Procedures for Analysis of Environmental Samples*. http://www2.epa.gov/sites/production/files/2015-06/documents/epa-emsl-33.pdf

6.2.9 EPA Method Rapid Radiochemical Method for Phosphorus-32 in Water for Environmental Restoration Following Homeland Security Events

Analyte(s)	CAS RN
Phosphorus-32	14596-37-3

Analysis Purpose: Qualitative analysis
Technique: Liquid scintillation

Method Developed for: Phosporus-32 in water
Method Selected for: SAM lists this method for qualitative analysis of drinking water samples.

Description of Method: A 100-mL water sample is filtered and phosphate carrier is added to the filtered sample. The solution is then passed through a cation exchange resin, followed by a Diphonix® resin, to remove interferences from cation radionuclides. The eluent is treated with a mixture of 10 mL of 30 % hydrogen peroxide and 10 mL of concentrated nitric acid, reduced to approximately 2 – 5 mL by heating, and quantitatively transferred to a liquid scintillation vial for counting. The Čerenkov photons from the P-32 beta (1710 keV, E_{max}) decay are detected using a calibrated liquid scintillation counter (LSC). Following counting of the sample, an aliquot of the final solution is used for yield determination by the inductively coupled plasma-atomic emission spectrometry (ICP-AES) method.

Special Considerations: SAM lists this method for rapid qualitative screening of drinking water samples. The method is not intended for use in compliance monitoring of drinking water.

Source: EPA, Office of Radiation and Indoor Air, National Air and Radiation Environmental Laboratory (NAREL). "Rapid Radiochemical Method for Phosphorus-32 in Water for Environmental Restoration Following Homeland Security Events." *Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events*. When published, this method will be posted at http://www2.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides.

6.2.10 EPA Method R4-73-014: Radioactive Phosphorus

Analyte(s)	CAS RN
Phosphorus-32	14596-37-3

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Low background alpha/beta counter

Method Developed for: Phosporus-32 in nuclear reactor solutions
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of water samples.

Description of Method: 200 mL or less of a water sample is acidified with nitric acid and carriers of phosphorus (standardized), cobalt, zirconium, silver and manganese are added. Hydroxides are precipitated by the addition of hydrogen peroxide and potassium hydroxide, and the hot solution is filtered through filter paper. Carriers of cobalt and zirconium are added to the filtrate, and the hydroxides are precipitated by the addition of hydrogen peroxide and potassium hydroxide. The solution is filtered and the hydroxides are discarded. The filtrate is acidified with hydrochloric acid, and phosphorous is precipitated as magnesium ammonium phosphate by the addition of a magnesium mixture and ammonium hydroxide. The magnesium ammonium phosphate is collected on a tared filter, dried, and weighed to determine the chemical yield. The precipitate is mounted and beta counted with a gas-flow proportional counter.

Source: EPA, EMSL. 1980. "Method R4-73-14: Radioactive Phosphorus." *Prescribed Procedures for Radiochemical Analysis of Nuclear Reactor Solutions*.
http://www2.epa.gov/sites/production/files/2015-06/documents/epa-r4-73-014.pdf

6.2.11 EPA Method: Determination of Radiostrontium in Food and Bioenvironmental Samples

Analyte(s)	CAS RN
Strontium-89	14158-27-1

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Low background alpha/beta counter

Method Developed for: Strontium-89 and strontium-90 in food, vegetation and tissue samples
Method Selected for: SAM lists this method for qualitative analysis of wipes and air filters and confirmatory analysis of wipes, air filters, soil and sediment and vegetation.

Description of Method: This method is use for the determination of strontium-89 and strontium-90 in various bio-environmental samples. A sample of 10 g or less is placed in a nickel crucible. Barium and strontium (standardized) carriers are added to the sample. Sodium hydroxide pellets and anhydrous sodium carbonate are added and mixed, and the sample is fused as a carbonate. The strontium-calcium carbonates are dissolved in hydrochloric acid, complexed with di-sodium EDTA and passed through a cation column where the strontium is absorbed, and the complexed calcium passes through. The strontium is eluted from the column and precipitated as a carbonate. The strontium carbonate is weighed and mounted on a planchet for beta counting with a low background gas-flow alpha beta counter. The chemical yield is determined gravimetrically, using calculations provided in the method.

Special Considerations: This method was developed for analysis of food, vegetation and tissue. Additional laboratory development and testing is necessary for application to soil, sediment, air filters and wipes.

Source: EPA, National Environmental Research Center. 1975. "Determination of Radiostrontium in Food and Bioenvironmental Samples." *Handbook of Radiochemical Methods*, EPA-680/4-75-001.
http://www2.epa.gov/sites/production/files/2015-06/documents/radiostrontium_in_food.pdf

6.2.12 EPA Method: Rapid Radiochemical Method for Americium-241 in Water for Environmental Restoration Following Homeland Security Events

Analyte(s)	CAS RN
Americium-241	14158-27-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Americium-241in water
Method Selected for: SAM lists this method for qualitative analysis of drinking water samples.

Description of Method: The method is based on a sequence of two chromatographic extraction resins. Americium is concentrated, isolated, and purified by removing interfering radionuclides as well as other components of the sample in order to prepare the americium fraction for counting by alpha spectrometry. The method utilizes vacuum-assisted flow to improve the speed of the separations. Prior to use of the extraction resins, the water sample is filtered as necessary to remove any insoluble fractions, equilibrated with americium-243 tracer, and concentrated by evaporation or calcium phosphate precipitation. The sample test source is prepared by microprecipitation with neodymium fluoride. Standard laboratory protocol for the use of an alpha spectrometer is used when the sample is ready for counting.

Special Considerations: SAM lists this method for rapid qualitative screening of drinking water samples. The method is not intended for use in compliance monitoring of drinking water.

Source: EPA, Office of Radiation and Indoor Air National Air and Radiation Environmental Laboratory (NAREL). 2010. "Rapid Radiochemical Method for Americium-241 in Water for Environmental Restoration Following Homeland Security Events." *Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events*, EPA 402-R-10-001. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-402-r-10-001.pdf

6.2.13 EPA Method: Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Water for Environmental Restoration Following Homeland Security Events

Analyte(s)	CAS RN
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Plutonium-238 and -239 in water
Method Selected for: SAM lists this method for qualitative analysis of drinking water samples.

Description of Method: This method is based on the sequential use of two chromatographic extraction resins to isolate and purify plutonium by removing interfering radionuclides as well as other components of the matrix in order to prepare the plutonium fraction for counting by alpha spectrometry. The method utilizes vacuum-assisted flow to improve the speed of the separations. Prior to using the extraction resins, a water sample is filtered as necessary to remove any insoluble fractions, equilibrated with plutonium-242 tracer, and concentrated by either evaporation or coprecipitation with calcium phosphate. The sample test source is prepared by microprecipitation with neodymium fluoride. Standard laboratory protocol for the use of an alpha spectrometer is used when the sample is ready for counting.

Special Considerations: SAM lists this method for rapid qualitative screening of drinking water samples. The method is not intended for use in compliance monitoring of drinking water.

Source: EPA, Office of Radiation and Indoor Air National Air and Radiation Environmental Laboratory (NAREL). 2010. "Rapid Radiochemical Method for Plutonium-238 and -239 in Water for Environmental Restoration Following Homeland Security Events." *Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events*, EPA 402-R-10-001. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-402-r-10-001.pdf

6.2.14 EPA Method: Rapid Radiochemical Method for Radium-226 in Water for Environmental Restoration Following Homeland Security Events

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Radium-226 in water
Method Selected for: SAM lists this method for qualitative analysis of drinking water samples.

Description of Method: A known quantity of radium-225 is used as the yield determinant in this analysis. The sample is initially digested using concentrated nitric acid, followed by volume reduction and conversion to the chloride salt using concentrated hydrochloric acid. The solution is adjusted to a neutral pH and batch equilibrated with manganese resin to separate radium from any radioactive and/or non-radioactive matrix constituents. Further selectivity is achieved using a column containing Diphonix® resin. The radium (including radium-226) eluted from the column is prepared for counting by microprecipitation with barium sulfate. Low-level measurements are performed by alpha spectrometry. The activity measured in the radium-226 region of interest is corrected for chemical yield based on the observed activity of the alpha peak at 7.07 MeV.

Special Considerations: SAM lists this method for rapid qualitative screening of drinking water samples. The method is not intended for use in compliance monitoring of drinking water.

Source: EPA, Office of Radiation and Indoor Air National Air and Radiation Environmental Laboratory (NAREL). 2010. "Rapid Radiochemical Method for Radium-226 in Water for Environmental Restoration Following Homeland Security Events." *Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events*, EPA 402-R-10-001. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-402-r-10-001.pdf

6.2.15 EPA Method: Rapid Radiochemical Method for Radiostrontium in Water for Environmental Restoration Following Homeland Security Events

Analyte(s)	CAS RN
Strontium-90	10098-97-2

Analysis Purpose: Qualitative analysis
Technique: Beta counting

Method Developed for: Strontium-90 in water
Method Selected for: SAM lists this method for qualitative analysis of drinking water samples.

Description of Method: Strontium is isolated from the sample matrix and purified from potentially interfering radionuclides and matrix constituents using a strontium-specific, rapid chemical separation procedure. The sample is equilibrated with strontium carrier, and concentrated by coprecipitation with strontium/barium carbonate. If insoluble residues are noted during acid dissolution steps, the residue and precipitate mixture is digested in 8 M nitric acid to solubilize strontium. The solution is passed through a Sr-Resin™ extraction chromatography column that selectively retains strontium while allowing most interfering radionuclides and matrix constituents to pass through to waste. If present in the sample, residual plutonium and several interfering tetravalent radionuclides are stripped from the column using an oxalic acid/ nitric acid rinse. Strontium is eluted from the column with 0.05 M nitric acid and taken to dryness in a tared, stainless steel planchet. The planchet containing the strontium nitrate precipitate is weighed to determine the strontium yield. The sample test source is promptly counted on a gas flow proportional counter to determine the beta emission rate, which is used to calculate the total radiostrontium activity.

Special Considerations: SAM lists this method for rapid qualitative screening of drinking water samples. The method is not intended for use in compliance monitoring of drinking water.

Source: EPA, Office of Radiation and Indoor Air National Air and Radiation Environmental Laboratory (NAREL). 2010. "Rapid Radiochemical Method for Radiostrontium in Water for Environmental Restoration Following Homeland Security Events." *Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events*, EPA 402-R-10-001. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-402-r-10-001.pdf

6.2.16 EPA Method: Rapid Radiochemical Method for Isotopic Uranium in Water for Environmental Restoration Following Homeland Security Events

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Uranium-234, -235 and -238 in water
Method Selected for: SAM lists this method for qualitative analysis of drinking water samples.

Description of Method: This method is based on the sequential elution of interfering radionuclides as well as other components of the sample matrix by extraction chromatography to isolate and purify uranium for counting by alpha spectrometry. The method utilizes vacuum assisted flow to improve the speed of the separations. Prior to the use of the extraction resins, a water sample is filtered as necessary to remove any insoluble fractions, equilibrated with uranium-232 tracer, and concentrated by either evaporation or coprecipitation with calcium phosphate. The sample test source is prepared by microprecipitation with neodymium fluoride. Standard laboratory protocol for the use of an alpha spectrometer is used when the sample is ready for counting.

Special Considerations: SAM lists this method for rapid qualitative screening of drinking water samples. The method is not intended for use in compliance monitoring of drinking water.

Source: EPA, Office of Radiation and Indoor Air National Air and Radiation Environmental Laboratory (NAREL). "Rapid Radiochemical Method for Isotopic Uranium in Water for Environmental Restoration Following Homeland Security Events." *Rapid Radiochemical Methods for Selected Radionuclides in*

Water for Environmental Restoration Following Homeland Security Events, EPA 402-R-10-001. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-402-r-10-001.pdf

6.2.17 EPA Method: Rapid Method for Acid Digestion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events

Analyte(s)	CAS RN
Americium-241	14158-27-1
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Radium-226	13982-63-3
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Americium-241, plutonium-238 and -239, radium-226, strontium-90, uranium-234, -235 and -238 in surface wipes and air filters
Method Selected for: SAM lists this method for qualitative analysis of surface wipe and air filter samples.

Description of Method: The method is based on the complete dissolution of both the filter material and deposited particulates. Glass-fiber filters (the siliceous filter as well as deposited silicates) are dissolved with direct application of hydrofluoric acid. The addition of nitric and hydrochloric acids facilitates dissolution of remaining solids. The sample digestate is taken to dryness and re-dissolved in nitric acid. Filters composed of organic materials, such as cellulose or polypropylene, are dry ashed in a 450 °C muffle furnace to destroy the organic filter material, then processed through the acid dissolution steps referenced above for non-organic filter material. Once sample dissolution is complete, it is re-dissolved in nitric acid solution. The sample is then processed for specific analyte determination using one of the following rapid methods contained in *Rapid Radiochemical Methods for Selected Radionuclides in Water for Environmental Restoration Following Homeland Security Events* (http://www2.epa.gov/sites/production/files/2015-07/documents/epa-402-r-10-001.pdf):

- Rapid Radiochemical Method for Americium-241 in Water for Environmental Remediation Following Homeland Security Events
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Water for Environmental Remediation Following Homeland Security Events
- Rapid Radiochemical Method for Isotopic Uranium in Water for Environmental Remediation Following Homeland Security Events
- Rapid Radiochemical Method for Radium-226 in Water for Environmental Remediation Following Homeland Security Events
- Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Water for Environmental Remediation Following Homeland Security Events

Special Considerations: This method is a gross pre-treatment technique, to be used prior to use of the appropriate rapid separation methods cited above. Filters that contain large amounts of particulate material may result in persistent undissolved particulates in the digestion process. These samples may

require repeated application of the digestion procedure to cause a complete dissolution of the particulates. If refractory constituents are suspected in the sampled particulates or the acidic digestion procedure is otherwise deemed to be ineffective because of refractory residuals or constituents, the alternate "Rapid Method for Sodium Carbonate Fusion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events" (Section 6.2.18) should be considered for sample preparation.

Source: EPA, Office of Radiation and Indoor Air National Air and Radiation Environmental Laboratory (NAREL). 2010. "Rapid Method for Acid Digestion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events." When published, this method will be posted at http://www2.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides.

6.2.18 EPA Method: Rapid Method for Sodium Carbonate Fusion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events

Analyte(s)	CAS RN
Americium-241	14158-27-1
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Radium-226	13982-63-3
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Americium-241, plutonium-238 and -239, radium-226, strontium-90, uranium-234, -235 and -238 in surface wipes and air filters
Method Selected for: SAM lists this method for qualitative analysis of surface wipe and air filter samples.

Description of Method: The method is based on the complete dissolution of both the filter or swipe material and the deposited particulates. Glass-fiber media and deposited particulates are destroyed by fusion with molten sodium carbonate in a nickel or platinum crucible. The resulting fusion cake is dissolved in hydrochloric acid. Filters composed of organic materials, such as cellulose or polypropylene are charred in a crucible to destroy the organic filter material. The resulting charred media and deposited particulates are destroyed by fusion with molten sodium carbonate in a nickel or platinum crucible. The resulting fusion cake is dissolved in hydrochloric acid. Once sample fusion is complete and the fusion cake is dissolved in hydrochloric acid, the sample is processed for specific analyte determination using one of the following rapid methods:

- Rapid Radiochemical Method for Americium-241 in Water for Environmental Remediation Following Homeland Security Events
- Rapid Radiochemical Method for Plutonium-238 and Plutonium-239/240 in Water for Environmental Remediation Following Homeland Security Events

- Rapid Radiochemical Method for Isotopic Uranium in Water for Environmental Remediation Following Homeland Security Events
- Rapid Radiochemical Method for Radium-226 in Water for Environmental Remediation Following Homeland Security Events
- Rapid Radiochemical Method for Total Radiostrontium (Sr-90) in Water for Environmental Remediation Following Homeland Security Events

Special Considerations: This method is a gross pre-treatment technique, to be used prior to use of the appropriate rapid separation methods cited. Filters that contain large amounts of particulate material may result in persistent undissolved particulates in the digestion process. These samples may require repeated application of the digestion procedure to cause a complete dissolution of the particulates.

Source: EPA, Office of Radiation and Indoor Air National Air and Radiation Environmental Laboratory (NAREL). 2010. "Rapid Method for Sodium Carbonate Fusion of Glass-Fiber and Organic/Polymeric Composition Filters and Swipes Prior to Isotopic Uranium, Plutonium, Americium, Strontium, and Radium Analyses for Environmental Remediation Following Homeland Security Events." When published, this method will be posted at http://www2.epa.gov/radiation/rapid-radiochemical-methods-selected-radionuclides.

6.2.19 EML HASL-300 Method Am-01-RC: Americium in Soil

Analyte(s)	CAS RN
Americium-241	14596-10-2
Californium-252	13981-17-4
Curium-244	13981-15-2

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Americium in soil
Method Selected for: SAM lists this method for confirmatory analysis of soil/sediment samples.

Description of Method: This method uses alpha spectrometry for determination of americium-241 in soil, and also can be applied for determination of californium-252 and curium-244. Americium is leached from soil with nitric acid and hydrochloric acid. Americium-243 is added as a tracer to determine chemical yield. The soil is processed through the plutonium separation steps using ion exchange resin according to Method Pu-11-RC. Americium is collected with a calcium oxalate precipitation and finally isolated and purified by ion exchange. Californium-252 and curium-244 are eluted with americium as americium is stripped off the column. After source preparation by microprecipitation, americium-241, californium-252 and curium-244 are determined by alpha spectrometry analysis. The counting period chosen depends on the sensitivity required of the measurement and the degree of uncertainty in the result that is acceptable. The lower limit of detection (LLD) for americium-241 is 0.5 mBq when counted for 1000 minutes. In cases where less than 100 g of sample is available, use of EML HASL-300 Method Pu-12-RC is recommended.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Am-01-RC: Americium in Soil." *EML Procedures Manual*, HASL-300, 28th Edition.
http://www2.epa.gov/sites/production/files/2015-06/documents/eml-am-01-rc.pdf

6.2.20 EML HASL-300 Method Am-04-RC: Americium in QAP Water and Air Filters - Eichrom's TRU Resin

Analyte(s)	CAS RN
Americium-241	14596-10-2
Californium-252	13981-17-4
Curium-244	13981-15-2

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Americium (but not lanthanides) in water and air filters
Method Selected for: SAM lists this method for confirmatory analysis of drinking water, aqueous/liquid samples, surface wipes, air filters and vegetation.

Description of Method: This method is specific to measurement of americium isotopes in samples that do not contain lanthanides, but also can be used for measurement of californium and curium. The method uses microprecipitation and determination by alpha spectrometry. Americium-243 is added to the sample to determine chemical yield. The sample is processed through separation steps using ion exchange resins. The eluate from the ion exchange column containing americium (and all other ions, except plutonium) is evaporated, redissolved, and loaded onto a Transuranic (TRU) Resin extraction column. Americium (and curium and californium, if present) is separated and purified on the column and finally stripped with dilute nitric acid stripping solution. Microprecipitation is used to prepare for alpha spectrometry. The method involves sample preparation steps from EML HASL-300 Method Pu-10-RC for water samples. The LLD for total americium is 0.3 mBq when counted for 1000 minutes.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Am-04-RC: Americium in QAP Water and Air Filters - Eichrom's TRU Resin." *EML Procedures Manual*, HASL-300, 28th Edition. http://www2.epa.gov/sites/production/files/2015-06/documents/eml-am-04-rc.pdf

6.2.21 EML HASL-300 Method Am-06-RC: Americium and/or Plutonium in Vegetation

Analyte(s)	CAS RN
Americium-241	14596-10-2
Californium-252	13981-17-4
Curium-244	13971-52-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Americium and/or plutonium in vegetation
Method Selected for: SAM lists this method for confirmatory analysis of vegetation.

Description of Method: Vegetation is either dry ashed in a ceramic crucible using a muffle furnace or wet ashed with nitric acid. Plutonium-236 and americium-243 tracers are added after dry ashing or before wet ashing. Wet ashing requires considerably more time and must be carefully monitored due to the

highly reactive nature of vegetation. The sample is further digested with hydrofluoric acid to dissolve silicate compounds. Plutonium is separated by ion exchange and determined by alpha spectrometry using the plutonium-236 tracer to determine the recovery. Americium (and californium-252 and curium-244, if present) is collected with a calcium oxalate precipitation and finally isolated and purified by ion exchange. After source preparation by microprecipitation, americium -241 (and californium-252 and curium-244, if present) is determined by alpha spectrometry using americium-243 tracer to provide recovery data.

Special Consideration: Polytetrafluoroethylene (PTFE) beakers must be used when digesting samples with hydrofluoric acid.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Am-06-RC: Americium and/or Plutonium in Vegetation." *EML Procedures Manual*, HASL-300, 28th Edition. http://www2.epa.gov/sites/production/files/2015-06/documents/eml-am-06-rc.pdf

6.2.22 EML HASL-300 Method Ga-01-R: Gamma Radioassay

Analyte(s)	CAS RN
Americium-241	14596-10-2
Cesium-137	10045-97-3
Cobalt-60	10198-40-0
Europium-154	15585-10-1
Iodine-131	10043-66-0
Iridium-192	14694-69-0
Molybdenum-99	14119-15-4
Ruthenium-103	13968-53-1
Ruthenium-106	13967-48-1
Selenium-75	14265-71-5
Select Mixed Fission Products	NA

Analysis Purpose: Qualitative and confirmatory analysis or gross gamma determination
Technique: Gamma spectrometry

Method Developed for: Gamma-ray emitting radionuclides in a variety of environmental matrices
Method Selected for: SAM lists this method for qualitative and/or confirmatory analysis of select gamma emitters in aqueous/liquid, soil/sediment, surface wipe, air filters and/or vegetation.

Description of Method: This method uses gamma spectrometry for the measurement of gamma photons emitted from radionuclides without separating them from the sample matrix. Samples are placed into a standard geometry for gamma counting, typically using an high purity Germanium [HP(Ge)] detector. Detectors such as Ge(Li) or NaI(Tl) also can be used. The sample is placed into a standard geometry for gamma counting. Soil samples and sludge are placed into an appropriately sized Marinelli beaker after drying and grinding the sample for homogenization. Air filters and surface wipes can be counted directly or pressed into a planchet and counted. Samples are counted long enough to meet the required sensitivity of measurement. For typical counting systems and sample types, activity levels of approximately 40 Bq are measured, and sensitivities as low as 0.002 Bq can be achieved for many nuclides. Because of electronic limitations, count rates higher than 2000 counts per second (cps) should be avoided. High activity samples may be diluted, reduced in size, or moved away from the detector (a limited distance) to reduce the count rate and allow for analysis. The method is applicable for analysis of samples that contain radionuclides emitting gamma photons with energies above approximately 20 keV for germanium (Ge) (both HP(Ge) and GeLi) detectors and above 50 keV for NaI(Tl) detectors.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Ga-01-R: Gamma Radioassay." *EML Procedures Manual*, HASL-300, 28th Edition.
http://www2.epa.gov/sites/production/files/2015-06/documents/eml-ga-01-r.pdf

6.2.23 EML HASL-300 Method Po-02-RC: Polonium in Water, Vegetation, Soil, and Air Filters

Analyte(s)	CAS RN
Polonium-210	1-3981-52-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Polonium in water, vegetation, soil and air filters
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of drinking water, aqueous/liquid, soil/sediment and/or vegetation samples.

Description of Method: This method uses alpha spectrometry for determination of polonium in water, vegetation, soil and air filter samples. Polonium equilibrated with polonium-208 or polonium-209 tracer is isolated from most other elements by coprecipitation with lead sulfide. The sulfide precipitate is dissolved in weak hydrochloric acid solution. Polonium is quantitatively deposited on a nickel disc, and the plated disc is counted on an alpha spectrometer to measure chemical yield and activity of the sample. The solution from the deposition may be retained and analyzed for polonium-210. When counted for 1000 minutes, the LLD for polonium is 1.0 mBq for water and 1.3 mBq for vegetation, soil and filters.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Po-02-RC: Polonium in Water, Vegetation, Soil, and Air Filters." *EML Procedures Manual*, HASL-300, 28th Edition.
http://www2.epa.gov/sites/production/files/2015-07/documents/eml-po-02-rc.pdf

6.2.24 EML HASL-300 Method Pu-12-RC: Plutonium and/or Americium in Soil or Sediments

Analyte(s)	CAS RN
Americium-241	14596-10-2
Californium-252	13981-17-4
Curium-244	13981-15-2

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Plutonium and americium in soil
Method Selected for: This method is listed in SAM for use when small soil and sediment sample sizes (≤100 g) will be analyzed.

Description of Method: A sample of soil of up to 100 g in size is equilibrated with americium-243 tracer. Contaminant isotopes are leached with nitric and hydrochloric acid. Plutonium is removed by ion exchange. The eluent from the plutonium separation is saved for determination of americium, curium and californium. Americium, curium and californium are collected with a calcium oxalate coprecipitation, isolated and purified by extraction chromatography. Microprecipitation is used to prepare the sample for analysis by alpha spectrometry of americium, curium and californium. The LLD for americium is 0.5 mBq when counted for 1000 minutes.

Special Considerations: In cases where only small sample sizes (≤100 g) will be analyzed, this method is recommended for confirmatory analysis. If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Pu-12-RC: Plutonium and/or Americium in Soil or Sediments." *EML Procedures Manual*, HASL-300, 28th Edition. http://www2.epa.gov/sites/production/files/2015-07/documents/eml-pu-12-rc.pdf

6.2.25 EML HASL-300 Method Ra-03-RC: Radium-226 in Soil, Vegetable Ash, and Ion Exchange Resin

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Radon emanation

Method Developed for: Radium-226 in soil, vegetation ash and ion exchange resin
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of vegetation.

Description of Method: Soil, vegetation ash or ion exchange resin are prepared for radon-222 emanation measurement. The sample is pretreated with nitric acid-hydrogen fluoride, fused with potassium fluoride and transposed to pyrosulfate. The cake is dissolved in dilute hydrochloric acid. Radium-barium sulfate is precipitated, filtered, and dissolved in alkaline EDTA. The chemical yield is determined with the γ-emitting tracer barium-133. The solution is transferred to a radon bubbler. Radon is de-emanated into an ionization chamber or scintillation cell, and counted using a counter with a photomultiplier.

Special Consideration: Use of platinum crucibles is required in this method.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Ra-03-RC: Radium 226 in Soil, Vegetable Ash, and Ion Exchange resin." *EML Procedures Manual*, HASL-300, 28th Edition. http://www2.epa.gov/sites/production/files/2015-07/documents/eml-ra-03-rc.pdf

6.2.26 EML HASL-300 Method Sr-03-RC: Strontium-90 in Environmental Samples

Analyte(s)	CAS RN
Strontium-90	10098-97-2

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Beta counting

Method Developed for: Strontium-90 in vegetation, water, air filters and soil
Method Selected for: SAM lists this method for confirmatory analysis of soil and sediment samples, vegetation, surface wipes and air filters.

Description of Method: Strontium is separated from calcium, other fission products and natural radioactive elements. Fuming nitric acid separations remove the calcium and most other interfering ions. Radium, lead and barium are removed with barium chromate. Traces of other fission products are scavenged with iron hydroxide. After strontium-90 and yttrium-90 equilibrium has been attained, yttrium-90 is precipitated as the hydroxide and converted to oxalate for counting on a low-background

gas proportional beta counter. Chemical yield is determined with strontium-85 tracer by counting in a gamma well detector.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Sr-03-RC: Strontium-90 in Environmental Samples." *EML Procedures Manual*, HASL-300, 28th Edition. http://www2.epa.gov/sites/production/files/2015-07/documents/eml-sr-03-rc.pdf

6.2.27 EML HASL-300 Method Tc-01-RC: Technetium-99 in Water and Vegetation

Analyte(s)	CAS RN
Technetium-99	14133-76-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Beta counting / Gamma spectrometry

Method Developed for: Technetium-99 in water and vegetation
Method Selected for: SAM lists this method for confirmatory analysis of vegetation.

Description of Method: Samples are wet ashed with nitric acid. After wet ashing is complete, samples are evaporated to the smallest volume possible with no salting out. The resulting solution is cooled, transferred to a 1-L beaker, and diluted to 800 mL with reagent water. The sample solution is then stirred and filtered with suction through a 15-cm glass fiber filter, and the filter is washed with water. The filter containing the silica and insoluble material is discarded. Technetium-99 is equilibrated with technetium-95m tracer in the wet ashing step. Technetium is separated from other elements by anion exchange and electro-deposition, and technetium-99 is beta counted. Gamma spectrometry measurement of technetium-95m tracer provides the chemical yield.

Special Consideration: Technetium-95m tracer is no longer readily available from the source cited in the method. If technetium-95m can not be obtained, technetium-99m tracer may be substituted.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Tc-01-RC: Technetium-99 in Water and Vegetation." *EML Procedures Manual*, HASL-300, 28th Edition. http://www2.epa.gov/sites/production/files/2015-07/documents/eml-tc-01-rc.pdf

6.2.28 EML HASL-300 Method Tc-02-RC: Technetium-99 in Water – TEVA® Resin

Analyte(s)	CAS RN
Technetium-99	14133-76-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Technetium-99 in water
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of drinking water samples.

Description of Method: The sample containing technetium-99 is mixed with technetium-95m added as a gamma-emitting tracer. The two isotopes of technetium are brought to an isotopic equilibrium and separated from other elements by ferrous and ferric hydroxide coprecipitation. The precipitate is dissolved with dilute nitric acid and passed through a commercially available resin column (TEVA® Resin) which is highly specific for technetium in the pertechnetate form. The resin is washed with dilute

nitric acid to remove possible interferences and then it is eluted directly into a suitable liquid scintillation cocktail. The sample is typically counted for 1 hour to simultaneously determine technetium-99 activity and the technetium-95m radiochemical yield. Quench/efficiency calibration curves need to be established for the liquid scintillation spectrometer for both technetium-95m and technetium-99.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method Tc-02-RC: Technetium-99 in Water – TEVA® Resin." *EML Procedures Manual*, HASL-300, 28th Edition. http://www2.epa.gov/sites/production/files/2015-07/documents/eml-tc-02-rc.pdf

6.2.29 EML HASL-300 Method U-02-RC: Isotopic Uranium in Biological and Environmental Materials

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Isotopic uranium in biological and environmental materials
Method Selected for: SAM lists this method for confirmatory analysis of vegetation.

Description of Method: Uranium from acid leached, dry-ashed and wet-ashed materials is equilibrated with uranium-232 tracer, and isolated by anion exchange chromatography. The separated uranium isotopes are microprecipitated for alpha spectrometry.

Special Considerations: For microprecipitation procedures, refer to HASL-300 Method G-03.

Source: EML, DOE (EML is currently part of the DHS). 1997. "HASL-300 Method U-02-RC: Isotopic Uranium in Biological and Environmental Materials." *EML Procedures Manual*, HASL-300, 28th Edition. http://www2.epa.gov/sites/production/files/2015-07/documents/eml-u-02-rc.pdf

6.2.30 DOE FRMAC Method Volume 2, Page 33: Gross Alpha and Beta in Air

Analysis Purpose: Gross alpha and gross beta determination
Technique: Alpha/Beta counting

Method Developed for: Gross alpha and beta in air
Method Selected for: SAM lists this method for gross alpha and gross beta determination in air filters, and for direct counting of surface wipes.

Description of Method: A thin-window gas-flow proportional counter is used for counting gross alpha and beta radioactivity. The method supplies an approximation of the alpha and beta activity present in the air or the removable surface activity dependent on the sample type. The method provides an indication of the presence of alpha and beta emitters, including the following SAM analytes:

- Americium-241 (CAS RN 14596-10-2) Alpha emitter
- Californium-252 (CAS RN 13981-17-4) Alpha emitter
- Cesium-137 (CAS RN 10045-97-3) Beta emitter
- Cobalt-60 (CAS RN 10198-40-0) Beta emitter

- Curium-244 (CAS RN 13981-15-2) Alpha emitter
- Europium-154 (CAS RN 15585-10-1) Beta emitter
- Iridium-192 (CAS RN 14694-69-0) Beta emitter
- Plutonium-238 (CAS RN 13981-16-3) Alpha emitter
- Plutonium-239 (CAS RN 15117-48-3) Alpha emitter
- Polonium-210 (CAS RN 13981-52-7) Alpha emitter
- Radium-226 (CAS RN 13982-63-3) Alpha emitter
- Ruthenium-103 (CAS RN 13968-53-1) Beta emitter
- Ruthenium-106 (CAS RN 13967-48-1) Beta emitter
- Strontium-90 (CAS RN 10098-97-2) Beta emitter
- Uranium-234 (CAS RN 13966-29-5) Alpha emitter
- Uranium-235 (CAS RN 15117-96-1) Alpha emitter
- Uranium-238 (CAS RN 7440-16-1) Alpha emitter

For this application, the procedure requires the use of thorium-230 for alpha counting efficiency and cesium-137 for beta counting efficiency in the calibration of the detector. An air filter or swipe sample is placed onto a planchet then counted for alpha and beta radioactivity. Activity is reported in activity units per volume of air sampled, as units of activity per surface area sampled, or as total units of activity in cases where sample collection information is not available.

Source: FRMAC. 1998. "Gross Alpha and Beta in Air." *FRMAC Monitoring and Analysis Manual – Sample Preparation and Analysis* - Volume 2, DOE/NV/11718-181 Vol. 2, UC-707, p. 33. http://www2.epa.gov/sites/production/files/2015-06/documents/frmac-vol2-pg33.pdf

6.2.31 DOE RESL Method P-2: ^{32}P Fish, Vegetation, Dry Ash, Ion Exchange

Analyte(s)	CAS RN
Phosphorus-32	14596-37-3

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Cerenkov counting with Liquid Scintillation

Method Developed for: Phosphorus-32 in fish and vegetation
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of soil, sediment, wipes, air filters and vegetation.

Description of Method: Samples up to 500 g are dry ashed at 550 ºC and dissolved in two portions of nitric acid. The sample is evaporated to half volume and transferred to a perchloric acid hood. Concentrated nitric acid and concentrated perchloric acid are added, and the sample is evaporated to dryness. The residue is dissolved in hydrochloric acid and filtered through a glass fiber filter. Iron-55 is removed by precipitation with cupferron. The solution containing phosphate is purified by passing it through anion and cation columns to remove possible contaminants. The purified phosphate is precipitated as magnesium ammonium phosphate, filtered onto a glass fiber filter, and dried. The magnesium ammonium phosphate is dissolved in nitric acid and transferred to a counting vial. Phopsphorus-32 is assayed by counting the Cerenkov radiation with a liquid scintillation counter.

Special Considerations: Laboratories using this method must have a designated perchloric acid fume hood. This method was developed for analysis of fish and vegetation. Additional development and testing is necessary for application to soil, sediment, wipes and air filters. Phosphorus and iron carrier must be added to matrices that do not contain mg quantities of both elements.

Source: RESL, DOE. 1977. "Method P-2: ^{32}P Fish, Vegetation, Dry Ash, Ion Exchange." *RESL Analytical Chemistry Branch Procedures Manual*, IDO-12096.
http://www2.epa.gov/sites/production/files/2015-07/documents/resl-p-2.pdf

6.2.32 DOE SRS Actinides and Sr-89/90 in Soil Samples

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15517-48-3
Strontium-89	14158-27-1
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry and beta counting

Method Developed for: Actinides and strontium-89 and -90 in soil samples
Method Selected for: SAM lists this method for qualitative analysis of soil and sediment samples.

Description of Method: Radioactive tracers are added to samples prior to wet ashing. Samples are fused at 600 °C using sodium hydroxide in zirconium crucibles. An iron hydroxide precipitation is performed. After dissolution by acidification of the precipitate, a lanthanum fluoride precipitation is used to further eliminate the sample matrix. The lanthanum fluoride precipitate is redissolved in nitric acid, boric acid, and aluminum nitrate. A column separation using TEVA®, TRU and DGA resins is applied to separate the actinides into three fractions: plutonium-neptunium, uranium and americium/curium. Plutonium-242 (or plutonium-236 if neptunium-237 is measured), thorium-229, americium-243 and uranium-232 are used as tracers to determine yield. The various fractions of actinides are eluted from the resin columns and precipitated with cesium fluoride, dried, and counted by alpha spectrometry. Strontium resin is used to separate strontium-89/90 for measurement by beta counting.

Special Considerations: Thorium-228, if present as a daughter of the uranium-232 tracer, will interfere with thorium-228 analysis. Self-cleaning uranium-232 tracer, with thorium-228 removed, is required if thorium isotopes are separated and measured with uranium.

Source: SRS, DOE. 2011. "Actinides and Sr-89/90 in Soil." *SRS Manual L3.23*, Procedure L3.23-10054.
http://www2.epa.gov/sites/production/files/2015-07/documents/l3.23-10054.pdf

6.2.33 DOE SRS Actinides and Sr-89/90 in Vegetation: Fusion Method

Analyte(s)	CAS RN
Americium-241	14596-10-2
Plutonium-238	13981-16-3
Plutonium-239	15517-48-3
Strontium-89	14158-27-1
Strontium-90	10098-97-2
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative analysis
Technique: Alpha spectrometry

Method Developed for: Actinides and strontium-89 and -90 in vegetation
Method Selected for: SAM lists this method for qualitative analysis of vegetation.

Description of Method: Radioactive tracers are added to samples prior to wet ashing. Samples are fused at 600 °C using sodium hydroxide in zirconium crucibles. An iron hydroxide precipitation is performed. After dissolution by acidification of the precipitate, a lanthanum fluoride precipitation is used to further eliminate the sample matrix. The lanthanum fluoride precipitate is redissolved in nitric acid, boric acid and aluminum nitrate. A column separation using commercially available resins (TEVA®, TRU and DGA) is applied to separate the actinides into three fractions: plutonium/neptunium, uranium and americium/curium. Plutonium-242 (or Plutonium-236 if neptunium-237 is measured), thorium -229, americium-243 and uranium-232 are used as tracers to determine yield. The various fractions of actinides are eluted from the resin columns and precipitated with cesium fluoride, dried, and counted by alpha spectrometry. Strontium resin is used to separate strontium-89/90 for measurement by beta counting.

Special Considerations: Thorium-228, if present as a daughter of uranium-232 tracer, will interfere with thorium-228 analysis. Self-cleaning uranium-232 tracer, with thorium-228 removed, is required if thorium isotopes are separated and measured with uranium.

Source: SRS, DOE. 2011. "Actinides and Sr-89/90 in Vegetation: Fusion Method." *SRS Manual L3.23*, Procedure L3.23-10055. http://www2.epa.gov/sites/production/files/2015-07/documents/l3.23-10055.pdf

6.2.34 ORISE Method AP1: Gross Alpha and Beta for Various Matrices

Analysis Purpose: Gross alpha and gross beta determination
Technique: Alpha/Beta counting

Method Developed for: Gross alpha and beta in water, soil, vegetation and other solids
Method Selected for: SAM lists this method for gross alpha and gross beta determination in soil/sediment and vegetation.

Description of Method: This method provides an indication of the presence of alpha and beta emitters, including the following SAM analytes:

- Americium-241 (CAS RN 14596-10-2) Alpha emitter
- Californium-252 (CAS RN 13981-17-4) Alpha emitter
- Cesium-137 (CAS RN 10045-97-3) Beta emitter
- Cobalt-60 (CAS RN 10198-40-0) Beta emitter

- Curium-244 (CAS RN 13981-15-2) Alpha emitter
- Europium-154 (CAS RN 15585-10-1) Beta emitter
- Iridium-192 (CAS RN 14694-69-0) Beta emitter
- Plutonium-238 (CAS RN 13981-16-3) Alpha emitter
- Plutonium-239 (CAS RN 15117-48-3) Alpha emitter
- Polonium-210 (CAS RN 13981-52-7) Alpha emitter
- Radium-226 (CAS RN 13982-63-3) Alpha emitter
- Ruthenium-103 (CAS RN 13968-53-1) Beta emitter
- Ruthenium-106 (CAS RN 13967-48-1) Beta emitter
- Strontium-90 (CAS RN 10098-97-2) Beta emitter
- Uranium-234 (CAS RN 13966-29-5) Alpha emitter
- Uranium-235 (CAS RN 15117-96-1) Alpha emitter
- Uranium-238 (CAS RN 7440-16-1) Alpha emitter

This procedure provides screening measurements to indicate whether specific analyses are required for water, soil, vegetation and other solids. Liquid samples are acidified, concentrated, dried in a planchet, and counted in a low-background proportional counter. Solid samples are dried and homogenized, and a known quantity is transferred to a planchet and counted in a low-background proportional counter.

Special Considerations: Volatile radionuclides will not be accurately determined using this procedure.

Source: ORISE, Oak Ridge Associated Universities (ORAU). 2001. "Method AP1: Gross Alpha and Beta for Various Matrices." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program*. http://www2.epa.gov/sites/production/files/2015-06/documents/orise-ap1.pdf

6.2.35 ORISE Method AP2: Determination of Tritium

Analyte(s)	CAS RN
Tritium (Hydrogen-3)	10028-17-8

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Tritium in soil, sediment, animal tissue, vegetation, smears and water samples
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of soil/sediment, surface wipes and vegetation.
Description of Method: The tritium in aqueous and solid samples is distilled using an Allihn condenser. For solid samples, an appropriate volume of laboratory reagent water is added to facilitate distillation. Certain solid samples may be refluxed to ensure distribution of any tritium that may be in the sample. The sample may be spiked with a standard tritium solution to evaluate quenching and counting efficiency. After the sample has been distilled, an aliquot of the distillate is added to a scintillation cocktail and the sample is counted using a liquid scintillation analyzer.

