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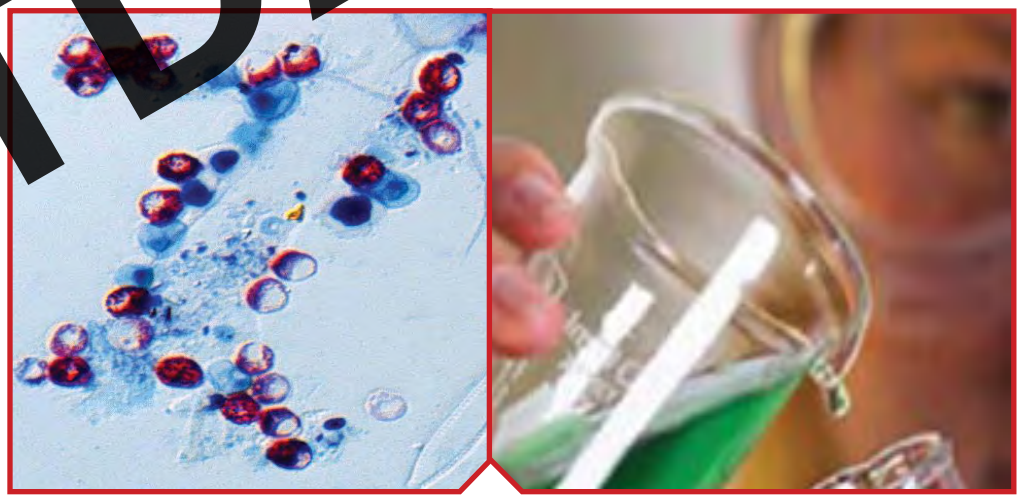
Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events

REVISION 4.0



SCIENCE

Office of Research and Development
National Homeland Security Research Center, Response Capability Enhancement

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Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events – Revision 4.0

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Disclaimer

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Use of This Document

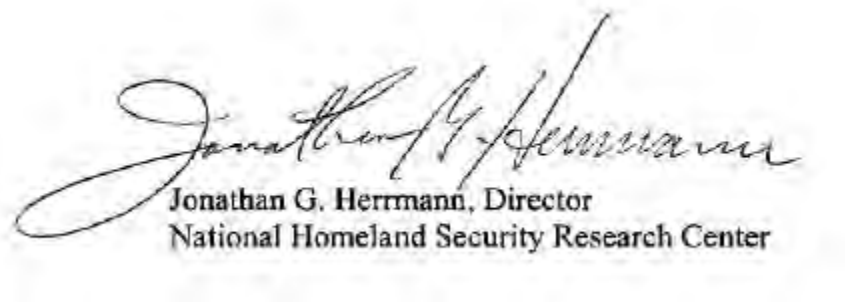
The information contained in this document represents the latest step in an ongoing effort of the Environmental Protection Agency's (EPA's) National Homeland Security Research Center (NHSRC) to provide standardized analytical methods for use by those laboratories tasked with performing confirmatory analyses of environmental samples in support of EPA restoration efforts following a homeland security incident. Although at this time, some of the methods listed have not been fully validated for a particular analyte (e.g., analytes not explicitly identified in the method) or sample type, the methods are considered to contain the most appropriate currently available techniques. 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 testing is needed, and adjustments may be required to accurately account for variations in analyte/sample type characteristics, environmental samples, and target risk levels. Many of the target analytes listed in this document have only recently become an environmental concern. EPA is actively pursuing development and validation of Standardized Analytical Protocols (SAPs) based on the methods listed, including optimization of procedures for measuring target analytes or agents. In those cases where method procedures are determined to be insufficient for a particular situation, EPA will provide guidance regarding appropriate actions. This will be an ongoing process as EPA will strive to establish a consistent level of validation for all listed analytes.

Foreword

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

The National Homeland Security Research Center (NHSRC) is EPA's organization for conducting research to facilitate protection and decontamination of indoor and outdoor areas and water infrastructure subject to chemical, biological, or radiological (CBR) terror attacks. NHSRC's research is designed to provide appropriate, effective, and validated technologies, methods, and guidance to understand the risks posed by CBR agents and to enhance our ability to detect, contain, and clean up in the event of an incident involving such agents. This document is intended to provide guidance for selecting methods that have a high likelihood of ensuring analytical consistency when laboratories are faced with a large scale environmental restoration crisis. At the same time, the document can be used as a tool to identify analytes that require further methods development and verification to ensure desired performance. NHSRC will also provide direct technical assistance to response personnel in the event of a CBR attack, as well as provide related interagency liaisons. This is most effectively accomplished by contacting EPA's Emergency Operations Center.

This publication has been produced as part of the NHSRC's long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.



Jonathan G. Herrmann, Director
National Homeland Security Research Center

Abbreviations and Acronyms

AA	Atomic Absorption
AdV40	Adenovirus 40
AdV41	Adenovirus 41
AEM	*Applied and Environmental Microbiology*
Amp-ELISA	Amplified-Enzyme-Linked Immunosorbent Assay
APHA	American Public Health Association
APHL	Association of Public Health Laboratories
AOAC	AOAC International (formerly the Association of Official Analytical Chemists)
ASE	Automated Soxhlet Extraction
ASM	American Society for Microbiology
ASTM	ASTM International (formerly the American Society for Testing and Materials)
ATP	2-aminothiophenol
AWWA	American Water Works Association
BCM	Biosynth Chromogen Medium
BCYE	Buffered Charcoal Yeast Extract
BGMK	Buffalo Green Monkey Kidney
BHT	Butylated hydroxytoluene
BS	Bismuth Sulfite
BLEB	Buffered *Listeria* Enrichment Broth
BMBL	Biosafety in Microbiological and Biomedical Laboratories
BSA	Bovine Serum Albumin
BSL	Biosafety Level
BZ	Quinuclidinyl benzilate
°C	Degrees Celsius
CA	Chocolate Agar
CAS RN	Chemical Abstracts Service Registry Number
CBR	Chemical, Biological, or Radiological
CCID	Coordinating Center for Infectious Diseases
CDC	Centers for Disease Control and Prevention
cDNA	Complementary Deoxyribonucleic Acid
CFR	Code of Federal Regulations
CFSAN	Center for Food Safety and Applied Nutrition
CFU	Colony Forming Unit
CIEIA	Competitive Inhibition Enzyme Immunoassay
CLLE	Continuous Liquid-Liquid Extraction
CLP	Contract Laboratory Program
CPE	Cytopathic Effect
cps	counts per second
CS	Tear gas; Chlorobenzylidene malonitrile
CVAA	Cold Vapor Atomic Absorption or 2-Chlorovinylarsonous acid
CVAFS	Cold Vapor Atomic Fluorescence Spectrometry
2,4-D	2,4-Dichlorophenoxyacetic Acid
DAPI	4',6-diamidino-2-phenylindole
DAS	Diacetoxyscipenol
DAS-HG-HSA	Diacetoxyscipenol Hemiglutarate Human Serum Albumin
DAS-HS-HRP	Diacetoxyscipenol Hemisuccinate Horseradish Peroxidase Conjugate
DB-1	100% Dimethylpolysiloxane
DBPR	Division of Bioterrorism Preparedness and Response
DHS	Department of Homeland Security
DIC	Differential Interference Contrast
DIG-ELISA	Digoxigenin Labeled Enzyme-Linked Immunosorbent Assay