Special Considerations: Other volatile radionuclides such as iodine and carbon isotopes may interfere and may require that the sample be made alkaline using solid sodium hydroxide before distillation. Organic impurities may interfere and may require the addition of an oxidizing agent to the sample as well as spiking the samples with a standard tritium solution. The addition of a standard tritium solution to each sample allows for counting efficiencies to be calculated for each individual sample.

Source: ORISE, ORAU. 2001. "Method AP2: Determination of Tritium." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program.*
http://www2.epa.gov/sites/production/files/2015-06/documents/orise-ap2.pdf

6.2.36 ORISE Method AP5: Determination of Technetium-99

Analyte(s)	CAS RN
Technetium-99	14133-76-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Technetium-99 in sediment, soil, smears and water at environmental levels
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of soil/sediment, surface wipe and air filter samples; and qualitative analysis of vegetation.

Description of Method: Solid samples are leached with dilute nitric acid. The leachates are passed through a commercially available resin column (TEVA® resin) which is highly specific for technetium in the pertechnetate form. The technetium is absorbed onto the extraction resin. The resin is added to a scintillation vial containing an appropriate cocktail and counted using a liquid scintillation analyzer. Most interfering beta emitting radionuclides (including carbon-14, phosphorus-32, sulfur-35, strontium-90, yttrium-90 and thorium-234) are effectively removed using TEVA® resin under the conditions in this procedure.

Special Considerations: Tritium may follow technetium due to the absorption of some tritium-labeled compounds by the resin. Possible tritium interferences are eliminated by setting the technetium counting window above the maximum energy of tritium beta particles.

Source: ORISE, ORAU. 2001. "Method AP5: Determination of Technetium-99." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program.* http://www2.epa.gov/sites/production/files/2015-06/documents/orise-ap5.pdf

6.2.37 ORISE Method AP11: Sequential Determination of the Actinides in Environmental Samples Using Total Sample Dissolution and Extraction Chromatography

Analyte(s)	CAS RN
Americium-241	14596-10-2
Californium-252	13981-17-4
Curium-244	13981-15-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Americium, curium, plutonium, neptunium, thorium and/or uranium in water and solid samples
Method Selected for: SAM recommends this method for confirmatory analysis when a sample exists in a refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem. In the event of refractory radioactive material, SAM recommends this method for both qualitative determination and confirmatory analysis of drinking water, aqueous/liquid, soil/sediment, surface wipes and air filter samples.

Description of Method: Solid and unfiltered aqueous samples (after evaporation to dryness) are dissolved completely by a combination of potassium hydrogen fluoride and pyrosulfate fusions. Filtered aqueous samples are evaporated to dryness followed by a pyrosulfate fusion. The fusion cake is dissolved and, for analyses requiring uranium only, two barium sulfate precipitations are performed, and the uranium is separated using EDTA. For all other analyses, one barium sulfate precipitation is performed and all alpha emitters are coprecipitated on barium sulfate. The barium sulfate is dissolved and the actinides are separated by extraction chromatography. An optional section is presented for the separation of americium from the lanthanides. All actinides are coprecipitated on cerium fluoride and counted with an alpha spectrometer system.

Source: ORISE, ORAU. 2001. "Method AP11: Sequential Determination of the Actinides in Environmental Samples Using Total Sample Dissolution and Extraction Chromatography." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program.* http://www2.epa.gov/sites/production/files/2015-06/documents/orise-ap11.pdf

6.2.38 ORISE Method Procedure #9: Determination of I-125 in Environmental Samples

Analyte(s)	CAS RN
Iodine-125	14158-31-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Gamma spectrometry

Method Developed for: Iodine-125 in environmental samples, such as soil, sediment, vegetation, water, milk, filters (air or water), etc.
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of drinking water, aqueous/liquid, soil/sediment, surface wipe, air filter and vegetation samples.

Description of Method: In this method a direct comparison is made between the sample and a source prepared from a National Institute of Standards and Technology (NIST) traceable standard. If it is known, either by the sample preparation procedure or by a qualitative analysis on some device (high resolution intrinsic planar detector) that iodine-125 is the only radionuclide contributing to the observed peak, then this method can be used as a rapid quantitative method.

The sample is prepared by matrix specific techniques and the final sample is placed in a 16-mL culture tube and counted in a 3" x 3" thin window sodium iodide (NaI) well detector attached to a pulse height analyzer. Iodine-125 gamma counting rate is determined in the 25 to 35 keV energy range by pulse height analysis. NIST traceable liquid standards are also counted in the same geometric configuration as the samples to determine iodine-125 counting efficiency.

Special Considerations: Due to the low photon energy of iodine-125, the Compton scattering and x-ray photons from other radionuclides may cause significant interferences in this procedure.

Source: ORISE, ORAU. 1995. "Procedure #9: Determination of I-125 in Environmental Samples." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program.* http://www2.epa.gov/sites/production/files/2015-06/documents/orise-procedure9-1995.pdf

6.2.39 ASTM Method D3084-05: Standard Practice for Alpha Spectrometry in Water

Analyte(s)	CAS RN
Americium-241	14596-10-2
Californium-252	13981-17-4
Curium-244	13981-15-2
Plutonium-238	13981-16-3
Plutonium-239	15117-48-3
Radium-226	13982-63-3

Analysis Purpose: Qualitative determination
Technique: Alpha spectrometry

Method Developed for: Alpha particle spectra in water
Method Selected for: SAM lists this method for qualitative determination of californium-252 and curium-244 in drinking water, surface wipes, air filters and vegetation; americium-241, californium-252, curium-244, and plutonium-238 and -239 in aqueous and liquid phase samples; and californium-252, curium-244 and radium-226 in soil and sediment.

Description of Method: This standard practice covers the process that is required to obtain well-resolved alpha spectra from water samples and discusses the associated problems. This practice is typically preceded with specific chemical separations and mounting techniques that are included in referenced methods. A chemical procedure is required to isolate and purify the radionuclides (see ASTM Methods D3865, *Standard Test Method for Plutonium in Water* and D3972, *Standard Test Method for Isotopic Uranium in Water by Radiochemistry*), and a radioactive tracer is added to determine yield. A source is prepared by employing electrodeposition, microprecipitation or evaporation (depositing the solution onto a stainless steel or platinum disc). Electrodeposition and microprecipitation are preferred. The source's radioactivity is then measured in an alpha spectrometer according to manufacturer's operating instructions. The counting period chosen depends on the sensitivity required of the measurement and the degree of uncertainty in the result that is acceptable.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 for sample preparation instead of the methods referenced in ASTM Method D3084.

Source: ASTM. 2005. "Method D3084-05: Standard Practice for Alpha Spectrometry in Water." *Annual Book of ASTM Standards*, Vol. 11.02. http://www.astm.org/Standards/D3084.htm

6.2.40 ASTM Method D3972-02: Standard Test Method for Isotopic Uranium in Water by Radiochemistry

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Confirmatory analysis
Technique: Alpha spectrometry

Method Developed for: Alpha-particle-emitting isotopes of uranium in water
Method Selected for: SAM lists this method for confirmatory analysis of drinking water samples.

Description of Method: Uranium is chemically separated from a water sample by coprecipitation with ferrous hydroxide followed by anion exchange, and electrodeposition. When suspended matter is present, an acid dissolution step is added to ensure that all of the uranium dissolves. The sample is acidified, and uranium-232 is added as an isotopic tracer to determine chemical yield. Uranium is coprecipitated from the sample with ferrous hydroxide. This precipitate is dissolved in concentrated hydrochloric acid, or is subjected to acid dissolution with concentrated nitric and hydrofluoric acids, if the hydrochloric acid fails to dissolve the precipitate. Uranium is separated from other radionuclides by adsorption on anion exchange resin, followed by elution with hydrochloric acid. The uranium is finally electrodeposited onto a stainless steel disc and counted using alpha spectrometry.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11.

Source: ASTM. 2002. "Method D3972-02: Standard Test Method for Isotopic Uranium in Water by Radiochemistry." *Annual Book of ASTM Standards*, Vol. 11.02. http://www.astm.org/DATABASE.CART/HISTORICAL/D3972-02.htm

6.2.41 ASTM Method D5811-08: Standard Test Method for Strontium-90 in Water

Analyte(s)	CAS RN
Strontium-90	10098-97-2

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Beta counting

Method Developed for: Strontium-90 in water samples
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of aqueous and liquid phase samples.

Description of Method: An aliquot of the sample is measured into a beaker, and strontium carrier is added. The sample is digested with nitric acid, sorbed on an ion exchange column, eluted, and evaporated to dryness. The residue is redissolved in nitric acid and then is selectively sorbed on a solid phase extraction column. Strontium is eluted with dilute nitric acid dried on a planchet, weighed, and counted for beta radiation.

Special Considerations: Significant amounts of stable strontium, if present in the sample, will interfere with the yield determination.

Source: ASTM. 2008. "Method D5811-08: Standard Test Method for Strontium-90 in Water." *Annual Book of ASTM Standards*, Vol. 11.02. http://www.astm.org/Standards/D5811.htm

6.2.42 ASTM Method D7168-05: Standard Test Method for Technetium-99 in Water by Solid Phase Extraction Disk

Analyte(s)	CAS RN
Technetium-99	14133-76-7

Analysis Purpose: Qualitative and confirmatory analysis
Technique: Liquid scintillation

Method Developed for: Technetium-99 in water samples
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of aqueous and liquid phase samples.

Description of Method: A measured aliquot of sample is transferred to a beaker and hydrogen peroxide is added to facilitate the formation of the extractable pertechnetate ion. The sample may be heated to oxidize organics, if suspected to be present. The entire sample is passed through a technetium-selective solid-phase extraction (SPE) disk onto which the pertechnetate is adsorbed. The disk is transferred to a liquid scintillation vial, scintillation cocktail is added, and the contents are well mixed. The beta-emission rate of the sample is determined by liquid scintillation spectrometry. Chemical yield corrections are determined by the method of standard additions.

Special Considerations: Suspended materials must be removed by filtration or centrifuging prior to processing the sample.

Source: ASTM. 2005. "Method D7168-05: Standard Test Method for Technetium-99 in Water by Solid Phase Extraction Disk." *Annual Book of ASTM Standards*, Vol. 11.02.
http://www.astm.org/Standards/D7168.htm

6.2.43 Standard Method 7110 B: Gross Alpha and Gross Beta Radioactivity (Total, Suspended, and Dissolved)

Analysis Purpose: Gross alpha and gross beta determination
Technique: Alpha/Beta counting

Method Developed for: Gross alpha and gross beta activity in water
Method Selected for: SAM lists this method for gross alpha and gross beta determination in aqueous/liquid samples.

Description of Method: This method allows for measurement of gross alpha and gross beta radiation in water samples. The method provides an indication of the presence of alpha and beta emitters, including the following SAM analytes:

- Americium-241 (CAS RN 14596-10-2) Alpha emitter
- Californium-252 (CAS RN 13981-17-4) Alpha emitter
- Cesium-137 (CAS RN 10045-97-3) Beta emitter
- Cobalt-60 (CAS RN 10198-40-0) Beta emitter
- Curium-244 (CAS RN 13981-15-2) Alpha emitter
- Europium-154 (CAS RN 15585-10-1) Beta emitter
- Iridium-192 (CAS RN 14694-69-0) Beta emitter
- Plutonium-238 (CAS RN 13981-16-3) Alpha emitter
- Plutonium-239 (CAS RN 15117-48-3) Alpha emitter
- Polonium-210 (CAS RN 13981-52-7) Alpha emitter
- Radium-226 (CAS RN 13982-63-3) Alpha emitter
- Ruthenium-103 (CAS RN 13968-53-1) Beta emitter
- Ruthenium-106 (CAS RN 13967-48-1) Beta emitter
- Strontium-90 (CAS RN 10098-97-2) Beta emitter
- Uranium-234 (CAS RN 13966-29-5) Alpha emitter
- Uranium-235 (CAS RN 15117-96-1) Alpha emitter
- Uranium-238 (CAS RN 7440-16-1) Alpha emitter

This method recommends using a thin-window gas-flow proportional counter for counting gross alpha and beta radioactivity. An internal proportional or Geiger counter may also be used. An aliquot of

sample is evaporated to a small volume and transferred to a tared counting pan. The sample residue is dried to constant weight, cooled, and reweighed to determine dry residue weight, then counted for alpha and beta radioactivity.

Special Considerations: Ground water samples containing elevated levels of dissolved solids will require use of smaller sample volumes.

Source: APHA, AWWA, and WEF. 2005. "Method 7110 B: Gross Alpha and Gross Beta Radioactivity (Total, Suspended, and Dissolved)." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

6.2.44 Standard Method 7120: Gamma-Emitting Radionuclides

Analyte(s)	CAS RN
Americium-241	14596-10-2
Cesium-137	10045-97-3
Cobalt-60	10198-40-0
Europium-154	15585-10-1
Iridium-192	14694-69-0
Ruthenium-103	13968-53-1
Ruthenium-106	13967-48-1
Selenium-75	14265-71-5

Analysis Purpose: Qualitative and confirmatory determination
Technique: Gamma spectrometry

Method Developed for: Gamma emitting radionuclides in water
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of select gamma emitters in aqueous/liquid samples.

Description of Method: The method uses gamma spectroscopy using either Ge detectors or NaI(Tl) crystals for the measurement of gamma photons emitted from radionuclides present in water. The method can be used for qualitative and confirmatory determinations with Ge detectors or semi-qualitative and semi-quantitative determinations (using NaI(Tl) detectors). Exact confirmation using NaI is possible for single nuclides or when the gamma emissions are limited to a few well-separated energies. A homogeneous water sample is placed into a standard geometry (normally a Marinelli beaker) for gamma counting. Sample portions are counted long enough to meet the required sensitivity of measurement. A radioactive standard, in the same geometry as the samples, containing a mixture of gamma energies from approximately 50 to 2000 keV is used for energy and efficiency calibration.

Source: APHA, AWWA, and WEF. 2005. "Method 7120: Gamma-Emitting Radionuclides." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

6.2.45 Standard Method 7500-Ra B: Radium: Precipitation Method

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Qualitative determination
Technique: Alpha counting

Method Developed for: Alpha-emitting isotopes of radium in water
Method Selected for: SAM lists this method for qualitative determination in aqueous/liquid samples.

Description of Method: This method is for determination of all alpha-emitting radium isotopes by alpha decay analysis. Lead and barium carriers are added to the sample containing alkaline citrate, then sulfuric acid is added to precipitate radium, barium and lead as sulfates. The precipitate is purified by washing with nitric acid, dissolving in alkaline EDTA, and re-precipitating as radium-barium sulfate after pH adjustment to 4.5. This slightly acidic EDTA keeps other naturally occurring alpha-emitters and the lead carrier in solution. Radium-223, -224 and -226 are identified by the rate of ingrowth of their daughter products in barium sulfate precipitate. The results are corrected by the rate of ingrowth of daughter products to determine radium activity. This method involves alpha counting by a gas-flow internal proportional counter, scintillation counter or thin end-window gas-flow proportional counter.

Source: APHA, AWWA, and WEF. 2005. "Method 7500-Ra B: Radium: Precipitation Method." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

6.2.46 Standard Method 7500-Ra C: Radium: Emanation Method

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Confirmatory determination
Technique: Alpha counting

Method Developed for: Soluble, suspended and total radium-226 in water
Method Selected for: SAM lists this method for confirmatory analysis of aqueous/liquid samples.

Description of Method: Radium in water is concentrated and separated from sample solids by coprecipitation with a relatively large amount of barium as the sulfate. The precipitate is treated to remove silicates, if present, and to decompose insoluble radium compounds, fumed with phosphoric acid to remove sulfite, and dissolved in hydrochloric acid. The completely dissolved radium is placed in a bubbler, which is then closed and stored for a period of several days to 4 weeks for ingrowth of radon. The bubbler is connected to an evacuation system and the radon gas is removed from the liquid by aeration and helium, dried with a desiccant, and collected in a counting cell. Four hours after radon collection, the cell is counted. The activity of the radon is equal to the radium concentration. The minimum detectable concentration depends on counter characteristics, background-counting rate of scintillation cell, cell efficiency, length of counting period, and contamination of apparatus and environment by radium-226. Without reagent purification, the overall reagent blank (excluding background) should be between 0.03 and 0.05 pCi radium-226, which may be considered the minimum detectable amount under routine conditions.

Source: APHA, AWWA, and WEF. 2005. "Method 7500-Ra C: Radium: Emanation Method." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

6.2.47 Standard Method 7500-U B: Uranium: Radiochemical Method

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative determination
Technique: Alpha counting

Method Developed for: Total uranium alpha activity in water
Method Selected for: SAM lists this method for qualitative determination in aqueous/liquid samples.

Description of Method: The sample is acidified with hydrochloric or nitric acid and boiled to eliminate carbonate and bicarbonate ions. Uranium is coprecipitated with ferric hydroxide and subsequently separated. The ferric hydroxide is dissolved, passed through an anion-exchange column, washed with acid, and the uranium is eluted with dilute hydrochloric acid. The acid eluate is evaporated to near dryness, the residual salt is converted to nitrate, and the alpha activity is counted by a gas-flow proportional counter or alpha scintillation counter.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11.

Source: APHA, AWWA, and WEF. 2005. "Method 7500-U B: Uranium: Radiochemical Method." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

6.2.48 Standard Method 7500-U C: Uranium: Isotopic Method

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Confirmatory determination
Technique: Alpha spectrometry

Method Developed for: Isotopic content of the uranium alpha activity; determining the differences among naturally occurring, depleted, and enriched uranium in water
Method Selected for: SAM lists this method for confirmatory analysis of aqueous/liquid samples.

Description of Method: This method is a radiochemical procedure for determination of the isotopic content of uranium alpha activity. The sample is acidified with hydrochloric or nitric acid and uranium-232 is added as an isotopic tracer. Uranium is coprecipitated with ferric hydroxide and subsequently separated from the sample. The ferric hydroxide precipitate is dissolved and the solution passed through an anion-exchange column. The uranium is eluted with dilute hydrochloric acid. The acid eluate is evaporated to near dryness, and the residual salt is converted to nitrate and electrodeposited onto a stainless steel disc and counted by alpha spectrometry.

Special Considerations: If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11.

Source: APHA, AWWA, and WEF. 2005. "Method 7500-U C: Uranium: Isotopic Method." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

6.2.49 Y-12 (DOE) Preparation of Samples for Total Activity Screening

Analyte(s)	CAS RN
Total Activity Screening	NA

Analysis Purpose: Total activity screening
Technique: Liquid scintillation
Method Developed for: Total activity screening
Method Selected for: SAM lists this method for gross total activity screening of drinking water, liquid and aqueous phase, soil and sediment, wipe, air filter and vegetation samples.

Description of Method: Aqueous sample aliquots that require no preparation are added directly to the scintillation cocktail. Solid and semi-solid sample aliquots are digested in nitric acid on a hot plate, cooled, filtered, and diluted to a specified volume. Oil sample aliquots are weighed directly into a tared counting vial. A specified volume of liquid scintillation cocktail is added to each vial and mixed with the sample aliquot. The samples are then counted for total activity.

Special Considerations: The method assumes 100% counting efficiencies for both beta and alpha emitters. Low energy beta emitters will not be counted at 100% efficiency, which can introduce a negative bias in the measurement.

Source: Y-12 (DOE). 2005. "Preparation of Samples for Total Activity Screening." Procedure Y50-AC-65-7230. http://www2.epa.gov/sites/production/files/2015-07/documents/y50-ac-65-7230.pdf

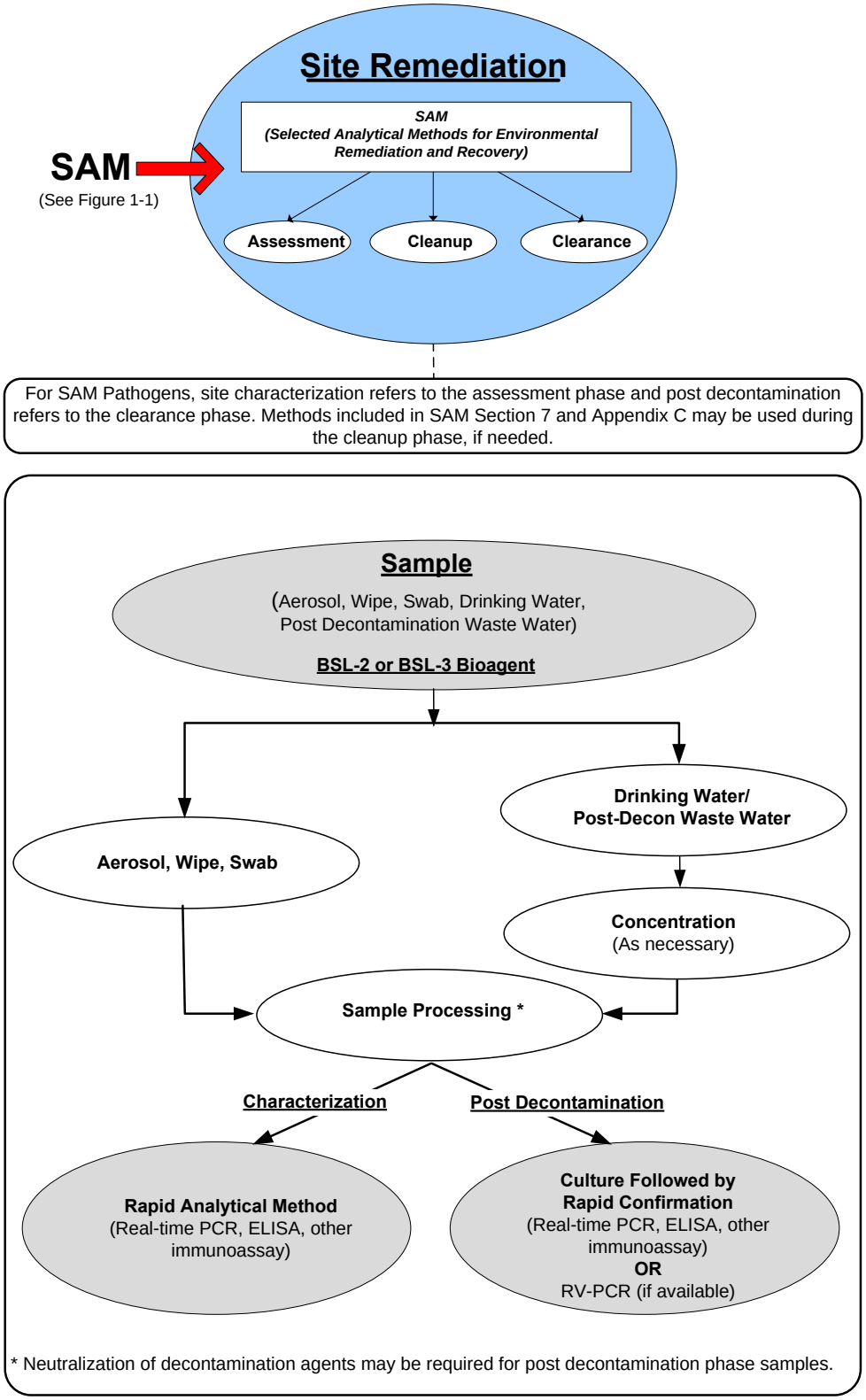
Section 7.0: Selected Pathogen Methods

Legislation and Presidential Directives give the U.S. EPA the responsibility to lead environmental and water clean up efforts associated with terrorist events and other disasters. This responsibility is listed in the Integrated Consortium of Laboratory Networks (ICLN) Federal Responsible Agency Matrix. Following a contamination event, it is assumed that the U.S. Centers for Disease Control and Prevention (CDC) and the Federal Bureau of Investigation (FBI) have completed the identification, confirmation and strain-level characterization of the bioagent/pathogen contaminant before EPA's remediation begins. The first phase of EPA's actions includes site characterization, to determine the extent and magnitude of contamination and guide remediation planning. Based on the results of sample analyses for site characterization, EPA will determine the approach for site decontamination. During the post decontamination (clearance) phase of remediation, samples are collected and analyzed to determine the efficacy of the decontamination treatment.

Based on lessons learned and experience gained through various interagency bioterrorism counter-measures programs, including the Interagency Biological Restoration Demonstration (IBRD), decision making during remediation includes scientific, public health, social, economic and political concerns. As part of this process, the analytical methods selected for use should aid in decision making in an effective and time-sensitive manner. The purpose of this section is to provide guidance to stakeholders in determining the appropriate methods for each remedial phase (site characterization and/or post decontamination) of a response to a biological event. Emphasis is given to environmental sample types that are most prevalently used to fulfill EPA's homeland security responsibilities (e.g., aerosols, surface wipes or swabs, drinking water and post decontamination waste water).

Selection of methods from Appendix C should be based on specific data and information needs, including consideration of the remediation phase and whether there is a need to determine either the presence of a pathogen, the viability of a pathogen or both. The flow chart in Figure 7-1 presents a summary of the sample types, overall steps in sample analysis, and analytical techniques that should be used to address pathogens during EPA site remediation activities following a contamination event. As depicted in Figure 7-1, for SAM Pathogens, site characterization refers to the assessment phase, decontamination refers to the cleanup phase, and post decontamination refers to the clearance phase.

Figure 7-1. Sample Analysis During Site Characterization and Post Decontamination Phases Following a Biological Contamination Event



Methods for Site Characterization Phase: Since decontamination of the affected site has to quickly follow the site characterization phase, rapid analytical methods should be selected to determine the extent and magnitude of contamination. It is assumed here that, prior to site characterization, the identity and viability of the pathogen have been determined by the CDC and FBI. Therefore, in most cases, the analytical methods selected for this phase may not have to determine whether the pathogen is viable. The methods should also provide a high throughput analytical capability, so that a large number of samples can be rapidly analyzed to determine the presence or absence of the pathogen and allow for site decontamination planning in a time-efficient manner. For most pathogens, such methods routinely include polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA) or other immunoassay-based methods. Depending on the pathogen, type of event and response, culture methods could be appropriate for use during site characterization. In certain cases, the viability determination of the pathogen within this phase may drive decontamination planning.

Methods for Post Decontamination Phase: It is extremely critical that the analytical methods used for sample analysis during the post decontamination phase be highly sensitive, specific, rapid, and able to determine pathogen viability. For post decontamination phase samples, neutralization or removal of the decontamination agent may be required prior to analysis. Traditional microbiological culture methods typically include plating on selective medium to determine the viability of the pathogen and to minimize or eliminate non-target growth. The absence of growth on the medium generally indicates the absence of live pathogen in the sample [with the exception of some pathogens which may become viable but non-culturable (VBNC)]. To minimize the analytical time needed to obtain results, typical colonies should be quickly analyzed to confirm the presence of the pathogen using reliable and rapid methods such as PCR, ELISA or other immunoassay-based methods, as opposed to time and labor intensive traditional biochemical and serological procedures. For *Bacillus anthracis*, the recently published Rapid Viability-PCR (RV-PCR) method may be used because it provides rapid and high throughput results in addition to viability determination (Letant *et al.* 2011[7], U.S. EPA Report 2010 [EPA/600/R-10/156][8]). However, if pathogen viability and concentration determinations are required for any samples, the culture method followed by rapid identification techniques (e.g., PCR, ELISA) should be used.

A list of methods that have been selected by the SAM Pathogen Methods Work Group for use in analyzing environmental samples for pathogens is provided in Appendix C. These methods should be used during remediation activities following a biological event. Appendix C is sorted alphabetically within pathogen categories (i.e., bacteria, viruses, protozoa and helminths). Protocols from peer-reviewed journal articles are listed where verified and/or validated methods for pathogens are not currently available. The literature references will be replaced as fully developed and validated protocols become available.

***Please note*:** This section provides guidance for selecting pathogen methods that have a high likelihood of assuring analytical consistency when laboratories analyze a large number of samples during remediation. Not all methods have been verified for the pathogen/sample type combination listed in Appendix C. Please refer to the specified method to identify analyte/sample type combinations for which the method has been verified. Any questions regarding information discussed in this section should be addressed to the appropriate contact(s) listed in Section 4.

Pathogens that are categorized as biosafety level (BSL)-4, such as hemorrhagic fever viruses and smallpox, will be handled only by reference laboratories with BSL-4 capability and are not included in

[7] Létant, S. E., Murphy, G.A., Alfaro, T. M., Avila, J. R., Kane, S. R., Raber, E., Bunt, T. M. and Shah, S. R. 2011. Rapid-Viability PCR Method for Detection of Live, Virulent *Bacillus anthracis* in Environmental Samples." Applied Environmental Microbiology, 77(18): 6570–6578.

[8] U.S. EPA. 2010. "Development and Verification of Rapid Viability Polymerase Chain Reaction (RV-PCR) Protocols for *Bacillus anthracis* – For Aapplication to Air Filters, Water and Surface Samples." EPA/600/R-10/156.

this document. All other pathogens should be handled using BSL-2 or BSL-3 containment and practices, as appropriate. If known, the BSL classification for each pathogen is provided in the method summaries in Sections 7.2 through 7.5. Pathogens that are considered to be solely of agricultural concern (i.e., animal and plant pathogens) are not currently included. However, such pathogens may be considered for possible inclusion in future revisions of SAM.

In addition to analytical methods, Appendix C lists commercially available spore strips, which may be used as general indicators that a decontamination process (e.g., fumigation) has been successful. Spore strip results, however, cannot replace negative-culture results as an indicator of decontamination efficacy. Culture-based methods have been selected for many of the pathogens; however, due to technical difficulty and time constraints, molecular techniques such as PCR will likely be used for viruses.

Some of the methods in Appendix C include multiple analytical techniques by inference. The analytical technique listed in Appendix C for each pathogen is intended to be a description of the predominant technique that is required to provide the data quality parameter (viability or detection and identification). This description does not preclude the use of other techniques that are within or referenced by the method. For example, a viability method or procedure listed as "culture" might include immunochemical or PCR-based assays for the identification of isolates. Several of the methods listed in Appendix C also include options such as the use of multiple cell culture media for primary isolation and a selection of a defined subset of biochemical tests for confirmation. To expedite time-to-results, however, isolates should be confirmed using rapid techniques (e.g., PCR, ELISA).

Appendix C includes the following information:

- **Pathogen(s).** A specific causative agent (e.g., viruses, bacteria) of disease.
- **Viability.** A microorganism's ability to grow and reproduce and/or cause infection.
- **Analytical technique.** An analytical procedure used to determine the identity, quantity and/or viability of a pathogen.
- **Analytical method.** A series of techniques which together isolate, concentrate and detect a microorganism or group of microorganisms. In some cases, a unique identifier or number is assigned to an analytical method by the method publisher. Analytical methods can be developed for various sample types, including:
  - **Aerosol (growth media, filters, liquids).** The recommended method/procedure for the pathogen of interest in air sample collectors such as growth media, filters or liquids.
  - **Particulate (swabs, wipes, filters).** The recommended method/procedure for the pathogen of interest in particulate sample collection tools such as swabs, wipes, Sponge-Sticks, and high-efficiency particulate air (HEPA) collecting socks and filters used for vacuum collection.
  - **Drinking water.** The recommended method/procedure for the pathogen of interest in potable water (concentrated and small volume grab samples).
  - **Post decontamination waste water.** The recommended method/procedure for the pathogen of interest in post decontamination waste water (concentrated and small volume grab samples).

Sample Processing: It is widely recognized in the scientific community that the processing of biologically contaminated environmental samples is one of the most challenging issues prior to sample analysis. Although details regarding sample processing are not within the scope of SAM, it is critical that end users and stakeholders select the most appropriate sample processing procedure for a given sample type and analytical method. It is highly unlikely that a single procedure will be applicable to all sample types and analytical methods. Inadequate sample processing may not only decrease recovery efficiency of biological targets (e.g., pathogen, deoxiribonucleic acid/ribonucleic acid [DNA/RNA], antigen/protein) from the samples, but also prevent accurate quantitation and high throughput. ***Note***: For post decontamination samples it may be necessary to neutralize the decontamination agent.

The methods listed in SAM attempt to address multiple environmental sample types, each with different physical, chemical and biological properties (e.g., pH, inhibitory substances and background microorganisms). In this edition, major emphasis is given to the environmental sample types that are most prevalently used to fulfill EPA's homeland security responsibilities following an event involving pathogen contaminants (e.g., aerosols, surface wipes or swabs, drinking water and post decontamination waste water). Other sample types may have to be analyzed, and for those sample types, specific requests should be sent to the SAM Pathogen Methods Lead and Alternate Lead (see Section 4).

7.1 General Guidelines

This section provides a general overview of how to identify the appropriate method(s) for a given pathogen as well as recommendations for quality control (QC) procedures.

For additional information on the properties of the pathogens listed in Appendix C, Toxicology Data Network (TOXNET) (http://toxnet.nlm.nih.gov/index.html), a cluster of databases on toxicology, hazardous chemicals and related areas maintained by the National Library of Medicine, is an excellent resource. Also informative are CDC's Emergency Preparedness and Response website (http://www.bt.cdc.gov/) and the U.S. Food and Drug Administration (FDA) Center for Food Safety and Applied Nutrition (CFSAN) 2012, "Bad Bug Book" (http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm296005.htm). Further research on pathogens is ongoing within EPA. Databases to manage this information are currently under development.

7.1.1 Standard Operating Procedures for Identifying Pathogen Methods

To determine the appropriate analytical method that is to be used for an environmental sample, locate the pathogen in Appendix C: Selected Pathogen Methods, under the "Pathogen(s)" column. After locating the pathogen, continue across the table and select an analytical technique. After an analytical technique has been chosen (e.g., culture, PCR, immunoassay), select the analytical method applicable to the sample type of interest (aerosols, surface wipes or swabs, drinking water and post decontamination waste water).

Once a method has been identified in Appendix C, the corresponding method summary can be found in Sections 7.2 through 7.5. Method summaries are listed in alphabetical order within each pathogen subcategory (i.e., bacteria, virus, protozoa, helminthes) and then by order of method selection hierarchy (see Figure 2-1), starting with EPA methods, followed by methods from other federal agencies, voluntary consensus standard bodies (VCSBs), and literature references. Where available, a direct link to the full text of the method is provided with the method summary. For additional information regarding sample preparation and analysis procedures available through consensus standards organizations, other federal agencies and journals, please use the source information provided in **Table 7-1**.

Table 7-1. Sources of Pathogen Methods

Name	Publisher	Reference
National Environmental Methods Index (NEMI)	EPA, U.S. Geological Survey (USGS)	http://www.nemi.gov
EPA Microbiology Home Page	EPA	http://www.epa.gov/nerlcwww/epamicrobiology.html
Information Collection Requirements Rule (ICR) Microbial Laboratory Manual	EPA ORD	http://www.epa.gov/nerlcwww/documents/icrmicro.pdf
EPA Manual of Methods for Virology	EPA	http://www2.epa.gov/sites/production/files/2015-07/documents/epa-600-4-84-013.pdf

Name	Publisher	Reference
Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage and Sludge	EPA, National Risk Management Research Laboratory (NRMRL)	http://water.epa.gov/scitech/wastetech/biosolids/upload/2007_05_31_625r92013_625R92013.pdf
U.S. Department of Agriculture (USDA) *Food Safety and Inspection Service (FSIS) Microbiology Laboratory Guidebook*	USDA FSIS	http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp
Bacteriological Analytical Manual (BAM)	FDA, CFSAN	http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm
Occupational Safety and Health Administration (OSHA) Methods	OSHA	http://www.osha.gov
National Institute for Occupational Safety and Health (NIOSH) Methods	NIOSH	http://www.cdc.gov/niosh/nmam/
Standard Methods for the Examination of Water and Wastewater, 21st Edition, 2005*	American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF)	http://www.standardmethods.org
*Annual Book of ASTM Standards**	ASTM International	http://www.astm.org
Applied and Environmental Microbiology*	American Society for Microbiology (ASM)	http://aem.asm.org/
Journal of Clinical Microbiology*	ASM	http://jcm.asm.org/
Clinical Microbiology Procedures Handbook, 3rd Edition, 2010*	ASM	http://estore.asm.org/viewitemdetails.asp?itemid=908
Molecular and Cellular Probes*	Elsevier	http://www.journals.elsevier.com/molecular-and-cellular-probes/
Canadian Journal of Microbiology*	NRC Research Press	http://www.nrcresearchpress.com/loi/cjm
Food and Environmental Virology*	Springer	http://www.springer.com/biomed/virology/journal/12560
Journal of Medical Virology*	Wiley InterScience	http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1096-9071
Journal of Virological Methods*	Elsevier	http://www.scimagojr.com/journalsearch.php?q=20241&tip=sid
Diagnostics Microbiology and Infectious Disease	Elsevier	http://www.elsevier.com/wps/find/journaldescription.cws_home/505759/description#description
Emerging Infectious Diseases	CDC	http://wwwnc.cdc.gov/eid/
Parasitology Research*	Springer	http://www.springer.com/biomed/medical+microbiology/journal/436
Journal of Parasitology*	American Society of Parasitologists	http://www.journalofparasitology.org/
Transactions of the Royal Society of Tropical Medicine and Hygiene*	The Royal Society of Tropical Medicine and Hygiene	http://www.elsevier.com/wps/find/journaldescription.cws_home/681019/description#description
Diagnostic Procedures in Veterinary Bacteriology and Mycology	Academic Press	http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1481267

Name	Publisher	Reference
Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases	ASM	http://www.asm.org/index.php/policy/sentinel-level-clinical-microbiology-laboratory-guidelines.html
Journal of Applied Microbiology*	Blackwell Publishing	http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1365-2672
Journal of Microbiological Methods*	Elsevier	http://www.sciencedirect.com/science/journal/01677012
Clinical Chemistry	American Association for Clinical Chemistry	http://www.clinchem.org/content/by/year
Antimicrobial Agents and Chemotherapy*	ASM	http://aac.asm.org/
Environmental Science and Technology*	American Chemical Society (ACS)	http://pubs.acs.org/journal/esthag

* Subscription and/or purchase required. ASM does not require a subscription or purchase 6 months after the publication date.

7.1.2 General QC Guidelines for Pathogen Methods

Generation of analytical data of known and documented quality is a critical factor in the accurate assessment of and appropriate response to emergency situations. The generation of data of sufficient quality requires that analytical laboratories: (1) have appropriately trained and proficient personnel; (2) acquire and maintain required supplies, equipment and reagents; (3) conduct the appropriate QC procedures to ensure that all measurement systems are in control and operating properly; (4) properly document all analytical results; (5) properly document analytical QC procedures and corrective actions; and (6) maintain personnel training and proficiency testing records.[9]

The level or amount of QC needed depends on the intended purpose of the data generated. Various levels of QC may be required if the data are generated for presence/absence determinations versus quantitative results. Specific data needs should be identified and QC requirements, based on those needs, applied consistently across laboratories when multiple laboratories are used. The individual methods listed, sampling and analytical protocols or contractual statements of work should be consulted to determine if additional QC procedures are required.

Method-specific QC requirements are described in many of the methods cited in this manual and will be included in protocols developed to address specific pathogen/sample type combinations of concern. In general, analytical QC requirements for pathogen methods include an initial demonstration of measurement system capability, as well as the capability of the laboratory and the analyst to perform the method with the required precision and accuracy. In addition, for molecular techniques (e.g., PCR) general guidelines are provided in EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples*.

Ongoing analysis of control samples to ensure the continued reliability of the analytical results should also be performed. At a minimum, the following QC analyses should be conducted on an ongoing basis:

- Media and reagent sterility checks
- Positive and negative controls
- Method blanks

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

- Reference matrix spikes to evaluate initial and ongoing method/analyst performance, if available
- Matrix spikes (where possible) to evaluate method performance in the sample type of interest
- Matrix spike duplicates (MSD) and/or sample replicates to assess method precision
- Instrument calibration checks and temperature controls

QC procedures and proper maintenance of ancillary laboratory equipment (e.g., thermometers, autoclaves) should be performed as frequently as necessary to ensure the reliability of analytical results.

***Please note*:** The appropriate points of contact identified in Section 4 should be consulted regarding appropriate quality assurance (QA)/QC procedures prior to sample analysis. These contacts should be consulted regarding any method deviations or modifications, sample problems or interferences, QC requirements, the use of alternative methods, or the need to address analytes or sample types other than those listed in SAM. As previously indicated, any deviations from the recommended method(s) should be reported immediately to ensure data comparability is maintained. Method deviations or modifications must be approved by the Environmental Response Laboratory Network (ERLN) or Water Laboratory Alliance (WLA) prior to use.

7.1.3 Safety and Waste Management

Laboratories should have a documented health and safety plan for handling samples that might contain target chemical, biological and/or radiological (CBR) contaminants. Laboratory staff should be trained in the safety procedures included in the plan and implement those procedures. Pathogens in samples taken from areas contaminated as the result of a homeland security event may be more hazardous than naturally occurring pathogens of the same genus and species. The pathogens may have been manufactured, engineered or treated to enhance dispersion or virulence characteristics. Laboratories should carefully consider implementing additional safety measures before agreeing to accept these samples.

In addition, many of the methods listed in Appendix C and summarized or cited in Sections 7.2 through 7.5 contain specific requirements, guidelines, or information regarding safety precautions that should be followed when handling or processing environmental samples and reagents. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. BSL-3 is applicable when performing manipulations of indigenous or exotic agents that can cause serious or potentially lethal disease and also have the potential for aerosol transmission. Whenever available, BSLs are provided in the method summaries in Sections 7.2 through 7.5. However, some pathogens that are normally handled at BSL-2 may require BSL-3 procedures and facilities if large volumes, high concentrations or potential aerosols are expected as a part of the analytical process. For more information on BSL practices and procedures, the following references should be consulted:

- CDC. 2009. *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 5th Edition. Available at: http://www.cdc.gov/biosafety/publications/bmbl5/
- CDC.2002. "Laboratory Security and Emergency Response Guidance for Laboratories Working with Select Agents," *Morbidity and Mortality Weekly Report*, Vol. 51, No. RR-19, 1 – 6, December 6, 2002. Available at: http://www.cdc.gov/mmwr/pdf/rr/rr5119.pdf
- Microbiology Biosafety for Level A Laboratories. Available at: http://www.bt.cdc.gov/documents/PPTResponse/table3bbiosafety.pdf
- Select Agent Rules and Regulations found at the National Select Agent Registry. Available at: http://www.selectagents.gov/ and http://www.selectagents.gov/regulations.html

The following sources provide information regarding waste management:

- EPA – Hazardous Waste Management (40 CFR part 260) and EPA Administered Permit Programs (40 CFR part 270). Available at: http://www.ecfr.gov/
- EPA. 2010. Laboratory Environmental Sample Disposal Information Document Companion to Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events (SAM) Revision 5. EPA/600/R-10/092. Available at: http://www2.epa.gov/sites/production/files/2015-06/documents/lesdid.pdf

Other resources that can be consulted for additional information include the following:

- OSHA – Hazardous Waste Operations and Emergency Response (29 CFR part 1910.120). Available at: http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9765
- OSHA – Occupational Exposure to Hazardous Chemicals in Laboratories (29 CFR part 1910.1450). Available at: http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10106
- OSHA – Respiratory Protection (29 CFR part 1910.134). Available at: http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=12716&p_table=STANDARDS
- DOT Hazardous Materials Shipment and Packaging (49 CFR parts 171–180). Available at: http://www.ecfr.gov/cgi-bin/text-idx?gp=&SID=994b04d45ee6d584ce676138929280b3&mc=true&tpl=/ecfrbrowse/Title49/49tab_02.tpl

7.1.4 Laboratory Response Network (LRN)

The LRN was created in accordance with the 1995 Presidential Directive 39, Policy on Counterterrorism, which established terrorism preparedness responsibilities for federal agencies. The LRN is primarily a national network of local, state, federal, military, food, agricultural, veterinary and environmental laboratories; however, additional LRN laboratories are located in strategic international locations. The CDC provides technical and scientific support to member laboratories as well as secure access to standardized procedures and reagents for rapid (4 – 6 hours) presumptive detection of biothreat agents and emerging infectious disease agents. The algorithm for a confirmed result is often a combination of one or more presumptive positive results from a rapid assay in combination with a positive result from one of the "gold standard" methods, such as culture. The standardized procedures, reagents and agent-specific algorithms are considered to be sensitive and are available only to LRN member laboratories. Thus, these procedures are not available to the general public and are not discussed in this document.

It is important to note that, in some cases, the procedures may not be fully developed or validated for each environmental sample type/pathogen combination listed in Appendix C, nor are all LRN member laboratories necessarily capable of analyzing all of the sample type/pathogen combinations. Except for *Coxiella burnetii*, culture methods are available for all of these pathogens as American Society for Microbiology's (ASM) Sentinel Laboratory Guidelines (http://www.asm.org/index.php?option=com_content&view=article&id=6342).

The agents identified below and listed in Appendix C are included in the U.S. Health and Human Services (HHS)/U.S. Department of Agriculture (USDA) select agent list and should be analyzed in accordance with appropriate regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121) and safety and BSL requirements (see CDC's BMBL, 5th Edition, http://www.cdc.gov/biosafety/publications/bmbl5/).

Select Agents Listed in Appendix C

Pathogen(s) [Disease]	Agent Category
Bacillus anthracis [Anthrax]	Bacteria
Brucella spp. [Brucellosis]	Bacteria
Burkholderia mallei [Glanders]	Bacteria
Burkholderia pseudomallei [Melioidosis]	Bacteria
Coxiella burnetii [Q-fever]	Bacteria
Francisella tularensis [Tularemia]	Bacteria
Yersinia pestis [Plague]	Bacteria

For additional information on the LRN, including selection of a laboratory capable of receiving and processing the specified sample type/pathogen, please use the contact information provided below or visit http://www.bt.cdc.gov/lrn/.

Centers for Disease Control and Prevention
Laboratory Response Branch
Division of Bioterrorism Preparedness and Response (DBPR)
National Center for the Prevention, Detection, and Control of Infectious Diseases (NCPDCID)
Coordinating Center for Infectious Diseases (CCID)
CDC
1600 Clifton Road NE, Mailstop C-18
Atlanta, GA 30333
Telephone: (866) 576-5227
E-mail: lrn@cdc.gov

Local public health laboratories, private laboratories and commercial laboratories with questions about the LRN should contact their state public health laboratory director or the Association of Public Health Laboratories (APHL) (contact information provided below).

Association of Public Health Laboratories
8515 Georgia Avenue, Suite 700
Silver Spring, MD 20910
Telephone: (240) 485-2745
Fax: (240) 485-2700
Website: http://www.aphl.org
E-mail: info@aphl.org

The following references and information sources provide additional information regarding Select Agents Culture Methods – LRN Sentinel Labs (website references for individual pathogens are included in their respective summaries):

- Avian Influenzae: http://www.asm.org/images/pdf/Clinical/Protocols/avianiinfluenza11-2008.pdf
- *Brucella*: http://www2.epa.gov/sites/production/files/2015-07/documents/asm-brucella.pdf
- *Burkholderia mallei and B. pseudomallei*: http://www.asm.org/images/pdf/Clinical/Protocols/bpseudomallei2008.pdf
- *Coxiella burnetii*: http://www2.epa.gov/sites/production/files/2015-07/documents/asm-cburnetti.pdf
- *Fransicella tularensis*: http://www.asm.org/images/pdf/Clinical/Protocols/tularemia.pdf

- *Yersinia pestis*: http://www2.epa.gov/sites/production/files/2015-07/documents/asm-ypestis.pdf

Sources:

ASM. 2010. Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases.
http://www.asm.org/index.php?option=com_content&view=article&id=6342

CDC. 2009. *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 5th Edition.
http://www.cdc.gov/biosafety/publications/bmbl5/

7.2 Method Summaries for Bacteria

Summaries of the analytical methods for bacteria listed in Appendix C are provided in Sections 7.2.1 through 7.2.16.

7.2.1 *Bacillus anthracis* [Anthrax] – BSL-3

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.2.1.1
Post Decontamination	RV-PCR	7.2.1.2
	Culture and Real-Time PCR	7.2.1.3

7.2.1.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (U.S. EPA, anticipated publication October 2012), referred to as the EPA BA Protocol for the remainder of SAM.

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).
Analytical Technique: Use real-time PCR [EPA BA Protocol (U.S. EPA, anticipated publication October 2012)].

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA

Protocol, Sections 9 and 11), and analyzed using the referenced target-specific PCR primers and probes and assay parameters. The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *B. anthracis* spores.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: *Bacillus anthracis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed [see CDC's BMBL, 5th Edition (CDC 2009)]. http://www.cdc.gov/biosafety/publications/bmbl5/).

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

7.2.1.2 Post Decontamination Sample Analyses (RV-PCR)

Note: Laboratories without RV-PCR capability should analyze samples according to the culture procedure provided in Section 7.2.1.3.

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).
Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use RV-PCR [EPA BA Protocol (U.S. EPA, anticipated publication October 2012)].

Description of Method: The RV-PCR procedure is a combination of a broth culture and real-time PCR. Culturing the sample allows the germination of *Bacillus anthracis* spores recovered from a processed sample. The real-time PCR provides rapid detection of *Bacillus anthracis*. By combining both culture and PCR the protocol allows for the detection of viable *Bacillus anthracis* spores. Prior to analysis, samples (air filter, wipe, Sponge-Stick, vacuum socks or filter, water) are processed using multiple extraction and wash steps. After brain heart infusion broth is added to the spores, an aliquot (Time 0 [T0]) is removed and stored at 4 °C. The remaining broth is then incubated for 9 hours at 36 °C. After the 9-hour incubation, an aliquot is removed (Time 9 [T9]). Both T0 and T9 aliquots then go through DNA extraction and purification followed by real-time PCR analysis. The cycle threshold (CT) values for the T0 and T9 aliquots are then compared. The difference in CT values between the T0 and T9 is used to detect viable *Bacillus anthracis* spores. A change (decrease) in the PCR CT ≥ 9 represents increased DNA amplification in the T9 aliquot relative to the T0 aliquot, which in turn, represents an increase in DNA as a result of the germination and growth of viable spores in the sample during the incubation period.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: *Bacillus anthracis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed [see BMBL, 5th Edition (CDC 2009)]. http://www.cdc.gov/biosafety/publications/bmbl5/).

Some laboratories may not have access to a positive control for this agent for culture analyses.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

7.2.1.3 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use culture and real-time PCR [EPA Protocol (U.S. EPA, anticipated publication October 2012)].

Description of Method: The culture procedure is based on the CDC Sentinel Procedure for *Bacillus anthracis.* Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the sample is streaked for isolation onto tryptic soy agar with 5% sheep's blood. Plates are incubated at 35 °C – 37 °C for 18 – 24 hours. Isolated typical colonies are resuspended in sterile distilled water. The bacterial suspensions are then heated at 95 °C – 98 °C to release the DNA from the cells (see Section 11). DNA extracts are then used in real-time PCR to confirm the presence of *Bacillus anthracis*. Combining the culture component with confirmation using real-time PCR analyses allows for detection and viability results within 24 – 30 hours as compared to traditional culture procedures that require a minimum of 48 hours.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: *Bacillus anthracis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should be followed [see BMBL, 5th Edition (CDC 2009)]
http://www.cdc.gov/biosafety/publications/bmbl5

Some laboratories may not have access to a positive control for this agent for culture analyses.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

7.2.2 *Brucella* spp. [Brucellosis] – BSL-3

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.2.2.1
Post Decontamination	Real-Time PCR/Immunoassay	7.1.4 [1]
	Culture and Real-Time PCR	7.2.2.2

[1] Standardized procedures, reagents and agent-specific algorithms are available to LRN member laboratories.

7.2.2.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011). All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for *Brucella* spp. [Journal of Microbiological Methods, 2008, 75(2): 375–378]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Microbiological Methods, 2008, 75(2): 375–378). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Brucella* spp.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: *Brucella* spp. are select agents requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed [see BMBL, 5th Edition (CDC 2009)].
http://www.cdc.gov/biosafety/publications/bmbl5/

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Hinić, V., Brodard, I., Thomann,A., Cvetnić, Ž., Makaya, P.V., Frey, J. and Abril, C. 2008. "Novel Identification and Differentiation of *Brucella melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* Suitable for Both Conventional and Real-time PCR Systems." Journal of Microbiological Methods, 75(2): 375–378.
http://www.sciencedirect.com/science/article/pii/S0167701208002522

7.2.2.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use culture ("Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species") and real-time PCR (Literature reference for *Brucella* spp. [Journal of Microbiological Methods, 2008 75(2): 375–378]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are plated directly on selective and non-selective agars and incubated at 35 °C (5–10% carbon dioxide) for up to 7 days. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Microbiological Methods, 2008 75(2): 375–378). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Brucella* spp.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: *Brucella* spp. are select agents requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also

be followed [see BMBL, 5th Edition (CDC 2009)].
http://www.cdc.gov/biosafety/publications/bmbl5/

Some laboratories may not have access to a positive control for this agent for culture analyses.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

ASM. 2004. "Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species."
http://www2.epa.gov/sites/production/files/2015-07/documents/asm-brucella.pdf

Hinić, V., Brodard, I., Thomann, A., Cvetnić, Ž., Makaya, P.V., Frey, J. and Abril, C. 2008. "Novel Identification and Differentiation of *Brucella melitensis*, *B. abortus*, *B. suis*, *B. ovis*, *B. canis*, and *B. neotomae* Suitable for Both Conventional and Real-Time PCR Systems." Journal of Microbiological Methods, 75(2): 375–378.
http://www.sciencedirect.com/science/article/pii/S0167701208002522

7.2.3 *Burkholderia mallei* [Glanders] – BSL-3 and *Burkholderia pseudomallei* [Melioidosis] – BSL-3

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.2.3.1
Post Decontamination	Real-Time PCR/Immunoassay	7.1.4 [1]
	Culture and Real-Time PCR	7.2.3.2

[1] Standardized procedures, reagents and agent-specific algorithms are available to LRN member laboratories.

7.2.3.1 Site Characterization Sample Analysis (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to

develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.

Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for *Burkholderia mallei* [Clinical Chemistry, 2006, 52(2): 307–310]; Literature reference for *Burkholderia pseudomallei* [Journal of Clinical Microbiology, 2006, 44(1): 85–90 and 2006, 44(8): 3028–3030]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Clinical Chemistry, 2006, 52(2): 307–310; Journal of Clinical Microbiology, 2006, 44(1): 85–90 and 2006, 44(8): 3028–3030). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Burkholderia mallei* and *Burkholderia pseudomallei*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: *Burkholderia mallei* and *Burkholderia pseudomallei* are select agents requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed [see BMBL, 5th Edition (CDC 2009)]. http://www.cdc.gov/biosafety/publications/bmbl5/

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.

http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Tomaso, H., Scholz, H.C., Al Dahouk, S., Eickhoff, M., Treu, T.M., Wernery, R., Wernery, U. and Neubauer, H. 2006. "Development of a 5'-Nuclease Real-Time PCR Assay Targeting fliP for the Rapid Identification of *Burkholderia mallei* in Clinical Samples." Clinical Chemistry, 52(2): 307–310. http://www.clinchem.org/content/52/2/307.full.pdf+html

Novak, R.T., Glass, M.B., Gee, J.E., Gal, D., Mayo, M.J., Currie, B.J. and Wilkins, P.P. 2006. "Development and Evaluation of a Real-Time PCR Assay Targeting the Type III Secretion System of *Burkholderia pseudomallei*." Journal of Clinical Microbiology, 44(1): 85–90. http://jcm.asm.org/content/44/1/85.full.pdf+html

Meumann, E.M., Novak, R.T., Gal, D., Kaestli, M.E., Mayo, M., Hanson,J.P., Spencer, E., Glass, M.B., Gee, J. E., Wilkins, P. P. and Currie, B.J. 2006. "Clinical Evaluation of a Type III Secretion System Real-Time PCR Assay for Diagnosing Melioidosis." Journal of Clinical Microbiology, 44(8): 3028–3030. http://jcm.asm.org/content/44/8/3028.full.pdf+html

7.2.3.2 Post Decontamination (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use culture (Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*) and real-time PCR (Literature reference for *Burkholderia mallei* [Clinical Chemistry, 2006, 52(2): 307–310]; Literature reference for

Burkholderia pseudomallei [Journal of Clinical Microbiology, 2006, 44(1): 85–90 and 2006, 44(8): 3028–3030]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are plated directly on sheep blood agar and incubated at 35 °C – 37 °C for 48 hours. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Clinical Chemistry, 2006, 52(2): 307–310; Journal of Clinical Microbiology, 2006, 44(1): 85–90 and 2006, 44(8): 3028–3030). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Burkholderia mallei* and *Burkholderia pseudomallei*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: *Burkholderia mallei* and *Burkholderia pseudomallei* are select agents requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed [see BMBL, 5th Edition (CDC 2009)]. http://www.cdc.gov/biosafety/publications/bmbl5/

Some laboratories may not have access to a positive control for this agent for culture analyses.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

ASM. 2008. "Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*."
http://www.asm.org/images/pdf/Clinical/Protocols/bpseudomallei2008.pdf

Tomaso, H., Scholz, H.C., Al Dahouk, S., Eickhoff, M., Treu, T.M., Wernery, R., Wernery,U. and Neubauer, H. 2006. "Development of a 5'-Nuclease Real-Time PCR Assay Targeting fliP for the Rapid Identification of *Burkholderia mallei* in Clinical Samples." Clinical Chemistry, 52(2): 307–310. http://www.clinchem.org/content/52/2/307.full.pdf+html

Novak, R.T., Glass, M.B., Gee, J.E., Gal, D., Mayo, M.J., Currie, B.J. and Wilkins, P.P. 2006. "Development and Evaluation of a Real-Time PCR Assay Targeting the Type III Secretion System of *Burkholderia pseudomallei*." Journal of Clinical Microbiology, 44(1):85–90. http://jcm.asm.org/content/44/1/85.full.pdf+html

Meumann, E.M., Novak, R.T., Gal, D., Kaestli, M.E., Mayo, M., Hanson,J.P., Spencer, E., Glass, M.B., Gee, J. E., Wilkins, P. P. and Currie, B.J. 2006. "Clinical Evaluation of a Type III Secretion System Real-Time PCR Assay for Diagnosing Melioidosis." Journal of Clinical Microbiology, 44(8): 3028–3030. http://jcm.asm.org/content/44/8/3028.full.pdf+html

7.2.4 *Campylobacter jejuni* [Campylobacteriosis] – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.2.4.1
Post Decontamination	Culture and Real-Time PCR	7.2.4.2

7.2.4.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for *Campylobacter jejuni* [Journal of Clinical Microbiology, 2010, 48(8): 2929–2933]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Clinical Microbiology, 2010, 48(8): 2929–2933). The use of

real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Campylobacter jejuni*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Cunningham, S.A., Sloan, L.M., Nyre, L.M., Vetter, E.A., Mandrekar, J. and Patel, R. 2010. "Three-Hour Molecular Detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* Species in Feces With Accuracy as High as That of Culture." Journal of Clinical Microbiology, 48(8): 2929–2933. http://jcm.asm.org/content/48/8/2929.full.pdf+html

7.2.4.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).
Analytical Technique: Use culture ["ISO 17795: Water quality – Detection and enumeration of thermotolerant *Campylobacter* species" (ISO 2005)] and real-time PCR (Literature reference for *Campylobacter jejuni* [Journal of Clinical Microbiology, 2010, 48(8): 2929-2933]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are inoculated into broth media and incubated, and then plated onto selective agar. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Clinical Microbiology, 2010, 48(8): 2929–2933). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Campylobacter jejuni*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

ISO. 2005. ISO 17795: Water quality – Detection and Enumeration of Thermotolerant *Campylobacter* Species, 2005.
http://www.iso.org/iso/iso_catalogue/catalogue_tc/catalogue_detail.htm?csnumber=42082

Cunningham, S.A., Sloan, L.M., Nyre, L.M., Vetter, E.A., Mandrekar, J. and Patel, R. 2010. "Three-Hour Molecular Detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* Species in Feces With Accuracy as High as That of Culture." Journal of Clinical Microbiology, 48(8): 2929–2933. http://jcm.asm.org/content/48/8/2929.full.pdf+html

7.2.5 *Chlamydophila psittaci* [Psittacosis] (formerly known as *Chlamydia psittaci*) – BSL-2; BSL-3 for Aerosols and Large Volumes

Remediation Phase	Analytical Technique	Section
Site Characterization	PCR	7.2.5.1
Post Decontamination	Tissue Culture and PCR	7.2.5.2

7.2.5.1 Site Characterization Sample Analyses (PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for *Chlamydophila psittaci* [Journal of Clinical Microbiology, 2000, 38(3): 1085–1093]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Clinical Microbiology, 2000, 38(3): 1085–1093). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Chlamydophila psittaci*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Madico, G., Quinn, T.C., Boman, J. and Gaydos, C.A. 2000. "Touchdown Enzyme Time Release-PCR for Detection and Identification of *Chlamydia trachomatis, C. pneumoniae, and C. psittaci* Using the 16S and 16S-23S Spacer rRNA Genes." Journal of Clinical Microbiology, 38(3): 1085–1093. http://jcm.asm.org/content/38/3/1085.full.pdf+html

7.2.5.2 Post Decontamination Sample Analyses (Tissue Culture and PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Analytical Technique: Use tissue culture and real-time PCR (Literature Reference for *Chlamydophila psittaci* [Journal of Clinical Microbiology, 2000, 38(3): 1085–1093]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are inoculated onto buffalo green monkey kidney (BGMK) cells to increase sensitivity. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Clinical Microbiology, 2000, 38(3): 1085–1093). The use of

real-time PCR analyses directly on isolates allows for rapid confirmation of *Chlamydophila psittaci*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Madico, G., Quinn, T.C., Boman, J. and Gaydos, C.A. 2000. "Touchdown Enzyme Time Release-PCR for Detection and Identification of *Chlamydia trachomatis, C. pneumoniae, and C. psittaci* Using the 16S and 16S-23S Spacer rRNA Genes." Journal of Clinical Microbiology, 38(3): 1085–1093. http://jcm.asm.org/content/38/3/1085.full.pdf+html

7.2.6 *Coxiella burnetii* [Q-fever] – BSL- 3

Remediation Phase	Analytical Technique	Section
Site Characterizaion	Real-Time PCR	7.2.6.1
Post Decontamination	Real-Time PCR/Immunoassay	7.1.4 [1]
	Tissue Culture and Real-Time PCR	7.2.6.2

[1] Standardized procedures, reagents and agent-specific algorithms are available to LRN member laboratories.

7.2.6.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to

develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.

Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for *Coxiella burnetii* [BMC Microbiology, 2008, 8:77]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (BMC Microbiology, 2008, 8:77). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Coxiella burnetii*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: *Coxiella burnetii* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed [see BMBL, 5th Edition (CDC 2009)].
http://www.cdc.gov/biosafety/publications/bmbl5/

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Panning, M., Kilwinski, J., Greiner-Fischer, S., Peters, M., Kramme, S., Frangoulidis, D., Meyer, H., Henning, K. and Drosten, C. 2008. "High Throughput Detection of *Coxiella burnetii* by Real-Time PCR With Internal Control System and Automated DNA Preparation." BMC Microbiology, 8:77. http://www.biomedcentral.com/1471-2180/8/77

7.2.6.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].
Analytical Technique: Use tissue culture (Literature reference for *Coxiella burnetii* [Antimicrobial Agents and Chemotherapy, 1991, 35(10): 2070–2077]) and real-time PCR (Literature reference for *Coxiella burnetii* [BMC Microbiology, 2008, 8:77]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are inoculated onto human erythroleukemia cells. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (BMC Microbiology, 2008, 8:77). The use of real-time PCR analyses directly on isolates allows for rapid confirmation of *Coxiella burnetii*.
At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: *Coxiella burnetii* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also

be followed [see BMBL, 5th Edition (CDC 2009)].
http://www.cdc.gov/biosafety/publications/bmbl5/

Some laboratories may not have access to a positive control for this agent for culture analyses.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Raoult, D. Torres, H. and Drancourt, M. 1991. "Shell-Vial Assay: Evaluation of a New Technique for Determining Antibiotic Susceptibility, Tested in 13 Isolates of *Coxiella burnetii*" Antimicrobial Agents and Chemotherapy, 35(10): 2070–2077.
http://aac.asm.org/content/35/10/2070.long

Panning, M., Kilwinski, J., Greiner-Fischer, S., Peters, M., Kramme, S., Frangoulidis, D., Meyer, H., Henning, K. and Drosten, C. 2008. "High Throughput Detection of *Coxiella burnetii* by Real-Time PCR With Internal Control System and Automated DNA Preparation." BMC Microbiology. 8:77. http://www.biomedcentral.com/1471-2180/8/77

7.2.7 *Escherichia coli* O157:H7 – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.2.7.1
Post Decontamination	Culture and Real-Time PCR	7.2.7.2

7.2.7.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus*

anthracis Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "EPA Standard Analytical Protocol for *Escherichia coli* O157:H7 in Water," EPA/600/R-10/056 (U.S. EPA 2010).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for *E. coli* O157:H7 [Environmental Science and Technology, 2011, 45(7): 2250–2256]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Environmental Science and Technology, 2011, 45(7): 2250–2256). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *E. coli* O157:H7.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. September 2010. "Standard Analytical Protocol for *Escherichia coli* O157:H7 in Water." EPA/600/R-10/056.
http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=498725

Sen, K., Sinclair, J.L., Boczek, L. and Rice, E.W. 2011. "Development of a Sensitive Detection Method for Stressed *E. coli* O157:H7 in Source and Finished Drinking Water by Culture-qPCR." Environmental Science and Technology, 45(6): 2250–2256.
http://pubs.acs.org/doi/abs/10.1021/es103365b

7.2.7.2 Post Decontamination (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "EPA Standard Analytical Protocol for *Escherichia coli* O157:H7 in Water," EPA/600/R-10/056 (U.S. EPA 2010).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use culture [Standard Analytical Protocol for *Escherichia coli* O157:H7 in Water, EPA/600/R-10/056 (EPA 2010)] and real-time PCR (Literature reference for *E. coli* O157:H7 [Environmental Science and Technology, 2011, 45(7): 2250–2256]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are cultured using multiple media and immunomagnetic separation (IMS). Typical isolates are then confirmed using biochemical and serological tests. To expedite time to results, isolates should be confirmed using real-time PCR analyses. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Environmental Science and Technology, 2011, 45(7): 2250–2256). The use of real-time PCR analyses allows for rapid confirmation of *E. coli* O157:H7.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. September 2010. "Standard Analytical Protocol for *Escherichia coli* O157:H7 in Water." EPA/600/R-10/056.
http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=498725

Sen, K., Sinclair, J.L., Boczek, L. and Rice, E.W. 2011. "Development of a Sensitive Detection Method for Stressed *E. coli* O157:H7 in Source and Finished Drinking Water by Culture-qPCR." Environmental Science and Technology, 45(7): 2250–2256.
http://pubs.acs.org/doi/abs/10.1021/es103365b

7.2.8 *Francisella tularensis* [Tularemia] – BSL-3

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.2.8.1
Post Decontamination	Real-Time PCR /Immunoassay	7.1.4 [1]
	Culture and Real-Time PCR	7.2.8.2

[1] Standardized procedures, reagents and agent-specific algorithms are available to LRN member laboratories.

7.2.8.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for *Francisella tularensis* [Journal of Clinical Microbiology, 2003, 41(12): 5492–5499]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Clinical Microbiology, 2003, 41(12): 5492–5499). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Francisella tularensis*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: *Francisella tularensis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed [see BMBL, 5th Edition (CDC 2009)]. http://www.cdc.gov/biosafety/publications/bmbl5/

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Versage, J., Severin, D.D.M., Chu, M.C. and Petersen, J.M. 2003. "Development of a Multitarget Real-Time TaqMan PCR Assay for Enhanced Detection of *Francisella tularensis* in Complex Specimens." Journal of Clinical Microbiology, 41(12): 5492–5499. http://jcm.asm.org/content/41/12/5492.full.pdf+html

7.2.8.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) and "Use of Acid Treatment and a Selective Medium to Enhance the Recovery of *Francisella tularensis* from Water" (Humrighouse *et al*. 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use **c**ulture ("Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Francisella tularensis*" CDC *et al.* 2001) and real-time PCR (Literature reference for *Francisella tularensis* [Journal of Clinical Microbiology, 2003, 41(12): 5492–5499]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are plated directly onto selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Clinical Microbiology, 2003, 41(12): 5492–5499). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Francisella tularensis*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: *Francisella tularensis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL

requirements should also be followed [see BMBL, 5th Edition (CDC 2009)].
http://www.cdc.gov/biosafety/publications/bmbl5/

Some laboratories may not have access to a positive control for this agent for culture analyses.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Humrighouse, B.W., Adcock, N.J. and Rice, E.W., 2011. "Use of Acid Treatment and a Selective Medium to Enhance the Recovery of *Francisella tularensis* from Water." Applied and Environmental Microbiology, 77(18): 6729–6732.
http://aem.asm.org/content/77/18/6729.full.pdf+html

CDC, ASM, and APHL. 2001. "Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Francisella tularensis*."
http://www.asm.org/images/pdf/Clinical/Protocols/tularemia.pdf

Versage, J.L., Severin, D.D, Chu, M.C. and Petersen, J.M. 2003. "Development of a Multitarget Real-Time TaqMan PCR Assay for Enhanced Detection of *Francisella tularensis* in Complex Specimens." Journal of Clinical Microbiology, 41(12): 5492–5499.
http://jcm.asm.org/content/41/12/5492.full.pdf+html

7.2.9 *Leptospira interrogans* [Leptospirosis] – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.2.9.1
Post Decontamination	Culture and Real-Time PCR	7.2.9.2

7.2.9.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to

develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.

Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for *Leptospira interrogans* [Molecular and Cellular Probes, 2005, 19(2): 111–117]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Molecular and Cellular Probes, 2005, 19(2): 111-117). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Leptospira interrogans*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Palaniappan, R.U.M., Chang, Y.F., Chang, C., Pan, M.J., Yang, C.W., Harpending, P., McDonough, S.P., Dubovi, E., Divers, T., Qu, J. and Roe, B. 2005. "Evaluation of Lig-based Conventional and Real Time PCR for the Detection of Pathogenic Leptospires." Molecular and Cellular Probes, 19(2): 111–117.
http://www.sciencedirect.com/science/article/pii/S0890850804000970

7.2.9.2 Post Decontamination Sample Analyses (Culture and Real-time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use culture (Standard Method 9260 I: *Leptospira*) and real-time PCR (Literature reference for *Leptospira interrogans* [Molecular and Cellular Probes, 2005, 19(2): 111–117]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are inoculated into selective broth media and incubated for up to six weeks at 30 °C. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Molecular and Cellular Probes, 2005, 19(2): 111–117). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Leptospira interrogans*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

APHA, AWWA and WEF. 2005. "Method 9260 I: *Leptospira*." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

Palaniappan, R.U.M., Chang, Y.F., Chang, C., Pan, M.J., Yang, C.W., Harpending, P., McDonough, S.P., Dubovi, E., Divers, T., Qu, J. and Roe, B. 2005. "Evaluation of Lig-based Conventional and Real Time PCR for the Detection of Pathogenic Leptospires." Molecular and Cellular Probes, 19(2): 111–117.
http://www.sciencedirect.com/science/article/pii/S0890850804000970

7.2.10 *Listeria monocytogenes* [Listeriosis] – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.2.10.1
Post Decontamination	Culture and Real-Time PCR	7.2.10.2

7.2.10.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR [Microbiology Laboratory Guidebook – Chapter MLG 8A.04 (USDA FSIS 2009)].

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Microbiology Laboratory Guidebook – Chapter MLG 8A.04, USDA, FSIS, 2009). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Listeria monocytogenes*.
At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

USDA, FSIS. 2009. "FSIS Procedure for the Use of a *Listeria monocytogenes* Polymerase Chain Reaction (PCR) Screening Test." Chapter MLG 8A.04 in *Microbiology Laboratory Guidebook*.
http://www2.epa.gov/sites/production/files/2015-07/documents/usda-mlg-8a.03.pdf

7.2.10.2 Post Decontamination Sample Analyses (Culture and Real-time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to

develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.

Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use culture [BAM–Chapter 10 (FDA CFSAN 2003)] and real-time PCR [Microbiology Laboratory Guidebook – Chapter MLG 8A.04 (USDA FSIS 2007)].

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are inoculated into broth media, incubated for 48 hours, and then plated onto selective agar. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Microbiology Laboratory Guidebook – Chapter MLG 8A.04 USDA, FSIS 2007). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Listeria monocytogenes*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Hitchins, A.D. and Jinneman, K. FDA, CFSAN. 2003. "Chapter 10 – Detection and Enumeration of Listeria monocytogenes in Foods." *Bacteriological Analytical Manual Online.* http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm071400.htm

USDA, FSIS. 2009. "FSIS Procedure for the Use of a *Listeria monocytogenes* Polymerase Chain Reaction (PCR) Screening Test." Chapter MLG 8A.04 in *Microbiology Laboratory Guidebook*. http://www2.epa.gov/sites/production/files/2015-07/documents/usda-mlg-8a.03.pdf

7.2.11 Non-typhoidal *Salmonella* (Not applicable to *S.* Typhi) [Salmonellosis] – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.2.11.1
Post Decontamination	Culture and Real-Time PCR	7.2.11.2

7.2.11.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Analytical Protocol for Non-Typhoidal *Salmonella* in Drinking Water and Surface Water" (U.S. EPA 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for non-typhoidal *Salmonella* [Environmental Science and Technology, 2011, 45(20): 8996–9002]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Environmental Science and Technology, 2011, 45(20): 8996–9002). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of non-typhoidal *Salmonella*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. June 2011. "Method 1200: Analytical Protocol for Non-Typhoidal *Salmonella* in Drinking Water and Surface Water."
http://owpubauthor.epa.gov/infrastructure/watersecurity/wla/upload/epa817r12004.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Jyoti, A., Vajpayee, P., Singh, G., Patel, C.B., Gupta, K.C. and Shanker, R. 2011. "Identification of Environmental Reservoirs of Nontyphoidal Salmonellosis: Aptamer-Assisted Bioconcentration and Subsequent Detection of *Salmonella Typhimurium* by Quantitative Polymerase Chain Reaction." Environmental Science and Technology, 45(20): 8996–9002.
http://pubs.acs.org/doi/abs/10.1021/es2018994

7.2.11.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1200: Analytical Protocol for Non-Typhoidal *Salmonella* in Drinking Water and Surface Water" (U.S. EPA 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use culture [Method 1682 (U.S. EPA 2006)] or "Analytical Protocol for Non-Typhoidal *Salmonella* in Drinking Water and Surface Water" (U.S. EPA 2011) and real-time PCR (Literature Reference for Non-Typhoidal *Salmonella* [Environmental Science and Technology, 2011, 45(20): 8996–9002]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are inoculated into broth media, incubated for 24 hours, and then plated onto multiple selective agars. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Environmental Science and Technology. 45(20): 8996–9002). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of Non-Typhoidal *Salmonella*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: This method will not detect *Salmonella* Typhi. MSRV and the elevated incubation temperature (42 °C) are inhibitory for *S.* Typhi.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium." http://www2.epa.gov/sites/production/files/2015-07/documents/epa-1682.pdf

U.S. EPA. 2011. "Method 1200: Analytical Protocol for Non-Typhoidal *Salmonella* in Drinking Water and Surface Water." http://owpubauthor.epa.gov/infrastructure/watersecurity/wla/upload/epa817r12004.pdf

Jyoti, A., Vajpayee, P., Singh, G., Patel, C.B., Gupta, K.C. and Shanker, R. 2011. "Identification of Environmental Reservoirs of Nontyphoidal Salmonellosis: Aptamer-Assisted Bioconcentration and Subsequent Detection of *Salmonella Typhimurium* by Quantitative Polymerase Chain Reaction." Environmental Science and Technology, 45(20): 8996–9002. http://pubs.acs.org/doi/abs/10.1021/es2018994

7.2.12 *Salmonella* Typhi [Typhoid fever] – BSL-2; BSL-3 for Aerosol Release

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.2.12.1
Post Decontamination	Culture and Real-Time PCR	7.2.12.2

7.2.12.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Standard Analytical Protocol for *Salmonella* Typhi in Drinking Water" (U.S. EPA 2010).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (CDC Laboratory Assay).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (CDC Laboratory Assay). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Salmonella* Typhi.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2010. "Standard Analytical Protocol for *Salmonella* Typhi in Drinking Water." EPA 600/R-10/133. http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=499264

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

CDC Laboratory Assay. "Triplex PCR for Detection of *S.* Typhi Using SmartCycler®." Contact: Dr. Eija Trees, CDC.

7.2.12.2 Post Decontamination (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Standard Analytical Protocol for *Salmonella* Typhi in Drinking Water" (U.S. EPA 2010).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use culture ("Standard Analytical Protocol for *Salmonella* Typhi in Drinking Water," EPA 600/R-10/133 (U.S. EPA 2010) and real-time PCR (CDC Laboratory Assay).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are inoculated into broth media, incubated for 24 hours, and then inoculated and plated onto multiple selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (CDC Laboratory Assay). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Salmonella* Typhi.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: This method is not recommended for non-typhoidal *Salmonella*.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. 2010. "Standard Analytical Protocol for *Salmonella* Typhi in Drinking Water." EPA 600/R-10/133. http://oaspub.epa.gov/eims/eimscomm.getfile?p_download_id=499264
CDC Laboratory Assay. "Triplex PCR for Detection of *S.* Typhi Using SmartCycler®." Contact: Dr. Eija Trees, CDC.

7.2.13 *Shigella* spp. [Shigellosis] – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.2.13.1
Post Decontamination	Culture and Real-Time PCR	7.2.13.2

7.2.13.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for *Shigella* spp. [Journal of Clinical Microbiology, 2010, 48(8): 2929–2933]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Clinical Microbiology, 2010, 48(8): 2929–2933). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Shigella* spp.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Cunningham, S.A., Sloan, L.M., Nyre, L.M., Vetter, E.A., Mandrekar, J. and Patel, R. 2010. "Three-Hour Molecular Detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* Species in Feces With Accuracy as High as That of Culture." Journal of Clinical Microbiology. 48(8): 2929–2933. http://jcm.asm.org/content/48/8/2929.full.pdf+html

7.2.13.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use culture (Standard Method 9260 E: *Shigella*) and real-time PCR (Literature reference for *Shigella* spp. [Journal of Clinical Microbiology, 2010, 48(8): 2929–2933]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are inoculated into broth media, incubated for 24 hours, and then plated onto multiple selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Clinical Microbiology, 2010, 48(8): 2929–2933). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Shigella* spp.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

APHA, AWWA and WEF. 2005. "Method 9260 Detection of Pathogenic Bacteria E: *Shigella*." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition.
http://www.standardmethods.org/
Cunningham, S.A., Sloan, L.M., Nyre, L.M., Vetter, E.A., Mandrekar, J., Patel, R. 2010. "Three-Hour Molecular Detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* Species in Feces With Accuracy as High as That of Culture." Journal of Clinical Microbiology. 48(8): 2929–2933.
http://jcm.asm.org/content/48/8/2929.full.pdf+html

7.2.14 *Staphylococcus aureus* – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.2.14.1
Post Decontamination	Culture and Real-Time PCR	7.2.14.2

7.2.14.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for *Staphylococcus aureus* [Journal of Food Protection, 2007, 70(12): 2855–2859]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Food Protection, 2007, 70(12): 2855–2859). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Staphylococcus aureus*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Chiang, Y.C, Fan, C.M., Liao, W.W., Lin, C.K. and Tsen, H.Y. 2007. "Real-Time PCR Detection of *Staphylococcus aureus* in Milk and Meat Using New Primers Designed From the Heat Shock Protein Gene htrA Sequence." Journal of Food Protection, 70(12): 2855–2859.
http://www.ingentaconnect.com/content/iafp/jfp/2007/00000070/00000012/art00023

7.2.14.2 Post Decontamination Sample Analyses (Culture and Real-time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.

Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use culture (Standard Method 9213 B: *Staphylococcus aureus*) and real-time PCR (Literature reference for *Staphylococcus aureus* [Journal of Food Protection, 2007, 70(12): 2855–2859]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples are inoculated into broth media, incubated for 24 hours, and then plated onto selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Food Protection, 2007, 70(12): 2855–2859). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Staphylococcus aureus*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Chiang, Y.C, Fan, C.M., Liao, W.W., Lin, C.K. and Tsen, H.Y. 2007. "Real-Time PCR Detection of *Staphylococcus aureus* in Milk and Meat Using New Primers Designed From the Heat Shock Protein Gene htrA Sequence." Journal of Food Protection. 70(12): 2855–2859. http://www.ingentaconnect.com/content/iafp/jfp/2007/00000070/00000012/art00023

APHA, AWWA and WEF. 2005. "Method 9213 B: *Staphylococcus aureus*." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

7.2.15 *Vibrio cholerae* [Cholera] – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.2.15.1
Post Decontamination	Culture and Real-Time PCR	7.2.15.2

7.2.15.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a

Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Standard Analytical Protocol for *Vibrio cholerae* O1 and O139 in Drinking Water and Surface Water" (U.S. EPA 2010).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for *Vibrio cholerae* [(Journal of Microbiological Methods, 2007, 68(2): 254–259]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Microbiological Methods, 2007, 68(2): 254–259). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Vibrio cholerae*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2010. Standard Analytical Protocol for *Vibrio cholerae* O1 and O139 in Drinking Water and Surface Water.

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Blackstone, G.M., Nordstrom, J.L., Bowen, M.D., Meyer, R.F., Imbro, P. and DePaola, A. 2007. "Use of a Real Time PCR Assay for Detection of the ctxA Gene of *Vibrio cholerae* in an Environmental Survey of Mobile Bay." Journal of Microbiological Methods, 68(2): 254–259. http://www.sciencedirect.com/science/article/pii/S016770120600248X

7.2.15.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Standard Analytical Protocol for *Vibrio cholerae* O1 and O139 in Drinking Water and Surface Water" (U.S. EPA 2010).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use culture (Standard Analytical Protocol for *Vibrio cholerae* O1 and O139 in Drinking Water and Surface Water, U.S. EPA, 2010) and real-time PCR (Literature reference for *Vibrio cholerae* [(Journal of Microbiological Methods, 2007, 68(2): 254–259]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are inoculated into enrichment broth, incubated for 8 hours, and then plated onto selective media. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Microbiological Methods, 2007, 68(2): 254–259). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Vibrio cholerae*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-

04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. October 2010. "Standard Analytical Protocol for *Vibrio cholerae* O1 and O139 in Drinking Water and Surface Water." EPA 600/R-10/139.
http://nepis.epa.gov/Adobe/PDF/P100978K.pdf

Blackstone, G.M., Nordstrom, J.L., Bowen, M.D., Meyer, R.F., Imbro, P. and DePaola, A. 2007. "Use of a Real Time PCR Assay for Detection of the ctxA Gene of *Vibrio cholerae* in an Environmental Survey of Mobile Bay." Journal of Microbiological Methods 68(2): 254–259.
http://www.sciencedirect.com/science/article/pii/S016770120600248X

7.2.16 *Yersinia pestis* [Plague] – BSL-3

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.2.16.1
Post Decontamination	Real-Time PCR /Immunoassay	7.1.4 [1]
	Culture and Real-Time PCR	7.2.16.2

[1] Standardized procedures, reagents and agent-specific algorithms are available to LRN member laboratories.