DIMP	Diisopropyl methylphosphonate
DMT	3,4-Dimercaptotoluene
DNA	Deoxyribonucleic Acid
2,4-DNPH	2,4-Dinitrophenylhydrazine
DoD	Department of Defense
DOE	Department of Energy
DOT	Department of Transportation
DPD	N,N-diethyl-*p*-phenylenediamine
DTPA	Diethylenetriamine-pentaacetate
EA2192	Diisopropylaminoethyl methylthiophosphonate
ECD	Electron Capture Detector
e-CFR	Electronic Code of Federal Regulations
ECL	Electrochemiluminescence
ED	Electron Diffraction or Ethyldichloroarsine
EDEA	N-Ethyldiethanolamine
EDL	Estimated Detection Limit
EDTA	Ethylenediaminetetraacetic acid
EDXA	Energy Dispersive X-ray Analysis
EEB	Enterohemorrhagic *E. coli* Enrichment Broth
EIA	Enzyme Immunoassay
ELCD	Electrolytic Conductivity Detector
ELISA	Enzyme-Linked Immunosorbent Assay
EMC	Emission Measurement Center
EMJH	Ellinghausen-McCullough Johnson Harris Formulation
EML	Environmental Measurements Laboratory
EMMI	Environmental Monitoring Methods Index
EMPA	Ethyl methylphosphonic acid
EMSL	Environmental Monitoring and Support Laboratory
EPA	Environmental Protection Agency
EQL	Estimated Quantitation Limit
ESI	Electrospray Ionization
ETV	Environmental Technology Verification
FA	Fluorescence Assay
FBI	Federal Bureau of Investigation
FDA	Food and Drug Administration
FEMS	Federation of European Microbiological Societies
FGI	Fluorescein derivative of *Conus geographus* α-conotoxin
FID	Flame Ionization Detector
FL	Fluorescence Detector
FPD	Flame Photometric Detector
FRET	Fluorescence Resonance Energy Transfer
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-MD	Gas Chromatography – Multi-Detector
GC-NPD	Gas Chromatography – Nitrogen-phosphorus Detector
GD	Soman

GE	1-Methylethyl ester ethylphosphonofluoridic acid or Genome Equivalent
Ge(Li)	Germanium (Lithium)
GESTIS	A German database (Gefahrstoffdaten banken) containing data and information on hazardous substances and products
GF	Cyclohexyl sarin
GFAA	Graphite Furnace Atomic Absorption Spectrophotometer or Graphite Furnace Atomic Absorption Spectrophotometry
GTC	Guanidinium Thiocyanate
HAdV	Human Adenoviruses
HASL	Health and Safety Laboratory, currently known as Environmental Measurements Laboratory (EML)
HAV	Hepatitis A Virus
HCoV	Human Coronavirus
HEV	Hepatitis E Virus
HEPA	High Efficiency Particulate Air (Filter)
HD	Sulfur mustard / mustard gas; bis(2-chloroethyl) sulfide
HHS	Health and Human Services
HMTD	Hexamethylenetriperoxidediamine
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
HN-1	Nitrogen mustard 1; bis(2-chloroethyl)ethylamine
HN-2	Nitrogen mustard 2; 2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine
HN-3	Nitrogen mustard 3; tris(2-chloroethyl)amine
HPGe	High Purity Germanium
HPLC	High Performance Liquid Chromatography
HPLC-FL	High Performance Liquid Chromatograph – Fluorescence
HPLC-MS	High Performance Liquid Chromatograph – Mass Spectrometer
HPLC-PDA	High Performance Liquid Chromatography – Photodiode Array Detector
HPLC-UV	High Performance Liquid Chromatography – Ultraviolet
HPLC-vis	High Performance Liquid Chromatography – visible
HRP	Horseradish Peroxidase
HV	High Volume
IC	Ion Chromatograph or Ion Chromatography
IC 20	Inhibitory Concentration – Concentration to inhibit 20%
IC 50	Inhibitory Concentration – Concentration to inhibit 50%
ICP	Inductively Coupled Plasma
ICP-AES	Inductively Coupled Plasma – Atomic Emission Spectrometry
ICP-MS	Inductively Coupled Plasma – Mass Spectrometry
ICR	Information Collection Requirements Rule or Information Collection Request
ID50	A dose which would be infectious to 50% of the population
IDL	Instrument Detection Limit
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 (U.S. EPA)
ISE	Ion Specific Electrode

ISG	Impregnated Silica Gel
ISO	International Organization for Standardization
L-1	Lewisite 1; 2-Chlorovinyldichloroarsine
L-2	Lewisite 2; bis(2-Chlorovinyl)chloroarsine
L-3	Lewisite 3; tris(2-Chlorovinyl)arsine
LB-M	Lim Benyesh-Melnick
LC	Liquid Chromatograph
LC/APCI-MS	Liquid Chromatography / Atmospheric Pressure Chemical Ionization-Mass Spectrometry
LC/ESI-MS	Liquid Chromatography / Electrospray Ionization-Mass Spectrometry
LC-MS	Liquid Chromatography – Mass Spectrometry
LC-MS-MS	Liquid Chromatography Tandem Mass Spectrometry
LC-TSP	Liquid Chromatography – Thermospray
LFD	Lateral Flow Device
LIA	Lysine Iron Agar
LLD	Lower Limit of Detection
LOD	Limit of Detection
LRN	Laboratory Response Network
LSE	Liquid-solid Extraction
Ltd.	A private company limited by shares
mAb	Monoclonal Antibody
MARLAP	Multi-Agency Radiological Laboratory Analytical Protocols
MCAWW	Methods for Chemical Analysis of Water and Waste (EPA/600/4-79/020)
MDL	Method Detection Limit
MF	Membrane Filtration
MIC	Methyl Isocyanate
MLD	Minimum Lethal Dose
MOPS	Morpholinepropanesulfonic Acid
MPA	Methylphosphonic acid
MPN	Most Probable Number
mRNA	Messenger Ribonucleic Acid
MS	Mass Spectrometer or Mass Spectrometry or Matrix Spike
MS-MS	Tandem Mass Spectometry
MSD	Matrix Spike Duplicate
MSE	Microscale Solvent Extraction
MSRV	Modified Semisolid Rappaport-Vassiliadis
MTBE	Methyl *tert*-butyl ether
MW	Molecular Weight
NA	Not Applicable
nAchR	Nicotinic Acetylcholine Receptor
NaI(Tl)	Thallium-Activated Sodium Iodide
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
NCTC	National Collection of Type Cultures
NEMI	National Environmental Methods Index
NERL	National Exposure Research Laboratory
NHSRC	National Homeland Security Research Center
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