7.2.16.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus*

anthracis Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).
Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for *Yersinia pestis* [Diagnostic Microbiology and Infectious Disease, 2006, 56(3): 261–268]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Diagnostic Microbiology and Infectious Disease, 2006, 56(3): 261–268). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Yersinia pestis*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control (purified nucleic acid), negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: *Yersinia pestis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed [see BMBL, 5th Edition (CDC 2009)].
http://www.cdc.gov/biosafety/publications/bmbl5/

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Woron, A.M., Nazarian, E.J., Egan, C., McDonough, K.A., Cirino, N.M., Limberger, R.J. and Musser, K.A. 2006. "Development and Evaluation of a 4-Target Multiplex Real-Time Polymerase Chain Reaction Assay for the Detection and Characterization of *Yersinia pestis*." Diagnostic Microbiology and Infectious Disease, 56(3): 261-268. http://www.dmidjournal.com/article/S0732-8893(06)00232-X/fulltext

7.2.16.2 Post Decontamination Sample Analyses (Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use culture ("Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Yersinia pestis,*" ASM, 2010) and real-time PCR (Literature reference for *Yersinia pestis* [Diagnostic Microbiology and Infectious Disease, 2006, 56(3): 261–268]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation procedures above), samples can be inoculated into enrichment broth prior to plating or plated directly on non-selective media and incubated for a minimum of three days. Confirmation is performed using real-time PCR. Target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Diagnostic Microbiology and Infectious Disease, 2006, 56(3): 261–268). The use of real-time PCR analyses directly on isolates (e.g., no biochemical/serological component) allows for rapid confirmation of *Yersinia pestis*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-

04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: *Yersinia pestis* is a select agent requiring regulatory compliance (42 CFR parts 72 and 73, and 9 CFR part 121); appropriate safety and BSL requirements should also be followed [see BMBL, 5th Edition (CDC 2009)].
http://www.cdc.gov/biosafety/publications/bmbl5/

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

ASM. 2010. "Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Yersinia pestis*."
http://www.asm.org/images/pdf/Clinical/Protocols/ypestis06-11-10.pdf

Woron, A.M., Nazarian, E.J., Egan, C., McDonough, K.A., Cirino, N.M., Limberger, R.J. and Musser, K.A. 2006. "Development and Evaluation of a 4-Target Multiplex Real-Time Polymerase Chain Reaction Assay for the Detection and Characterization of *Yersinia pestis*." Diagnostic Microbiology and Infectious Disease, 56(3): 261–268.
http://www.dmidjournal.com/article/S0732-8893(06)00232-X/fulltext

7.3 Method Summaries for Viruses

Summaries of the analytical methods for viruses listed in Appendix C are provided in Sections 7.3.1 through 7.3.10.

7.3.1 Adenoviruses: Enteric and Non-enteric (A-F) – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.3.1.1
Post Decontamination	Tissue Culture and Real-Time PCR	7.3.1.2

7.3.1.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012). ***Note***: Since the concentration of adenovirus using the Nanoceram® filter has not been optimized, use of the 1MDS filter is recommended.

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for adenoviruses [Applied and Environmental Microbiology, 2005, 71(6): 3131–3136]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific real-time PCR primers and probes and assay parameters (Applied and Environmental Microbiology, 2005, 71(6): 3131–3136). The use of real-time PCR analyses directly on samples (e.g., no tissue culture component) allows for rapid detection of adenoviruses.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Jothikumar, N., Cromeans, T.L., Hill, V.R., Lu, X., Sobsey, M.D. and Erdman, D.D. 2005. "Quantitative Real-Time PCR Assays for Detection of Human Adenoviruses and Identification of Serotypes 40 and 41." Applied and Environmental Microbiology, 71(6): 3131–3136. http://aem.asm.org/content/71/6/3131.full.pdf+html

7.3.1.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012). ***Note***: Since the concentration of adenovirus using the Nanoceram® filter has not been optimized, use of the 1MDS filter is recommended.

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615(U.S. EPA 2012).

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use tissue culture ["Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012)] and real-time PCR (Literature reference for adenoviruses [Applied and Environmental Microbiology, 2005, 71(6): 3131–3136]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples should be cultured to assess viability [Method 1615 (U.S. EPA 2012)]. For confirmation, target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific real-time PCR primers and probes and assay parameters (Applied and Environmental Microbiology, 2005, 71(6): 3131–3136).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: For the viability assessment of adenoviruses 40 and 41, given that they can be difficult to grow in culture, cell lines such as G293 or CaCo-2 may be considered when these viruses are suspected to be present.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Jothikumar, N., Cromeans, T.L., Hill, V.R., Lu, X., Sobsey, M.D. and Erdman, D.D. 2005. "Quantitative Real-Time PCR Assays for Detection of Human Adenoviruses and Identification of Serotypes 40 and 41." Applied and Environmental Microbiology, 71(6): 3131–3136.
http://aem.asm.org/content/71/6/3131.full.pdf+html

7.3.2 Astroviruses – BSL not specified

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.2.1
Post Decontamination	Integrated Cell Culture and Real-Time Reverse Transcription-PCR	7.3.2.2

7.3.2.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription- PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012). ***Note***: Since the concentration of astrovirus using the Nanoceram® filter has not been optimized, use of the 1MDS filter is recommended.

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time reverse transcription-PCR (Literature reference for astroviruses [Canadian Journal of Microbiology, 2004, 50(4): 269–278]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Canadian Journal of Microbiology, 2004, 50(4): 269–278). The use of real-time reverse transcription-PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of astroviruses.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Grimm, A.C., Cashdollar, J.L., Williams, F.P. and Fout, G.S. 2004. "Development of an Astrovirus RT-PCR Detection Assay for Use With Conventional, Real-Time, and Integrated Cell Culture/RT-PCR." Canadian Journal of Microbiology, 50(4): 269–278.
http://www.nrcresearchpress.com/doi/abs/10.1139/w04-012

7.3.2.2 Post Decontamination Sample Analyses (Integrated Cell Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists these procedures for detection and viability assessment in aerosols, surface wipes or swabs, drinking water and post decontamination waste water. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012). ***Note***: Since the concentration of astrovirus using the Nanoceram® filter has not been optimized, use of the 1MDS filter is recommended.

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).
Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use integrated cell culture and real-time reverse transcription-PCR (Literature Reference for Astroviruses [Canadian Journal of Microbiology, 2004, 50(4): 269–278]).

Description of Method: The method is a real-time reverse transcription-PCR procedure that can be integrated with cell culture (CaCo-2 cells) to enhance sensitivity. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), concentrated samples are analyzed directly or indirectly, after cell culture, by a two-step real-time reverse transcription-PCR (i.e., reverse transcription followed by real-time PCR) assay using astrovirus-specific primers and probes and assay parameters (Canadian Journal of Microbiology, 2004, 50(4): 269–278).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Grimm, A.C., Cashdollar, J.L., Williams, F.P. and Fout, G.S. 2004. "Development of an Astrovirus RT-PCR Detection Assay for Use With Conventional, Real-Time, and Integrated Cell Culture/RT-PCR." Canadian Journal of Microbiology, 50(4): 269–278. http://www.nrcresearchpress.com/doi/abs/10.1139/w04-012

7.3.3 Caliciviruses: Noroviruses – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.3.1
Post Decontamination	No method available to determine viable virus after decontamination	7.3.3.2

7.3.3.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types included in SAM, other than water.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012).

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).
.
Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time reverse transcription-PCR [EPA Method 1615 (U.S. EPA 2012)].

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters [EPA Method 1615 (U.S. EPA 2012)].

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-

04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

7.3.3.2 Post Decontamination Sample Analyses

No method available to determine viable virus after decontamination.

7.3.4 Caliciviruses: Sapovirus – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.4.1
Post Decontamination	Tissue Culture and Real-Time Reverse Transcription-PCR	7.3.4.2

7.3.4.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.

Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.

Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a

Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012).

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time reverse transcription-PCR **(**Literature Reference for sapoviruses [Journal of Medical Virology, 2006, 78(10): 1347–1353]).

Description of Method: The method is a TaqMan®-based real-time reverse transcriptase PCR assay that can detect four of the five distinct sapovirus genogroups (GI–GV) using a multiplex assay. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Medical Virology, 2006, 78(10): 1347–1353).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Oka, T., Katayama, K., Hansman, G.S., Kageyama, T., Ogawa, S., Wu, F.T., White, P.A. and Takeda, N. 2006. "Detection of Human Sapovirus by Real-Time Reverse Transcription-Polymerase Chain Reaction." Journal of Medical Virology, 78(10): 1347–1353. http://cat.inist.fr/?aModele=afficheN&cpsidt=18099754

7.3.4.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types included in SAM, other than water.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012).

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use tissue culture (Literature reference for Sapovirus [Archives of Virology, 1991, 120(1-2): 115–122]) and real-time reverse transcription-PCR **(**Literature Reference for sapoviruses [Journal of Medical Virology, 2006, 78(10): 1347–1353]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples should be cultured using LL-PK cells supplemented with intestinal contents preparation (ICP) to assess viability (Archives of Virology, 1991, 120(1-2): 115–122). For confirmation target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific real-time PCR primers and probes and assay parameters (Journal of Medical Virology, 2006, 78(10): 1347–1353).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis. Culture procedure is for porcine sapovirus and may not be appropriate for all strains of sapoviruses.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

Parwani, A.V., Flynn, W.T., Gadfield, K.L and Saif L.J. 1991. "Serial Propagation of Porcine Enteric Calicivirus in a Continuous Cell Line. Effect of Medium Supplementation With Intestinal Contents or Enzymes." Archives of Virology, 120(1-2): 115–122. http://www.springerlink.com/content/u3v0041507k032h1/

Oka, T., Katayama, K., Hansman, G.S., Kageyama, T., Ogawa, S., Wu, F.T., White, P.A. and Takeda, N. 2006. "Detection of Human Sapovirus by Real-Time Reverse Transcription-Polymerase Chain Reaction." Journal of Medical Virology, 78(10): 1347–1353. http://cat.inist.fr/?aModele=afficheN&cpsidt=18099754

7.3.5 Coronaviruses: Severe Acute Respiratory Syndrome (SARS) -associated Human Coronavirus (HCoV) – BSL-2; BSL-3 for Propagation

Remediation Phase	Analytical Technique	Section
Site Characterization	Reverse Transcription-PCR	7.3.5.1
Post Decontamination	Tissue Culture and Reverse Transcription-PCR	7.3.5.2

7.3.5.1 Site Characterization Sample Analyses (Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use reverse transcription-PCR (Literature Reference for Coronavirus: SARS [Journal of Virological Methods, 2004, 122(1): 29–36]).

Description of Method: This method uses a conventional single-tube reverse transcription-PCR procedure using the Stratagene Robocycler®. End-point amplicon analysis is by electrophoresis and subsequent visualization. The assay can detect the SARS-HCoV as well as several other human respiratory coronaviruses (HCoV-OC43 and HCoV-229E). Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Virological Methods, 2004, 122(1): 29–36).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

Adachi, D., Johnson. G., Draker, R., Ayers, M., Mazzulli, T., Talbot, P.J. and Tellier, R. 2004. "Comprehensive Detection and Identification of Human Coronaviruses, Including the SARS-Associated Coronavirus, With a Single RT-PCR Assay." Journal of Virological Methods, 122(1): 29–36. http://www.sciencedirect.com/science/article/pii/S0166093404002162

7.3.5.2 Post Decontamination Sample Analyses (Tissue Culture and Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use tissue culture (Literature reference for Coronavirus: SARS [Applied Biosafety, 2007, 12(2): 100–108]) and reverse transcription-PCR (Literature Reference for Coronavirus: SARS [Journal of Virological Methods, 2004, 122(1): 29–36]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are inoculated onto Vero cell monolayers; the cells are examined for cytopathic effects (CPE) to assess viability (Applied Biosafety, 2007, 12(2): 100–108). For confirmation, target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Virological Methods, 2004, 122(1): 29–36).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Pagat, A., Seux-Goepfert, R., Lutsch, C., Lecouturier, V., Saluzzo, J. and Kusters, I.C. 2007. "Evaluation of SARS-Coronavirus Decontamination Procedures." Applied Biosafety 12(2): 100–108. https://my.absa.org/tiki-download_file.php?fileId=3490

Adachi, D., Johnson. G., Draker, R., Ayers, M., Mazzulli, T., Talbot, P.J. and Tellier, R. 2004. "Comprehensive Detection and Identification of Human Coronaviruses, Including the SARS-Associated Coronavirus, With a Single RT-PCR Assay." Journal of Virological Methods. 122(1): 29–36. http://www.sciencedirect.com/science/article/pii/S0166093404002162

7.3.6 Hepatitis E Virus (HEV) – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.6.1
Post Decontamination	Tissue Culture and Real-Time Transcription-PCR	7.3.6.2

7.3.6.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types included in SAM, other than water.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012). ***Note***: Since the concentration of hepatitis E virus using the Nanoceram® filter has not been evaluated, use of the 1MDS filter is recommended.

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time reverse transcription-PCR **(**Literature Reference for Hepatitis E Virus [Journal of Virological Methods, 2006, 131(1): 65–71]).

Description of Method: The method uses a TaqMan® real-time reverse transcription-PCR assay using the R.A.P.I.D.® PCR systems to detect and quantitate all four major HEV genotypes. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Virological Methods, 2006, 131(1): 65–71).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Jothikumar, N., Cromeans, T.L., Robertson, B.H., Meng, X.J. and Hill, V.R. 2006. "A Broadly Reactive One-Step Real-Time RT-PCR Assay for Rapid and Sensitive Detection of Hepatitis E Virus." Journal of Virological Methods, 131(1): 65–71.
http://cat.inist.fr/?aModele=afficheN&cpsidt=17367357

7.3.6.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time Reverse Transcription-PCR)

Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012). ***Note***: Since the concentration of hepatitis E virus using the Nanoceram® filter has not been evaluated, use of the 1MDS filter is recommended.

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for

virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use tissue culture (Literature reference for Hepatitis E Virus [FEMS Immunology Medical Microbiology, 2009, 56(1): 73–79]) and real-time reverse transcription-PCR **(**Literature Reference for Hepatitis E Virus [Journal of Virological Methods, 2006, 131(1): 65–71]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are inoculated onto HPG11 cells; the cells are examined for CPEs to assess viability [Federation of European Microbiological Societies (FEMS) Immunology Medical Microbiology, 2009, 56(1): 73–79]. For confirmation target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Virological Methods, 2006, 131(1): 65–71).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Zaki, M., Foud, M.F. and Mohamed, A. F. 2009. "Value of Hepatitis E Virus Detection by Cell Culture Compared With Nested PCR and Serological Studies by IgM and IgG." FEMS Immunology Medical Microbiology, 56(1): 73–79.
http://onlinelibrary.wiley.com/doi/10.1111/j.1574-695X.2009.00552.x/pdf

Jothikumar, N., Cromeans, T.L., Robertson, B.H., Meng, X.J. and Hill, V.R. 2006. "A Broadly Reactive One-Step Real-Time RT-PCR Assay for Rapid and Sensitive Detection of Hepatitis E Virus." Journal of Virological Methods, 131(1): 65–71.
http://cat.inist.fr/?aModele=afficheN&cpsidt=17367357

7.3.7 Influenza H5N1 virus – BSL-3

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.7.1
Post Decontamination	Isolation of H5N1 virus should not be performed except at the Influenza Division, CDC.	7.3.7.2

7.3.7.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time reverse transcription-PCR (Literature Reference for Influenza H5N1 [Emerging Infectious Diseases, 2005, 11(8): 1303–1305]).

Description of Method: This is a two-step, real-time reverse transcriptase-PCR multiplex assay. The assay is specific for the H5 subtype. ***Note***: Influenza H5N1 virus samples are to be handled with BSL-3 containment and practices. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Emerging Infectious Diseases, 2005, 11(8): 1303–1305).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

Ng, E.K.O., Cheng, P.K.C., Ng, A.Y.Y., Hoang, T.L. and Lim, W.W.L. 2005. "Influenza A H5N1 Detection." Emerging Infectious Diseases, 11(8): 1303–1305. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3320469/

ASM. 2008. "Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: Avian Influenza A H5N1." http://www.asm.org/images/pdf/Clinical/Protocols/avianiinfluenza11-2008.pdf

7.3.7.2 Post Decontamination Sample Analyses

Isolation of H5N1 virus should not be performed except at the Influenza Division, CDC.

7.3.8 Picornaviruses: Enteroviruses – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.8.1
Post Decontamination	Tissue Culture	7.3.8.2

7.3.8.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types included in SAM, other than water.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012). Sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).

Analytical Technique: Use real-time reverse transcription-PCR ["Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012)].

Description of Method: The method uses a TaqMan® real-time reverse transcriptase-PCR assay to detect and quantify enteroviruses. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified, and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Method 1615, U.S. EPA 2012).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* SporesFrom Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

7.3.8.2 Post Decontamination Sample Analyses (Tissue Culture)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types included in SAM, other than water.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012).

Sample types other than water should be processed as follows: (A) For virus recovery, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).

Analytical Technique: Use tissue culture with serum neutralization ("Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," U.S. EPA 2012).

Description of Method: This method describes procedures for determining infectivity and quantifying enteroviruses using BGMK cells. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), aliquots of the sample are used to inoculate BGMK cells. Cell culture flasks are examined for evidence of CPE for a total of 14 days [Method 1615 (U.S. EPA 2012)].

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

7.3.9 Picornaviruses: Hepatitis A Virus (HAV) – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.9.1
Post Decontamination	Integrated Cell Culture and Real-Time Reverse Transcription-PCR	7.3.9.2

7.3.9.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription- PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012).

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time reverse transcription-PCR (Literature Reference for Enteric Viruses [Journal of Food Protection, 2011, 74(10): 1756–1761]).

Description of Method: The method is a multiplex real-time reverse transcription-PCR procedure optimized for the simultaneous detection of enteroviruses, HAV, reoviruses and rotaviruses. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Food Protection, 2011, 74(10): 1756–1761).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Hyeon, J. Y, Chon, J.Y, Park, C., Lee, J.B., Choi, I.S., Kim, M.S. and Seo, K.H. 2011. "Rapid Detection Method for Hepatitis A Virus from Lettuce by a Combination of Filtration and Integrated Cell Culture-Real-Time Reverse Transcription PCR," Journal of Food Protection, 74(10): 1756–1761. http://www.ncbi.nlm.nih.gov/pubmed/22004827

7.3.9.2 Post Decontamination Sample Analyses (Integrated Cell Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types included in SAM, other than water.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012).

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use integrated cell culture and real-time reverse transcription-PCR (Literature Reference for Hepatitis A Virus [Journal of Food Protection, 2011, 74(10):1756–1761]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are inoculated onto fetal rhesus monkey kidney (FRhK-4) cells, and the cells are examined for CPE to assess viability. For confirmation, target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Food Protection, 2011, 74(10): 1756–1761).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Hyeon, J. Y, Chon, J.Y, Park, C., Lee, J.B., Choi, I.S., Kim, M.S. and Seo, K.H. 2011. "Rapid Detection Method For Hepatitis A Virus From Lettuce by a Combination of Filtration and Integrated Cell Culture-Real-Time Reverse Transcription PCR." Journal of Food Protection, 74(10): 1756–1761. http://www.ncbi.nlm.nih.gov/pubmed/22004827

7.3.10 Reoviruses: Rotavirus (Group A) – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time Reverse Transcription-PCR	7.3.10.1
Post Decontamination	Tissue Culture and Real-Time Reverse Transcription-PCR	7.3.10.2

7.3.10.1 Site Characterization Sample Analyses (Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus

and Norovirus Occurrence in Water by Culture and RT-qPCR," U.S. EPA, 2012. ***Note***: Since the concentration of reoviruses using the Nanoceram® filter has not been optimized, use of the 1MDS filter is recommended.

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012).

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time reverse transcription-PCR (Literature Reference for Rotavirus (Group A) [Journal of Virological Methods, 2009, 155(2): 126–131]).

Description of Method: The method is used to detect rotavirus using a one-step real-time reverse-transcription PCR. Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Virological Methods, 2009, 155(2): 126–131).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Jothikumar, N., Kang, G. and V.R. Hill. 2009. "Broadly Reactive TaqMan® Assay for Real-Time RT-PCR Detection of Rotavirus in Clinical and Environmental Samples." Journal of Virological Methods, 155(2): 126–131.
http://www.sciencedirect.com/science/article/pii/S0166093408003571

7.3.10.2 Post Decontamination Sample Analyses (Tissue Culture and Real-Time Reverse Transcription-PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types included in SAM, other than water.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR" (U.S. EPA 2012). ***Note***: Since the concentration of reoviruses using the Nanoceram® filter has not been optimized, use of the 1MDS filter is recommended.

All sample types other than water should be processed as follows: (A) For virus recovery from samples, follow the EPA BA Protocol (Section 11: sample processing and elution); and (B) for virus concentration following recovery, follow relevant steps from Section 11.2.3 of Method 1615 (U.S. EPA 2012). Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use tissue culture [Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR (U.S. EPA 2012)] and real-time reverse transcription-PCR (Literature Reference for Enteric Viruses (Journal of Virological Methods, 2009, 155(2): 126–131).

Description of Method: This method describes procedures for determining infectivity and quantifying enteroviruses using BGMK cells. Following appropriate sample preparation (see Sample Preparation Procedures above), aliquots of the sample are used to inoculate BGMK cells. Cell culture flasks are examined for evidence of CPE for a total of 14 days [Method 1615 (U.S. EPA 2012)]. For confirmation, target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific PCR primers and probes and assay parameters (Journal of Virological Methods, 2009, 155(2): 126–131).

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control*

Guidance for Laboratories Performing PCR Analyses on Environmental Samples (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Special Considerations: Appropriate RNAse inhibitors should be included during sample processing and analysis.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2012. "Method 1615: Enterovirus and Norovirus Occurrence in Water by Culture and RT-qPCR," EPA/600/R-10/181. http://www.epa.gov/nerlcwww/documents/Method1615v1_1.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Jothikumar, N., Kang, G. and V.R. Hill. 2009. "Broadly Reactive TaqMan® Assay for Real-Time RT-PCR Detection of Rotavirus in Clinical and Environmental Samples." Journal of Virological Methods, 155(2): 126–131. http://www.sciencedirect.com/science/article/pii/S0166093408003571

7.4 Method Summaries for Protozoa

Summaries of the analytical methods for protozoa listed in Appendix C are provided in Sections 7.4.1 through 7.4.4.

7.4.1 *Cryptosporidium* spp. [Cryptosporidiosis] – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.4.1.1
	IMS/ Fluorescence assay (FA)	7.4.1.2 [1]
	IMS/FA	7.4.1.3 [1]
Post Decontamination	Cell Culture Immunofluorescence (IFA)	7.4.1.4

[1] Methods 1622 and 1623 include the same sample processing and analytical procedures for *Cryptosporidium*; either method could be used.

7.4.1.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Real-Time PCR for Quantification of *Giardia* and *Cryptosporidium* in Environmental Water Samples and Sewage," (Guy *et al*. 2003) and "Development of Procedures for Direct Extraction of *Cryptosporidium* DNA from Water Concentrates and for Relief of PCR Inhibitors" (Jiang *et al*. 2005).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Analytical Technique: Use real-time PCR (Literature References for *Cryptosporidium* spp. [Applied and Environmental Microbiology, 2003, 69(9): 5178–5185 and Applied and Environmental Microbiology, 2005, 71(3): 1135–1141]).

Description of Method: Following appropriate sample preparation (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified, and analyzed using the referenced target-specific real-time PCR primers and probes and assay parameters (Applied and Environmental Microbiology, 2003, 69(9): 5178–5185 and Applied and Environmental Microbiology, 2005, 71(3): 1135–1141). The use of real-time PCR analyses directly on samples (e.g., no culture component) allows for rapid detection of *Cryptosporidium* spp.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Guy, R.A., Payment, P., Krull, U.J. and Horgen, P.A. 2003. "Real-Time PCR for Quantification of *Giardia* and *Cryptosporidium* in Environmental Water Samples and Sewage." Applied and Environmental Microbiology, 69(9): 5178–5185.
http://aem.asm.org/content/69/9/5178.full.pdf+html

Jiang, J., Alderisio, K.A., Singh, A. and Xiao, L. 2005. "Development of Procedures for Direct Extraction of *Cryptosporidium* DNA from Water Concentrates and for Relief of PCR Inhibitors." Applied and Environmental Microbiology. 71(3): 1135–1141.
http://aem.asm.org/content/71/3/1135.full.pdf+html

7.4.1.2 Site Characterization Sample Analyses (Immunomagnetic Separation/Fluorescence Assay [IMS/FA])

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for detection in aerosols, surface wipes or swabs, drinking water and post decontamination waste water. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types included in SAM, other than water.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA" (U.S. EPA 2005).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Analytical Technique: Use immunomagnetic separation and fluorescence assay microscopy ("Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA," U.S. EPA 2005).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are centrifuged to pellet the oocysts, and the supernatant fluid is aspirated. A solution containing anti-*Cryptosporidium* antibodies conjugated to magnetic beads is added to the pellet and mixed. The oocyst magnetic bead complex is separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts. The oocysts are stained on well slides with fluorescently labeled monoclonal antibodies (mAbs) and 4',6-diamidino-2-phenylindole (DAPI). The stained sample is examined using fluorescence and differential interference contrast (DIC) microscopy. Qualitative analysis is performed by scanning each slide well for objects that meet

the size, shape, and fluorescence characteristics of *Cryptosporidium* oocysts. Quantitative analysis is performed by counting the total number of objects on the slide confirmed as oocysts. This method is not intended to determine viability, species, or infectivity of the oocysts.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control, matrix spike/matrix spike duplicate (MS/MSD) and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed as stipulated in the method.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. 2005. "Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA." EPA 815-R-05-001. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-1622.pdf

7.4.1.3 Site Characterization Sample Analyses (IMS/FA)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for detection in aerosols, surface wipes or swabs, drinking water and post decontamination waste water. Further research is needed to develop comprehensive pathogen-specific procedures for environmental sample types included in SAM, other than water.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA" (U.S. EPA 2005).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Analytical Technique: Use IMS and FA microscopy ["Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA" (U.S. EPA 2005)].

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are centrifuged to pellet the oocysts and cysts, and the supernatant fluid is aspirated. A solution containing anti-*Cryptosporidium* and anti-*Giardia* antibodies conjugated to magnetic beads is added to the pellet and mixed. The oocyst and cyst magnetic bead complex is separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts and cysts. The oocysts and cysts are stained on well slides with fluorescently labeled mAbs and DAPI. The stained sample is examined using fluorescence and DIC microscopy. Qualitative analysis is performed by scanning each slide well for objects that meet the size, shape, and fluorescence characteristics of *Cryptosporidium* oocysts and *Giardia* cysts. Quantitative analysis is performed by counting the total number of objects on the slide confirmed as oocysts or cysts.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control, MS/MSD and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. 2005. "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." EPA 815-R-05-002. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-1623.pdf

7.4.1.4 Post Decontamination Sample Analyses (Cell Culture Immunofluorescence [IFA])

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.

Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Analytical Technique: Use cell culture IFA (Literature reference for *Cryptosporidium* spp. [Canadian Journal of Microbiology, 2007, 53(5): 656–663]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are used to inoculate HCT-8 monolayers and incubated. Following incubation the monolayers are examined using IFA to determine the number of viable oocysts present in the sample. The use of cell culture IFA analyses is a cost effective and expedient alternative to mouse infectivity assays to determine in vitro infectivity of *Cryptosporidium* oocysts.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Bukhari, Z., Holt, D.M., Ware, M.W. and Schaefer III, F.W. 2007. "Blind Trials Evaluating In Vitro Infectivity of *Cryptosporidium* Oocysts Using Cell Culture Immunofluorescence." Canadian Journal of Microbiology, 53(5): 656–663.
http://www.nrcresearchpress.com/doi/abs/10.1139/W07-032?url_ver=Z39.88-2003&rfr_id=ori:rid:crossref.org&rfr_dat=cr_pub%3dpubmed

7.4.2 *Entamoeba histolytica* – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.4.2.1
Post Decontamination	Cell Culture	7.4.2.2

7.4.2.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Analytical Technique: Use real-time PCR (Literature reference for *Entamoeba histolytica* [Journal of Clinical Microbiology, 2005, 43(5): 2168–2172].

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified, and analyzed using the referenced target-specific real-time PCR primers and probes and assay parameters (Journal of Clinical Microbiology, 2005, 43(5): 2168–2172). The use of real-time PCR analyses directly on samples allows for rapid detection of *Entamoeba histolytica*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Roy, S., Kabir, M., Mondal, D., Ali, I.K.M., Petri Jr., W.A. and Haque, R. 2005. "Real-Time-PCR Assay for Diagnosis of *Entamoeba histolytica* Infection." Journal of Clinical Microbiology, 43(5): 2168–2172. http://jcm.asm.org/content/43/5/2168.full.pdf+html

7.4.2.2 Post Decontamination Sample Analyses (Cell Culture)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below

Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.

Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011)..

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Analytical Technique: Use cell culture (Literature Reference for *Entamoeba histolytica* (Journal of Parasitology, 1972, 58(2): 306–310).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), *Entamoeba histolytica* cysts are placed in a modified trypticase-panmede liver digest-serum medium and incubated for 10 hours. Live amoebae excyst through a rupture in the cyst wall, whereas non-viable amoebae remain encysted. Microscopic examination of an aliquot of the incubated excystation culture allows calculation of the percent of empty (live) cysts and full (dead) cysts in a population.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Stringert, R.P. 1972. "New Bioassay System for Evaluating Percent Survival of *Entamoeba histolytica* Cysts." The Journal of Parasitology, 58(2): 306–310.
http://www.jstor.org/discover/10.2307/3278094?uid=3739704&uid=2129&uid=2&uid=70&uid=4&uid=3739256&sid=47698759181407

7.4.3 *Giardia* spp. [Giardiasis] – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.4.3.1
	IMS/FA	7.4.3.2
Post Decontamination	Cell Culture	7.4.3.3

7.4.3.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Real-Time PCR for Quantification of *Giardia* and *Cryptosporidium* in Environmental Water Samples and Sewage" (Guy *et al*. 2003).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Analytical Technique: Use real-time PCR (Literature reference for *Giardia* [Applied and Environmental Microbiology, 2003, 69(9): 5178–5185]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified, and analyzed using the referenced target-specific real-time PCR primers and probes and assay parameters (Applied and Environmental Microbiology, 2003, 69(9): 5178–5185). The use of real-time PCR analyses directly on samples allows for rapid detection of *Giardia*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Guy, R.A., Payment, P., Krull, U.J. and Horgen, P.A. 2003. "Real-Time PCR for Quantification of *Giardia* and *Cryptosporidium* in Environmental Water Samples and Sewage." Applied and Environmental Microbiology, 69(9): 5178–5185.
http://aem.asm.org/content/69/9/5178.full.pdf+html

7.4.3.2 Site Characterization Sample Analyses (IMS/FA)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.

Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA" (U.S. EPA 2005).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Analytical Technique: Use IMS and FA microscopy ["Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA" (U.S. EPA 2005)].

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), samples are centrifuged to pellet the oocysts and cysts, and the supernatant fluid is aspirated. A solution containing anti-*Cryptosporidium* and anti-*Giardia* antibodies conjugated to magnetic beads is added to the pellet and mixed. The oocyst and cyst magnetic bead complex is separated from the extraneous materials using a magnet, and the extraneous materials are discarded. The magnetic bead complex is then detached from the oocysts and cysts. The oocysts and cysts are stained on well slides with fluorescently labeled mAbs and DAPI. The stained sample is examined using fluorescence and DIC microscopy. Qualitative analysis is performed by scanning each slide well for objects that meet the size, shape, and fluorescence characteristics of *Cryptosporidium* oocysts and *Giardia* cysts. Quantitative analysis is performed by counting the total number of objects on the slide confirmed as oocysts or cysts.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control, MS/MSD and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. 2005. "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." EPA 815-R-05-002. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-1623.pdf

7.4.3.3 Post Decontamination Sample Analyses (Cell Culture)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA" (U.S. EPA 2005).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Analytical Technique: Use cell culture (Literature Reference for *Giardia* spp. [Transactions of the Royal Society of Tropical Medicine and Hygiene, 1983, 77(4): 487–488]).

Description of Method: Procedures are described for analysis of cell culture samples and may be adapted for assessment of aerosols, surface wipes or swabs and water samples (see Sample Preparation Procedures above). Trypticase-yeast-iron-serum medium supplemented with bovine bile and additional cysteine is used to isolate and culture *Giardia lamblia*. *G. lamblia* is incubated for intervals of 72 and 96 hours at 36 °C in borosilicate glass tubes. The cells form a dense, adherent monolayer on the surface of the glass or are observed swimming through the liquid medium.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.

http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2005. "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." EPA 815-R-05-002. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-1623.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Keister, D. 1983. "Axenic Culture of *Giardia lamblia* in TYI-S-33 Medium Supplemented With Bile." Transactions of the Royal Society of Tropical Medicine and Hygiene, 77(4): 487–488. http://www.sciencedirect.com/science/article/pii/0035920383901207

7.4.4 *Toxoplasma gondii* [Toxoplasmosis] – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.4.4.1
Post Decontamination	Cell Culture	7.4.4.2

7.4.4.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA" (U.S. EPA 2005).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Analytical Technique: Use real-time PCR (Literature reference for *Toxoplasma gondii* [Applied and Environmental Microbiology, 2009, 75(11): 3477–3483]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified, and analyzed using the referenced target-specific real-time PCR primers and probes and assay parameters (Applied and Environmental Microbiology, 2009, 75(11): 3477–3483). The use of real-time PCR analyses directly on samples allows for rapid detection of *Toxoplasma gondii*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146. http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359. http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103. http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. 2005. "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." EPA 815-R-05-002. http://www2.epa.gov/sites/production/files/2015-07/documents/epa-1623.pdf

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Yang, W., Lindquist, H.D. A., Cama, V., Schaefer III, F.W., Villegas, E., Fayer, R., Lewis, E.J., Feng, Y. and Xiao, L. 2009. "Detection of *Toxoplasma gondii* Oocysts in Water Sample Concentrates by Real-Time PCR." Applied and Environmental Microbiology, 75(11): 3477–3483. http://aem.asm.org/content/75/11/3477.full.pdf+html

7.4.4.2 Post Decontamination Sample Analyses (Cell Culture)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA" (U.S. EPA 2005).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Analytical Technique: Use cell culture (Literature Reference for *Toxoplasma gondii* [Journal of Microbiological Methods, 81(3): 219–225]).

Description of Method: Samples are subjected to a series of mechanical and chemical digestion steps to release sporozoites from the *Toxoplasma gondii* oocysts and then inoculated onto confluent fibroblast monolayers. Inoculated monolayers are then incubated undisturbed for ten days to allow for plaque formation. After ten days, the monolayers are fixed, stained with crystal violet, and examined for plaque formation. The literature reference also includes a qPCR procedure to determine viability of *Toxoplasma gondii* oocysts, however, it may not be appropriate depending on the type of disinfection used during remediation.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Villegas, E. N., Augustine, S.A., Villegas, L. F., Ware, M.W., See, M. J., Lindquist, H.D.A., Schaefer, III, F. W. and Dubey, J.P. 2010. "Using Quantitative Reverse Transcriptase PCR and Cell Culture Plaque Assays to Determine Resistance of *Toxoplasma gondii* Oocysts to Chemical Sanitizers." Journal of Microbiological Methods, 81(3): 219–225.
http://www.sciencedirect.com/science/article/pii/S0167701210001107

7.5 Method Summaries for Helminths

Summaries of the analytical methods for helminths listed in Appendix C is provided in Section 7.5.1.

7.5.1 *Baylisascaris procyonis* [Raccoon roundworm fever] – BSL-2

Remediation Phase	Analytical Technique	Section
Site Characterization	Real-Time PCR	7.5.1.1
Post Decontamination	Embryonation of Eggs and Microscopy	7.5.1.2

7.5.1.1 Site Characterization Sample Analyses (Real-Time PCR)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below

Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.

Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Evaluation of a Molecular Beacon Real-time PCR Assay for Detection of *Baylisascaris procyonis* in Different Soil Types and Water Samples" (Gatcombe *et al.* 2010).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction by bead beating and purification for all sample types should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use real-time PCR (Literature reference for *Baylisascaris procyonis* [Parasitology Research, 2010, 106: 499–504]).

Description of Method: Following the appropriate sample preparation procedure (see Sample Preparation Procedures above), the target nucleic acid should be extracted, purified (EPA BA Protocol, Section 9.2), and analyzed using the referenced target-specific real-time PCR primers and probes and assay parameters (Parasitology Research, 2010, 106: 499–504). The use of real-time PCR analyses directly on samples (e.g., no embryonation or microscopic examination) allows for rapid detection of *Baylisascaris procyonis*.

At a minimum, the following QC checks should be performed and evaluated when using this protocol: positive control, negative control, external inhibition control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed. PCR QC checks should be performed according to EPA's *Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* (EPA 815-B-

04-001) document at: http://www2.epa.gov/sites/production/files/2015-07/documents/epa-qaqc-pcr.pdf, or consult the points of contact identified in Section 4.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA and CDC. 2011. "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water." EPA 600/R-11/103.
http://cfpub.epa.gov/si/si_public_record_report.cfm?address=nhsrc/&dirEntryId=238310

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

Gatcombe, R.R., Jothikumar, N., Dangoudoubiyam, S., Kazacos, K.R. and Hill, V.R. 2010. "Evaluation of a Molecular Beacon Real-time PCR Assay for Detection of *Baylisascaris procyonis* in Different Soil Types and Water Samples," Parasitology Research, 106:499–504.
http://www.springerlink.com/content/k8t3581t07n82562

7.5.1.2 Post Decontamination Sample Analyses (Embryonation of Eggs and Microscopy)

Method: This method includes a combination of sample preparation and analysis procedures as summarized below.
Method Selected for: SAM lists this method for analysis of aerosols, surface wipes or swabs, drinking water and post decontamination waste water sample types. Further research is needed to develop comprehensive pathogen-specific procedures for different environmental sample types included in SAM.
Sample Preparation: Particulate samples (surface swabs and wipes) should be prepared according to "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces" (Hodges *et al*. 2010) or "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces" (Rose *et al.* 2011).

Water samples should be processed according to "Comparison of Ultrafiltration Techniques for Recovering Biothreat Agents in Water" (U.S. EPA and CDC 2011) or "Evaluation of a molecular beacon real-time PCR assay for detection of *Baylisascaris procyonis* in different soil types and water samples" (Gatcombe *et al.* 2010).

All other environmental sample types should be processed according to procedures within the EPA BA Protocol [U.S. EPA (anticipated publication October 2012)].

Nucleic acid extraction and purification should be performed according to procedures within the EPA BA Protocol (Protocol Section 9.2).

Analytical Technique: Use microscopy and embryonation of eggs (*U.S. EPA Environmental Regulations and Technology,* 2003, EPA/625/R-92/013).

Description of Method: The protocol describes procedures for analysis of solid and wastewater samples. Samples are processed by blending with buffered water containing a surfactant. The blend is screened to remove large particles, the solids in the screened portion are allowed to settle out, and the supernatant is decanted. The sediment is subjected to density gradient centrifugation using magnesium sulfate. This flotation procedure yields a layer likely to contain *Ascaris* and other parasite eggs, if present in the sample. Small particulates are removed by a second screening on a small mesh size screen. The resulting concentrate is incubated until control helminth eggs are fully embryonated. The concentrate is then microscopically examined for the categories of helminth ova on a counting chamber.

At a minimum, the following QC checks should be performed and evaluated: positive control, negative control and blank. Ongoing analysis of QC samples to ensure reliability of the analytical results should also be performed.

Sources:

Hodges, L.R., Rose, L.J., O'Connell, H. and Arduino, M.J. 2010. "National Validation Study of a Swab Protocol for the Recovery of *Bacillus anthracis* Spores From Surfaces." Journal of Microbiological Methods, 81(2): 141–146.
http://www.sciencedirect.com/science/article/pii/S0167701210000692

Rose L.J., Hodges, L, O'Connell, H. and Noble-Wang, J. 2011. "National Validation Study of a Cellulose Sponge-Wipe Processing Method for Use After Sampling *Bacillus anthracis* Spores From Surfaces." Applied Environmental Microbiology, 77(23): 8355–8359.
http://aem.asm.org/content/77/23/8355.full.pdf+html

U.S. EPA. [Anticipated publication October 2012] "Protocol for Detection of *Bacillus anthracis* in Environmental Samples During the Remediation Phase of an Anthrax Event" (EPA BA Protocol).

U.S. EPA. 2003. "Appendix I: Test Method for Detecting, Enumerating, and Determining the Viability of Ascaris Ova in Sludge." *U.S. EPA Environmental Regulations and Technology: Control of Pathogens and Vector Attractions in Sewage Sludge*, EPA/625/R-92/013.
http://www2.epa.gov/sites/production/files/2015-07/documents/epa-625-r-92-013.pdf

Section 8.0: Selected Biotoxin Methods

A list of methods or procedures to be used in analyzing environmental samples for biotoxin contaminants is provided in Appendix D. These methods should be used to support remediation activities (site assessment through clearance) following a contamination incident. Procedures have been compiled for each biotoxin that may need to be identified and/or quantified following a contamination incident. Analytical procedures are not currently available for all the analyte-sample type combinations included in this document. Future research needs include identification of additional methods as well as development and testing of the procedures listed. Appendix D is sorted alphabetically by analyte, within each of two analyte types (i.e., protein and small molecule).