NoV	Norovirus
NOS	Not Otherwise Specified
NPD	Nitrogen-phosphorus Detector
NRC	Nuclear Regulatory Commission
NRMRL	National Risk Management Research Laboratory
nS	nano Siemens
NTIS	National Technical Information Service
NTU	Nephelometric Turbidity Units
OAQPS	U.S. EPA Office of Air Quality Planning and Standards
OAR	U.S. EPA Office of Air and Radiation
ORAU	Oak Ridge Associated Universities
ORISE	Oak Ridge Institute for Science and Education
ORD	U.S. EPA Office of Research and Development
ORF	Open Reading Frame
OSWER	U.S. EPA Office of Solid Waste and Emergency Response
OSHA	Occupational Safety and Health Administration
OVS	OSHA Versatile Sampler
OW	U.S. EPA Office of Water
OXA	Oxford Medium
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
PFE	Pressurized Fluid Extraction
PFIB	Perfluoroisobutylene
PFIB-ATP	PFIB with 2-aminothiophenol
PFIB-DMT	PFIB with 3,4-Dimercaptotoluene
PID50	50% Pig Infectious Dose
PMPA	Pinacolyl methyl phosphonic acid
1,2-PP	1-(2-pyridyl)piperazine
PubMED	PubMED is a service of the U.S. National Library of Medicine (www.pubmed.gov), containing citations from scientific journals
PUF	Polyurethane Foam
PVC	Polyvinyl Chloride
QA	Quality Assurance
QAP	Quality Assessment Program
QC	Quality Control
qPCR	Quantitative Polymerase Chain Reaction
®	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
RLAB	Regional Laboratory
RNA	Ribonucleic Acid
rpm	Revolutions per Minute
rRNA	Ribosomal Ribonucleic Acid
RTECS	Registry of Toxic Effects of Chemical Substances
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAED	Select Area Electron Diffraction

SAM	Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events
SAP	Standardized Analytical Protocol
SARS	Severe Acute Respiratory Syndrome
SaV	Sapovirus
SBA	Sheep Blood Agar
SIM	Selective Ion Monitoring
SM	*Standard Methods for the Examination of Water and Wastewater*
SPE	Solid-Phase Extraction
spp.	Species (plural)
STEC	Shiga-toxigenic *E. coli*
STEL	Short Term Exposure Limit
STX	Saxitoxin
Stx	Shiga Toxin
Stx-1	Shiga Toxin Type 1
Stx-2	Shiga Toxin Type 2
SW	Solid Waste
TATP	Triacetone triperoxide
TBD	To Be Determined
TCBS	Thiosulfate-Citrate-Bile Salts-Sucrose
TC-SMAC	Tellurite Cefixime Sorbitol MacConkey Agar
TCLP	Toxicity Characteristic Leaching Procedure
TDG	Thiodiglycol
TEA	Triethanolamine
TEM	Transmission Electron Microscope or Microscopy
TETR	Touchdown Enzyme Time Release
TFA	Trifluoroacetic acid
™	Trademark
TM	Test Methods or Thayer-Martin
1,3,5-TNB	1,3,5-Trinitrobenzene
2,4,6-TNT	2,4,6-Trinitrotoluene
TO	Toxic Organic
TOFMS	Time-of-Flight Mass Spectrometry
TOXNET	Toxicology Data Network
TRU	Transuranic
TSB	Tryptic Soy Broth
TSAye	Trypticase™ Soy Agar with yeast extract
TSI	Triple Sugar Iron
TSP	Thermospray
TSP-MS	Thermopray – Mass Spectrometry
TTN	Technical Transfer Network
TTX	Tetrodotoxin
UF	Ultrafiltration
U.S.	United States
USDA	United States Department of Agriculture
USGS	United States Geological Survey
UV	Ultraviolet
UVM	University of Vermont
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

VOCs	Volatile Organic Compounds
VR	Methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester (R 33)
VX	O-ethyl-S-(2-diisopropylaminoethyl)methylphosphonothiolate
WEF	Water Environment Federation
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate

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Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events

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Section 1.0: Introduction

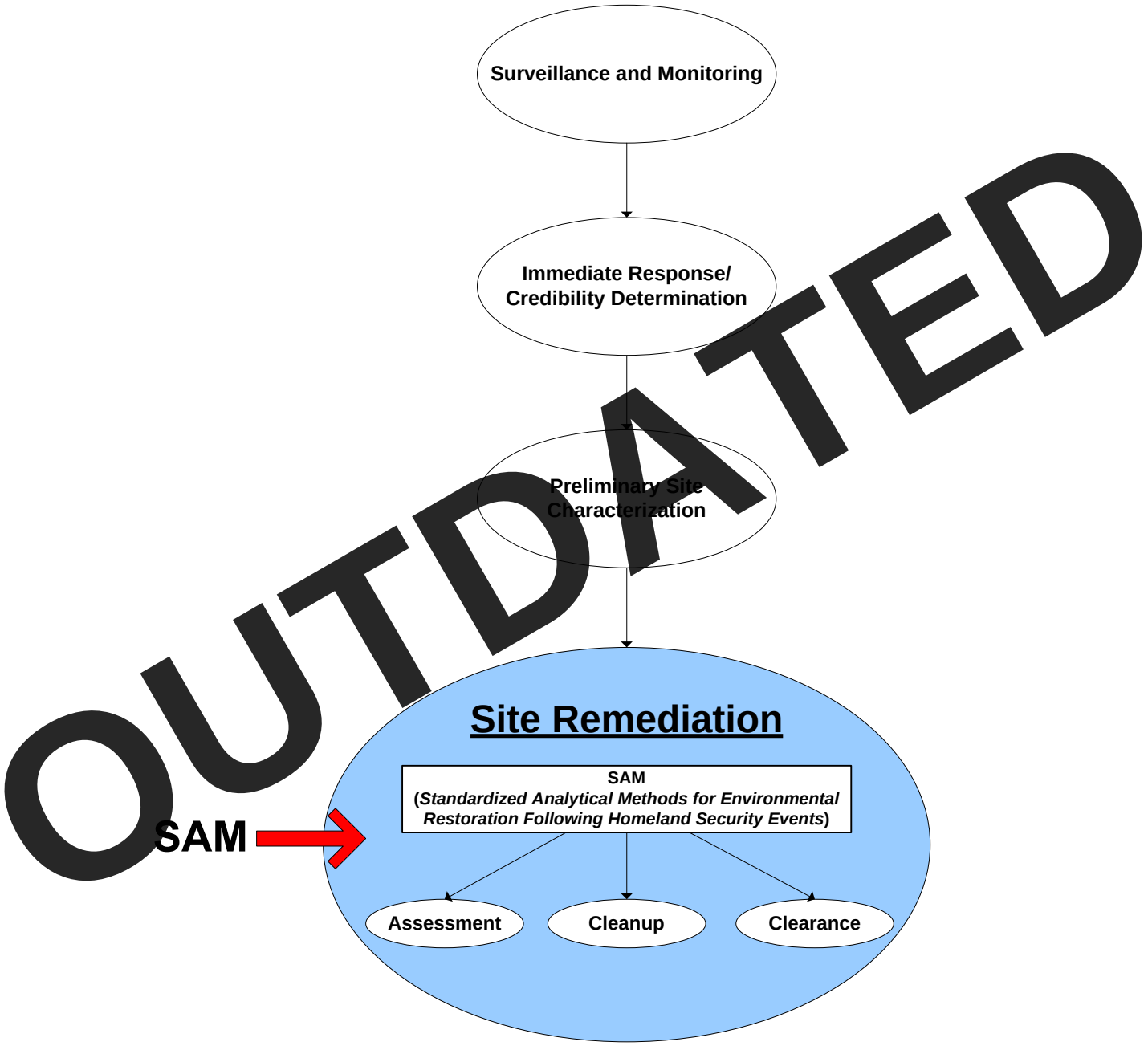
After the terrorist attacks of September 11, 2001 and the anthrax attacks in the fall of 2001, federal and state personnel provided response, recovery, and remediation under trying circumstances, including unprecedented demand on their capabilities to analyze environmental samples. In reviewing these events, the Environmental Protection Agency (EPA) identified several areas where the country could better prepare itself in the event of future terrorist incidents. The need to improve the nation's laboratory capacity and capability to analyze environmental samples following a homeland security event was one of the most important areas identified.

In response, EPA formed the Homeland Security Laboratory Capacity Workgroup 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 Workgroup consists of representatives from the 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 Pollution Prevention and Toxics, and several EPA regional offices.

A critical area identified by the workgroup was the need for a list of analytical methods to be used by all laboratories when analyzing homeland security event samples and, in particular, when analysis of many samples is required over a short period of time. Having standardized 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. Standardized methods would also improve the follow-up activities of validating results, evaluating data, and making decisions. To this end, workgroup 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 *Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events* (SAM) provides information for analytical methods to be applied during the "Site Remediation" phase.

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 and other federal agencies to review methods and, if necessary, revise the methods listed. 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. For analytes where limited laboratory testing/experience exists, such as chemical warfare agents, methods were selected based on their applicability to similar chemicals (e.g., nerve agents and some pesticides). In these cases, laboratory studies to test the ability of the selected method to measure the target analyte(s) are either underway or planned. **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, EPA's National Homeland Security Research Center (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 future 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), and U.S. Geological Survey (USGS). Methodologies were considered for chemical and biological agents of concern in the types of environmental samples that would be anticipated. The primary objective of this effort was to identify appropriate SAM Analytical Methods Subteam consensus methods that represent a balance between providing existing, documented, determinative techniques and providing consistent and valid analytical results.

A survey of available confirmatory analytical methods for approximately 120 biological and chemical analytes was conducted using existing resources including the following:

- National Environmental Methods Index (NEMI) and NEMI-Chemical, Biological, and Radiological (NEMI-CBR)
- Environmental Monitoring Method Index (EMMI)
- EPA Test Methods Index
- EPA Office of Solid Waste SW-846 Methods
- EPA Microbiology 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*
- PubMED Literature Database

In September 2004, EPA published *Standardized Analytical Methods for Use During Homeland Security Events, Revision 1.0* (SAM, Revision 1.0, EPA/600/R-04/126), which provided a list of analytical and

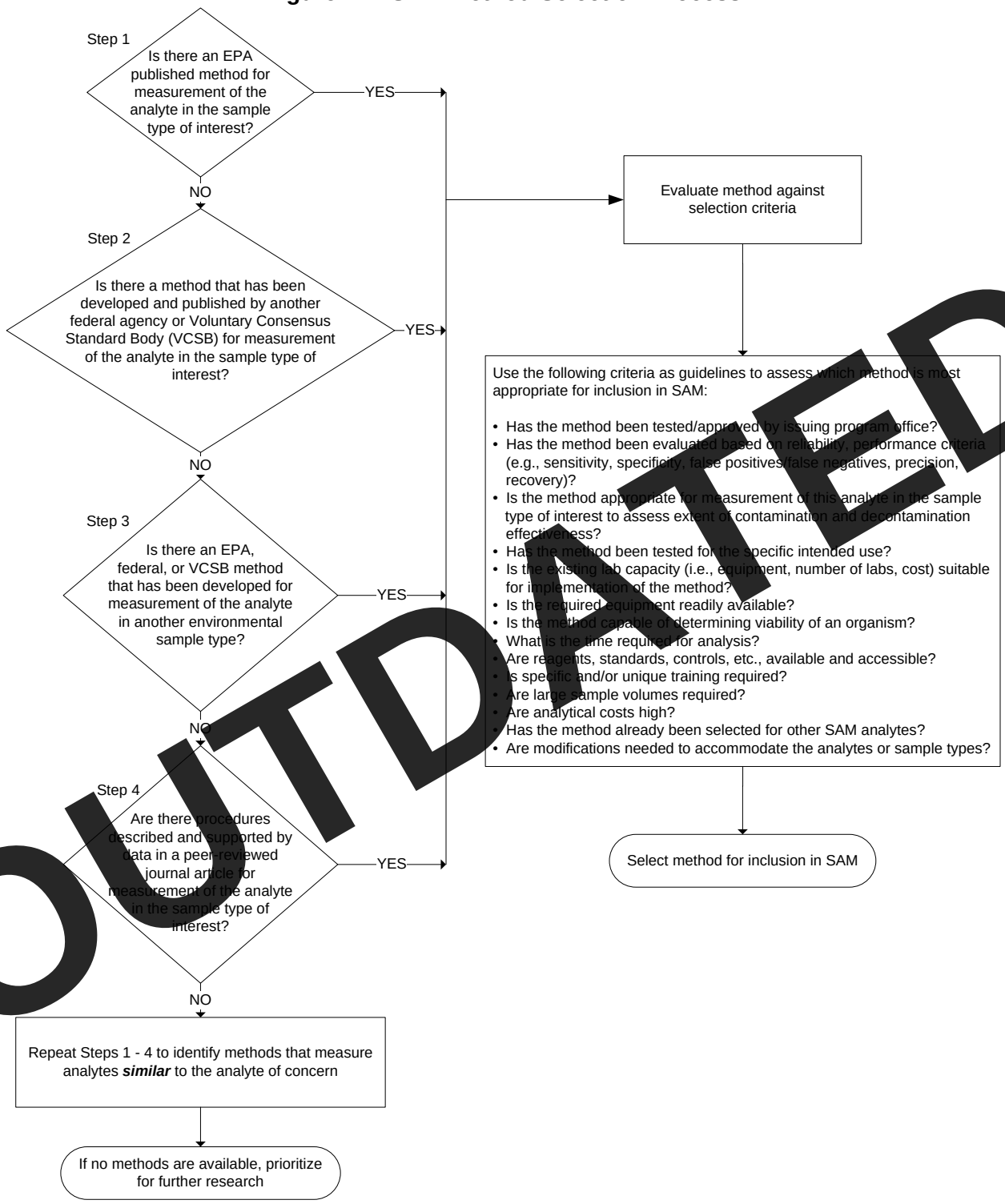
sample preparation methods that were selected for measurement of 82 chemical analytes in aqueous/liquid, solid, oily solid, and air samples, and 27 biological analytes in water, dust, and aerosol samples. During 2005, SAM was expanded to include radioisotopes, several persistent chemical warfare agent degradation products, a drinking water sample type, methods for determination of the viability of biological organisms, and a separate section for biotoxin analytes. Where necessary, the methods included in SAM Revision 1.0 were updated to reflect more recent or appropriate methodologies. Similar efforts to those used for method selection during development of SAM Revision 1.0 were undertaken to select and include methods for measurement of radioisotopes and chemical warfare agent degradation products in all sample types, for measurement of CBR analytes in drinking water, and to determine the viability of biological organisms. These additional analytes and the corresponding methods selected were included in SAM Revision 2.0.