Please note: This section provides guidance for selecting biotoxin methods that have a high likelihood of assuring analytical consistency when laboratories are faced with a large-scale environmental remediation crisis. Not all methods have been verified for the analyte/sample type combination listed in Appendix D. Please refer to the specified method to identify analyte/sample type combinations that have been verified. Any questions regarding information discussed in this section should be addressed to the appropriate contact(s) listed in Section 4.

Appendix D provides the following information:

- **Analyte(s).** The compound or compound(s) of interest.
- **Chemical Abstracts Service Registry Number (CAS RN) / Description.** A unique identifier for substances that provides an unambiguous way to identify a toxin or toxin isoform when there are many possible systematic, generic or trivial names and/or a brief statement describing the toxin.
- **Analysis type.** Tests are either for presumptive identification, confirmatory identification or biological activity determination; tests types are described below.
- **Analytical Technique.** An analytical instrument or technique used to determine the quantity and identification of compounds or components in a sample.
- **Analytical Method.** The recommended method or procedure, and the corresponding publisher.
- **Aerosol (filter/cassette or liquid impinger).** The recommended method/procedure to measure the analyte of interest in air sample collection media such as filter cassettes and liquid impingers.
- **Solid (soil, powder).** The recommended method/procedure to measure the analyte of interest in solid samples such as soil and powders.
- **Particulate (swabs, wipes, filters).** The recommended method/procedure to measure the analyte of interest in particulate sample collection media such as swabs, wipes and dust-collecting socks used with vacuum collection.
- **Liquid/water.** The recommended method/procedure to measure the analyte of interest in liquid and water samples.
- **Drinking water.** The recommended method/procedure to measure the analyte of interest in drinking water samples.

Depending on site- and event-specific goals, a determination of whether contaminant concentrations are above pre-existing levels may be necessary. Such determinations could involve investigations of background levels at potentially uncontaminated locations in close proximity to the site, using methods listed in Appendix D. Other means might include examination of historical information regarding contaminant occurrence. For example, periodic episodes of some of the biotoxins (such as microcystins and other algal toxins) have been detected and measured in surface waters throughout the United States by methods similar to those in Appendix D (http://toxics.usgs.gov/highlights/algal_toxins/). When using historical data, knowledge of the analytical methods and techniques used would be necessary, particularly in terms of their similarity in performance and quality control (QC) to the methods listed in Appendix D.

The "analysis type" listed for each biotoxin method in Appendix D is intended to address: (1) the level of certainty of results and (2) the remediation phase (e.g., site mapping, assessment, clearance). A tiered approach (i.e., algorithm) may be used when implementing the analysis types, particularly when needed to address a large number of samples. For example, methods identified as presumptive, which are generally more rapid than confirmatory methods, might be used during the initial stages of remediation to evaluate the extent of contamination once a contamination event and the type of contamination are known. Presumptive methods also might be used to identify samples that should be analyzed using confirmatory methods. In turn, the results of the confirmatory methods might be used to select samples to be analyzed by applicable biological activity methods, which tend to be much slower and less available than the confirmatory methods. Note that the use of the terms "presumptive" and "confirmatory" in this document is not intended to redefine or supersede the Laboratory Response Network's (LRN) use of the terms. The terms as used by the LRN are described in Section 8.1.4.

Many of the presumptive methods are immunoassays, which may be adapted for large-scale sample processing while maintaining an appropriate level of analytical certainty. Confirmatory methods are generally more time consuming and expensive and are intended to provide results with a high level of certainty. Confirmatory methods should be considered for use when: (1) presumptive analysis indicates the presence of the biotoxin, (2) a smaller subset of samples requires processing, or (3) as required for a tiered approach to remediation. Methods that address biological activity tend to be even more time consuming and expensive, and are intended to provide a high level of certainty in corroborating other assay results. Depending on the goals of the remediation phase, biological activity methods may be needed because biotoxins may be detectable but inactive; therefore, these assays may also provide information about potential impact on human safety.

For small molecule biotoxins in Appendix D, the presence of intact compound structure is an indication of biological activity; therefore, the confirmatory method listed for these biotoxins also serves as a measure of biological activity. For protein biotoxins, biological activity may be determined directly using *in vivo* (e.g., mouse bioassay) or *in vitro* (e.g., enzymatic activity) methods. However, biological availability (i.e., biotoxin accessibility to site of action) and activity are both required to elicit toxicity and some *in vitro* methods may not address both parameters.

Numerous analytical techniques using a variety of instrumentation (e.g., high performance liquid chromatography-mass spectrometer [HPLC-MS], immunoassay [enzyme-linked immunosorbent assay (ELISA)], immunoassay [lateral flow device (LFD)], electrochemiluminescence (ECL)-based, enzyme immunoassay [EIA]) have been cited in Appendix D. It is recognized that new reports describing advances in procedures for analysis of biotoxins frequently appear in the literature, and commercially available equipment for these analyses are evolving rapidly. Accordingly, the individual techniques and methods listed in Appendix D are to be regarded as a starting point. The biotoxin methods points of contact listed in Section 4.0 of SAM should be consulted for additional information regarding currently available methods.

The presence of disinfectants (e.g., chlorine) and/or preservatives added during water sample collection to slow degradation (e.g., pH adjustors, de-chlorinating agents) could possibly affect analytical results. When present, the impact of these agents on method performance should be evaluated, if not previously determined. Additional research on biotoxin contaminants is ongoing within EPA and includes impact of disinfectants and preservatives.

EPA's NHSRC is working on a sample collection document that is intended as a companion to SAM. This sample collection document will provide information regarding sampling container/media, preservation, holding time, sample size and shipping and is intended to complement the laboratory analytical methods that are the focus of the SAM document.

8.1 General Guidelines

This section provides a general overview of how to identify the appropriate method(s) for a given biotoxin as well as recommendations for QC procedures.

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

Additional resources include:

- *Defense Against Toxin Weapons*, published by the U.S. Army Medical Research Institute of Infectious Diseases (http://www.usamriid.army.mil/education/defensetox/toxdefbook.pdf) contains information regarding sample collection, toxin analysis and identification, as well as decontamination and water treatment.
- Select agent rules and regulations are found at the National Select Agent Registry (http://www.selectagents.gov/).
- The Centers for Disease Control and Prevention (CDC) has additional information regarding select agent toxins (http://www.cdc.gov/od/sap/sap/toxinamt.htm)
- See Syracuse Research Corporation's (SRC) PHYSPROP and Chemfate, part of the Environmental Fate Database supported by EPA (http://srcinc.com/what-we-do/product.aspx?id=133)
- INCHEM contains both chemical and toxicity information(http://www.inchem.org/)
- The Registry of Toxic Effects of Chemical Substances (RTECS) database can be accessed via the National Institute for Occupational Safety and Health (NIOSH) website at http://www.cdc.gov/niosh/rtecs/default.html for toxicity information.
- The Forensic Science and Communications Journal published by the Laboratory Division of the Federal Bureau of Investigation (FBI). See http://www.fbi.gov/about-us/lab/forensic-science-communications

8.1.1 Standard Operating Procedures for Identifying Biotoxin Methods

To determine the appropriate method that is to be used on an environmental sample, locate the biotoxin of concern in Appendix D: Selected Biotoxin Methods under the "Analyte(s)" column. After locating the biotoxin, continue across the table and identify the appropriate analysis type. After an analysis type has been chosen, find the analytical technique (e.g., immunoassay) and analytical method applicable to the sample type of interest (aerosol, solid, particulate, liquid or drinking water) corresponding to that particular analyte.

Once a method has been identified in Appendix D, the corresponding method summary can be found in Sections 8.2.1 through 8.3.12. Method summaries are listed first by alphabetical order within each biotoxin subcategory (i.e., protein and small molecule) and then in order of method selection hierarchy (see Figure 2-1), starting with EPA methods, followed by methods from other federal agencies, voluntary consensus standard bodies (VCSBs) and journal articles. Where available, a direct link to the full text of the method is provided with the method summary. For additional information on sample preparation procedures and methods available through consensus standards organizations, please use the contact information provided in **Table 8-1**.

Table 8-1. Sources of Biotoxin Methods

Name	Publisher	Reference
Food and Drug Administration (FDA), Bacteriological Analytical Manual Online	FDA	http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm
Official Methods of Analysis of AOAC International*	AOAC International	http://www.aoac.org
National Environmental Methods Index (NEMI)	EPA, U.S. Geological Survey (USGS)	http://www.nemi.gov/
Pharmacology & Toxicology*	Blackwell Synergy	http://www.blackwell-synergy.com/loi/pto
Analytical Biochemistry*	Science Direct	http://www.sciencedirect.com/
Biochemical Journal*	Portland Press Ltd.	http://www.biochemj.org/
Journal of Medicinal Chemistry*	American Chemical Society (ACS)	http://www.acs.org/
Journal of Food Protection*	International Association for Food Protection	http://www.foodprotection.org/
Journal of Chromatography B*	Elsevier Science Publishers	http://www.elsevier.com/
Biomedical Chromatography*	John Wiley And Sons Ltd	http://www.wiley.com/
Environmental Health Perspectives*	National Institute of Environmental Health Sciences	http://www.niehs.nih.gov/
Toxicon*	Elsevier Science Publishers	http://www.journals.elsevier.com/toxicon/
Federation of European Microbiological Societies (FEMS) Microbiology Letters*	Wiley-Blackwell	http://www.wiley.com/
International Journal of Food Microbiology*	Elsevier Science Publishers	http://www.elsevier.com/
Rapid Communications in Mass Spectrometry *	John Wiley And Sons Ltd.	http://www.wiley.com/
Journal of AOAC International*	AOAC International	http://www.aoac.org
Analyst*	Royal Society of Chemistry	http://www.rsc.org/
Journal of Pharmaceutical and Biomedical Analysis*	Elsevier Science Publishers	http://www.elsevier.com/
Journal of Clinical Microbiology	American Society for Microbiology (ASM)	http://www.asm.org/
Journal of Clinical Laboratory Analysis*	John Wiley And Sons Ltd.	http://www.wiley.com/
Journal of Analytical Toxicology*	S. Tinsley Preston	http://www.jatox.com/
Lateral Flow Immunoassay Kits	Environmental Technology Verification (ETV) Program	http://www.epa.gov/etv/
Journal of Agricultural and Food Chemistry*	ACS Publications	http://pubs.acs.org/

Name	Publisher	Reference
Applied and Environmental Microbiology (AEM)*	ASM	http://aem.asm.org/
Journal of Chemical Health and Safety*	Elsevier Science Publishers	http://www.elsevier.com/

* Subscription and/or purchase required.

8.1.2 General QC Guidelines for Biotoxin Methods

Having data of known and documented quality is critical so that public officials can accurately assess the activities that may be needed in remediating a site during and following emergency situations. Having such data requires that laboratories: (1) conduct the necessary QC to ensure that measurement systems are in control and operating properly; (2) properly document results of the analyses; and (3) properly document measurement system evaluation of the analysis-specific QC.[10] Ensuring data quality also requires that laboratory results are properly evaluated and the results of the data quality evaluation are included within the data report when transmitted to decision makers.

The level or amount of QC needed often depends on the intended purpose of the data that are generated. Various levels of QC may be required if the data are generated during presence/absence determinations versus confirmatory analyses. The specific needs for data generation should be identified. QC requirements and data quality objectives (DQOs) should be derived based on those needs and should be applied consistently across laboratories when multiple laboratories are used. For example, during rapid sample screening, minimal QC samples (e.g., blanks, replicates) and documentation might be required to ensure data quality. Sample analyses for environmental evaluation during site assessment through site clearance, such as those identified in this document, might require increased QC (e.g., demonstrations of method sensitivity, precision and accuracy).

While method-specific QC requirements may be included in many of the procedures that are cited in this document, and will be referenced in any SAPs developed to address specific analytes and sample types of concern, the following describes a minimum set of QC samples and procedures that should be conducted for all analyses. Individual methods, sampling and analysis protocols or contractual statements of work also should be consulted to determine any additional QC that may be needed. QC tests should be run as frequently as necessary to ensure the reliability of analytical results. In general, sufficient QC includes an initial demonstration of measurement system capability as well as ongoing assessments to ensure the continued reliability of the analytical results.

Examples of sufficient QC for the presumptive tests listed in Appendix D include:

- Method blanks
- Positive test samples / negative test samples
- Calibration check samples
- Use of test kits and reagents prior to expiration date
- Accurate temperature controls

Examples of sufficient QC for the confirmatory tests listed in Appendix D include:

- Demonstration that the measurement system is operating properly
  - Initial calibration
  - Method blanks

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

- Demonstration of measurement system suitability for intended use
  - Precision and recovery (verify measurement system has adequate accuracy)
  - Analyte/sample type/level of concern-specific QC samples (verify that measurement system has adequate sensitivity at levels of concern)

- Demonstration of continued measurement system reliability
  - Matrix spike/matrix spike duplicates (MS/MSDs) recovery and precision
  - QC samples (system accuracy and sensitivity at levels of concern)
  - Continuing calibration verification
  - Method blanks

Please note: The appropriate point of contact identified in Section 4 should be consulted regarding appropriate quality assurance (QA)/QC procedures prior to sample analysis. These contacts will consult with the EPA Environmental Response Laboratory Network (ERLN) and Water Laboratory Alliance (WLA) coordinators responsible for laboratory activities during the specific event to ensure QA/QC procedures are performed consistently across laboratories. EPA program offices will be responsible for ensuring that the QA/QC practices are implemented.

8.1.3 Safety and Waste Management

It is imperative that safety precautions be used during collection, processing, and analysis of environmental samples. Laboratories should have a documented health and safety plan for handling samples that may contain target chemical, biological and/or radiological (CBR) contaminants, and laboratory staff should be trained in and implement the safety procedures included in the plan. In addition, many of the methods summarized or cited in Section 8.2 contain some specific requirements, guidelines or information regarding safety precautions that should be followed when handling or processing environmental samples and reagents. These methods also provide information regarding waste management. Other resources that can be consulted for additional information include the following:

- American Biological Safety Association, Risk Group Classifications for Infectious Agents. Available at: http://www.absa.org/riskgroups/index.html
- CDC. 2009. *Biosafety in Microbiological and Biomedical Laboratories* (BMBL), 5th Edition. Available at: http://www.cdc.gov/biosafety/publications/bmbl5/index.htm
- Fleming, D.O. and Hunt, D.L. (editors). 2006. Biological Safety: Principles and Practices, 4th Ed. American Society for Microbiology (ASM) Press: Herndon, VA
- CDC – 42 CFR part 72. Interstate Shipment of Etiologic Agents
- CDC – 42 CFR part 73. Select Agents and Toxins
- DOT – 49 CFR part 172. Hazardous Materials Table, Special Provisions, Hazardous Materials Communications, Emergency Response Information, and Training Requirements
- EPA – 40 CFR part 260. Hazardous Waste Management System: General. Available at: http://www.ecfr.gov/
- EPA – 40 CFR part 270. EPA Administered Permit Programs: The Hazardous Waste Permit Program. Available at: http://www.access.gpo.gov/nara/cfr/waisidx_07/40cfr270_07.html
- Occupational Safety and Health Administration (OSHA) – 29 CFR part 1910.1450. Occupational Exposure to Hazardous Chemicals in Laboratories Available at: http://www.gpo.gov/fdsys/pkg/CFR-2014-title29-vol6/pdf/CFR-2014-title29-vol6-sec1910-1450.pdf
- OSHA – 29 CFR part 1910.120. Hazardous Waste Operations and Emergency Response

- U.S. Department of Agriculture (USDA) – 9 CFR part 121. Possession, Use, and Transfer of Select Agents and Toxins

The Electronic Code of Federal Regulations (e-CFR) is available at http://www.ecfr.gov/

8.1.4 Laboratory Response Network (LRN)

The LRN was created in accordance with Presidential Decision Directive 39, which established terrorism preparedness responsibilities for federal agencies. The LRN is primarily a national network of local, state, federal, military, food, agricultural, veterinary and environmental laboratories; however, additional LRN laboratories are located in strategic international locations. The CDC provides technical and scientific support to member laboratories as well as secure access to standardized procedures and reagents for rapid (within 4 to 6 hours) presumptive detection of biothreat agents and emerging infectious disease agents. These rapid presumptive assays are part of agent-specific algorithms of assays which lead to a confirmed result. The algorithm for a confirmed result is often a combination of one or more presumptive positive results from a rapid assay in combination with a positive result from one of the "gold standard" methods, such as culture. The standardized procedures, reagents and agent-specific algorithms are considered to be sensitive and are available only to LRN member laboratories. Thus, these procedures are not available to the general public and are not discussed in this document.

Many of the biotoxins listed in SAM are select agents. Additional information on select agents and regulations may be obtained at the National Select Agent Registry at: http://www.selectagents.gov/. Relevant to the purposes of analytical methods, SAM users should note that some of these agents are not regulated if the amount under the control of a principal investigator does not exceed, at any time, the amounts indicated in the table at: http://www.selectagents.gov/PermissibleToxinAmounts.html.

For additional information on the LRN, including selection of a laboratory capable of receiving and processing the specified sample type/analyte, please use the contact information provided below or visit http://www.bt.cdc.gov/lrn/.

Centers for Disease Control and Prevention
Laboratory Response Branch
Division of Bioterrorism Preparedness and Response (DBPR)
National Center for Prevention, Detection, and Control of Infectious Diseases (NCPDCID) Coordinating Center for Infectious Diseases (CCID)
1600 Clifton Road NE, Mailstop C-18
Atlanta, GA 30333
Telephone: (866) 576-5227
E-mail: lrn@cdc.gov

Local public health laboratories, private, and commercial laboratories with questions about the LRN should contact their state public health laboratory director or the Association of Public Health Laboratories (APHL) (contact information provided below).

Association of Public Health Laboratories
8515 Georgia Avenue, Suite 700
Silver Spring, MD 20910
Telephone: (240) 485-2745
Fax: (240) 485-2700
Web site: www.aphl.org
E-mail: info@aphl.org

8.2 Method Summaries for Protein Biotoxins

Summaries of the analytical methods for protein biotoxins listed in Appendix D are provided in Sections 8.2.1 through 8.2.5. These sections contain summary information, extracted from the selected methods. The full version of the method should be consulted prior to sample analysis.

Each summary contains a brief description of the method, intended method application, performance data (if available), and a link to or source for obtaining a full version of the method.

8.2.1 Abrin

Abrin – CAS RN: 1393-62-0.
Description: Glycoprotein consisting of a deadenylase (25–32 kDa A chain) and lectin (35 kDa B chain); an agglutinin (A2B2) may be present in crude preparations.
Abrine – CAS RN: 526-31-8
Description: Small molecule, indole alkaloid marker for abrin.

Method	Analytical Technique	Section
Journal of Food Protection. 2008. 71(9): 1868–1874	Immunoassay (ELISA, ECL-based)	8.2.1.1
Journal of Agricultural and Food Chemistry. 2008. 56(23): 11139–11143	LC-MS-MS	8.2.1.2
Pharmacology & Toxicology. 2001. 88(5): 255–260	Ribosome inactivation assay	8.2.1.3
Analytical Biochemistry. 2008. 378: 87–89	Enzyme activity	8.2.1.4

8.2.1.1 Literature Reference for Abrin (Journal of Food Protection. 2008. 71(9): 1868–1874)

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay (ELISA, ECL-based)
Method Developed for: Abrin in food
Method Selected for: SAM lists these procedures for presumptive analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Procedures are described for using mouse monoclonal antibodies (mAbs) and rabbit-derived polyclonal antibodies prepared against a mixture of abrin isozymes for three separate ELISA and ECL-based assays in food products. The three assays vary by use of antibody combination (e.g., assay configuration): (1) polyclonal (capture)/polyclonal (detection) ELISA, (2) polyclonal/monoclonal ELISA and (3) polyclonal/monoclonal ECL assay. The LODs, with purified Abrin C and various abrin extracts in buffer, are between 0.1 and 0.5 ng/mL for all three assays. The LOD for abrin spiked into food products ranged from 0.1 to 0.5 ng/mL, using the ECL assay. The LOD for abrin spiked into food products for the ELISA assays ranged between 0.5 and 10 ng/mL depending on the antibody combination. In all cases, the LODs were less than the concentration at which abrin may pose a health concern.

Special Considerations: Crude preparations of abrin may also contain agglutinins that are unique to rosary peas and that can cross-react in the immunoassays. Addition of non-fat milk powder to the sample buffer may eliminate false-positive results (Dayan-Kenigsberg, J., Bertocchi, A. and Garber, E.A.E. 2008. "Rapid Detection of Ricin in Cosmetics and Elimination of Artifacts Associated With Wheat Lectin." Journal of Immunological Methods. 336(2): 251–254). http://www.sciencedirect.com/science/journal/00221759

Source: Garber, E.A.E., Walker, J.L. and O'Brien, T.W. 2008. "Detection of Abrin in Food Using Enzyme-Linked Immunosorbent Assay and Electrochemiluminescence Technologies." Journal of Food Protection. 71(9): 1868–1874. http://www.ingentaconnect.com/content/iafp/jfp/2008/00000071/00000009/art00015

8.2.1.2 Literature Reference for Abrin by Abrine Detection (Journal of Agricultural and Food Chemistry. 2008. 56(23): 11139-11143)

Analysis Purpose: Complementary presumptive for abrin
Analytical Technique: LC-MS-MS

Method Developed for: Abrine in beverages
Method Selected for: SAM lists these procedures for complementary presumptive analysis of abrin by abrine detection in aerosol, solid, particulate, liquid and water samples. Abrine, an alkaloid present in equal concentrations with abrin in rosary peas (*Abrus precatorius* L.), is found in crude preparations of abrin and may be an indicator of abrin contamination. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Procedures are described for sample extraction by solid-phase extraction (SPE) or liquid-liquid extraction, followed by tandem mass spectrometry. The method was verified in beverages (bottled water, cola, juice drink, 1% low fat milk, bottled tea) spiked with abrine at either 0.5µg/mL or 0.05µg/mL. These samples were prepared for LC-MS-MS by either an optimized SPE procedure or a liquid-liquid extraction procedure. For SPE, optimal abrine recoveries were achieved with sample pH adjusted to 2 – 6 with formic acid, inclusion of a water/methanol (95/5, v/v) washing step prior to elution, and use of a Strata-X SPE cartridge. Liquid-liquid extraction was with an equal volume (2 mL) of acetonitrile/water (75/25, v/v). Differences in recovery between the two extraction methods were determined using the two-sided Student's *t* test, assuming equal variance. At 0.5 µg/mL, recovery of abrine by SPE was significantly higher ($P < 0.01$) for water and juice drink as compared to liquid-liquid extraction, but no significant differences were observed for cola and tea. At 0.05 µg/mL, the differences in recovery of abrine in water, tea, cola and juice drink were highly statistically different ($P < 0.001$), with better recoveries for the optimized SPE procedure. The method had a MDL of 0.025 µg/mL and LOQ of 0.05 µg/mL. Storage stability was also tested for abine at 10 µg/mL in a water/methanol stock solution (90/10, v/v) at three temperatures (0 °C, 4 °C and 23 °C). Aliquots were analyzed in triplicate at 0, 1, 7 and 21 days after sample preparation. There was no statistically significant difference between abrine standards stored at the three temperatures at 21 days and no loss of abrine concentration.

Special Considerations: The biotoxin methods points of contact listed in Section 4.0 of SAM should be consulted for additional information regarding water and drinking water analyses.

Source: Owens, J. and Koester, C. 2008. "Quantitation of Abrine, an Indole Alkaloid Marker of the Toxic Glycoproteins Abrin, by Liquid Chromatography/Tandem Mass Spectrometry When Spiked into Various Beverages." Journal of Agriculture and Food Chemistry. 56(23): 11139–11143. http://pubs.acs.org/doi/abs/10.1021/jf802471y

8.2.1.3 Literature Reference for Abrin and Ricin (Analytical Biochemistry. 2008. 378(1): 87-89)

Analysis Purpose: Biological activity
Analytical Technique: Enzyme activity
Method Developed for: Jequirity seed (abrin) and castor bean (ricin) extracts in buffer

Method Selected for: SAM lists these procedures for biological activity analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This *in vitro* assay is a ribonucleic acid (RNA) N-glycosidase enzyme activity assay for the detection of purified abrin and ricin toxins (Types I and II) or in jequirity seed (abrin) and castor bean (ricin) extracts. Synthetic biotinylated RNA substrates with varied loop sequences are cleaved by either the ricin or abrin toxin and the RNA products are hybridized to ruthenylated-oligodeoxynucleotides to generate an ECL signal. Assays require incubation for 2 hours at 48 °C. Commercially available ECL-based reagents and RNase inactivators are used. Control experiments for the jequirity seed experiments and the distinct GdAA/GdAGA ratio for the castor bean assay demonstrate lack of non-specific cleavage for the assay. The undiluted castor bean extract contained 22.0 ± 0.7 mg/mL total protein and 4.1 ± 0.3 mg/mL ricin equivalents as determined by standard protein determination and by ECL immunoassay assays respectively. The undiluted jequirity seed extract was similarly assayed, with a resultant 21.6 ± 0.6 mg/mL total protein and 3.7 ± 0.3 µg/mL equivalents of toxin. Dilutions were performed to determine effective signal-to-background ratio and the linear range for calculation of toxin activity. Resultant calculations for ricin activity equivalents in the undiluted castor bean extract were equivalent to those obtained with the ECL immunoassays: 4.4 ± 0.2 mg/mL activity equivalents. In contrast, the undiluted jequirity seed extract contained a calculated level of 740 ± 50 µg/mL activity equivalents, which greatly exceeded the immunoassay-based value.

Special Considerations: This enzyme activity assay does not test for cell binding; cell culture assays are being developed to test for cell binding but are not currently available. The only readily available assay to test for both the cell binding and enzymatic activity of the intact (whole) toxin is the mouse bioassay.

Source: Keener, W.K., Rivera, V.R., Cho, C.R., Hale, M.L., Garber, E.A.E. and Poli, M.A. 2008. "Identification of the RNA N-glycosidase Activity of Ricin in Castor Bean Extracts by an Electrochemiluminescence-Based Assay." Analytical Biochemistry. 378(1): 87–89. http://www.sciencedirect.com/science/journal/00032697

8.2.1.4 Literature Reference for Abrin, Shiga Toxin, and Shiga-like Toxins (Pharmacology Toxicology. 2001. 88(5): 255–260)

Analysis Purpose: Confirmatory for abrin; biological activity for shiga and shiga-like toxins
Analytical Technique: Ribosome inactivation assay

Method Developed for: Abrin in phosphate buffered saline (PBS)
Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Procedures are described for measuring the biological activity of ribosome-inactivating proteins using a microtiter plate format for detection of abrin in PBS. Nuclease-treated rabbit reticulocyte lysate containing luciferase messenger ribonucleic acid (mRNA) is used to measure toxin activity via inhibition of protein synthesis. The relative biological activity is determined by comparing luminescence levels in treated samples versus those of untreated controls. The amount of luciferase translated, as measured by luminescence, is inversely proportional to the toxin concentration. Linear dose response curves are generated for abrin, with a 50% inhibition of translation at 0.5 nanomolar (nM). Coupling this procedure, or a modification of this procedure, with an immunoassay will provide more information regarding the specificity and toxicity of the target biotoxin.

Special Considerations: For abrin, as well as shiga and shiga-like toxins, this assay does not test for cell binding; cell culture assays are being developed to test for cell binding but are not currently available. The only readily available assay to test for both the cell binding and enzymatic activity of the intact (whole) toxin is the mouse bioassay.

Source: Hale, M.L. 2001. "Microtiter-Based Assay for Evaluating the Biological Activity of Ribosome-Inactivation Proteins." Pharmacology Toxicology. 88(5): 255–260. http://www3.interscience.wiley.com/journal/120703798/abstract

8.2.2 Botulinum Neurotoxins (Serotypes A, B, E, F)

Botulinum neurotoxins – Description: Protein composed of ~100 kDa heavy chain and ~50 kDa light chain; can be complexed with hemagglutinin and non-hemagglutinin components for total molecular weight (MW) of ~900 kDa.
SNAP-25 – Description: Synaptosomal-associated protein 25; 25 kDa membrane-associated protein cleaved by botulinum neurotoxin Serotypes A, C and E
VAMP 2 – Description: Vesicle-associated membrane protein 2 (also known as synaptobreven 2); cleaved by botulinum neurotoxin Serotypes B, D, F and G

Method	Analytical Technique	Section
LRN	Immunoassay, Immunoassay (ELISA) and Mouse bioassay	8.1.4
FDA, Bacteriological Analytical Manual Online, January 2001, Chapter 17, *Clostridium botulinum*	Immunoassay (ELISA) and Mouse bioassay	8.2.2.1
Journal of Chemical Health and Safety. 2008. 15(6): 14–19	Endopep-MS	8.2.2.2
Lateral Flow Device Immunoassay Kits	Immunoassay (LFD)	8.2.2.3

8.2.2.1 FDA, Bacteriological Analytical Manual Online, Chapter 17, 2001: Botulinum Neurotoxins

Analysis Purpose: Confirmatory and biological activity
Analytical Technique: Immunoassay (ELISA) and mouse bioassay

Method Developed for: Botulinum neurotoxins (Serotypes A, B, E, F) in food
Method Selected for: SAM lists this procedure for confirmation and biological activity assessment in aerosol samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: An amplified-enzyme-linked immunosorbent assay (amp-ELISA) and a digoxigenin-labeled enzyme-linked immunosorbent assay (DIG-ELISA) are described for the detection of Types A, B, E and F botulinum neurotoxins in food products. The amp-ELISA method uses goat anti-A or E, rabbit anti-B or horse anti-F serum to capture the toxins in a 96-well plate, and a corresponding biotinylated goat antitoxin to detect the toxin. Visualization is with streptavidin-alkaline phosphatase. The DIG-ELISA method is a modification of the amp-ELISA method, with digoxigenin-labeled antitoxin IgG's substituted for the streptavidin-alkaline phosphatase. Toxin can be detected at approximately 10 minimum lethal doses (MLD)/mL (0.12 to 0.25 ng/mL). High concentration samples (greater than 10,000 MLD/mL) may give a positive absorbance for more than one toxin type. Further dilution of the sample will remove cross-reactivity.

The mouse bioassay detects biologically active toxin using a three part approach: toxin screening; toxin titer; and finally, toxin neutralization using monovalent antitoxin sera. Samples are

prepared by centrifugation for suspended solids under refrigeration, or solids are extracted with an equal volume of pH 6.2 gel-phosphate buffer and then centrifuged. Toxins from nonproteolytic strains of *C. botulinum* may need trypsin activation to be detected. Serial dilutions of untreated and trypsin-treated sample fluids are injected in separate pairs of mice intraperitoneally (i.p.). Mice are also injected with heated, untreated, undiluted sample. Death of mice, along with symptoms of botulism, confirms presence of botulinum toxin. After calculation of an MLD, dilute monovalent antitoxin sera types A, B, E and F are injected into mice 30 minutes to 1 hour before challenging them with the i.p. injection of each dilution that gave the highest MLD from the toxic preparation.

Special Considerations: Immunoassays with botulinum toxins may produce variable results with uncomplexed forms of toxin.

Source: FDA, Center for Food Safety and Applied Nutrition (CFSAN). 2001. "Chapter 17 – *Clostridium botulinum*." *Bacteriological Analytical Manual Online.* http://www2.epa.gov/sites/production/files/2015-07/documents/fda-bam-chap17.pdf

8.2.2.2 Literature Reference for Botulinum Neurotoxins by SNAP-25 and VAMP 2 Cleavage Product Detection (Journal of Chemical Health and Safety. 2008. 15(6): 14–19)

Analysis Purpose: Complementary presumptive for botulinum neurtotoxins
Analytical Technique: LC-MS

Method Developed for: Botulinum neurotoxins Serotypes A, B, E and F in clinical samples (stool, serum)
Method Selected for: SAM lists these procedures for complementary presumptive analysis of botulinum neurotoxins by SNAP-25 and VAMP 2 cleavage product detection in aerosol samples. SNAP-25 and VAMP 2 function as substrates for botulinum neurotoxins and may be an indicator of botulinum neurotoxin contamination. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Procedures are described for antibody-based sample extraction, followed by synthetic peptide cleavage and high resolution matrix-assisted laser-desorption ionization (MALDI) time-of-flight (TOF)-MS. The method is verified for stool and serum clinical samples obtained from an exposed individual. Botulinum neurotoxin Serotypes A, B, D and E are obtained from Metabiologics (Madison, Wisconsin) and used as positive controls. Rabbit polyclonal antibodies specific for Serotypes A, B, E and F are also obtained from Metabiologics and are coupled to Dynabeads® Protein G beads. A cocktail of protease inhibitors and 20 µL of beads are added to 100 µL of stool sample. The mixture is incubated for two hours at 37 °C, washed in buffer, followed by a water wash. A 500-µL serum sample is added to 100 µL of beads and similarly incubated and washed. Protease inhibitors are not required for serum samples. After antibody isolation, the bead-extracted sample is incubated in a reaction buffer with synthetic peptide substrates specific for Serotypes A, B, E and F. Samples are incubated at 37 °C for four hours. A 2-µL aliquot of the reaction mixture supernatant is mixed with 18 µL of a matrix solution and 0.5 µL of the resultant mixture is placed on a 192-spot MALDI plate. Mass spectra are collected from 650 to 4500 *m/z* in the positive ion reflector mode on either an Applied Biosystems™ 4700 Proteomics Analyzer or an Applied Biosystems™ 4800 TOF/TOF. Cleavage product peaks specific for Serotypes A, B, E and F can be for observed for the positive controls and positive stool and serum samples. Negative controls do not show these peaks.

Special Considerations: Additional detector platforms are available such as described in "Development of an *In Vitro* Activity Assay as an Alternative to the Mouse Bioassay for Clostridium botulinum Neurotoxin Type A," 2008. Applied and Environmental Microbiology.

74(14): 4309–4313. (http://aem.asm.org/content/74/14/4309.short). Fluorescence resonance energy transfer (FRET) based assays are also available as commercial products (http://www.biosentinelpharma.com/).

Source: Barr, J.R., Kalb, S.R., Moura, H. and Pirkle, J.L. 2008. "Biological Monitoring of Exposure to Botulinum Neurotoxins." Journal of Chemical Health and Safety. 15(6): 14–19. http://www.sciencedirect.com/science/article/pii/S1871553208000418

8.2.2.3 EPA Environmental Technology Verification (ETV) Program Reports – Lateral Flow Immunoassay Kits

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay (LFD)

Method Developed for: Botulinum neurotoxins (Types A, B) and ricin in buffer or water samples
Method Selected for: SAM lists these procedures for presumptive analysis in aerosol samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water.

Description of Method: Test strips are self-contained, qualitative assays for screening environmental samples for the presence of botulinum toxin and ricin. After the sample is collected, it is transferred onto the test strip where dye-labeled antibodies detect trace amounts of the contaminant, as indicated by the presence of two bands in the test result window. After 15 minutes, the results are read visually. Botulinum neurotoxin Type A can be detected at 5 mg/L and Type B at 4 mg/L, 33% of the time. Ricin toxin can be detected at 20 mg/L, with no cross-reactivity to certain substances (i.e., lectin from soybeans).

An alternative immunochromatographic LFD also can be used. This device uses two antibodies in combination to specifically detect target antigen in solution. When a sufficient amount of target antigen is present, the colloidal gold label accumulates in the sample window on a test strip, forming a visible reddish-brown colored line. The presence of two bands indicates a positive reading. Botulinum neurotoxin Type A can be detected at 0.01 mg/L and Type B at 0.5 mg/L, with no false negatives detected when interferents are present. Ricin toxin can be detected at 0.035 mg/L, with 88% accuracy.

LFD immunoassay kits have been evaluated by the EPA ETV Program using BADD™ and BioThreat Alert® test strips for the detection of botulinum neurotoxins Types A and B and ricin. Reports and information associated with these evaluations are available at: http://www2.epa.gov/sites/production/files/2015-07/documents/etv-badd091904.pdf (BADD™ test strips) and http://www2.epa.gov/sites/production/files/2015-07/documents/etv-biothreat092104.pdf (BioThreat Alert® test strips).

Special Considerations: Immunoassays with botulinum toxins may produce variable results with uncomplexed form of toxin. Addition of non-fat milk powder to the sample buffer may eliminate false-positive results (Dayan-Kenigsberg, J., Bertocchi, A. and Garber, E.A.E. 2008. "Rapid Detection of Ricin in Cosmetics and Elimination of Artifacts Associated With Wheat Lectin." Journal of Immunological Methods. 336(2): 251–254). http://www.sciencedirect.com/science/article/pii/S0022175908001671

Source: ETV. 2006. http://www.epa.gov/etv/

8.2.3 Ricin (Ricinine)

Ricin – CAS RN: 9009-86-3.
Description: 60 kDa glycoprotein consisting of a deadenylase (~32 kDa A chain) and lectin (~34 kDa B chain); an agglutinin of MW 120 kDa may be present in crude preparations.
Ricinine – CAS RN: 5254-40-3.
Description: Small molecule, alkaloid marker for ricin.

Method	Analytical Technique	Section
LRN	Immunoassay	8.1.4
Analytical Biochemistry. 2008. 378: 87–89	Enzyme activity	8.2.1.3
LFD Immunoassay Kits	Immunoassay (LFD)	8.2.2.2
Journal of AOAC International. 2008. 91(2): 376–382	Immunoassay (ECL)	8.2.3.1
Journal of Analytical Toxicology. 2005. 29: 149–155	LC-MS	8.2.3.2

8.2.3.1 Literature Reference for Ricin (Journal of AOAC International. 2008. 91(2): 376–382)

Analysis Purpose: Confirmatory
Analytical Technique: Immunoassay (ECL)

Method Developed for: Ricin for food products
Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This immunoassay is for the detection of various concentrations of purified ricin in food products (e.g., juice, dairy products, vegetables, bakery products, condiments). The immunoassay uses ECL detection in a 96-well plate format with a monoclonal capture antibody against ricin (19A-2C6) and either a polyclonal or monoclonal detector antibody. The samples and detector antibodies can be added sequentially or in combination during the capture step. Using the polyclonal antibody, ricin was detected at concentrations as low as 0.04 ng/mL. Simultaneous addition of sample and detector antibody allowed for a shortened procedure with only a single 20 minute incubation with no false negatives caused by "hook" effects at high concentrations of ricin. Quantitation can be performed either with the sequential procedure or with the simultaneous procedure if it is know that the ricin concentration is not in the "hook" region. The simultaneous procedure should not be used when a sample contains constituents that may react with the ruthenium tag. Polyclonal/monoclonal antibodies are commercially available as an ELISA test kit.

Special Considerations: Crude preparations of ricin may also contain agglutinins that are unique to castor beans and that can cross-react in the immunoassays.

Source: Garber, E.A.E. and O'Brien, T. W. 2008. "Detection of Ricin in Food Using Electrochemiluminescence-Based Technology." Journal of AOAC International. 91(2): 376–382. http://aoac.publisher.ingentaconnect.com/content/aoac/jaoac/2008/00000091/00000002/art00016

8.2.3.2 Literature Reference for Ricin by Ricinine Detection (Journal of Analytical Toxicology. 2005. 29(3): 149–155)

Analysis Purpose: Complementary presumptive for ricin
Analytical Technique: LC-MS

Method Developed for: Ricinine in human and rat urine samples
Method Selected for: SAM lists these procedures for complementary presumptive analysis of ricin by ricinine detection in aerosol, solid, particulate, liquid and water samples. Ricinine, an alkaloid component of castor beans, is found in crude preparations of ricin, and may be an indicator of ricin contamination. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Procedures are described for sample extraction by SPE, isocratic HPLC, followed by electrospray ionization (ESI) tandem mass spectrometry. For MS analyses, protonated molecular ions are selected in the multiple reaction monitoring mode and quantified by isotope dilution with $^{13}C_6$-labeled ricinine as the internal reference. Urine pools enriched with ricinine at two concentrations were used as quality controls for validation of the method in urine samples. The calculated LOD was 0.04 ng/mL. In addition to the validation with urine samples, testing was performed on a single human urine sample (forensic), a crude ricin preparation, and urine samples from an animal ricinine exposure study. For the human urine sample, the concentration of ricinine was measured to be 4.24 ng/mL. After a series of simple extraction and filtration steps to provide a crude castor bean preparation, the final ricinine level was 502 ng/mL. For the animal exposure study, rats were injected with ricinine at 1, 5 and 10 mg/kg, with mean 24-hour urine concentrations of 1010, 6364 and 17,152 ng/mL, respectively. Mean 48-hour urine concentrations were 40, 324 and 610 mg/mL. Stability of ricinine in human urine was also tested, with ricinine found to be stable in human urine samples when heated at 90 °C for 1 hour and when stored at 25 °C and 5 °C for 3 weeks.

Special Considerations: The following updated literature reference adds the analyte abrine for detection of select agent abrin: Rudolph C. Johnson, Yingtao Zhou, Ram Jain, Sharon W. Lemire, Shannon Fox, Pat Sabourin and John R. Barr. 2009. "Quantification of L-Abrine in Human and Rat Urine: A Biomarker for the Toxin Abrin." Journal of Analytical Toxicology, 33, (2), 77−84.

Source: Johnson, R.C., Lemire, S.W., Woolfitt, A.R., Ospina, M., Preston, K.P, Olson, C.T. and Barr, J.R. 2005. "Quantification of Ricinine in Rat and Human Urine: A Biomarker for Ricin Exposure." Journal of Analytical Toxicology. 29(3): 149–155.
http://jat.oxfordjournals.org/content/29/3/149.full.pdf+html

8.2.4 Shiga and Shiga-like Toxins (Stx, Stx-1, Stx-2)

CAS RN: 75757-64-1 (Stx).
Description: Protein composed of one ~32 kDa A chain and five 7.7 kDa B chains.

Method	Analytical Technique	Section
Pharmacology & Toxicology. 2001. 88(5): 255–260	Ribosome inactivation assay	8.2.1.4
FDA, Bacteriological Analytical Manual Online, January 2001, Appendix 1, Rapid Methods for Detecting Foodborne Pathogens	Immunoassay (ELISA)	8.2.4.1
Journal of Clinical Microbiology. 1997. 35 (8): 2051 – 2054	Immunoassay (EIA)	8.2.4.2

8.2.4.1 FDA, Bacteriological Analytical Manual Online, Appendix 1, 2001: Rapid Methods for Detecting Foodborne Pathogens

Analysis Purpose: Confirmatory
Analytical Technique: Immunoassay (ELISA)

Method Developed for: Shiga and shiga-like toxins in food
Method Selected for: SAM lists this manual for presumptive analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental samples.

Description of Method: Shiga toxin (Stx) is produced by *Shigella dysenteriae* and Shiga-like toxins (Shiga toxin Types 1 [Stx-1] and 2 [Stx-2]) are produced by various Shiga-toxigenic *E. coli* (STEC). An ELISA is described for the detection of these toxins. Diluted samples are added to microwells coated with an anti-Shiga toxin capture antibody. After incubation at room temperature, a wash is performed to remove unbound material. A second anti-Shiga toxin antibody is added for detection and incubation continued at room temperature. A wash is performed to remove unbound antibody. Enzyme conjugated anti-IgG visualization antibody, directed against the species from which the second anti-Shiga toxin antibody was derived, is added and the plate incubated then rinsed. Substrate is added, and after incubation to develop the color, stop solution is added. The results are interpreted spectrophotometrically.

Source: FDA, CFSAN. 2001. "Rapid Methods for Detecting Foodborne Pathogens." *Bacteriological Analytical Manual Online.* http://www2.epa.gov/sites/production/files/2015-07/documents/fda-bam-appendix1.pdf

8.2.4.2 Literature Reference for Shiga and Shiga-like Toxins (Journal of Clinical Microbiology. 1997. 35 (8): 2051 – 2054)

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay (EIA)

Method Developed for: Shiga toxin in clinical samples
Method Selected for: SAM lists these procedures for presumptive analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Procedures are described for a rapid EIA for the detection of Stx-1 and Stx-2 using a commercially available kit. Fecal samples are assayed for Shiga toxin using the EIA kit with overnight enrichment (500 specimens) and using a sorbitol-MacConkey's (sMac) culture method (474 specimens). Samples producing positive results by EIA kit and/or sMac culture are confirmed by Vero cell cytotoxicity assay using a 96-well format. Samples positive by EIA kit and negative by sMac culture are recultured by the mitomycin immunoblot procedure to isolate organisms and retested for confirmation by Vero cell cytotoxicity assay. The sMac culture method had a sensitivity and specificity of 60% and 100%, respectively, for detection of Shiga toxin producing *E. coli* O157:H7. EIA kit sensitivity and specificity are 100% and 99.7%, respectively. The EIA kit is also capable of detecting Shiga toxin in cultures negative for the *E. coli* O157:H7 serotype (e.g., *E. coli* serotypes O26:NM and 6:H–).

Source: Kehl, K.S., Havens, P., Behnke, C.E. and Acheson, D.W.K. 1997. "Evaluation of the Premier EHEC Assay for Detection of Shiga Toxin-Producing *Escherichia coli*." Journal of Clinical Microbiology. 35(8): 2051–2054. http://jcm.asm.org/cgi/reprint/35/8/2051.pdf

8.2.5 Staphylococcal Enterotoxins (SEA, SEB, SEC)

CAS RNs: 37337-57-8 (SEA), 39424-53-8 (SEB), 39424-54-9 (SEC)
Description: Monomeric protein of ~ 28 kDa (SEB), monomeric proteins of ~ 27–27.5 kDa (SEA and SEC)

Method	Analytical Technique	Section
LRN	Immunoassay	8.1.4
AOAC Official Method 993.06	Immunoassay (EIA)	8.2.5.1
Applied and Environmental Microbiology. 1997. 63(6): 2361–2365	T-cell proliferation assay	8.2.5.2

8.2.5.1 AOAC Official Method 993.06: Staphylococcal Enterotoxins in Selected Foods

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay (EIA)

Method Developed for: Staphylococcal enterotoxins in selected foods
Method Selected for: SAM lists this method for presumptive analysis of staphylococcal enterotoxins Type B (SEB) in aerosol samples, and Types A (SEA) and C (SEC) in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This method is an EIA using a mixture of high-affinity capture antibodies for identification of toxin(s) in food samples. Samples are prepared by dilution in Tris buffer, centrifugation, and filtration of the supernatant through a syringe, with adjustment to a final pH of 7.0 to 8.0. Samples are incubated in 96-well plates with the mixture of antibodies conjugated to horseradish peroxidase (HRP), and visualized with a peroxidase substrate. Assay results are determined visually or using a microtiter plate reader. Test is considered positive for staphylococcal enterotoxins if absorbance is >0.200 and is considered negative if absorbance is ≤0.200. Specific toxin serotypes are not differentiated. This method detects from 1.3 to 3.3 ng/mL staphylococcal enterotoxin in extracts prepared from food containing 4 to 10 ng/mL staphylococcal enterotoxin.

Source: AOAC International. 1994. "Method 993.06: Staphylococcal Enterotoxins in Selected Foods." *Official Methods of Analysis of AOAC International*. 16th Edition. 4th Revision; Vol. I. http://www.aoac.org/

8.2.5.2 Literature Reference for Staphylococcal Enterotoxins Types A, B, and C (Applied and Environmental Microbiology. 1997. 63(6): 2361–2365)

Analysis Purpose: Biological activity
Analytical Technique: T-cell proliferation assay

Method Developed for: Staphylococcal enterotoxin Type A (SEA) in selected foods
Method Selected for: SAM lists this method for biological activity assessment of staphylococcal enterotoxins Type B in aerosol samples, and Types A and C in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This method is a T-cell proliferation assay using lymphocytes in a 96-well plate format for identification of staphylococcal enterotoxin(s) in food samples. Lymphocytes are prepared from heparinized Lewis rat blood or human blood using Ficoll-Paque™. Cells are divided into aliquots at 0.5×10^5 to 1.0×10^5 cell per well in 100 µL culture medium into a U-bottomed 96-well tissue culture plate. Food samples (potato salad, canned mushrooms, hot dogs, dry milk) are homogenized in PBS (1:1, wt/wt), centrifuged, the supernatants diluted 1:10 in PBS, and added directly to sample wells containing lymphocytes. Varying concentrations of SEA can be used as a standard curve. The treated samples are added to the lymphocytes and incubated for two to five days at 37 °C. On the last day either 1 µCi of [methyl-^{3}H] thymidine or 20 µL of Alamar blue is added to the well. After 24 hours, supernatant is either harvested onto glass fiber filters and the beta-radioactivity counted or the color reaction of the Alamar blue treated wells is read on a plate reader at 570 nm. Both human and rat lymphocytes produce strong T-cell proliferation in response to SEA. The radioactive assay shows a significant level of proliferation ($P < 0.05$) as compared to control medium at levels as

low as 0.1 pg SEA per well. The Alamar blue assay detects SEA at 1 ng per well. Diluted food samples without SEA do not induce T-cell proliferation.

Special Considerations: This method was developed for SEA in selected foods and has not been tested with SEB and SEC or in other sample types. However, because the T-cell proliferation assay is not antigen specific, the method may be appropriate for SEB and SEC, both of which have superantigen T-cell proliferation activity. This assay cannot identify the specific superantigen nor can it assess emetic activity; additional testing to determine specificity and assess toxin activity should be performed.

Source: Rasooly, L., Rose, N.R., Shah, D.B. and Rasooly, A. 1997. "In Vitro Assay of *Staphylococcus aureus* Enterotoxin A Activity in Food." Applied and Environmental Microbiology. 63(6): 2361–2365. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC168529/

8.3 Method Summaries for Small Molecule Biotoxins

Summaries of the analytical methods for small molecule biotoxins listed in Appendix D are provided in Sections 8.3.1 through 8.3.12. These sections contain summary information only, extracted from the selected methods. The full version of the method should be consulted prior to sample analysis. Each summary contains a brief description of the method, intended method application, performance data (if available), and a link to or source for obtaining a full version of the method.

8.3.1 Aflatoxin (Type B1)

CAS RN: 27261-02-5

Method	Analytical Technique	Section
AOAC Official Method 991.31	Immunoassay (column) and HPLC-FL	8.3.1.1

8.3.1.1 AOAC Official Method 991.31: Aflatoxins in Corn, Raw Peanuts, and Peanut Butter

Analysis Purpose: Presumptive and confirmatory
Analytical Technique: Immunoassay (column) and high performance liquid chromatography-fluorescence (HPLC-FL)

Method Developed for: Aflatoxins (Type B1) in corn, raw peanuts and peanut butter
Method Selected for: SAM lists this method for presumptive and confirmatory analyses in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: This method is for the detection of aflatoxins in agricultural products. The sample is extracted with methanol-water (7 + 3), filtered, diluted with water, and applied to an affinity column containing mAbs specific for aflatoxins B1, B2 (CAS RN 22040-96-6), G1 (CAS RN 1385-95-1), and G2 (CAS RN 7241-98-7). Antibody-bound aflatoxins are removed from the column with methanol. For detection and quantitation of total aflatoxins, fluorescence measurement after reaction with bromine solution is performed. For individual aflatoxins, fluorescence detection and postcolumn iodine derivatization are performed and quantitation is by LC. Method performance was characterized using various commodities (e.g., corn) at aflatoxin levels over a range of 10 to 30 ng/g. This method was originally designed for the analysis of aflatoxins (B_1, B_2, G_1 and G_2) in samples where cleanup was necessary to remove food components, such as fats and proteins; the cleanup procedure may not be necessary for analysis of water samples.

Special Considerations: AOAC Official Method 994.08: Aflatoxin in Corn, Almonds, Brazil Nuts, Peanuts, and Pistachio Nuts, (AOAC International. 1998. *Official Methods of Analysis of AOAC International*, 16th Edition. 4th Revision, Vol. II. http://www.aoac.org/) may be used as a complementary HPLC-FL method in order to provide more flexibility for analyses.

Source: AOAC International. 1994. "Method 991.31: Aflatoxins in Corn, Raw Peanuts, and Peanut Butter." *Official Methods of Analysis of AOAC International*. 16th Edition. 4th Revision; Vol. II. http://www.aoac.org/

8.3.2 α-Amanitin

CAS RN: 23109-05-9

Method	Analytical Technique	Section
Journal of Chromatography B. 1991. 563(2): 299–311	HPLC amperometric detection	8.3.2.1
Journal of Food Protection. 2005. 68(6): 1294–1301	Immunoassay (ELISA)	8.3.2.2

8.3.2.1 Literature Reference for α-Amanitin (Journal of Chromatography B. 1991. 563(2): 299–311)

Analysis Purpose: Confirmatory
Analytical Technique: HPLC with amperometric detection

Method Developed for: α-Amanitin in plasma
Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Procedures are described for the selective determination in human plasma of α-amanitin using HPLC with amperometric detection. After extraction of plasma with disposable C_{18} silica cartridges, the extracts are separated by isocratic reversed-phase chromatography using a macroporous polystyrene-divinylbenzene column and a mobile phase of 0.05 M phosphate buffer-acetonitrile (91:9) at pH 9.5. Amperometric detection is performed by applying an oxidation potential as low as +350 mV (vs. Ag/AgCl) to a glassy carbon electrode, in a thin-layer flow-cell. The linear range for alpha-amanitin is 3 to 200 ng/mL, and the relative LOD in plasma is 2 ng/mL at a signal-to-noise ratio of 2. The intra-assay precision has been evaluated at levels of 10 and 200 ng/mL.

Source: Tagliaro, F., Schiavon, G., Bontempelli, G., Carli, G. and Marigo, M. 1991. "Improved High-Performance Liquid Chromatographic Determination with Amperometric Detection of Alpha-amanitin in Human Plasma Based on its Voltammetric Study." Journal of Chromatography B. 563(2): 299–311. http://www.ncbi.nlm.nih.gov/pubmed/2055993

8.3.2.2 Literature Reference for α-Amanitin, T-2 Mycotoxin (Journal of Food Protection. 2005. 68(6): 1294–1301)

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay (ELISA)

Method Developed for: α-Amanitin, ricin and T-2 mycotoxin in food and beverages
Method Selected for: SAM lists these procedures for presumptive analysis of α-amanitin and T-2 toxin in aerosol, solid, particulate, liquid and water samples and for confirmatory analysis of ricin in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Commercially available ELISAs are described and assessed for detection of ricin, amanitin and T-2 toxin at levels below those described as a health concern in food samples. Solid food samples are prepared by washing the sample with sodium phosphate buffer followed by dilution with phosphate-buffered saline. Liquid beverage samples are prepared by dilution in sodium phosphate buffer. Amanitin samples are similarly prepared using water instead of buffer, and T-2 toxin samples are similarly prepared using 35% methanol in water instead of buffer. The prepared samples are used with commercially obtained ELISA kits according to the manufacturer's directions, except for the incorporation of an eight-point calibration curve and reading the plates at both 405 and 650 nm after 26 minutes of incubation at 37 °C. This assay detects ricin in food products at 0.01 µg/mL with acceptable background levels. Amanitin can be detected in food products at 1 µg/g with the ELISA. Background responses occurred, but at less than the equivalent of 0.5 ppm for amanitin. The ELISA kit will successfully detect T-2 toxin at targeted levels of 0.2 µg/g. The ELISA kit successfully detects T-2 toxin at targeted levels of 0.2 µg/g; the immunoassay for T-2 toxin, however, shows significant background responses and varies up to 0.1 ppm.

Source: Garber, E.A.E., Eppley, R.M., Stack, M.E., McLaughlin, M.A. and Park, D.L. 2005. "Feasibility of Immunodiagnostic Devices for the Detection of Ricin, Amanitin, and T-2 Toxin in Food." Journal of Food Protection. 68(6): 1294–1301.
http://www.ingentaconnect.com/content/iafp/jfp/2005/00000068/00000006/art00027

8.3.3 Anatoxin-a

CAS RN: 64285-06-9

Method	Analytical Technique	Section
Biomedical Chromatography. 1996. 10: 46–47	HPLC-FL (precolumn derivatization)	8.3.3.1

8.3.3.1 Literature Reference for Anatoxin-a (Biomedical Chromatography. 1996. 10(1): 46–47)

Analysis Purpose: Confirmatory
Analytical Technique: HPLC-FL (precolumn derivatization)

Method Developed for: Anatoxin-a in potable water
Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water.

Description of Method: Procedures are described for HPLC analysis with fluorimetric detection of anatoxin-a in water samples after derivatization with 7-fluoro-4-nitro-2,1,3-benzoxadiazole (NBD-F). Samples are extracted at pH 7 with SPE using a weak cation exchanger. The toxin is eluted with methanol containing 0.2% trifluoroacetic acid (TFA). Samples are evaporated, reconstituted with acetonitrile, and re-evaporated prior to derivatization. This procedure detects anatoxin-a at concentrations of 0.1 µg/L with a good linear calibration.

Source: James, K.J. and Sherlock, I.R. 1996. "Determination of the Cyanobacterial Neurotoxin, Anatoxin-a, by Derivatisation Using 7-Fluoro-4-Nitro-2,1,3-Benzoxadiazole (NBD-F) and HPLC Analysis With Fluorimetric Detection." Biomedical Chromatography. 10(1): 46–47.
http://www3.interscience.wiley.com/journal/18562/abstract

8.3.4 Brevetoxins (B form)

CAS RN: 79580-28-2

Method	Analytical Technique	Section
Environmental Health Perspectives. 2002. 110(2): 179–185	Immunoassay (ELISA)	8.3.4.1
Toxicon. 2004. 43(4): 455–465	HPLC-MS-MS	8.3.4.2

8.3.4.1 Literature Reference for Brevetoxins (Environmental Health Perspectives. 2002. 110(2): 179–185)

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay (ELISA)

Method Developed for: Brevetoxins in shellfish
Method Selected for: SAM lists these procedures for presumptive analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Procedures are described for a competitive ELISA used to detect brevetoxins in shellfish. The assay uses goat anti-brevetoxin antibodies in combination with a three-step signal amplification process: (1) secondary biotinylated antibodies; (2) streptavidin-HRP conjugate; and (3) chromogenic enzyme substrate. Sample preparation for liquids is dilution in PBS. Sample preparation for solids (oysters) is homogenization in PBS, or extraction in acetone. The working range for the assay is 0.2 to 2.0 ng/mL for diluted and undiluted liquid samples, and 0.2 to 2.0 ng/mL for solid samples, corresponding to 0.8 to 8.0 µg brevetoxins/100.0 g shellfish. The method has been compared to the mouse bioassay and is equivalent in sensitivity.

Source: Naar, J., Bourdelais, A., Tomas, C., Kubanek, J., Whitney, P.L., Flewelling, L., Steidinger, K., Lancaster, J. and Badan, D.G. 2002. "A Competitive ELISA to Detect Brevetoxins from *Karenia brevis* (Formerly *Gymnodinium breve*) in Seawater, Shellfish, and Mammalian Body Fluid." Environmental Health Perspectives. 110(2): 179–185.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1240733/

8.3.4.2 Literature Reference for Brevetoxins (Toxicon. 2004. 43(4): 455–465)

Analysis Purpose: Confirmatory
Analytical Technique: High performance liquid chromatography tandem mass spectrometry (HPLC-MS-MS)

Method Developed for: Brevetoxins in shellfish
Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Shellfish sample homogenates are extracted with acetone, and centrifuged. The supernatants are combined, evaporated, and re-solubilized in 80% methanol. Following a wash with 95% n-hexane, the methanolic layer is evaporated, and the residue re-solubilized in 25% methanol and applied to a C_{18} SPE column. Analytes are eluted with 100% methanol, evaporated, and re-solubilized in methanol for analysis. Analysis of prepared samples is performed using HPLC-MS-MS with a mobile phase of water and acetonitrile with acetic acid. Analytes are detected by an MS with ESI interface. Brevetoxins are extensively metabolized, with many sub-forms. This method describes multiple liquid chromatography/electrospray ionization mass spectrometry (LC-ESI-MS) profiles for metabolites of brevetoxins from oysters.

Source: Wang, Z., Plakas, S.M., El Said, K.R., Jester, E.L., Granade, H.R. and Dickey, R.W. 2004. "LC/MS Analysis of Brevetoxin Metabolites in the Eastern Oyster (*Crassostrea virginica*)." Toxicon. 43(4): 455–465. http://cat.inist.fr/?aModele=afficheN&cpsidt=15668117

8.3.5 α-Conotoxin

CAS RN: 156467-85-5

Method	Analytical Technique	Section
Biochemical Journal. 1997. 328: 245–250	Immunoassay (Solution phase binding assay)	8.3.5.1
Journal of Medicinal Chemistry. 2004. 47(5): 1234–1241	HPLC-MS	8.3.5.2

8.3.5.1 Literature Reference for α-Conotoxin (Biochemical Journal. 1997. 328(1): 245–250)

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay (solution phase binding assay)

Method Developed for: Purified α-Conotoxin GI in phosphate buffer
Method Selected for: SAM lists these procedures for presumptive analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: A biologically active fluorescein derivative of *Conus geographus* α-conotoxin (FGI) is used in solution-phase-binding assays with two purified *Torpedo californica* monoclonal antibodies (mAbs) to detect the toxin in laboratory samples. For competitive ligand-displacement spin-column assays, FGI was premixed with various dilutions of unlabelled ligands and then incubated with the two mAbs (5A1 and 8D2) at room temperature. Fluorescence is measured in ratio mode using cuvettes with excitation and emission monochromators set at gamma = 490 nm and gamma = 525 nm, respectively. The binding of FGI to the mAbs had apparent dissociation constants of 10 to 100 nM. The binding specificity and epitopes recognized by the two mAbs against α-conotoxin GI are also characterized. Competitive displacement assays showed that both mAbs specifically bound α-conotoxin GI with high avidity. Cross-reactivity with α-conotoxins M1 and S1 was not observed for either mAb in a direct ELISA. With spin-column assay, however, 5A1, but not 8D2, cross-reacted at a low level (100 – 300-fold less avid) with these α-conotoxins. An antibody/α-conotoxin GI molar ratio of 1:1 afforded complete protection in mouse lethal assays.

Source: Ashcom, J.D. and Stiles, B.G. 1997. "Characterization of α-Conotoxin Interactions With the Nicotinic Acetylcholine Receptor and Monoclonal Antibodies." Biochemical Journal. 328(1): 245–250. http://www2.epa.gov/sites/production/files/2015-07/documents/bj-328-pgs245-250.pdf

8.3.5.2 Literature Reference for α-Conotoxin (Journal of Medicinal Chemistry. 2004. 47(5): 1234–1241)

Analysis Purpose: Confirmatory
Analytical Technique: HPLC-MS

Method Developed for: *Conus anemone* venom (α-Conotoxins AnIA, AnIB, and AnIC) in buffer
Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Procedures are discussed for the detection of peptides within the α-conotoxin molecular mass range using an HPLC-MS. A crude extract of *Conus anemone* venom sample is made using 30% acetonitrile/water acidified with 0.1% TFA, with the insoluble portion of the sample removed by centrifugation. A portion of the sample extract is fractionated by size-exclusion chromatography in order to prepare a sample containing small peptides in the range of 1000 to 2500 Da. Chromatography conditions are elution with 30% acetonitrile / 0.048% TFA at a flow rate of 0.5 mL/minute, with detection at 214 nm. Three sulfated α-conotoxins (AnIA, AnIB and AnIC) can be identified by LC-MS that are within the molecular mass range of other α-conotoxins (i.e., 1400–2200 Da). Peptides can be quantified by reversed-phase HPLC using an external reference standard for each peptide.

Source: Loughnan, M.L., Nicke, A., Jones, A., Adams, D.J., Alewood, P.F. and Lewis, R.J. 2004. "Chemical and Functional Identification and Characterization of Novel Sulfated Alpha-conotoxins from the Cone Snail *Conus anemone*." Journal of Medicinal Chemistry. 47(5): 1234–1241. http://pubs.acs.org/cgi-bin/abstract.cgi/jmcmar/2004/47/i05/abs/jm031010o.html

8.3.6 Cylindrospermopsin

CAS RN: 143545-90-8

Method	Analytical Technique	Section
FEMS Microbiology Letters. 2002. 216: 159-164	HPLC-PDA	8.3.6.1
ELISA Kits for Cylindrospermopsin	Immunoassay (ELISA)	8.3.6.2

8.3.6.1 Literature Reference for Cylindrospermopsin (FEMS Microbiology Letters. 2002. 216(2): 159–164)

Analysis Purpose: Confirmatory
Analytical Technique: High performance liquid chromatography – Photodiode array detector (HPLC-PDA)

Method Developed for: Cylindrospermopsin in eutrophic waters
Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water.

Description of Method: Cylindrospermopsin is detected using HPLC with photodiode array detector (PDA) in environmental waters. The suggested solvent range for cylindrospermopsin is below 50% methanol and 30% acetonitrile. Complex samples (culture medium) are purified on a C_{18} column with a linear gradient of 1 to 12% (v/v) methanol/water over 24 minutes at 40 °C, with monitoring at 262 nm. The use of C_{18} columns for environmental waters is suggested for removal of the large number of organic compounds that may be present. This method detects and recovers cylindrospermopsin from spiked environmental water samples at 1 µg/L.

Source: Metcalf, J.S., Beattie, K.A., Saker, M.L. and Codd, G.A. 2002. "Effects of Organic Solvents on the High Performance Liquid Chromatographic Analysis of the Cyanobacterial Toxin Cylindrospermopsin and Its Recovery from Environmental Eutrophic Waters by Solid Phase Extraction." FEMS Microbiology Letters. 216(2): 159–164.
http://cat.inist.fr/?aModele=afficheN&cpsidt=14002569

8.3.6.2 ELISA Kits for Cylindrospermopsin

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay (ELISA)

Method Developed for: Cylindrospermopsin in ground water, surface water and well water
Method Selected for: SAM lists these procedures for presumptive analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water.

Description of Method: Cylindrospermopsin is detected using a colorimetric immunoassay (competitive ELISA) procedure. A sample (0.05 mL), enzyme conjugate (cylindrospermopsin-HRP), and an antibody solution containing rabbit anti-cylindrospermopsin antibodies are added to plate wells containing immobilized sheep anti-rabbit antibodies. Both the cylindrospermopsin (if present) in the sample and cylindrospermopsin-HRP conjugate compete in solution to bind to the rabbit anti-cylindrospermopsin antibodies in proportion to their respective concentrations. The anti-cylindrospermopsin antibody-target complexes are then bound to the immobilized sheep anti-rabbit antibodies on the plate. After incubation, the unbound molecules are washed and decanted. A specific substrate is then added which is converted from a colorless to a blue solution by the HRP enzyme conjugate solution. The reaction is terminated with the addition of a dilute acid. The concentration of cylindrospermopsin in the sample is determined photometrically by comparing sample absorbance to the absorbance of the calibrators (standards) at a specific wavelength (450 nm). The applicable concentration range is 0.4–2.0 µg/L, with a minimum detection level of 0.4 µg/L.

Source: NEMI. 2006. https://www.nemi.gov/methods/keyword/?keyword_search_field=ELISA

8.3.7 Diacetoxyscirpenol (DAS)

CAS RN: 2270-40-8

Method	Analytical Technique	Section
International Journal of Food Microbiology. 1988. 6(1): 9–17	Immunoassay (ELISA)	8.3.7.1
Rapid Communications in Mass Spectrometry. 2006. 20(9): 1422–1428	LC/APCI-MS	8.3.7.2

8.3.7.1 Literature Reference for Diacetoxyscirpenol (DAS) (International Journal of Food Microbiology. 1988. 6(1): 9–17)

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay (ELISA)

Method Developed for: DAS in food
Method Selected for: SAM lists these procedures for presumptive analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: An ELISA is used for the detection of DAS in food samples. Antibodies against DAS are obtained after immunization of rabbits with DAS-hemiglutarate-human serum albumin (DAS-HG-HSA), and a DAS-hemisuccinate-HRP conjugate (DAS-HS-HRP) is prepared by an ester method for use as enzyme-labeled toxin in the competitive assay. The detection limit for DAS using this assay is approximately 10 pg/mL. This assay will cross-react related toxins. The relative cross-reactivities of the assay are 597.5, 5.2, 100.0, 2.5 and 1.5% for 3 alpha-acetyl-DAS, DAS, T-2 toxin, neosolaniol and 15-acetoxyscirpenol, respectively.

Source: Klaffer, U., Martlbauer, E. and Terplan, G. 1988. "Development of a Sensitive Enzyme-Linked Immunosorbent Assay for the Detection of Diacetoxyscirpenol." International Journal of Food Microbiology. 6(1): 9–17.
http://www.sciencedirect.com/science/article/pii/0168160588900797

8.3.7.2 Literature Reference for Diacetoxyscirpenol (DAS) and T-2 Mycotoxin (Rapid Communications in Mass Spectrometry. 2006. 20(9): 1422–1428)

Analysis Purpose: Confirmatory
Analytical Technique: Liquid chromatography/atmospheric pressure chemical ionization mass spectrometry (LC/APCI-MS)

Method Developed for: DAS and T-2 mycotoxin in food
Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: A LC/APCI-MS procedure based on TOF-MS, with a real-time reference mass correction, is used for simultaneous determination of *Fusarium* mycotoxins (to include DAS and T-2 mycotoxin) in foodstuffs. Mycotoxin samples are extracted with acetonitrile/water (85:15) and centrifuged, and the supernatant is applied to a column for cleanup. Prepared samples are separated by liquid chromatography with an aqueous mobile phase of ammonium acetate and methanol detection is provided in exact mass chromatograms with a mass window of 0.03 Th. The limits of detection range from 0.1 to 6.1 ng/g in analyzed foodstuffs.

Source: Tanaka, H., Takino, M., Sugita-Konishi, Y. and Tanaka, T. 2006. "Development of Liquid Chromatography/Time-of-Flight Mass Spectrometric Method for the Simultaneous Determination of Trichothecenes, Zearalenone, and Aflatoxins in Foodstuffs." Rapid Communications in Mass Spectrometry. 20(9): 1422–1428.
http://cat.inist.fr/?aModele=afficheN&cpsidt=17697070

8.3.8 Microcystins (Principal isoforms: LA, LR, LW, RR, YR)

CAS RNs: 96180-79-9 (LA), 101043-37-2 (LR), 157622-02-1 (LW), 111755-37-4 (RR), 101064-48-6 (YR)

Method	Analytical Technique	Section
Journal of AOAC International. 2001. 84(4): 1035–1044	Immunoassay (ELISA) /Phosphatase assay	8.3.8.1
Analyst. 1994. 119(7): 1525–1530	HPLC-PDA	8.3.8.2

8.3.8.1 Literature Reference for Microcystins (Journal of AOAC International. 2001. 84(4): 1035–1044)

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay (ELISA)/Phosphatase assay

Method Developed for: Microcystins-LA, -LR, -LW, -RR, -YR in algae products
Method Selected for: SAM lists these procedures for presumptive analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: ELISA and protein phosphatase inhibition assays are used to detect microcystins in blue-green algae products. Solid samples are prepared by homogenization in methanol (75% in water), with centrifugation to remove solids. Immunoassays are performed on

the prepared samples using a commercially available ELISA test kit as described by the manufacturer. Samples are quantitated by comparison with a microcystins-LR standard curve. Quantitation with the colorimetric protein phosphatase inhibition assay is based on a comparison with a microcystin-LR standard curve. ELISA and phosphatase assay results agree over a concentration range of 0.5 to 35 µg/g. Neither assay is specific for a particular isoform.
Source: Lawrence, J.F., Niedzwiadek, B., Menard, C., Lau, B.P., Lewis, D., Kuper-Goodman, T., Carbone, S. and Holmes, C. 2001. "Comparison of Liquid Chromatography/Mass Spectrometry, ELISA, and Phosphatase Assay for the Determination of Microcystins in Blue-Green Algae Products." Journal of AOAC International. 84(4): 1035–1044.
http://cat.inist.fr/?aModele=afficheN&cpsidt=1135453

8.3.8.2 Literature Reference for Microcystins (Analyst. 1994. 119(7): 1525–1530)

Analysis Purpose: Confirmatory
Analytical Technique: HPLC-PDA

Method Developed for: Microcystins-LA, -LR, -LW, -RR, -YR in raw and treated waters
Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water.

Description of Method: Procedures are discussed to test the presence of microcystin-LR, -LY, -LW, -LF (CAS RN 154037-70-4), and -RR in treated and untreated water samples. Cyanobacterial cells are separated from the water by filtration through 110-mm glass fiber grade C (GF/C) discs. The cellular components collected on the discs are extracted three times with methanol; the collected extraction fluids are combined and dried. The residue is resuspended in methanol and analyzed by HPLC-PDA. The liquid portion of the filtered water sample is subjected to trace enrichment using a C_{18} SPE cartridge, followed by identification and determination by HPLC-PDA. This procedure can detect microcystin concentrations as low as 250 ng/L and is the basis of the World Health Organization (WHO) method for the detection of microcystins.

Source: Lawton, L.A., Edwards, C. and Codd, G.A. 1994. "Extraction and High-Performance Liquid Chromatographic Method for the Determination of Microcystins in Raw and Untreated Waters." Analyst. 119(7): 1525–1530.
http://www.rsc.org/Publishing/Journals/AN/article.asp?doi=AN9941901525

8.3.9 Picrotoxin

CAS RN: 124-87-8

Method	Analytical Technique	Section
Journal of Pharmaceutical and Biomedical Analysis. 1989. 7(3): 369–375	HPLC	8.3.9.1

8.3.9.1 Literature Reference for Picrotoxin (Journal of Pharmaceutical & Biomedical Analysis. 1989. 7(3): 369–375)

Analysis Purpose: Confirmatory
Analytical Technique: HPLC

Method Developed for: Picrotoxin in serum
Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Procedures are described for quantification of the two components of picrotoxin (picrotin [CAS RN 21416-53-5] and picrotoxinin [CAS RN 17617-45-7]) in serum samples. Serum samples are prepared by washing with *n*-hexane, followed by extraction with chloroform. The chloroform is evaporated and the sample is reconstituted in acetonitrile-1 mM ammonium acetate buffer (pH 6.4) 34:66 (v/v) for assay by reversed-phase HPLC. The effluent is monitored at 200 nm, and quantification is based on peak-height ratio of analyte to the internal standard. A linear response is obtained for both analytes (picrotin and picrotoxinin) in the range 0.2 to 20.0 µg/mL.

Source: Soto-Otero, R., Mendez-Alvarez, E., Sierra-Paredes, G., Galan-Valiente, J., Aguilar-Veiga, E. and Sierra-Marcuno, G. 1989. "Simultaneous Determination of the Two Components of Picrotoxin in Serum by Reversed-Phase High-Performance Liquid Chromatography With Application to a Pharmacokinetic Study in Rats." Journal of Pharmaceutical & Biomedical Analysis. 7(3): 369–375. http://www.sciencedirect.com/science/article/pii/0731708589801049

8.3.10 Saxitoxins

CAS RNs: 35523-89-8 (STX) and various congeners

Method	Analytical Technique	Section
Journal of AOAC International. 1995. 78: 528–532	HPLC-FL (post column derivatization)	8.3.10.1
ELISA Kits for Saxitoxin	Immunoassay (ELISA)	8.3.10.2

8.3.10.1 Literature Reference for Saxitoxin (Journal of AOAC International. 1995. 78(2): 528–532)

Analysis Purpose: Confirmatory
Analytical Technique: HPLC-FL (post column derivatization)

Method Developed for: Saxitoxins (STX, NEOSTX, GTX, dcGTX, dcSTX) in shellfish
Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Procedures are described to detect multiple analogues of saxitoxin in shellfish using ion-interaction chromatography on a silica-based reversed-phase (C_8) column with postcolumn periodate oxidation and FL detection. Toxin groups of different net charges are determined separately by isocratic elution using either sodium 1-heptanesulfonate in ammonium phosphate (GTX-1, GTX-6, dcGTX2, dcGTX3) or sodium 1-heplanesulfonate in ammonium phosphate and acetonitrile (STX [CAS RN 35523-89-8], neoSTX [CAS RN 64296-20-4], dcSTX [CAS RN 58911-04-9]). For biological sample types, a cleanup procedure using a C_{18} SPE cartridge is effective in preventing false peaks. High sensitivity with detection limits ranging from 20 to 110 fmol are achieved as a result of reduced band broadening and optimized reaction conditions. This method, when applied to low-toxicity shellfish, gives higher values than the standard mouse bioassay.

Special Considerations: AOAC is in the process of publishing multi-laboratory tested alternate procedures for high through-put saxitoxin analysis that may be available in the near future. For single laboratory tested procedures and more details, see Van Dolah *et. al.*: Journal of AOAC International. Vol. 92, No. 6. 2009. 1705

Source: Oshima, Y. 1995. "Postcolumn Derivatization Liquid Chromatographic Method for Paralytic Shellfish Toxins." Journal of AOAC International. 78(2): 528–532. http://cat.inist.fr/?aModele=afficheN&cpsidt=3469391

8.3.10.2 ELISA Kits for Saxitoxins

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay (ELISA)

Method Developed for: STX in water and solid samples (e.g., shellfish)
Method Selected for: SAM lists these procedures for presumptive analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types other than water.

Description of Method: Saxitoxin is detected using a colorimetric immunoassay (competitive ELISA) procedure. A sample (0.05 mL), enzyme conjugate (saxitoxin-HRP), and an antibody solution containing rabbit anti-saxitoxin antibodies are added to plate wells containing immobilized sheep anti-rabbit antibodies. Both the saxitoxin (if present) in the sample and saxitoxin-HRP conjugate compete in solution to bind to the rabbit anti-saxitoxin antibodies in proportion to their respective concentrations. The anti-saxitoxin antibody-target complexes are then bound to the immobilized sheep anti-rabbit antibodies on the plate. After incubation, the unbound molecules are washed and decanted. A specific substrate is then added which is converted from a colorless to a blue solution by the HRP enzyme conjugate solution. The reaction is terminated with the addition of a dilute acid. The concentration of saxitoxin in the sample is determined photometrically by comparing sample absorbance to the absorbance of the calibrators (standards) at a specific wavelength (450 nm). The applicable concentration range is 0.015–0.4 ng/mL, with a minimum detection level of 0.015 ng/mL.

Special Considerations: Cross-reactivity is observed with the following saxitoxin types: dcSTX (29%), GTX 2, 3, and 5B (23%), sulfo GTX 1 and 2 (2.0%, dcGTX 2 and 3 (1.4%), NEOSTX (1.3%), dcNEOSTX (0.6%), GTX 1 and 4 (<0.2%). High concentrations (e.g., above 0.1 ng/mL for toxins with >20% cross-reactivity) may provide an indication that they are present. The vendor of this kit indicates that it provides "screening results… positive samples requiring some action should be confirmed by an alternative method."

AOAC is in the process of publishing multi-laboratory tested alternate procedures for high through-put saxitoxin analysis that may be available in the near future. For single laboratory tested procedures and more details, see Van Dolah *et. al*.: Journal of AOAC International. Vol. 92. No. 6. 2009. 1705.

Source: NEMI. 2006. https://www.nemi.gov/methods/keyword/?keyword_search_field=ELISA

8.3.11 T-2 Mycotoxin

CAS RN: 21259-20-1

Method	Analytical Technique	Section
Journal of Food Protection. 2005. 68(6): 1294–1301	Immunoassay (ELISA)	8.3.2.2
Rapid Communications in Mass Spectrometry. 2006. 20(9): 1422–1428	LC/APCI-MS	8.3.7.2

See Sections 8.3.2.2 and 8.3.7.2 for information on immunoassay (ELISA) and LC/APCI-MS procedures for T-2 Mycotoxin.

8.3.12 Tetrodotoxin

CAS RN: 9014-39-5

Method	Analytical Technique	Section
Analytical Biochemistry. 2001. 290: 10-17	LC/ESI-MS	8.3.12.1
Journal of Clinical Laboratory Analysis. 1992. 6: 65–72	Immunoassay [competitive inhibition enzyme immunoassay (CIEIA)]	8.3.12.2

8.3.12.1 Literature Reference for Tetrodotoxin (Analytical Biochemistry. 2001. 290(1): 10–17)

Analysis Purpose: Confirmatory
Analytical Technique: LC/ESI-MS

Method Developed for: Tetrodotoxin (TTX) from puffer fish and newt tissues
Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Procedures are described for LC/ESI-MS analysis of TTXs in tissue samples from puffer fish and newts by a combination of chromatography on a reversed-phase column with long carbon chains (C30) and with the mobile phase containing an ion pair reagent (ammonium heptafluorobutyrate). The relationship between the amount of applied standard TTX and its peak area on the mass chromatogram (m/z 320) shows good linearity over a range of 50 to 1000 pmol. The detection limit of TTX in the selective ion monitoring (SIM) mode is estimated to be 0.7 pmol, with a signal to noise ratio of 2:1.

Source: Shoji, Y., Yotsu-Yamashita, M., Miyazawa, T. and Yasumoto, T. 2001. "Electrospray Ionization Mass Spectrometry of Tetrodotoxin and its Analogs: Liquid Chromatography/Mass Spectrometry, Tandem Mass Spectrometry, and Liquid Chromatography/Tandem Mass Spectrometry." Analytical Biochemistry. 290(1): 10–17.
http://www.sciencedirect.com/science/article/pii/S0003269700949534

8.3.12.2 Literature Reference for Tetrodotoxin (Journal of Clinical Laboratory Analysis. 1992. 6(2): 65–72)

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay (CIEIA)

Method Developed for: Tetrodotoxin in buffer
Method Selected for: SAM lists these procedures for presumptive analysis in aerosol, solid, particulate, liquid and water samples. Further research is needed to adapt and verify the procedures for environmental sample types.

Description of Method: Procedures are described for a CIEIA for tetrodotoxin in biological samples. An anti-TTX mAb, designated T20G10, is directly labeled with alkaline phosphatase for use in the assay. Sensitivities of 6 to 7 ng/mL (IC 50) and 2 to 3 ng/mL (IC 20) are achieved.

Source: Raybould, T.J., Bignami, G.S., Inouye, L.K., Simpson, S.B., Byrnes, J.B., Grothaus, P.G. and Vann, D.C. 1992. "A Monoclonal Antibody-Based Immunoassay for Detecting Tetrodotoxin in Biological Samples." Journal of Clinical Laboratory Analysis. 6(2): 65–72.
http://www3.interscience.wiley.com/journal/112131435/abstract

Section 9.0: Conclusions

SAM is intended for use by EPA and EPA-contracted and -subcontracted laboratories; it also can be used by other agencies and laboratory networks, such as the Integrated Consortium of Laboratory Networks (ICLN). The information also can be found on the SAM website (www.epa.gov/sam), which provides searchable links to supporting information based on SAM analytes and the analytical methods listed. Methods listed in Appendix A (chemical methods), Appendix B (radiochemical methods), Appendix C (pathogen methods) and Appendix D (biotoxin methods) are recommended for use in assessment of the extent of contamination and the effectiveness of decontamination following an intentional or unintentional contamination event.

The primary objective of this document is to identify appropriate methods that represent a balance between providing existing, documented, techniques and providing consistent and valid analytical results. The method selected for each analyte/sample type combination was deemed the most general, appropriate, and broadly applicable of available methods by a group of technical experts in each appropriate field. The selected methods are subject to change following further research to improve methods or following the development of new methods. The contacts listed in Section 4.0 encourage the scientific community to inform them of any such method improvements.