During 2006, SAM was revised further to incorporate analytes included on updated federal agency lists, provide additional or more current method listings for target analytes, incorporate explosives into the chemical analytes listing, combine identification and viability methods information for pathogens, and address comments from EPA Science Advisory Board's Homeland Security Advisory Committee[1] to clarify the intended use of the document. These changes were included in SAM Revision 3.0 (*Standardized Analytical Methods for Environmental Restoration Following Homeland Security Events*, February 2007 / EPA/600/R-07/015). SAM Revision 3.0 included a new title to emphasize the intended use of SAM methods for analysis during environmental restoration activities. Following publication of SAM Revision 3.0, SAM workgroups updated the document to include the addition of several chemical analytes, one radionuclide, and one biotoxin, along with corresponding selected methods, and provided the updated documents as SAM Revision 3.1 (November 2007 / EPA/600/R-07/136). In 2007, NHSRC also developed a Web-based version of the SAM document to allow users and other stakeholders to search for specific needs and to submit questions and comments regarding the information.

NHSRC plans to continue convening SAM Technical Workgroups at least once per year, to evaluate and, if necessary, update the analytes and methods that are listed. This version, SAM Revision 4.0, reflects the addition of several chemical and radiochemical analytes as well as the addition of a wipe sample type for chemical analytes and several polymerase chain reaction (PCR) methods for pathogens.

[1]EPA Science Advisory Board's Homeland Security Advisory Committee: http://yosemite.epa.gov/sab/sabpeople.nsf/WebCommittees/BOARD

Figure 2-1. SAM Method Selection Process



Section 3.0: Scope and Application

The premise and purpose of this document is to standardize the analytical methods that will be used in cases when multiple laboratories are called on to analyze environmental samples following a homeland security event. The document also is intended as a tool that will be available to assist state and local laboratories in planning for and analyzing environmental samples following a homeland security event. 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), and
- Confirm effectiveness of decontamination in support of site clearance decisions.

The methods provided are limited to ones 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 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 or should be analyzed in specially designed, highly secure facilities. This document also is not intended to provide information regarding sample collection activities or equipment. Methods for addressing these needs are and will be the subject of other efforts.

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 in numerical order by the developing agency, throughout Sections 5.2 (chemical methods), 6.2 (radiochemical methods), 7.2 (pathogen methods), and 8.2 (biotoxin methods).

It is important to note that, in some cases, the methods included in this document have not been fully validated for the analyte/sample type combination(s) for which they have been selected. The information contained in this document represents the latest step in an ongoing effort by EPA's NHSRC to provide standardized analytical methods for use by those laboratories tasked with performing confirmatory analyses on environmental samples in support of EPA restoration efforts following a homeland security incident. Although at this time, some of the methods listed have not been fully validated for a particular analyte (e.g., analytes not explicitly identified in the method) or sample type, the methods are considered to contain the most appropriate currently available techniques. Unless a published method listed in this document states specific applicability to the analyte/sample type combination for which it has been selected, it should be assumed that method testing is needed, and adjustments may be required to accurately account for variations in analyte characteristics, environmental samples, and target risk levels. Many of the target analytes listed in this document have only recently become an environmental concern. EPA is actively pursuing development and validation of Standardized Analytical Protocols (SAPs) based on the methods listed, including optimization of procedures for measuring target analytes or agents. In those cases where method procedures are determined to be insufficient for a particular situation, EPA will provide guidance regarding appropriate actions. This will be an ongoing process as EPA will strive to establish a consistent level of validation for all listed analytes.

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 guidance regarding appropriate actions (see list of contacts in Section 4). Where further development and testing are necessary, EPA is developing and validating SAPs based on the methods that are listed in this document. Once validation is complete, data regarding the resulting method performance and data quality objectives will be available. The SAM document and corresponding SAPs will be reviewed frequently. EPA plans to continue to update the SAM document 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 guidance regarding issues that currently are not addressed by this document. Any deviations from the methods referenced in this document should be coordinated with the appropriate point(s) of contact identified in Section 4.

Participants in the chemical, radiochemical, pathogen, and biotoxin work groups, including representatives from the U.S. EPA, CDC, FDA, DHS, FBI, DoD, USDA, and USGS evaluated the suitability of existing methodologies and selected this set of methods for use by those laboratories that support EPA environmental restoration efforts in an emergency. EPA recognizes that this advanced selection of such methods may pose potential risks, including the following:

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

To address these potential risks as soon as possible, EPA plans to take several steps. These include the following:

- Developing and specifying measurement quality objectives for all analyte/sample type combinations listed in this document. This includes required minimum standards of accuracy (bias and precision) and sensitivity for the analysis of samples that support the data quality needs of the particular stage of the emergency response/recovery process);
- Specifying guidance for ensuring the analytical methods listed provide results that are consistent with and support their intended use as indicated in SAM;
- Working with other government agencies and the private sector to establish a laboratory network to ensure that laboratories, selected to assist EPA and its federal, state, and local partners in responding to homeland security events, have the requisite expertise and systems to perform this type of testing; and
- Continuing to work with multiple agencies and stakeholders to update SAM and supporting documents periodically.