Since publication of SAM Revision 1.0 in September 2004, NHSRC has continued to convene technical work groups to evaluate and, if necessary, update the analytes and methods that are listed. Details regarding changes that have been incorporated into each revision of SAM are provided in Attachment 1. SAM 2012 also reflects a title change agreed to by stakeholders (i.e., EPA's NHSRC, WSD and WLA, Office of Emergency Response and ERLN, ORIA and Regional Offices) during a 2010 SAM Summit, to better reflect SAM's focus on providing selected analytical methods for use across multiple laboratories during environmental remediation and recovery. This current revision (SAM 2012) includes the addition of vegetation as a sample type under the radiochemistry sections, the addition of method applicability tiers to Appendix A (Selected Chemical Methods), several new methods added or replaced for currently listed chemical analytes, clarification of immunoassay methods listed for biotoxin analytes, the addition of restructured pathogen sections to more clearly define scope and application.

Appendix A: Selected Chemical Methods

SAM 2012 Appendix A: Selected Chemical Methods

The fitness of a method for an intended use is related to site-specific data quality objectives (DQOs) for a particular environmental remediation activity. These selected chemical methods have been assigned the tiers (below) to indicate a level of method usability for the specific analyte and sample type. The assigned tiers reflect the conservative view for DQOs involving timely implementation of methods for analysis of a high number of samples (such that multiple laboratories are necessary), low limits of identification and quantification, and appropriate quality control.

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

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

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

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
Acephate	30560-19-1	LC-MS-MS	Sample Prep	Adapted from J. Chromatogr. A, (2007) 1154(1): 3-25	II	Adapted from Chromatographia, 63(5/6): 233-237	II	538 (EPA OW)	I	Adapted from J. Chromatogr. A, (2007) 1154(1): 3-25	III	Adapted from J. Chromatogr. A, (2007) 1154(1): 3-25	III
			Determinative										
Acrylamide	79-06-1	HPLC	Sample Prep	Water extraction	III	8316 (EPA SW-846)	II	8316 (EPA SW-846)	II	PV2004 (OSHA)	I	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8316 (EPA SW-846)								8316 (EPA SW-846)	
Acrylonitrile	107-13-1	HPLC / GC-MS	Sample Prep	5035A (EPA SW-846)	II	524.2 (EPA OW)	II	524.2 (EPA OW)	II	PV2004 (OSHA)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8260C (EPA SW-846)								8260C (EPA SW-846)	
Aldicarb (Temik)	116-06-3	HPLC	Sample Prep	8318A (EPA SW-846)	II	D7645-10 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846)	III
			Determinative									8318A (EPA SW-846)	
Aldicarb sulfone	1646-88-4	HPLC	Sample Prep	8318A (EPA SW-846)	II	D7645-10 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative									8318A (EPA SW-846)	
Aldicarb sulfoxide	1646-87-3	HPLC	Sample Prep	8318A (EPA SW-846)	III	D7645-10 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative									8318A (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
Allyl alcohol	107-18-6	GC-MS	Sample Prep	5035A (EPA SW-846)	II	5030C (EPA SW-846)	II	5030C (EPA SW-846)	II	TO-15[1] (EPA ORD)	III	Not of concern	NA
			Determinative	8260C (EPA SW-846)		8260C (EPA SW-846)		8260C (EPA SW-846)					
4-Aminopyridine	504-24-5	HPLC	Sample Prep	8330B (EPA SW-846)	III	3535A/8330B (EPA SW-846)	III	3535A/8330B (EPA SW-846)	III	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative			8330B (EPA SW-846)		8330B (EPA SW-846)				8330B (EPA SW-846)	
Ammonia	7664-41-7	Visible spectrophotometry	Sample Prep	Not of concern	NA	4500-NH_3 B (SM)	I	350.1 (EPA OW)	I	6015 (NIOSH)	I	Not of concern	NA
			Determinative			4500- NH_3 G (SM)							
Ammonium metavanadate (analyze as total vanadium)	7803-55-6	ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010C/6020A (EPA SW-846)						IO-3.4/IO-3.5 (EPA ORD)		6010C/6020A (EPA SW-846)	
Arsenic, Total	7440-38-2	ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010C/6020A (EPA SW-846)						IO-3.4/IO-3.5 (EPA ORD)		6010C/6020A (EPA SW-846)	
Arsenic trioxide (analyze as total arsenic)	1327-53-3	ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010C/6020A (EPA SW-846)						IO-3.4/IO-3.5 (EPA ORD)		6010C/6020A (EPA SW-846)	
Arsine (analyze as total arsenic in non-air samples)	7784-42-1	GFAA / ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	200.7/200.8 (EPA OW)	I	6001 (NIOSH)	I	9102 (NIOSH)	I
			Determinative	6010C/6020A (EPA SW-846)								6010C/6020A (EPA SW-846)	
Asbestos	1332-21-4	TEM	Sample Prep	D5755-03 (soft surfaces-microvac) (ASTM)	III	Not of concern	NA	Not of concern	NA	10312:1995 (ISO)	I	D6480-05 (hard surfaces-wipes) (ASTM)	I
			Determinative										
Boron trifluoride	7637-07-2	ISE	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	ID216SG (OSHA)	I	Not of concern	NA
			Determinative										
Brodifacoum	56073-10-0	HPLC	Sample Prep	3541/3545A (EPA SW-846)	III	D7644-10 (ASTM)	II	D7644-10 (ASTM)	II	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8321B (EPA SW-846)								8321B (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
Bromadiolone	28772-56-7	HPLC / LC-MS-MS	Sample Prep	3541/3545A (EPA SW-846)	III	D7644-10 (ASTM)	II	D7644-10 (ASTM)	II	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8321B (EPA SW-846)								8321B (EPA SW-846)	
BZ [Quinuclidinyl benzilate]	6581-06-2	HPLC	Sample Prep	3541/3545A (EPA SW-846)	III	3520C/3535A (EPA SW-846)	II	3520C/3535A (EPA SW-846)	II	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8321B (EPA SW-846)		8321B (EPA SW-846)		8321B (EPA SW-846)				8321B (EPA SW-846)	
Calcium arsenate (analyze as total arsenic)	7778-44-1	ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010C/6020A (EPA SW-846)						IO-3.4/IO-3.5 (EPA ORD)		6010C/6020A (EPA SW-846)	
Carbofuran (Furadan)	1563-66-2	HPLC / LC-MS-MS	Sample Prep	8318A (EPA SW-846)	II	D7645-10 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846)	III
			Determinative									8318A (EPA SW-846)	
Carbon disulfide	75-15-0	GC-MS	Sample Prep	5035A (EPA SW-846)	I	5030C (EPA SW-846)	I	524.2 (EPA OW)	I	TO-15 (EPA ORD)	I	Not of concern	NA
			Determinative	8260C (EPA SW-846)		8260C (EPA SW-846)							
Carfentanil	59708-52-0	HPLC	Sample Prep	3541/3545A (EPA SW-846)	III	3520C/3535A (EPA SW-846)	III	3520C/3535A (EPA SW-846)	III	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8321B (EPA SW-846)		8321B (EPA SW-846)		8321B (EPA SW-846)				8321B (EPA SW-846)	
Chlorfenvinphos	470-90-6	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	I	3520C/3535A (EPA SW-846)	I	3520C/3535A (EPA SW-846)	I	TO-10A (EPA ORD)	II	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Chlorine	7782-50-5	Visible spectrophotometry	Sample Prep	Not of concern	NA	4500-Cl G (SM)	I	4500-Cl G (SM)	I	Adapted from Analyst (1999) 124(12): 1853-1857	II	Not of concern	NA
			Determinative							4500-Cl G (SM)			
2-Chloroethanol	107-07-3	GC-MS / GC-FID	Sample Prep	5035A (EPA SW-846)	II	5030C (EPA SW-846)	II	5030C (EPA SW-846)	II	2513 (NIOSH)	I	Not of concern	NA
			Determinative	8260C (EPA SW-846)		8260C (EPA SW-846)		8260C (EPA SW-846)					

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
3-Chloro-1,2-propanediol	96-24-2	GC-MS	Sample Prep	Adapted from Eur. J. Lipid Sci. Technol. (2011) 113: 345-355	II	Adapted from J. Chromatogr. A (2000) 866: 65-77	II	Adapted from J. Chromatogr. A (2000) 866: 65-77	II	TO-10A[2] (EPA ORD)	III	Adapted from Eur. J. Lipid Sci. Technol. (2011) 113: 345-355	III
			Determinative										
Chloropicrin	76-06-2	GC-MS / GC-ECD	Sample Prep	3570 (EPA SW-846)	II	551.1 (EPA OW)	I	551.1 (EPA OW)	I	PV2103 (OSHA)	I	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D[3] (EPA SW-846)								8270D[3] (EPA SW-846)	
Chlorosarin	1445-76-7	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	TO-10A[2] (EPA ORD)	III[13]	3570/8290A Appendix A (EPA SW-846)	III[13]
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Chlorosoman	7040-57-5	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	TO-10A[2] (EPA ORD)	III[13]	3570/8290A Appendix A (EPA SW-846)	III[13]
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
2-Chlorovinylarsonous acid (2-CVAA) (degradation product of Lewisite) (analyze as total arsenic)	85090-33-1	ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010C/6020A (EPA SW-846)						IO-3.4/IO-3.5 (EPA ORD)		6010C/6020A (EPA SW-846)	
Chlorpyrifos	2921-88-2	GC-MS	Sample Prep	3570 (EPA SW-846)	II	3511 (EPA SW-846)	II	525.2 (EPA OW)	II	TO-10A (EPA ORD)	I	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)						8270D (EPA SW-846)	
Chlorpyrifos oxon	5598-15-2	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III	3520C/3535A (EPA SW-846)	III	525.2 (EPA OW)	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)						8270D (EPA SW-846)	
Crimidine	535-89-7	GC-MS	Sample Prep	3570 (EPA SW-846)	II	3511 (EPA SW-846)	II	3511 (EPA SW-846)	II	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D[4] (EPA SW-846)		8270D[4] (EPA SW-846)		8270D[4] (EPA SW-846)				8270D[4] (EPA SW-846)	
Cyanide, Amenable to chlorination	NA	Visible spectrophotometry	Sample Prep	3135.2I (EPA RLAB)	I	3135.2I (EPA RLAB)	I	3135.2I (EPA RLAB)	I	Not of concern	NA	3135.2I (EPA RLAB)	III
			Determinative										
Cyanide, Total	57-12-5	Visible spectrophotometry	Sample Prep	ISM01.3 CN (EPA CLP)	I	ISM01.3 CN (EPA CLP)	I	335.4 (EPA OW)	I	6010 (NIOSH)	I	ISM01.3 CN (EPA CLP)	III
			Determinative										

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
Cyanogen chloride	506-77-4	GC-MS/GC-ECD	Sample Prep	Adapted from Encyclopedia of Anal. Chem. (2006) DOI: 10.1002/9780 470027318.a0809	II	Adapted from Encyclopedia of Anal. Chem. (2006) DOI: 10.1002/9780 470027318.a0809	II	Adapted from Encyclopedia of Anal. Chem. (2006) DOI: 10.1002/9780 470027318.a0809	II	TO-15 (EPA ORD)	III	Not of concern	NA
			Determinative										
Cyclohexyl sarin (GF)	329-99-7	GC-MS	Sample Prep	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*
			Determinative										
1,2-Dichloroethane (degradation product of HD)	107-06-2	GC-MS	Sample Prep	5035A (EPA SW-846)	I	5030C (EPA SW-846)	I	524.2 (EPA OW)	I	TO-15 (EPA ORD)	I	Not of concern	NA
			Determinative	8260C (EPA SW-846)		8260C (EPA SW-846)							
Dichlorvos	62-73-7	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	I	3535A (EPA SW-846)	I	525.2 (EPA OW)	I	TO-10A (EPA ORD)	I	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)						8270D (EPA SW-846)	
Dicrotophos	141-66-2	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	I	3535A (EPA SW-846)	I	3535A (EPA SW-846)	I	TO-10A (EPA ORD)	I	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Diesel range organics	NA	GC-FID	Sample Prep	3541/3545A (EPA SW-846)	I	3520C/3535A (EPA SW-846)	I	3520C/3535A (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative	8015C (EPA SW-846)		8015C (EPA SW-846)		8015C (EPA SW-846)				8015C (EPA SW-846)	
Diisopropyl methylphosphonate (DIMP) (degradation product of GB)	1445-75-6	HPLC / LC-MS-MS	Sample Prep	E2866-12 (ASTM)	II	D7597-09 (ASTM)	II	538 (EPA OW)	I	TO-10A[2] (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	II
			Determinative									8321B (EPA SW-846)	
Dimethylphosphite	868-85-9	GC-MS	Sample Prep	3570 (EPA SW-846)	II	Not of concern	NA	Not of concern	NA	TO-10A (EPA ORD)	II	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)								8270D (EPA SW-846)	
Dimethylphosphoramidic acid (degradation product of GA)	33876-51-6	HPLC	Sample Prep	3541/3545A (EPA SW-846)	III	3535A (EPA SW-846)	III	3535A (EPA SW-846)	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8321B (EPA SW-846)		8321B (EPA SW-846)		8321B (EPA SW-846)				8321B (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
Diphacinone	82-66-6	HPLC	Sample Prep	3541/3545A (EPA SW-846)	III	D7644-10 (ASTM)	II	D7644-10 (ASTM)	II	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8321B (EPA SW-846)								8321B (EPA SW-846)	
Disulfoton	298-04-4	GC-MS / GC-FPD	Sample Prep	3570 (EPA SW-846)	II	525.2 (EPA OW)	II	525.2 (EPA OW)	II	5600 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)								8270D (EPA SW-846)	
Disulfoton sulfone oxon[5]	2496-91-5	GC-MS / GC-FPD	Sample Prep	3541/3545A (EPA SW-846)	III	525.2 (EPA OW)	III	525.2 (EPA OW)	III	5600 (NIOSH)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8270D (EPA SW-846)								8270D (EPA SW-846)	
Disulfoton sulfoxide	2497-07-6	GC-MS / GC-FPD	Sample Prep	3541/3545A (EPA SW-846)	III	525.2 (EPA OW)	II	525.2 (EPA OW)	II	5600 (NIOSH)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8270D (EPA SW-846)								8270D (EPA SW-846)	
Disulfoton sulfoxide oxon[5]	2496-92-6	GC-MS / GC-FPD	Sample Prep	3541/3545A (EPA SW-846)	III	525.2 (EPA OW)	III	525.2 (EPA OW)	III	5600 (NIOSH)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8270D (EPA SW-846)								8270D (EPA SW-846)	
1,4-Dithiane (degradation product of HD)	505-29-3	GC-MS	Sample Prep	3570 (EPA SW-846)	II	3511 (EPA SW-846)	II	3511 (EPA SW-846)	II	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
EA2192 [S-2-(diisopropylamino)ethyl methylphosphonothioic acid] (hydrolysis product of VX)	73207-98-4	HPLC	Sample Prep	3541/3545A (EPA SW-846)	III	3535A (EPA SW-846)	III	3535A (EPA SW-846)	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8321B (EPA SW-846)		8321B (EPA SW-846)		8321B (EPA SW-846)				8321B (EPA SW-846)	
Ethyl methylphosphonic acid (EMPA) (degradation product of VX)	1832-53-7	HPLC / LC-MS-MS	Sample Prep	E2866-12 (ASTM)	II	D7597-09 (ASTM)	II	D7597-09 (ASTM)	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	II
			Determinative									8321B (EPA SW-846)	
Ethyldichloroarsine (ED)	598-14-1	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III	3535A (EPA SW-846)	III	3535A (EPA SW-846)	III	TO-15 (EPA ORD)	III	9102 (NIOSH)	III
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
N-Ethyldiethanolamine (EDEA) (degradation product of HN-1)	139-87-7	HPLC / LC-MS-MS	Sample Prep	3541/3545A (EPA SW-846)	III	D7599-09 (ASTM)	II	D7599-09 (ASTM)	III	TO-10A (EPA ORD)	III	EPA 600/R-11/143 (EPA/NIOSH)	II
			Determinative	8321B (EPA SW-846)									
Ethylene oxide	75-21-8	GC-MS	Sample Prep	5035A (EPA SW-846)	II	5030C (EPA SW-846)	II	5030C (EPA SW-846)	II	TO-15 (EPA ORD)	I	Not of concern	NA
			Determinative	8260C (EPA SW-846)		8260C (EPA SW-846)		8260C (EPA SW-846)					
Fenamiphos	22224-92-6	GC-MS	Sample Prep	3570 (EPA SW-846)	II	3511 (EPA SW-846)	II	525.2 (EPA OW)	I	TO-10A (EPA ORD)	II	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)						8270D (EPA SW-846)	
Fentanyl	437-38-7	HPLC	Sample Prep	3541/3545A (EPA SW-846)	III	3520C/3535A (EPA SW-846)	II	3520C/3535A (EPA SW-846)	II	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8321B (EPA SW-846)		8321B (EPA SW-846)		8321B (EPA SW-846)				8321B (EPA SW-846)	
Fluoride	16984-48-8	IC-conductivity detection	Sample Prep	Not of concern	NA	300.1, Rev 1.0 (EPA OW)	I	300.1, Rev 1.0 (EPA OW)	I	Not of concern	NA	Not of concern	NA
			Determinative										
Fluoroacetamide	640-19-7	GC-MS	Sample Prep	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	II	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	II	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	II	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	III	Adapted from J. Chromatogr. B (2008) 876(1): 103-108	III
			Determinative										
Fluoroacetic acid and fluoroacetate salts (analyze as fluoroacetate ion)	NA	LC-MS	Sample Prep	Adapted from J. Chromatogr. A (2007) 1139: 271-278	III	Adapted from J. Chromatogr. B (2010) 878: 1045-1050	III	Adapted from J. Chromatogr. B (2010) 878: 1045-1050	III	S301-1 (NIOSH)	III	Adapted from J. Chromatogr. A (2007) 1139: 271-278	III
			Determinative							Adapted from J. Chromatogr. A (2007) 1139: 271-278			
2-Fluoroethanol	371-62-0	GC-MS / GC-FID	Sample Prep	5035A (EPA SW-846)	III	5030C (EPA SW-846)	III	5030C (EPA SW-846)	III	2513 (NIOSH)	III	Not of concern	NA
			Determinative	8260C (EPA SW-846)		8260C (EPA SW-846)		8260C (EPA SW-846)					
Formaldehyde	50-00-0	FGC-ECD / HPLC	Sample Prep	8315A (EPA SW-846)	I	8315A (EPA SW-846)	I	556.1 (EPA OW)	I	2016 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846)	III
			Determinative									8315A (EPA SW-846)	
Gasoline range organics	NA	GC-FID	Sample Prep	5035A (EPA SW-846)	I	5030C (EPA SW-846)	I	5030C (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative	8015C (EPA SW-846)		8015C (EPA SW-846)		8015C (EPA SW-846)				8015C (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4	HPLC	Sample Prep	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative			8330B (EPA SW-846)		8330B (EPA SW-846)				8330B (EPA SW-846)	
Hexamethylenetriperoxidediamine (HMTD)	283-66-9	LC-MS	Sample Prep	8330B (EPA SW-846)	II	3535A/8330B (EPA SW-846)	II	3535A/8330B (EPA SW-846)	II	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	Adapted from Analyst (2001) 126:1689-1693		Adapted from Analyst (2001) 126:1689-1693		Adapted from Analyst (2001) 126:1689-1693				Adapted from Analyst (2001) 126:1689-1693	
Hydrogen bromide	10035-10-6	IC-conductivity detection	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	7903 (NIOSH)	I	Not of concern	NA
			Determinative										
Hydrogen chloride	7647-01-0	IC-conductivity detection	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	7903 (NIOSH)	I	Not of concern	NA
			Determinative										
Hydrogen cyanide	74-90-8	Visible spectrophotometry	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	6010 (NIOSH)	I	Not of concern	NA
			Determinative										
Hydrogen fluoride	7664-39-3	IC-conductivity detection	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	7903[6] (NIOSH)	I	Not of concern	NA
			Determinative										
Hydrogen sulfide	7783-06-4	IC-conductivity detection	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	6013 (NIOSH)	I	Not of concern	NA
			Determinative										
Isopropyl methylphosphonic acid (IMPA) (degradation product of GB)	1832-54-8	HPLC / LC-MS-MS	Sample Prep	E2866-12 (ASTM)	II	D7597-09 (ASTM)	II	D7597-09 (ASTM)	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	II
			Determinative									8321B (EPA SW-846)	
Kerosene	64742-81-0	GC-FID	Sample Prep	5035A (EPA SW-846)	I	5030C (EPA SW-846)	I	5030C (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative	8015C (EPA SW-846)		8015C (EPA SW-846)		8015C (EPA SW-846)				8015C (EPA SW-846)	
Lead arsenate (analyze as total arsenic)	7645-25-2	ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010C/6020A (EPA SW-846)						IO-3.4/IO-3.5 (EPA ORD)		6010C/6020A (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
Lewisite 1 (L-1) [7] [2-chlorovinyldichloroarsine] (analyze as total arsenic)	541-25-3	ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010C/6020A (EPA SW-846)						IO-3.4/IO-3.5 (EPA ORD)		6010C/6020A (EPA SW-846)	
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine] (analyze as total arsenic)	40334-69-8	ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010C/6020A (EPA SW-846)						IO-3.4/IO-3.5 (EPA ORD)		6010C/6020A (EPA SW-846)	
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine] (analyze as total arsenic)	40334-70-1	ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010C/6020A (EPA SW-846)						IO-3.4/IO-3.5 (EPA ORD)		6010C/6020A (EPA SW-846)	
Lewisite oxide (degradation product of Lewisite) (analyze as total arsenic)	1306-02-1	ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010C/6020A (EPA SW-846)						IO-3.4/IO-3.5 (EPA ORD)		6010C/6020A (EPA SW-846)	
Mercuric chloride (analyze as total mercury)	7487-94-7	Visible spectrophotometry / CVAA / CVAFS	Sample Prep	7473 [8] (EPA SW-846)	I	245.1 [9] (EPA OW)	I	245.1 (EPA OW)	I	Not of concern	NA	9102 (NIOSH)	I
			Determinative									7473 [8] (EPA SW-846)	
Mercury, Total	7439-97-6	Visible spectrophotometry / CVAA / CVAFS	Sample Prep	7473 [8] (EPA SW-846)	I	245.1 [9] (EPA OW)	I	245.1 (EPA OW)	I	IO-5 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative									7473 [8] (EPA SW-846)	
Methamidophos	10265-92-6	LC-MS-MS	Sample Prep	Adapted from J. Chromatogr. A (2007) 1154(1): 3-25	II	Adapted from Chromatographia (2006) 63(5/6): 233-237	II	538 (EPA OW)	I	Adapted from J. Chromatogr. A (2007) 1154(1): 3-25	III	Adapted from J. Chromatogr. A (2007) 1154(1): 3-25	III
			Determinative										
Methomyl	16752-77-5	HPLC / LC-MS-MS	Sample Prep	8318A (EPA SW-846)	II	D7645-10 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846)	III
			Determinative									8318A (EPA SW-846)	
Methoxyethylmercuric acetate (analyze as total mercury)	151-38-2	Visible spectrophotometry / CVAA / CVAFS	Sample Prep	7473 [8] (EPA SW-846)	I	245.1 [9] (EPA OW)	I	245.1 (EPA OW)	I	IO-5 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative									7473 [8] (EPA SW-846)	
Methyl acrylonitrile	126-98-7	HPLC / GC-MS	Sample Prep	5035A (EPA SW-846)	II	524.2 (EPA OW)	II	524.2 (EPA OW)	II	PV2004 (OSHA)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8260C (EPA SW-846)								8260C (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
Methyl fluoroacetate (analyze as fluoroacetate ion)	453-18-9	LC-MS	Sample Prep	J. Chromatogr. A (2007) 1139: 271-278	III	J. Chromatogr. B (2010) 878: 1045-1050	III	J. Chromatogr. B (2010) 878: 1045-1050	III	S301-1 (NIOSH)	III	J. Chromatogr. A (2007) 1139: 271-278	III
			Determinative							J. Chromatogr. A (2007) 1139: 271-278			
Methyl hydrazine	60-34-4	Visible spectrophotometry/ HPLC-UV	Sample Prep	3541/3545A (EPA SW-846)	III	J. Chromatogr. (1993) 617: 157-162	II	J. Chromatogr. (1993) 617: 157-162	II	3510 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	J. Chromatogr. (1993) 617: 157-162								J. Chromatogr. (1993) 617: 157-162	
Methyl isocyanate	624-83-9	HPLC	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	OSHA 54	I	Not of concern	NA
			Determinative										
Methyl paraoxon	950-35-6	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III	3535A (EPA SW-846)	III	3535A (EPA SW-846)	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Methyl parathion	298-00-0	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	I	3535A (EPA SW-846)	I	3535A (EPA SW-846)	I	TO-10A (EPA ORD)	I	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Methylamine	74-89-5	HPLC	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	OSHA 40	I	Not of concern	NA
			Determinative										
N-Methyldiethanolamine (MDEA) (degradation product of HN-2)	105-59-9	HPLC / LC-MS-MS	Sample Prep	3541/3545A (EPA SW-846)	III	D7599-09 (ASTM)	II	D7599-09 (ASTM)	III	TO-10A (EPA ORD)	III	EPA 600/R-11/143 (EPA/NIOSH)	II
			Determinative	8321B (EPA SW-846)									
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	TO-10A[2] (EPA ORD)	III[13]	3570/8290A Appendix A (EPA SW-846)	III[13]
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Methylphosphonic acid (MPA) (degradation product of VX, GB, & GD)	993-13-5	HPLC	Sample Prep	E2866-12 (ASTM)	II	D7597-09 (ASTM)	II	D7597-09 (ASTM)	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	II
			Determinative									8321B (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
Mevinphos	7786-34-7	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	I	3535A (EPA SW-846)	I	525.2 (EPA OW)	I	TO-10A (EPA ORD)	II	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)						8270D (EPA SW-846)	
Monocrotophos	6923-22-4	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	I	3535A (EPA SW-846)	I	3535A (EPA SW-846)	I	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	TO-10A (EPA ORD)	III[13]	3570/8290A Appendix A (EPA SW-846)	III[13]
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine]	51-75-2	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	TO-10A (EPA ORD)	III[13]	3570/8290A Appendix A (EPA SW-846)	III[13]
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	TO-10A (EPA ORD)	III[13]	3570/8290A Appendix A (EPA SW-846)	III[13]
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Mustard, sulfur / Mustard gas (HD)	505-60-2	GC-MS	Sample Prep	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*
			Determinative										
Nicotine compounds (analyze as nicotine)	54-11-5	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	II	3535A (EPA SW-846)	II	3535A (EPA SW-846)	II	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0	HPLC	Sample Prep	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative			8330B (EPA SW-846)		8330B (EPA SW-846)				8330B (EPA SW-846)	
Osmium tetroxide (analyze as total osmium)	20816-12-0	ICP-AES	Sample Prep	3050B (EPA SW-846)	II	200.7/200.8 (EPA OW)	II	200.7/200.8 (EPA OW)	II	IO-3.1 (EPA ORD)	II	9102 (NIOSH)	III
			Determinative	6010C (EPA SW-846)						IO-3.4 (EPA ORD)		6010C (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
Oxamyl	23135-22-0	HPLC / LC-MS-MS	Sample Prep	8318A (EPA SW-846)	II	D7645-10 (ASTM)	II	531.2 (EPA OW)	I	5601 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846)	III
			Determinative									8318A (EPA SW-846)	
Paraoxon	311-45-5	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III	3520C/3535A (EPA SW-846)	III	3520C/3535A (EPA SW-846)	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Paraquat	4685-14-7	HPLC-UV/LC-MS-MS	Sample Prep	Adapted from J. Chromatogr. A (2008) 1196-1197: 110-116	II	549.2 (EPA OW)	I	549.2 (EPA OW)	I	Not of concern	NA	Adapted from J. Chromatogr. A (2008) 1196-1197: 110-116	III
			Determinative										
Parathion	56-38-2	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	I	3520C/3535A (EPA SW-846)	I	3520C/3535A (EPA SW-846)	I	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Pentaerythritol tetranitrate (PETN)	78-11-5	HPLC	Sample Prep	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative			8330B (EPA SW-846)		8330B (EPA SW-846)				8330B (EPA SW-846)	
Phencyclidine	77-10-1	GC-MS	Sample Prep	3570 (EPA SW-846)	II	3511 (EPA SW-846)	II	3511 (EPA SW-846)	II	TO-10A (EPA ORD)	II	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Phorate	298-02-2	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	I	3535A (EPA SW-846)	I	3535A (EPA SW-846)	I	TO-10A (EPA ORD)	II	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Phorate sulfone	2588-04-7	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III	3535A (EPA SW-846)	III	3535A (EPA SW-846)	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Phorate sulfone oxon[5]	2588-06-9	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III	3535A (EPA SW-846)	III	3535A (EPA SW-846)	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
Phorate sulfoxide	2588-03-6	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III	3535A (EPA SW-846)	III	3535A (EPA SW-846)	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Phorate sulfoxide oxon[5]	2588-05-8	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III	3535A (EPA SW-846)	III	3535A (EPA SW-846)	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Phosgene	75-44-5	GC-NPD	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	OSHA 61	I	Not of concern	NA
			Determinative										
Phosphamidon	13171-21-6	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	I	3520C/3535A (EPA SW-846)	I	3520C/3535A (EPA SW-846)	I	TO-10A (EPA ORD)	II	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Phosphine	7803-51-2	Visible spectrophotometry	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	6002 (NIOSH)	I	Not of concern	NA
			Determinative										
Phosphorus trichloride	7719-12-2	Visible spectrophotometry	Sample Prep	Not of concern	NA	Not of concern	NA	Not of concern	NA	6402 (NIOSH)	I	Not of concern	NA
			Determinative										
Pinacolyl methyl phosphonic acid (PMPA) (degradation product of GD)	616-52-4	HPLC / LC-MS-MS	Sample Prep	E2866-12 (ASTM)	II	D7597-09 (ASTM)	II	D7597-09 (ASTM)	III	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	II
			Determinative									8321B (EPA SW-846)	
Propylene oxide	75-56-9	GC-MS / GC-FID	Sample Prep	5035A (EPA SW-846)	II	5030C (EPA SW-846)	II	5030C (EPA SW-846)	II	1612 (NIOSH)	I	Not of concern	NA
			Determinative	8260C (EPA SW-846)		8260C (EPA SW-846)		8260C (EPA SW-846)					
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	TO-10A (EPA ORD)	III[13]	3570/8290A Appendix A (EPA SW-846)	III[13]
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Sarin (GB)	107-44-8	GC-MS	Sample Prep	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*
			Determinative										

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
Sodium arsenite (analyze as total arsenic)	7784-46-5	ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010C/6020A (EPA SW-846)						IO-3.4/IO-3.5 (EPA ORD)		6010C/6020A (EPA SW-846)	
Sodium azide (analyze as azide ion)	26628-22-8	IC CD	Sample Prep	Adapted from J. Forensic Sci. (1998) 43(1): 200-202 [10]	II	Adapted from J. Forensic Sci. (1998) 43(1): 200-202 [10]	II	Adapted from J. Forensic Sci. (1998) 43(1): 200-202 [10]	II	ID-211 (OSHA)	I	ID-211 (OSHA)	I
			Determinative	300.1, Rev 1.0 [11] (EPA OW)		300.1, Rev 1.0 [11] (EPA OW)		300.1, Rev 1.0 [11] (EPA OW)					
Soman (GD)	96-64-0	GC-MS	Sample Prep	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*
			Determinative										
Strychnine	57-24-9	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	I	3535A (EPA SW-846)	I	3535A (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Tabun (GA)	77-81-6	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III[13]	3535A (EPA SW-846)	III[13]	3535A (EPA SW-846)	III[13]	TO-10A (EPA ORD)	III[13]	3570/8290A Appendix A (EPA SW-846)	III[13]
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Tetraethyl pyrophosphate (TEPP)	107-49-3	GC-MS	Sample Prep	3570 (EPA SW-846)	II	3511 (EPA SW-846)	II	3511 (EPA SW-846)	II	TO-10A (EPA ORD)	II	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
Tetramethylenedisulfotetramine (TETS)	80-12-6	GC-MS	Sample Prep	3570 (EPA SW-846)	II	3511 (EPA SW-846)	II	EPA 600/R-11/091 (EPA/CDC)	II	TO-10A (EPA ORD)	II	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D[3] (EPA SW-846)		8270D[3] (EPA SW-846)						8270D[3] (EPA SW-846)	
Thallium sulfate (analyze as total thallium)	10031-59-1	ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010C/6020A (EPA SW-846)						IO-3.4/IO-3.5 (EPA ORD)		6020A/6010C (EPA SW-846)	
Thiodiglycol (TDG) (degradation product of HD)	111-48-8	HPLC / LC-MS-MS	Sample Prep	E2787-11 (ASTM)	II	D7598-09 (ASTM)	II	D7598-09 (ASTM)	III	TO-10A (EPA ORD)	III	E2838-11 (ASTM)	II
			Determinative										
Thiofanox	39196-18-4	HPLC	Sample Prep	3541/3545A (EPA SW-846)	III	D7645-10 (ASTM)	II	538 (EPA OW)	I	5601 (NIOSH)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8321B (EPA SW-846)								8321B (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
1,4-Thioxane (degradation product of HD)	15980-15-1	GC-MS	Sample Prep	3570 (EPA SW-846)	II	3511 (EPA SW-846)	II	3511 (EPA SW-846)	II	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	II
			Determinative	8270D[12] (EPA SW-846)		8270D[12] (EPA SW-846)		8270D[12] (EPA SW-846)				8270D[12] (EPA SW-846)	
Titanium tetrachloride (analyze as total titanium)	7550-45-0	ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	Not of concern	NA	Not of concern	NA	Not of concern	NA	9102 (NIOSH)	III
			Determinative	6010C/6020A (EPA SW-846)								6010C/6020A (EPA SW-846)	
Triethanolamine (TEA) (degradation product of HN-3)	102-71-6	HPLC / LC-MS-MS	Sample Prep	3541/3545A (EPA SW-846)	III	D7599-09 (ASTM)	II	D7599-09 (ASTM)	III	TO-10A (EPA ORD)	III	EPA 600/R-11/143 (EPA/NIOSH)	II
			Determinative	8321B (EPA SW-846)									
Trimethyl phosphite	121-45-9	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III	Not of concern	NA	Not of concern	NA	TO-10A (EPA ORD)	III	3570/8290A Appendix A (EPA SW-846)	III
			Determinative	8270D[3] (EPA SW-846)								8270D[3] (EPA SW-846)	
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4	HPLC	Sample Prep	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative			8330B (EPA SW-846)		8330B (EPA SW-846)				8330B (EPA SW-846)	
2,4,6-Trinitrotoluene (2,4,6-TNT)	118-96-7	HPLC	Sample Prep	8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	3535A/8330B (EPA SW-846)	I	Not of concern	NA	3570/8290A Appendix A (EPA SW-846)	I
			Determinative			8330B (EPA SW-846)		8330B (EPA SW-846)				8330B (EPA SW-846)	
Vanadium pentoxide (analyze as total vanadium)	1314-62-1	ICP-AES / ICP-MS	Sample Prep	3050B (EPA SW-846)	I	200.7/200.8 (EPA OW)	I	200.7/200.8 (EPA OW)	I	IO-3.1 (EPA ORD)	I	9102 (NIOSH)	I
			Determinative	6010C/6020A (EPA SW-846)						IO-3.4/IO-3.5 (EPA ORD)		6010C/6020A (EPA SW-846)	
VE [phosphonothioic acid, ethyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21738-25-0	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	TO-10A (EPA ORD)	III[13]	3570/8290A Appendix A (EPA SW-846)	III[13]
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	TO-10A (EPA ORD)	III[13]	3570/8290A Appendix A (EPA SW-846)	III[13]
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples		Aqueous Liquid Samples		Drinking Water Samples		Air Samples		Wipes	
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	3520C/3535A (EPA SW-846)	III[13]	TO-10A (EPA ORD)	III[13]	3570/8290A Appendix A (EPA SW-846)	III[13]
			Determinative	8270D (EPA SW-846)		8270D (EPA SW-846)		8270D (EPA SW-846)				8270D (EPA SW-846)	
VX [O-ethyl-S-(2-diisopropylaminoethyl)methyl-phosphonothiolate]	50782-69-9	GC-MS	Sample Prep	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*	CWA Protocol (EPA NHSRC)	*
			Determinative										
White phosphorus	12185-10-3	GC-NPD / GC-FPD	Sample Prep	7580 (EPA SW-846)	I	7580 (EPA SW-846)	I	7580 (EPA SW-846)	I	7905 (NIOSH)	I	3570/8290A Appendix A (EPA SW-846)	III
			Determinative									7580 (EPA SW-846)	

* Only laboratories approved under the ERLN umbrella are designated for handling the CWA standards needed for this method. For access to the nearest ERLN laboratory specially trained and equipped for CWA analysis, contact the EPA Headquarters Emergency Operations Center (EPA/HQ-EOC) at 202-564-3850.

Footnotes

[1] If problems occur when using this method, TO-10A should be used.

[2] If problems occur when using this method, the canister Method TO-15 should be used.

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

[4] If problems occur when using this method, SW-846 Method 8321B should be used as the Determinative method. Sample preparation methods should remain the same.

[5] If problems occur during measurement of oxon compounds, analysts should consider use of procedures included in Kamal, A. *et al*., "Oxidation of selected organophosphate pesticides during chlorination of simulated drinking water." Water Research. 2009. 43(2): 522–534. http://www.sciencedirect.com/science/journal/00431354.

[6] If problems occur when using this method, NIOSH Method 7906 should be used.

[7] Laboratory testing is currently under way for speciation of Lewisite 1 using GC-MS techniques.

[8] If equipment is not available or problems occur when analyzing solid and wipe samples, use CVAA Method 7471B (EPA SW-846).

[9] If problems occur when using EPA Method 245.1 for these analytes during preparation and analysis of aqueous liquid samples, refer to EPA Method 7470A (SW-846).

[10] Water extraction, filtration and acidification steps from the Journal of Forensic Science. 1998. 43(1): 200-202 should be used for the preparation of solid samples. Filtration and acidification steps from this journal should be used for preparation of aqueous liquid and drinking water samples.

[11] If analyses are problematic, refer to column manufacturer for alternate conditions.

[12] If problems occur when using this method, SW-846 Method 8260C and appropriate corresponding sample preparation procedures (i.e., 5035A for solid samples, and 5030C for aqueous liquid and drinking water samples) should be used.

[13] Data are not available for this analyte/sample type using this method. However, the referenced SW-846 method or the CWA Protocol may be applicable.