EPA recognizes that having data of known and documented quality is critical. Public officials can accurately assess the activities that may be needed in remediating a site during and following emergency situations. Data must be of sufficient quality to support decision making. Quality control (QC), however, takes time. Time is often critical in emergency-related activities where there will be tremendous pressure to conduct sampling and analytical operations quickly and efficiently. While reduced levels of QC might be tolerated during the rapid screening stage of emergency response, implementation of analytical methods for risk assessment and site release will require a higher and more appropriate level of QC. Many of the methods listed in this document include QC requirements for collecting and analyzing samples. These QC requirements may or may not be appropriate for addressing emergency response

situations, and may be adjusted as necessary to maximize data and decision quality. Specific QC 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).

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. These contacts should be consulted regarding any method deviations or modifications, sample problems or interferences, QC requirements, or the use of potential alternative methods. 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.

General	
Oba Vincent - *Primary* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 569-7456 vincent.oba@epa.gov	Rob Rothman - *Alternate* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 569-7187 rothman.rob@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	
Tonya Nichols - *Primary* National Homeland Security Research Center U.S. EPA ORD (NG16) 26 West Martin Luther King Jr. Drive Cincinnati, OH 45268 (513) 569-7805 nichols.tonya@epa.gov	Sanjiv Shah - *Alternate* National Homeland Security Research Center U.S. EPA ORD - 8801RR 1200 Pennsylvania Avenue, NW Washington, DC 20460 (202) 564-9522 shah.sanjiv@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 1200 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 emergency. 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.
- **Non-aqueous liquid/organic solid samples.** The recommended method/procedure to identify and measure the analyte of interest in non-aqueous liquid/organic phase samples. An organic solid sample is a solid that completely dissolves in an organic solvent and leaves no solid residue.
- **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.

5.1 General Guidance

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 QC procedures.

For additional information on the properties of the chemicals listed in Appendix A, 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 Physprop and Chemfate, part of the Environmental Fate Database supported by EPA. http://www.syrres.com/esc/databases.htm
- INCHEM at 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 NIOSH Web site at http://www.cdc.gov/niosh/rtecs/vz72d288.html#JWIDAW for toxicity information.
- EPA's Integrated Risk Information System (IRIS): http://www.epa.gov/iris/ contains toxicity information.
- *Forensic Science and Communications* published by the Laboratory Division of the FBI. http://www.fbi.gov/hq/lab/fsc/current/backissu.htm
- European Chemicals Bureau Toxicology and Chemical Substances: http://ecb.jrc.it and http://ecb.jrc.it/testing-methods/ containing information regarding European Directive 67/548/EEC and Annex V

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: Chemical Methods under. 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, non-aqueous liquid/organic solid, aqueous liquid, drinking water, air, or wipe). In some cases, two methods (sample preparation and determinative) are needed to complete sample analysis.

Sections 5.2.1 through 5.2.86 below provide summaries of the sample preparation and determinative methods listed in Appendix A. Once a method has been identified in Appendix A, **Table 5-1** can be used to locate the method summary.

Table 5-1. Chemical Methods and Corresponding Text Section Numbers

Analyte	CAS RN	Method	Section
Acrylamide	79-06-1	3570 (EPA SW-846)	5.2.22
		8290A Appendix A (EPA SW-846)	5.2.38
Acrylonitrile	107-13-1	8316 (EPA SW-846)	5.2.40
		PV2004 (OSHA)	5.2.75

Analyte	CAS RN	Method	Section
Aldicarb (Temik)	116-06-3	531.2 (EPA OW)	5.2.12
		3570 (EPA SW-846)	5.2.22
Aldicarb sulfone	1646-88-4	8290A Appendix A (EPA SW-846)	5.2.38
Aldicarb sulfoxide	1646-87-3	8318A (EPA SW-846)	5.2.41
		5601 (NIOSH)	5.2.57
Allyl alcohol	107-18-6	3585 (EPA SW-846)	5.2.25
		5030C (EPA SW-846)	5.2.26
		5035A (EPA SW-846)	5.2.27
		8260C (EPA SW-846)	5.2.36
		TO-15 (EPA ORD)	5.2.51
4-Aminopyridine	504-24-5	3535A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.22
		8290A Appendix A (EPA SW-846)	5.2.38
		8330B (EPA SW-846)	5.2.43
Ammonia	7664-41-7	350.1 (EPA OW)	5.2.8
		6015 (NIOSH)	5.2.63
		4500-NH_3 B (SM)	5.2.80
		4500-NH_3 G (SM)	5.2.81
Ammonium metavanadate	7803-55-6	200.7 (EPA OW)	5.2.2
		200.8 (EPA OW)	5.2.3
		3031 (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
Arsenic, Total	7440-38-2	6010C (EPA SW-846)	5.2.28
		6020A (EPA SW-846)	5.2.29
Arsenic trioxide	1327-53-3	IO-3.1 (EPA ORD)	5.2.46
		IO-3.4 (EPA ORD)	5.2.47
		IO-3.5 (EPA ORD)	5.2.48
		9102 (NIOSH)	5.2.68
Arsine	7784-42-1	200.7 (EPA OW)	5.2.2
		200.8 (EPA OW)	5.2.3
		3050B (EPA SW-846)	5.2.17
		7010 (EPA SW-846)	5.2.30
		6001 (NIOSH)	5.2.58
		9102 (NIOSH)	5.2.68
Asbestos	1332-21-4	D5755-03 (ASTM)	5.2.77
		D6480-99 (ASTM)	5.2.78
		10312:1995 (ISO)	5.2.79
Boron trifluoride	7637-07-2	ID216SG (OSHA)	5.2.74
Brodifacoum	56073-10-0	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
Bromadiolone	28772-56-7	3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.38
		8321B (EPA SW-846)	5.2.42

Analyte	CAS RN	Method	Section
BZ (Quinuclidinyl benzilate)	6581-06-2	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.38
		8321B (EPA SW-846)	5.2.42
		TO-10A (EPA ORD)	5.2.50
Calcium arsenate	7778-44-1	200.7 (EPA OW)	5.2.2
		200.8 (EPA OW)	5.2.3
		3031 (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		6010C (EPA SW-846)	5.2.28
		6020A (EPA SW-846)	5.2.29
		IO-3.1 (EPA ORD)	5.2.46
		IO-3.4 (EPA ORD)	5.2.47
		IO-3.5 (EPA ORD)	5.2.48
		9102 (NIOSH)	5.2.68
Carbofuran (Furadan)	1563-66-2	531.2 (EPA OW)	5.2.12
		3570 (EPA SW-846)	5.2.22
		8290A Appendix A (EPA SW-846)	5.2.38
		8318A (EPA SW-846)	5.2.41
		5601 (NIOSH)	5.2.57
Carbon disulfide	75-15-0	524.2 (EPA OW)	5.2.10
		3585 (EPA SW-846)	5.2.25
		5030C (EPA SW-846)	5.2.26
		5035A (EPA SW-846)	5.2.27
		8260C (EPA SW-846)	5.2.36
		TO-15 (EPA ORD)	5.2.51
Chlorfenvinphos	470-90-6	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Chlorine	7782-50-5	4500-Cl G (SM)	5.2.82
		Analyst. 1999. 124: 1853–1857	5.2.83
2-Chloroethanol	107-07-3	3585 (EPA SW-846)	5.2.25
		5030C (EPA SW-846)	5.2.26
		5035A (EPA SW-846)	5.2.27
		8260C (EPA SW-846)	5.2.36
		2513 (NIOSH)	5.2.54

Analyte	CAS RN	Method	Section
3-Chloro-1,2-propanediol	96-24-2	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Chloropicrin	76-06-2	551.1 (EPA OW)	5.2.14
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		PV2103 (OSHA)	5.2.76
Chlorosarin Chlorosoman	1445-76-7 7040-57-5	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
2-Chlorovinylarsonous acid (CVAA)	85090-33-1	200.7 (EPA OW)	5.2.2
		200.8 (EPA OW)	5.2.3
		3031 (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		6010C (EPA SW-846)	5.2.28
		6020A (EPA SW-846)	5.2.29
		IO-3.1 (EPA ORD)	5.2.46
		IO-3.4 (EPA ORD)	5.2.47
		IO-3.5 (EPA ORD)	5.2.48
		9102 (NIOSH)	5.2.68
Chlorpyrifos	2921-88-2	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50

Analyte	CAS RN	Method	Section
Crimidine	535-89-7	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
Cyanide, Amenable to chlorination	NA	RLAB Method 3135.2I	5.2.45
Cyanide, Total	57-12-5	335.4 (EPA OW)	5.2.7
		ILM05.3 CN (EPA CLP)	5.2.44
		6010 (NIOSH)	5.2.61
Cyanogen chloride	506-77-4	3585 (EPA SW-846)	5.2.25
		5030C (EPA SW-846)	5.2.26
		5035A (EPA SW-846)	5.2.27
		8260C (EPA SW-846)	5.2.36
		TO-15 (EPA ORD)	5.2.51
Cyclohexyl sarin (GF)	329-99-7	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
1,2-Dichloroethane	107-06-2	524.2 (EPA OW)	5.2.10
		3585 (EPA SW-846)	5.2.25
		5030C (EPA SW-846)	5.2.26
		5035A (EPA SW-846)	5.2.27
		8260C (EPA SW-846)	5.2.36
		TO-15 (EPA ORD)	5.2.51
Dichlorvos	62-73-7	525.2 (EPA OW)	5.2.11
		3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Dicrotophos	141-66-2	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50

Analyte	CAS RN	Method	Section
Diesel Range Organics	NA	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8015C (EPA SW-846)	5.2.35
		8290A Appendix A (EPA SW-846)	5.2.38
Diisopropyl methylphosphonate (DIMP)	1445-75-6	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.38
		8321B (EPA SW-846)	5.2.42
		TO-10A (EPA ORD)	5.2.50
Dimethylphosphite	868-85-9	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Dimethylphosphoramidic acid	33876-51-6	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.38
		8321B (EPA SW-846)	5.2.42
		TO-10A (EPA ORD)	5.2.50
Diphacinone	82-66-6	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.38
		8321B (EPA SW-846)	5.2.42
Disulfoton Disulfoton sulfoxide	298-04-4 2497-07-6	525.2 (EPA OW)	5.2.11
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		5600 (NIOSH)	5.2.56

Analyte	CAS RN	Method	Section
1,4-Dithiane	505-29-3	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
EA2192 [Diisopropylaminoethyl methyl-thiophosphonate] Ethyl methylphosphonic acid (EMPA)	73207-98-4 1832-53-7	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.38
		8321B (EPA SW-846)	5.2.42
		TO-10A (EPA ORD)	5.2.50
Ethyldichloroarsine (ED)	598-14-1	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		TO-15 (EPA ORD)	5.2.51
		9102 (NIOSH)	5.2.68
N-Ethyldiethanolamine (EDEA)	139-87-7	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.38
		8321B (EPA SW-846)	5.2.42
		TO-10A (EPA ORD)	5.2.50
Ethylene oxide	75-21-8	3585 (EPA SW-846)	5.2.25
		5030C (EPA SW-846)	5.2.26
		5035A (EPA SW-846)	5.2.27
		8260C (EPA SW-846)	5.2.36
		TO-15 (EPA ORD)	5.2.51
Fenamiphos	22224-92-6	525.2 (EPA OW)	5.2.11
		3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50

Analyte	CAS RN	Method	Section
Fentanyl	437-38-7	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.38
		8321B (EPA SW-846)	5.2.42
		TO-10A (EPA ORD)	5.2.50
Fluoride	16984-48-8	300.1, Rev 1.0 (EPA OW)	5.2.6
Fluoroacetamide	640-19-7	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Fluoroacetic acid and fluoroacetate salts	NA	300.1, Rev 1.0 (EPA OW)	5.2.6
		3570 (EPA SW-846)	5.2.22
		8290A Appendix A (EPA SW-846)	5.2.38
		S301-1 (NIOSH)	5.2.69
		Analytical Letters, 1994, 27 (14), 2703-2718	5.2.84
Formaldehyde	50-00-0	3570 (EPA SW-846)	5.2.22
		8290A Appendix A (EPA SW-846)	5.2.38
		8315A (EPA SW-846)	5.2.39
		2016 (NIOSH)	5.2.53
Gasoline Range Organics	NA	3570 (EPA SW-846)	5.2.22
		3585 (EPA SW-846)	5.2.25
		5030C (EPA SW-846)	5.2.26
		5035A (EPA SW-846)	5.2.27
		8015C (EPA SW-846)	5.2.35
		8290A Appendix A (EPA SW-846)	5.2.38
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4	3535A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.22
Hexamethylenetriperoxidediamine (HMTD)	283-66-9	8290A Appendix A (EPA SW-846)	5.2.38
		8330B (EPA SW-846)	5.2.43
Hydrogen bromide Hydrogen chloride	10035-10-6 7647-01-0	7903 (NIOSH)	5.2.65
Hydrogen cyanide	74-90-8	6010 (NIOSH)	5.2.61
Hydrogen fluoride	7664-39-3	7903 (NIOSH)	5.2.65
Hydrogen sulfide	7783-06-4	6013 (NIOSH)	5.2.62