Appendix B: Selected Radiochemical Methods

SAM 2012 Appendix B: Selected Radiochemical Methods

Analyte Class	Determinative Technique	Drinking Water Samples	Aqueous and Liquid Phase Samples	Soil and Sediment Samples	Surface Wipes	Air Filters	Vegetation Samples
Gross Alpha	Alpha / Beta counting	900.0 (EPA)	7110 B (SM)	AP1 (ORISE)	FRMAC, Vol 2, pg. 33 (DOE)	FRMAC, Vol 2, pg. 33 (DOE)	AP1 (ORISE)
Gross Beta	Alpha / Beta counting	900.0 (EPA)	7110 B (SM)	AP1 (ORISE)	FRMAC, Vol 2, pg. 33 (DOE)	FRMAC, Vol 2, pg. 33 (DOE)	AP1 (ORISE)
Gamma	Gamma spectrometry	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Select Mixed Fission Products[1]	Gamma spectrometry	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Total Activity Screening	Liquid scintillation	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)	Preparation of Samples for Total Activity Screening (Y-12)

Analyte(s)	CAS RN	Determinative Technique	Drinking Water Samples		Aqueous and Liquid Phase Samples		Soil and Sediment Samples		Surface Wipes		Air Filters		Vegetation Samples	
			Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory
Americium-241[4]	14596-10-2	Alpha spectrometry	Rapid Radiochemical Method for Am-241[3] (EPA)	Am-04-RC (HASL-300)	D3084-05 (ASTM)	Am-04-RC (HASL-300)	Actinides and Sr-89/90 in Soil Samples (DOE SRS)	Am-01-RC[5] (HASL-300)	Rapid methods* for acid or fusion digestion (EPA)	Am-04-RC (HASL-300)	Rapid methods* for acid or fusion digestion (EPA)	Am-04-RC (HASL-300)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	Am-06-RC (HASL-300)
		Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Californium-252[4]	13981-17-4	Alpha spectrometry	D3084-05 (ASTM)	Am-04-RC (HASL-300)	D3084-05 (ASTM)	Am-04-RC (HASL-300)	D3084-05 (ASTM)	Am-01-RC[5] (HASL-300)	D3084-05 (ASTM)	Am-04-RC (HASL-300)	D3084-05 (ASTM)	Am-04-RC (HASL-300)	D3084-05 (ASTM)	Am-06-RC (HASL-300)
Cesium-137	10045-97-3	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Cobalt-60	10198-40-0	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Curium-244[4]	13981-15-2	Alpha spectrometry	D3084-05 (ASTM)	Am-04-RC (HASL-300)	D3084-05 (ASTM)	Am-04-RC (HASL-300)	D3084-05 (ASTM)	Am-01-RC[5] (HASL-300)	D3084-05 (ASTM)	Am-04-RC (HASL-300)	D3084-05 (ASTM)	Am-04-RC (HASL-300)	D3084-05 (ASTM)	Am-06-RC (HASL-300)
Europium-154	15585-10-1	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Iodine-125	14158-31-7	Gamma spectrometry	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)	Procedure #9[6] (ORISE)	Procedure #9[6] (ORISE)	Procedure #9 (ORISE)	Procedure #9 (ORISE)
Iodine-131	10043-66-0	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R[6] (HASL-300)	Ga-01-R[6] (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Iridium-192	14694-69-0	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Molybdenum-99	14119-15-4	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)

Analyte(s)	CAS RN	Determinative Technique	Drinking Water Samples		Aqueous and Liquid Phase Samples		Soil and Sediment Samples		Surface Wipes		Air Filters		Vegetation Samples	
			Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory
Phosphorus-32	14596-37-3	Liquid scintillation / Beta counting	Rapid Radiochemical Method for P-32 in water[3] (EPA)	R4-73-014 (EPA)	R4-73-014 (EPA)	R4-73-014 (EPA)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)	RESL P-2 (DOE)
Plutonium-238[4]	13981-16-3	Alpha spectrometry	Rapid Radiochemical Method for Pu[3] (EPA)	EMSL-33 (EPA)	D3084-05 (ASTM)	EMSL-33 (EPA)	Actinides and Sr-89/90 in Soil Samples (DOE SRS)	EMSL-33 (EPA)	Rapid methods* for acid or fusion digestion (EPA)	EMSL-33 (EPA)	Rapid methods* for acid or fusion digestion (EPA)	EMSL-33 (EPA)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	Am-06-RC (HASL-300)
Plutonium-239[4]	15117-48-3	Alpha spectrometry	Rapid Radiochemical Method for Pu[3] (EPA)	EMSL-33 (EPA)	D3084-05 (ASTM)	EMSL-33 (EPA)	Actinides and Sr-89/90 in Soil Samples (DOE SRS)	EMSL-33 (EPA)	Rapid methods* for acid or fusion digestion (EPA)	EMSL-33 (EPA)	Rapid methods* for acid or fusion digestion (EPA)	EMSL-33 (EPA)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	Am-06-RC (HASL-300)
Polonium-210	13981-52-7	Alpha spectrometry	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)	Method 111 (EPA)	Method 111 (EPA)	Method 111 (EPA)	Method 111 (EPA)	Po-02-RC (HASL-300)	Po-02-RC (HASL-300)
Radium-226	13982-63-3	Alpha spectrometry / Radon emanation	Rapid Radiochemical Method for Ra-226[3] (EPA)	903.1 (EPA)	7500-Ra B (SM)	7500-Ra C (SM)	D3084-05 (ASTM)	EMSL-19 (EPA)	Rapid methods* for acid or fusion digestion (EPA)	EMSL-19 (EPA)	Rapid methods* for acid or fusion digestion (EPA)	EMSL-19 (EPA)	Ra-03-RC (HASL-300)	Ra-03-RC (HASL-300)
Ruthenium-103	13968-53-1	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Ruthenium-106	13967-48-1	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Selenium-75	14265-71-5	Gamma spectrometry	901.1 (EPA)	901.1 (EPA)	7120 (SM)	7120 (SM)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)	Ga-01-R (HASL-300)
Strontium-89	14158-27-1	Beta counting	905.0 (EPA)	905.0 (EPA)	905.0 (EPA)	905.0 (EPA)	Actinides and Sr-89/90 in Soil Samples (DOE SRS)	Strontium in Food and Bioenvironmental Samples (EPA)	Strontium in Food and Bioenvironmental Samples (EPA)		Strontium in Food and Bioenvironmental Samples (EPA)		Actinides and Sr-89/90 in Vegetation (DOE SRS)	Strontium in Food and Bioenvironmental Samples (EPA)
Strontium-90	10098-97-2	Beta counting	Rapid Radiochemical Methods for Sr-90[3] (EPA)	905.0 (EPA)	D5811-08 (ASTM)	D5811-08 (ASTM)	Actinides and Sr-89/90 in Soil Samples (DOE SRS)	Sr-03-RC (HASL-300)	Rapid methods* for acid or fusion digestion (EPA)	Sr-03-RC (HASL-300)	Rapid methods* for acid or fusion digestion (EPA)	Sr-03-RC (HASL-300)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	Sr-03-RC (HASL-300)
Technetium-99	14133-76-7	Liquid scintillation / beta counting	Tc-02-RC (HASL-300)	Tc-02-RC (HASL-300)	D7168-05 (ASTM)	D7168-05 (ASTM)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	AP5 (ORISE)	Tc-01-RC (HASL-300)
Tritium (Hydrogen-3)	10028-17-8	Liquid scintillation	906.0 (EPA)	906.0 (EPA)	906.0 (EPA)	906.0 (EPA)	AP2 (ORISE)	AP2 (ORISE)	AP2 (ORISE)	AP2 (ORISE)	Not applicable[7]	Not applicable[7]	AP2 (ORISE)	AP2 (ORISE)
Uranium-234[4]	13966-29-5	Alpha spectrometry	Rapid Radiochemical Method for U[3] (EPA)	D3972-02 (ASTM)	7500-U B[8] (SM)	7500-U C (SM)	Actinides and Sr-89/90 in Soil Samples (DOE SRS)	EMSL-33 (EPA)	Rapid methods* for acid or fusion digestion (EPA)	EMSL-33 (EPA)	Rapid methods* for acid or fusion digestion (EPA)	EMSL-33 (EPA)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	U-02-RC (HASL-300)

Analyte(s)	CAS RN	Determinative Technique	Drinking Water Samples		Aqueous and Liquid Phase Samples		Soil and Sediment Samples		Surface Wipes		Air Filters		Vegetation Samples	
			Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory	Qualitative Determination[2]	Confirmatory
Uranium-235[4]	15117-96-1	Alpha spectrometry	Rapid Radiochemical Method for U[3] (EPA)	D3972-02 (ASTM)	7500-U B[8] (SM)	7500-U C (SM)	Actinides and Sr-89/90 in Soil Samples (DOE SRS)	EMSL-33 (EPA)	Rapid methods* for acid or fusion digestion (EPA)	EMSL-33 (EPA)	Rapid methods* for acid or fusion digestion (EPA)	EMSL-33 (EPA)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	U-02-RC (HASL-300)
Uranium-238[4]	7440-61-1	Alpha spectrometry	Rapid Radiochemical Method for U[3] (EPA)	D3972-02 (ASTM)	7500-U B[8] (SM)	7500-U C (SM)	Actinides and Sr-89/90 in Soil Samples (DOE SRS)	EMSL-33 (EPA)	Rapid methods* for acid or fusion digestion (EPA)	EMSL-33 (EPA)	Rapid methods* for acid or fusion digestion (EPA)	EMSL-33 (EPA)	Actinides and Sr-89/90 in Vegetation (DOE SRS)	U-02-RC (HASL-300)

Footnotes

[1] Please note that this category does not cover all fission products. In addition to the specific radionuclides listed in this appendix, gamma-ray spectometry with a high resoution HP(Ge) detector will identify and quantify fission products with gamma rays in the energy range of 30 keV to 2000 keV. The sensitivity will be dependent on the detector efficiency and the gamma-ray emission probabilities (branching ratio) for the specific radionuclide.

[2] In those cases where the same method is listed for qualitative determination and confirmatory analysis, qualitative determination can be performed by application of the method over a shorter count time than that used for confirmatory analysis.

[3] SAM lists this method for rapid qualitative screening of drinking water samples. The method is not intended for use in compliance monitoring of drinking water.

[4] If it is suspected that the sample exists in refractory form (i.e., non-digestible or dissolvable material after normal digestion methods) or if there is a matrix interference problem, use ORISE Method AP11 for qualitative determination or confirmatory analysis of alpha radioactivity.

[5] In cases where only small sample volumes (≤100 g) are available, use HASL-300 Method Pu-12-RC.

[6] This procedure should be used only for filters specifically designed for iodine.

[7] Because tritium is not sampled using traditional air filters, this matrix is not applicable.

[8] This method was developed for measurement of total uranium and does not distinguish between uranium isotopes.

* These rapid methods describe wipe and air filter digestion procedures, and include references to the analyte-specific separation procedures listed for rapid analysis of drinking water samples, to be used to complete anlaysis of the digested samples.

Appendix C: Selected Pathogen Methods

SAM 2012 Appendix C: Selected Pathogen Methods

Not all methods have been evaluated for each pathogen/sample type/environmental matrix combination in Appendix C. Each laboratory using these methods must operate a formal quality assurance program and, at a minimum, analyze appropriate quality control samples (Section 7.1.2). Also, if required, a modification or an appropriate replacement method may be warranted for a specific pathogen/sample type/environmental matrix or a combination thereof. Additionally, the SAM Pathogen primary and alternate points of contact should be consulted for additional guidance (Section 4.0, Points of Contact).

Note: If viability determinations are needed (e.g., for post decontamination phase samples), a viability-based procedure (such as culture) should be used. Rapid analysis techniques (such as PCR, immunoassays) without culture are preferred for determination of extent and magnitude of contamination (e.g., for site characterization phase samples). Please see Figure 7-1.

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method			
			Aerosol (growth media, filter, liquid)	Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)	Drinking Water	Post Decontamination Waste Water
Bacteria						
Bacillus anthracis (BA) [Anthrax]	Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	EPA BA Protocol (Anticipated publication October 2012)			
	Real-time PCR/ RV-PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	EPA BA Protocol (Anticipated publication October 2012)			
Brucella spp. (*B. abortus, B. melitensis, B. suis*) [Brucellosis]	Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents ff Bioterrorism and Emerging Infectious Diseases: *Brucella species*			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Hinić *et al*. 2008. J. Microbiol. Methods. 75(2): 375-378.			

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method			
			Aerosol (growth media, filter, liquid)	Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)	Drinking Water	Post Decontamination Waste Water
Burkholderia mallei [Glanders] and *Burkholderia pseudomallei* [Melioidosis]	Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Tomaso *et al*. 2006. Clin. Chem. 52(2): 307-310, Novak *et al*. 2006. J. Clin. Microbiol. 44(1): 85-90, and Meumann *et al*. 2006 J. Clin. Microbiol. 44(8): 3028-3030			
Campylobacter jejuni [Campylobacteriosis]	Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	ISO 17795			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Cunningham *et al*. 2010. J. Clin. Microbiol. 48(8): 2929-2933.			
Chlamydophila psittaci (formerly known as *Chlamydia psittaci*) [Psittacosis]	Tissue culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Madico *et al*. 2000. J. Clin. Microbiol. 38(3): 1085-1093.			
	PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Madico *et al*. 2000. J. Clin. Microbiol. 38(3): 1085-1093.			

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method			
			Aerosol (growth media, filter, liquid)	Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)	Drinking Water	Post Decontamination Waste Water
Coxiella burnetii [Q-fever]	Tissue Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Raoult *et al*. 1991. Antimicrob. Agents Chemother. 35(10): 2070-2077.			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Panning et al. 2008. BMC Microbiol. 8:77.			
Escherichia coli O157:H7	Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Protocol (EPA/600/R-10/056)	
		Analytical Technique	EPA Protocol (EPA/600/R-10/056)			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Protocol (EPA/600/R-10/056)	
		Analytical Technique	Sen *et al*. 2011. Environ. Sci. Technol. 45(7): 2250-2256.			
Francisella tularensis [Tularemia]	Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) and Humrighouse *et al.* 2011. Appl. Environ. Microbiol. 77(18): 6729-6732.	
		Analytical Technique	CDC, ASM and APHL: Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Francisella tularensis*			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Versage *et al*. 2003. J. Clin. Microbiol. 41(12): 5492–5499.			

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method			
			Aerosol (growth media, filter, liquid)	Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)	Drinking Water	Post Decontamination Waste Water
Leptospira [Leptospirosis]	Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Standard Method 9260 I: *Leptospira*			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Palaniappan *et al*. 2005. Mol. Cell Probes. 19(2): 111-117.			
Listeria monocytogenes [Listeriosis]	Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	FDA, CFSAN. 2003. Bacteriological Analytical Manual Online.			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	USDA, FSIS. 2009. Microbiology Laboratory Guidebook MLG 8A.04.			
Non-typhoidal *Salmonella* (Not applicable to *S.* Typhi) [Salmonellosis]	Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) and EPA Method 1200 (EPA 817-R-12-004)	
		Analytical Technique	EPA Method 1682 (EPA-821-R-06-14) or EPA Analytical Protocol for Non-Typhoidal *Salmonella* in Drinking Water and Surface Water			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) and EPA Method 1200 (EPA 817-R-12-004)	
		Analytical Technique	Jyoti *et al*. Environ. Sci. Technol. 45(20): 8996-9002.			

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method			
			Aerosol (growth media, filter, liquid)	Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)	Drinking Water	Post Decontamination Waste Water
Salmonella Typhi [Typhoid fever]	Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA protocol (EPA 600/R-10/133)	
		Analytical Technique	EPA Protocol (EPA 600/R-10/133)			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA protocol (EPA 600/R-10/133)	
		Analytical Technique	CDC Laboratory Assay			
Shigella spp. [Shigellosis]	Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Standard Method 9260 E: *Shigella*			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Cunningham *et al*. 2010. J. Clin. Microbiol. 48(8): 2929-2933.			
Staphylococcus aureus	Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Standard Method 9213 B: *Staphylococcus aureus*			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Chiang *et al*. 2007. J. Food Prot. 70(12): 2855-2859.			

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method			
			Aerosol (growth media, filter, liquid)	Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)	Drinking Water	Post Decontamination Waste Water
Vibrio cholerae O1 and O139 [Cholera]	Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Protocol (EPA 600/R-10/139)	
		Analytical Technique	EPA Protocol (EPA 600/R-10/139)			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Protocol (EPA 600/R-10/139)	
		Analytical Technique	Blackstone *et al*. 2007. J. Microbiol. Methods. 68(2): 254-259.			
Yersinia pestis [Plague]	Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Yersinia pestis*			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Woron *et al*. 2006. Diagn. Micr. Infec. Dis. 56(3): 261-268.			
Viruses						
Adenoviruses: Enteric and non-enteric (A-F)	Tissue Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	EPA Method 1615 (EPA/600/R-10/181)			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	Jothikumar *et al*. 2005. Appl. Environ. Microbiol. 71(6): 3131–3136			

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method			
			Aerosol (growth media, filter, liquid)	Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)	Drinking Water	Post Decontamination Waste Water
Astroviruses	Integrated Cell Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	Grimm *et al*. 2004. Can. J. Microbiol. 50(4): 269-278			
	Real-time reverse transcription-PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	Grimm *et al*. 2004. Can. J. Microbiol. 50(4): 269-278			
Caliciviruses: Noroviruses	Real-time reverse transcription-PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	EPA Method 1615 (EPA/600/R-10/181)			
Caliciviruses: Sapovirus	Tissue Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	Parwani *et al*. 1991. Arch. Virol. 120(1-2):115-122.			
	Real-time reverse transcription-PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	Oka *et al*. 2006. J. Med. Virol. 78(10): 1347-1353			

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method			
			Aerosol (growth media, filter, liquid)	Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)	Drinking Water	Post Decontamination Waste Water
Coronaviruses: SARS-associated human coronavirus	Tissue Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Pagat *et al*. 2007. Applied Biosafety 12(2): 100-108.			
	Reverse transcription-PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Adachi *et al*. 2004. J. Virol. Methods. 122(1): 29-36			
Hepatitis E virus (HEV)	Tissue Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	Zaki *et al*. 2009. FEMS Immunol. Med. Mic. 56: 73-79.			
	Real-time reverse transcription-PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	Jothikumar *et al*. 2006. J. Virol. Methods. 131(1): 65-71			
Influenza H5N1 virus	Real-time reverse transcription-PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Ng *et al*. 2005. Emerg. Infect. Dis. 11(8): 1303-1305			

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method			
			Aerosol (growth media, filter, liquid)	Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)	Drinking Water	Post Decontamination Waste Water
Picornaviruses: Enteroviruses	Tissue Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	EPA Method 1615 (EPA/600/R-10/181)			
	Reverse transcription-PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	EPA Method 1615 (EPA/600/R-10/181)			
Picornaviruses: Hepatitis A virus (HAV)	Integrated Cell Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	Hyeon *et al*. 2011. J. Food Prot. 74(10):1756-1761			
	Real-time Reverse Transcription-PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	Hyeon *et al*. 2011. J. Food Prot. 74(10):1756-1761			
Reoviruses: Rotavirus (Group A)	Tissue Culture	Sample Preparation	EPA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	EPA Method 1615 (EPA/600/R-10/181)			
	Real-time reverse transcription-PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1615 (EPA/600/R-10/181)	
		Analytical Technique	Jothikumar *et al*. 2009. J. Virol. Methods. 155(2): 126-131			

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method			
			Aerosol (growth media, filter, liquid)	Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)	Drinking Water	Post Decontamination Waste Water
Protozoa						
Cryptosporidium spp. [Cryptosporidiosis]	Cell Culture IFA	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Bukhari *et al*. 2007. Can. J. Microbiol. 53(5): 656-663.			
	IMS/FA	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) and EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623 (EPA 815-R-05-002)	
		Analytical Technique	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623 (EPA 815-R-05-002)			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103), or Guy *et al*. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185, and Jiang *et al*. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141.	
		Analytical Technique	Guy *et al*. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185 and Jiang *et al*. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141.			
Entamoeba histolytica	Cell Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Stringert *et al*. 1972. J Parasitol. 58(2): 306-310.			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103)	
		Analytical Technique	Roy *et al*. 2005. J. Clin. Microbiol. 43(5): 2168-2172.			

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method			
			Aerosol (growth media, filter, liquid)	Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)	Drinking Water	Post Decontamination Waste Water
Giardia spp. [Giardiasis]	Cell Culture	Sample Preparation	EPA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1623 (EPA 815-R-05-002)	
		Analytical Technique	Keister *et al*. 1983. T. Roy. Soc. Trop. Med. H. 77(4): 487-488.			
	IMS/FA	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or EPA Method 1623 (EPA 815-R-05-002)	
		Analytical Technique	EPA Method 1623 (EPA 815-R-05-002)			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or Guy *et al*. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185.	
		Analytical Technique	Guy *et al*. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185.			
Toxoplasma gondii [Toxoplasmosis]	Cell Culture	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	Villegas *et al*. 2010. J. Microbiol. Methods 81(3): 219 – 225.	
		Analytical Technique	Villegas *et al*. 2010. J. Microbiol. Methods 81(3): 219 – 225.			
	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Method 1623 (EPA 815-R-05-002)	
		Analytical Technique	Yang *et al*. 2009. Appl. Environ. Microbiology. 75(11): 3477-3483.			

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method			
			Aerosol (growth media, filter, liquid)	Particulate (swabs, wipes, Sponge-Sticks, vacuum socks and filters)	Drinking Water	Post Decontamination Waste Water
Helminths						
Baylisascaris procyonis [Raccoon roundworm infection]	Real-time PCR	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or Gatcombe *et al*. 2010. Parasitol. Res. 106:499-504.	
		Analytical Technique	Gatcombe *et al*. 2010. Parasitol. Res. 106:499 – 504.			
	Embryonation of Eggs and Microscopy	Sample Preparation	EPA BA Protocol (Anticipated publication October 2012)	Hodges *et al*. 2010. J. Microbiol. Methods. 81(2):141-146, or Rose *et al*. 2011. Appl. Environ. Microbiol. 77(23):8355-8359, or EPA BA Protocol (Anticipated publication October 2012)	EPA Report (EPA 600/R-11/103) or Gatcombe *et al*. 2010. Parasitol. Res. 106:499-504.	
		Analytical Technique	EPA Document (EPA/625/R-92/013)			
General Remediation Efficacy						
Biological indicator (spore) strips	Culture		Manufacturers' Instructions			

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Appendix D: Selected Biotoxin Methods

SAM 2012 Appendix D: Selected Biotoxin Methods

Note: The presence of disinfectants (e.g., chlorine) and/or preservatives added during water sample collection to slow degradation (e.g., pH adjustors, de-chlorinating agents) could possibly affect analytical results. When present, the impact of these agents on method performance should be evaluated if not previously determined.

Analyte(s)	CAS RN / Description	Analysis Type[1]	Analytical Technique	Aerosol (filter/cassette, liquid impinger)	Solid (soil, powder)	Particulate (swabs, wipes, dust socks)	Liquid Water	Drinking Water
Protein								
Abrin	1393-62-0 (abrin) / Glycoprotein consisting of a deadenylase (25-32 kDa A chain) and lectin (35 kDa B chain); an agglutinin (A2B2) may be present in crude preparations 526-31-8 (abrine) / small molecule, abrin marker	Presumptive	Immunoassay (ELISA, ECL-based)[2]	Adapted from Journal of Food Protection 71(9): 1868-1874	Adapted from Journal of Food Protection 71(9): 1868-1874	Adapted from Journal of Food Protection 71(9): 1868-1874	Adapted from Journal of Food Protection 71(9): 1868-1874	Adapted from Journal of Food Protection 71(9): 1868-1874
		Complementary Presumptive (abrine)	LC-MS-MS	Adapted from Journal of Agricultural and Food Chemistry 56(23): 11139-11143	Adapted from Journal of Agricultural and Food Chemistry 56(23): 11139-11143	Adapted from Journal of Agricultural and Food Chemistry 56(23): 11139-11143	Adapted from Journal of Agricultural and Food Chemistry 56(23): 11139-11143	Adapted from Journal of Agricultural and Food Chemistry 56(23): 11139-11143
		Confirmatory	Ribosome inactivation assay	Adapted from Pharmacology & Toxicology 88(5): 255-260	Adapted from Pharmacology & Toxicology 88(5): 255-260	Adapted from Pharmacology & Toxicology 88(5): 255-260	Adapted from Pharmacology & Toxicology 88(5): 255-260	Adapted from Pharmacology & Toxicology 88(5): 255-260
		Biological Activity	Enzyme activity[3]	Adapted from Analytical Biochemistry 378(1): 87-89	Adapted from Analytical Biochemistry 378(1): 87-89	Adapted from Analytical Biochemistry 378(1): 87-89	Adapted from Analytical Biochemistry 378(1): 87-89	Adapted from Analytical Biochemistry 378(1): 87-89
Botulinum neurotoxins (Serotoypes A, B, E, F)	Protein composed of ~100 kDa heavy chain and ~50 kDa light chain; can be complexed with hemagglutinin and non-hemagglutinin components for total MW of ~900 kDa SNAP-25, VAMP 2 / botulinum neurotoxin markers	Presumptive	Immunoassay (LFD)[4]	Adapted from EPA Environmental Technology Verification report	**LRN** If analysis for this agent is required in solid, particulate, or liquid samples, contact the LRN at (404) 639-2790 for information of the closest LRN laboratory capable of receiving and processing the sample. The terms presumptive and confirmatory as used for LRN methods are described in Section 8.1.4.			
		Complementary Presumptive (SNAP25, VAMP 2)	LC-MS	Adapted from Journal of Chemical Health and Safety 15(6): 14-19				
		Confirmatory	Immunoassay[4] (ELISA)	Adapted from FDA Bacteriological Analytical Manual, Chapter 17				
		Biological Activity	Mouse Bioassay	Adapted from FDA Bacteriological Analytical Manual, Chapter 17				

Analyte(s)	CAS RN / Description	Analysis Type[1]	Analytical Technique	Aerosol (filter/cassette, liquid impinger)	Solid (soil, powder)	Particulate (swabs, wipes, dust socks)	Liquid Water	Drinking Water
Ricin	9009-86-3 (ricin) / 60 kDa glycoprotein composed of two subunits (~32 kDa A chain and ~34 kDa B chain); an agglutinin of MW 120 kDa may be present in crude preparations 5254-40-3 (ricinine) / small molecule, ricin marker	Presumptive	Immunoassay (LFD)[2]	Adapted from EPA Environmental Technology Verification report	**LRN** If analysis for this agent is required in solid, particulate, or liquid samples, contact the LRN at (404) 639-2790 for information of the closest LRN laboratory capable of receiving and processing the sample. The terms presumptive and confirmatory as used for LRN methods are described in Section 8.1.4.			
		Complementary Presumptive (ricinine)	LC-MS	Adapted from Journal of Analytical Toxicology 29(3): 149-155	Adapted from Journal of Analytical Toxicology 29(3): 149-155	Adapted from Journal of Analytical Toxicology 29(3): 149-155	Adapted from Journal of Analytical Toxicology 29(3): 149-155	Adapted from Journal of Analytical Toxicology 29(3): 149-155
		Confirmatory	Immunoassay (ECL)	Adapted from Journal of AOAC International 91(2): 376-382	Adapted from Journal of AOAC International 91(2): 376-382	Adapted from Journal of AOAC International 91(2): 376-382	Adapted from Journal of AOAC International 91(2): 376-382	Adapted from Journal of AOAC International 91(2): 376-382
		Biological Activity	Enzyme activity[3]	Adapted from Analytical Biochemistry 378(1): 87-89	Adapted from Analytical Biochemistry 378(1): 87-89	Adapted from Analytical Biochemistry 378(1): 87-89	Adapted from Analytical Biochemistry 378(1): 87-89	Adapted from Analytical Biochemistry 378(1): 87-89
Shiga and Shiga-like Toxins (Stx, Stx-1, Stx-2)	75757-64-1 (Stx) / Protein composed of one ~32 kDa A chain and five 7.7 kDa B chains	Presumptive	Enzyme immunoassay (EIA)	Adapted from Journal of Clinical Microbiology 35(8): 2051-2054	Adapted from Journal of Clinical Microbiology 35(8): 2051-2054	Adapted from Journal of Clinical Microbiology 35(8): 2051-2054	Adapted from Journal of Clinical Microbiology 35(8): 2051-2054	Adapted from Journal of Clinical Microbiology 35(8): 2051-2054
		Confirmatory	Immunoassay (ELISA)	Adapted from FDA Bacteriological Analytical Manual, Appendix 1	Adapted from FDA Bacteriological Analytical Manual, Appendix 1	Adapted from FDA Bacteriological Analytical Manual, Appendix 1	Adapted from FDA Bacteriological Analytical Manual, Appendix 1	Adapted from FDA Bacteriological Analytical Manual, Appendix 1
		Biological Activity	Ribosome inactivation assay[3]	Adapted from Pharmacology & Toxicology 88(5): 255-260	Adapted from Pharmacology & Toxicology 88(5): 255-260	Adapted from Pharmacology & Toxicology 88(5): 255-260	Adapted from Pharmacology & Toxicology 88(5): 255-260	Adapted from Pharmacology & Toxicology 88(5): 255-260
Staphylococcal enterotoxins (SEB)	39424-53-8 (SEB) / Monomeric protein of ~ 28 kDa	Presumptive	Enzyme Immunoassay (EIA)	Adapted from 993.06 (AOAC)	**LRN** If analysis for this agent is required in solid, particulate, or liquid samples, contact the LRN at (404) 639-2790 for information of the closest LRN laboratory capable of receiving and processing the sample. The terms presumptive and confirmatory as used for LRN methods are described in Section 8.1.4.			
		Confirmatory	TBD	TBD	TBD	TBD	TBD	TBD
		Biological Activity	T-cell proliferation assay	Adapted from Applied and Environmental Microbiology 63(6): 2361-2365	Adapted from Applied and Environmental Microbiology 63(6): 2361-2365	Adapted from Applied and Environmental Microbiology 63(6): 2361-2365	Adapted from Applied and Environmental Microbiology 63(6): 2361-2365	Adapted from Applied and Environmental Microbiology 63(6): 2361-2365
Staphylococcal enterotoxins (SEA, SEC)	37337-57-8 (SEA) 39424-54-9 (SEC) / Monomeric proteins of ~ 27-27.5 kDa	Presumptive	Enzyme Immunoassay (EIA)	Adapted from 993.06 (AOAC)	Adapted from 993.06 (AOAC)	Adapted from 993.06 (AOAC)	Adapted from 993.06 (AOAC)	Adapted from 993.06 (AOAC)
		Confirmatory	TBD	TBD	TBD	TBD	TBD	TBD
		Biological Activity	T-cell proliferation assay	Adapted from Applied and Environmental Microbiology 63(6): 2361-2365	Adapted from Applied and Environmental Microbiology 63(6): 2361-2365	Adapted from Applied and Environmental Microbiology 63(6): 2361-2365	Adapted from Applied and Environmental Microbiology 63(6): 2361-2365	Adapted from Applied and Environmental Microbiology 63(6): 2361-2365

Analyte(s)	CAS RN / Description	Analysis Type[1]	Analytical Technique	Aerosol (filter/cassette, liquid impinger)	Solid (soil, powder)	Particulate (swabs, wipes, dust socks)	Liquid Water	Drinking Water
Small Molecule								
Aflatoxin (Type B1)	27261-02-5	Presumptive	Immunoassay (column)	Adapted from 991.31 (AOAC)	Adapted from 991.31 (AOAC)	Adapted from 991.31 (AOAC)	Adapted from 991.31 (AOAC)	Adapted from 991.31 (AOAC)
		Confirmatory	HPLC-FL	Adapted from 991.31 (AOAC)	Adapted from 991.31 (AOAC)	Adapted from 991.31 (AOAC)	Adapted from 991.31 (AOAC)	Adapted from 991.31 (AOAC)
α-Amanitin	23109-05-9	Presumptive	Immunoassay (ELISA)	Adapted from Journal of Food Protection 68(6): 1294-1301	Adapted from Journal of Food Protection 68(6): 1294-1301	Adapted from Journal of Food Protection 68(6): 1294-1301	Adapted from Journal of Food Protection 68(6): 1294-1301	Adapted from Journal of Food Protection 68(6): 1294-1301
		Confirmatory	HPLC amperometric detection	Adapted from Journal of Chromatography 563(2): 299-311	Adapted from Journal of Chromatography 563(2): 299-311	Adapted from Journal of Chromatography 563(2): 299-311	Adapted from Journal of Chromatography 563(2): 299-311	Adapted from Journal of Chromatography 563(2): 299-311
Anatoxin-a	64285-06-9	Presumptive	TBD	TBD	TBD	TBD	TBD	TBD
		Confirmatory	HPLC-FL (precolumn derivatization)	Adapted from Biomedical Chromatography B 10(1): 46-47	Adapted from Biomedical Chromatography B 10(1): 46-47	Adapted from Biomedical Chromatography B 10(1): 46-47	Adapted from Biomedical Chromatography B 10(1): 46-47	Adapted from Biomedical Chromatography B 10(1): 46-47
Brevetoxins (B form)	79580-28-2	Presumptive	Immunoassay (ELISA)	Adapted from Environmental Health Perspectives 110(2): 179-185	Adapted from Environmental Health Perspectives 110(2): 179-185	Adapted from Environmental Health Perspectives 110(2): 179-185	Adapted from Environmental Health Perspectives 110(2): 179-185	Adapted from Environmental Health Perspectives 110(2): 179-185
		Confirmatory	HPLC-MS-MS	Adapted from Toxicon 43(4): 455-465	Adapted from Toxicon 43(4): 455-465	Adapted from Toxicon 43(4): 455-465	Adapted from Toxicon 43(4): 455-465	Adapted from Toxicon 43(4): 455-465
α-Conotoxin	156467-85-5	Presumptive	Immunoassay (solution phase binding assay)	Adapted from Biochemical Journal 328(1): 245-250	Adapted from Biochemical Journal 328(1): 245-250	Adapted from Biochemical Journal 328(1): 245-250	Adapted from Biochemical Journal 328(1): 245-250	Adapted from Biochemical Journal 328(1): 245-250
		Confirmatory	HPLC-MS	Adapted from Journal of Medicinal Chemistry 47(5): 1234-1241	Adapted from Journal of Medicinal Chemistry 47(5): 1234-1241	Adapted from Journal of Medicinal Chemistry 47(5): 1234-1241	Adapted from Journal of Medicinal Chemistry 47(5): 1234-1241	Adapted from Journal of Medicinal Chemistry 47(5): 1234-1241
Cylindrospermopsin	143545-90-8	Presumptive	Immunoassay (ELISA)	Adapted from ELISA kits for Cylindrospermopsin	Adapted from ELISA kits for Cylindrospermopsin	Adapted from ELISA kits for Cylindrospermopsin	Adapted from ELISA kits for Cylindrospermopsin	Adapted from ELISA kits for Cylindrospermopsin
		Confirmatory	HPLC-PDA	Adapted from FEMS Microbiology Letters 216(2): 159-164	Adapted from FEMS Microbiology Letters 216(2): 159-164	Adapted from FEMS Microbiology Letters 216(2): 159-164	Adapted from FEMS Microbiology Letters 216(2): 159-164	Adapted from FEMS Microbiology Letters 216(2): 159-164
Diacetoxyscirpenol (DAS)	2270-40-8	Presumptive	Immunoassay (ELISA)	Adapted from International Journal of Food Microbiology 6(1): 9-17	Adapted from International Journal of Food Microbiology 6(1): 9-17	Adapted from International Journal of Food Microbiology 6(1): 9-17	Adapted from International Journal of Food Microbiology 6(1): 9-17	Adapted from International Journal of Food Microbiology 6(1): 9-17
		Confirmatory	LC/APCI-MS	Adapted from Rapid Communications in Mass Spectrometry 20(9): 1422-1428	Adapted from Rapid Communications in Mass Spectrometry 20(9): 1422-1428	Adapted from Rapid Communications in Mass Spectrometry 20(9): 1422-1428	Adapted from Rapid Communications in Mass Spectrometry 20(9): 1422-1428	Adapted from Rapid Communications in Mass Spectrometry 20(9): 1422-1428

Analyte(s)	CAS RN / Description	Analysis Type[1]	Analytical Technique	Aerosol (filter/cassette, liquid impinger)	Solid (soil, powder)	Particulate (swabs, wipes, dust socks)	Liquid Water	Drinking Water
Microcystins Principal isoforms: LA, LR, LW, RR, YR	96180-79-9 (LA) 101043-37-2 (LR) 157622-02-1 (LW) 111755-37-4 (RR) 101064-48-6 (YR)	Presumptive	Immunoassay (ELISA)/ Phosphatase assay	Adapted from Journal of AOAC International 84(4): 1035-1044	Adapted from Journal of AOAC International 84(4): 1035-1044	Adapted from Journal of AOAC International 84(4): 1035-1044	Adapted from Journal of AOAC International 84(4): 1035-1044	Adapted from Journal of AOAC International 84(4): 1035-1044
		Confirmatory	HPLC-PDA	Adapted from Analyst 119(7): 1525-1530	Adapted from Analyst 119(7): 1525-1530	Adapted from Analyst 119(7): 1525-1530	Adapted from Analyst 119(7): 1525-1530	Adapted from Analyst 119(7): 1525-1530
Picrotoxin	124-87-8	Presumptive	Immunoassay	TBD	TBD	TBD	TBD	TBD
		Confirmatory	HPLC	Adapted from Journal of Pharmaceutical and Biomedical Analysis 7(3): 369-375	Adapted from Journal of Pharmaceutical and Biomedical Analysis 7(3): 369-375	Adapted from Journal of Pharmaceutical and Biomedical Analysis 7(3): 369-375	Adapted from Journal of Pharmaceutical and Biomedical Analysis 7(3): 369-375	Adapted from Journal of Pharmaceutical and Biomedical Analysis 7(3): 369-375
Saxitoxins Principal isoforms: Saxitoxin (STX) Neosaxitoxin (NEOSTX) Gonyautoxin (GTX) Decarbamoylgonyautoxin (dcGTX) Decarbamoylsaxitoxin (dcSTX)	35523-89-8 (STX) 64296-20-4 (NEOSTX) 77462-64-7 (GTX) None given (dcGTX) 58911-04-9 (dcSTX)	Presumptive	Immunoassay (ELISA)	Adapted from ELISA kits for Saxitoxins	Adapted from ELISA kits for Saxitoxins	Adapted from ELISA kits for Saxitoxins	Adapted from ELISA kits for Saxitoxins	Adapted from ELISA kits for Saxitoxins
		Confirmatory	HPLC-FL (post column derivatization)	Adapted from Journal of AOAC International 78(2): 528-532	Adapted from Journal of AOAC International 78(2): 528-532	Adapted from Journal of AOAC International 78(2): 528-532	Adapted from Journal of AOAC International 78(2): 528-532	Adapted from Journal of AOAC International 78(2): 528-532
T-2 Mycotoxin	21259-20-1	Presumptive	Immunoassay (ELISA)	Adapted from Journal of Food Protection 68(6): 1294-1301	Adapted from Journal of Food Protection 68(6): 1294-1301	Adapted from Journal of Food Protection 68(6): 1294-1301	Adapted from Journal of Food Protection 68(6): 1294-1301	Adapted from Journal of Food Protection 68(6): 1294-1301
		Confirmatory	LC/APCI-MS	Adapted from Rapid Communications in Mass Spectrometry 20(9): 1422-1428	Adapted from Rapid Communications in Mass Spectrometry 20(9): 1422-1428	Adapted from Rapid Communications in Mass Spectrometry 20(9): 1422-1428	Adapted from Rapid Communications in Mass Spectrometry 20(9): 1422-1428	Adapted from Rapid Communications in Mass Spectrometry 20(9): 1422-1428
Tetrodotoxin	9014-39-5	Presumptive	Immunoassay (CIEIA)	Adapted from Journal of Clinical Laboratory Analysis 6(2): 65-72	Adapted from Journal of Clinical Laboratory Analysis 6(2): 65-72	Adapted from Journal of Clinical Laboratory Analysis 6(2): 65-72	Adapted from Journal of Clinical Laboratory Analysis 6(2): 65-72	Adapted from Journal of Clinical Laboratory Analysis 6(2): 65-72
		Confirmatory	LC/ESI-MS	Adapted from Analytical Biochemistry 290(1): 10-17	Adapted from Analytical Biochemistry 290(1): 10-17	Adapted from Analytical Biochemistry 290(1): 10-17	Adapted from Analytical Biochemistry 290(1): 10-17	Adapted from Analytical Biochemistry 290(1): 10-17

[1] Descriptions for presumptive, confirmatory and biological activity assays are provided in Section 8.0.

[2] Crude preparations of ricin and abrin may also contain agglutinins that are unique to castor beans and rosary peas, respectively, and that can cross-react in the immunoassays.

[3] This assay does not test for cell binding; cell culture assays are being developed to test for cell binding but are not currently available. The only readily available assay to test for both the cell binding and enzymatic activity of the intact (whole) toxin is a mouse bioassay.

[4] Immunoassays may produce variable results with uncomplexed form of toxin.

Attachment 1:
SAM Revisions and Supporting Documents

SAM is updated periodically to incorporate revisions to the list of target analytes and environmental sample types, and to provide the most recent analytical methods and procedures. The table below provides information regarding additional changes that were incorporated into each revision of SAM, since publication of SAM Revision 1.0 in September 2004.

SAM Revisions Tracking Table		
SAM Revision	**Publication Date**	**Changes incorporated summary**
SAM Revision 1.0	September 2004	*Standardized Analytical Methods for Use During Homeland Security Events* Included chemical and biological contaminants
SAM Revision 2.0	September 2005	Added: • Radiochemicals • Several persistent CWA degradation products • Separate drinking water sample type for chemicals and radiochemicals • Viability determination methods for pathogens • Separate section for biotoxins
SAM Revision 3.0	February 2007	Added explosive chemicals Combined identification and viability methods for pathogens Added drinking water sample type for pathogens Title changed to: *Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events (SAM)*
SAM Revision 3.1	November 2007	Developed a SAM website, to provide the SAM document and a format for searching and linking to SAM methods by analyte and sample type.
SAM Revision 4.0	September 2008	Added: • Wipe samples for chemistry analytes • Added PCR methods for pathogens
SAM Revision 5.0	September 2009	Added separate drinking water sample type to biotoxins
SAM 2010 (Revision 6.0)	October 2010	• Removed non-aqueous liquid sample type from chemistry • Temporary removal of pathogens
SAM 2012	July 2012	• Changed title to: *Selected Analytical Methods for Environmental Remediation and Recovery* (SAM) – 2012 • Added vegetation sample type, newly available rapid methods, and total activity screening procedure for radiochemistry analytes • Re-introduced pathogen methods with restructuring to clarify method applications for site characterization and post remediation • Assigned applicability tiers to chemistry methods to indicate the extent of data available that support use of each method for analyte/sample type pairs

The following documents and tools have been developed by EPA to provide information regarding a contamination event. The information included in the documents is intended to be complementary to information provided in the analytical methods listed in SAM. As additional documents containing similar complementary information become available, they will be added to the list contained in this Attachment.

- Searchable SAM Web site at: www.epa.gov/sam/

- "Guidelines for Development of Sample Collection Plans for Radiochemical Analytes in Environmental Matrices Following Homeland Security Events," EPA/600/R-08/128, February 2009. http://www2.epa.gov/sites/production/files/2015-07/documents/guide_for_developing_sample_collection_plans_for_radiochemical_analytes.pdf

- "Sample Collection Procedures for Radiochemistry Analytes in Environmental Matrices," EPA/600/R-12/566, July 2012. http://www2.epa.gov/sites/production/files/2015-07/documents/sample_collection_procedures_for_radiochemical_analytes.pdf

- "Sample Collection Information Document for Pathogens and Biotoxins – Companion to SAM Revision 5.0," EPA/600/R-09/074, June 2010. http://www2.epa.gov/sites/production/files/2015-07/documents/scid_pathogens_and_biotoxins.pdf

- "Sample Collection Information Document for Chemical and Radiochemical Analytes – Companion to SAM 2012," EPA/600/R-14/215, September 2014. http://www.epa.gov/sam/pdfs/SCID_Chemical_and_Radiochemicals.pdf

- "Rapid Screening and Preliminary Identification Techniques and Methods – Companion to SAM Revision 5.0," EPA/600/R-10/090, September 2010. http://www2.epa.gov/sites/production/files/2015-07/documents/rapid_screening_and_preid.pdf

- "Laboratory Environmental Sample Disposal Information Document – Companion to SAM Revision 5.0," EPA/600/R-10/092, September 2010. http://www2.epa.gov/sites/production/files/2015-06/documents/lesdid.pdf