Analyte	CAS RN	Method	Section
Isopropyl methylphosphonic acid (IMPA)	1832-54-8	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.38
		8321B (EPA SW-846)	5.2.42
		TO-10A (EPA ORD)	5.2.50
Kerosene	64742-81-0	3570 (EPA SW-846)	5.2.22
		3585 (EPA SW-846)	5.2.25
		5030C (EPA SW-846)	5.2.26
		5035A (EPA SW-846)	5.2.27
		8015C (EPA SW-846)	5.2.35
		8290A Appendix A (EPA SW-846)	5.2.38
Lead arsenate	7645-25-2	200.7 (EPA OW)	5.2.2
		200.8 (EPA OW)	5.2.3
		3031 (EPA SW-846)	5.2.16
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]	541-25-3	3050B (EPA SW-846)	5.2.17
		6010C (EPA SW-846)	5.2.28
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]	40334-69-8	6020A (EPA SW-846)	5.2.29
		IO-3.1 (EPA ORD)	5.2.46
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]	40334-70-1	IO-3.4 (EPA ORD)	5.2.47
		IO-3.5 (EPA ORD)	5.2.48
Lewisite oxide	1306-02-1	9102 (NIOSH)	5.2.68
Mercury, Total	7439-97-6	245.2 (EPA OW)	5.2.4
		7473 (EPA SW-846)	5.2.33
		IO-5 (EPA ORD)	5.2.49
		9102 (NIOSH)	5.2.68
Methamidophos	10265-92-6	3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Methomyl	16752-77-5	531.2 (EPA OW)	5.2.12
		3570 (EPA SW-846)	5.2.22
		8290A Appendix A (EPA SW-846)	5.2.38
		8318A (EPA SW-846)	5.2.41
		5601 (NIOSH)	5.2.57
Methoxyethylmercuric acetate	151-38-2	245.2 (EPA OW)	5.2.4
		7473 (EPA SW-846)	5.2.33
		IO-5 (EPA ORD)	5.2.49
		9102 (NIOSH)	5.2.68

Analyte	CAS RN	Method	Section
Methyl acrylonitrile	126-98-7	3570 (EPA SW-846)	5.2.22
		8290A Appendix A (EPA SW-846)	5.2.38
		8316 (EPA SW-846	5.2.40
		PV2004 (OSHA)	5.2.75
Methyl fluoroacetate	453-18-9	300.1, Rev 1.0 (EPA OW)	5.2.6
		3570 (EPA SW-846)	5.2.22
		8290A Appendix A (EPA SW-846)	5.2.38
		S301-1 (NIOSH)	5.2.69
		Analytical Letters, 1994, 27 (14): 2703-2718	5.2.84
Methyl hydrazine	60-34-4	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		3510 (NIOSH)	5.2.55
Methyl isocyanate	624-83-9	OSHA 54	5.2.71
Methyl parathion	298-00-0	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Methylamine	74-89-5	OSHA 40	5.2.70
N-Methyldiethanolamine (MDEA)	105-59-9	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.38
		8321B (EPA SW-846)	5.2.42
		TO-10A (EPA ORD)	5.2.50
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50

Analyte	CAS RN	Method	Section
Methylphosphonic acid (MPA)	993-13-5	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.38
		8321B (EPA SW-846)	5.2.42
		TO-10A (EPA ORD)	5.2.50
Mevinphos	7786-34-7	525.2 (EPA OW)	5.2.11
		3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)-ethylamine]	538-07-8	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)-methylamine]	51-75-2	3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)-amine]	555-77-1	8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Mustard, sulfur / Mustard gas (HD)	505-60-2	3570 (EPA SW-846)	5.2.22
		3571 (EPA SW-846)	5.2.23
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Nicotine compounds	54-11-5	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0	3535A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.22
		8290A Appendix A (EPA SW-846)	5.2.38
		8330B (EPA SW-846)	5.2.43

Analyte	CAS RN	Method	Section
Organophosphate pesticides, NOS	NA	507 (EPA OW)	5.2.9
		614 (EPA OW)	5.2.15
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		5600 (NIOSH)	5.2.56
Osmium tetroxide	20816-12-0	252.2 (EPA OW)	5.2.5
		3050B (EPA SW-846)	5.2.17
		6010C (EPA SW-846)	5.2.28
		IO-3.1 (EPA ORD)	5.2.46
		IO-3.4 (EPA ORD)	5.2.47
		9102 (NIOSH)	5.2.68
Oxamyl	23135-22-0	531.2 (EPA OW)	5.2.12
		3570 (EPA SW-846)	5.2.22
		8290A Appendix A (EPA SW-846)	5.2.38
		8318A (EPA SW-846)	5.2.41
		5601 (NIOSH)	5.2.57
Paraquat	4685-14-7	549.2 (EPA OW)	5.2.13
Parathion	56-38-2	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Pentaerythritol tetranitrate (PETN)	78-11-5	3535A (EPA SW-846)	5.2.19
		3570 (EPA SW-846)	5.2.22
		8290A Appendix A (EPA SW-846)	5.2.38
		8330B (EPA SW-846)	5.2.43
Perfluoroisobutylene (PFIB)	382-21-8	OSHA 61	5.2.72
Phencyclidine	77-10-1	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50

Analyte	CAS RN	Method	Section
Phenol	108-95-2	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Phorate	298-02-2	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Phosgene	75-44-5	OSHA 61	5.2.72
Phosphamidon	13171-21-6	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Phosphine	7803-51-2	6002 (NIOSH)	5.2.59
Phosphorus trichloride	7719-12-2	6402 (NIOSH)	5.2.64
Pinacolyl methyl phosphonic acid (PMPA)	616-52-4	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.38
		8321B (EPA SW-846)	5.2.42
		TO-10A (EPA ORD)	5.2.50
Propylene oxide	75-56-9	3585 (EPA SW-846)	5.2.25
		5030C (EPA SW-846)	5.2.26
		5035A (EPA SW-846)	5.2.27
		8260C (EPA SW-846)	5.2.36
		1612 (NIOSH)	5.2.52

Analyte	CAS RN	Method	Section
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Sarin	107-44-8	3570 (EPA SW-846)	5.2.22
		3571 (EPA SW-846)	5.2.23
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Semivolatile Organic Compounds, NOS	NA	525.2 (EPA OW)	5.2.11
		3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Sodium arsenite	7784-46-5	200.7 (EPA OW)	5.2.2
		200.8 (EPA OW)	5.2.3
		3031 (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		6010C (EPA SW-846)	5.2.28
		6020A (EPA SW-846)	5.2.29
		IO-3.1 (EPA ORD)	5.2.46
		IO-3.4 (EPA ORD)	5.2.47
		IO-3.5 (EPA ORD)	5.2.48
		9102 (NIOSH)	5.2.68
Sodium azide	26628-22-8	300.1, Rev 1.0 (EPA OW)	5.2.6
		3580A (EPA SW-846)	5.2.24
		ID-211 (OSHA)	5.2.73
		Journal of Forensic Sciences, 1998. 43(1): 200–202	5.2.86
Soman (GD)	96-64-0	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50

Analyte	CAS RN	Method	Section
Strychnine	57-24-9	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
Sulfur dioxide	7446-09-5	6004 (NIOSH)	5.2.60
Sulfur trioxide	7446-11-9	Method 8 (EPA)	5.2.1
Tabun (GA)	77-81-6	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Tear gas (CS) [chlorobenzylidene malonitrile]	2698-41-1	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Tetraethyl pyrophosphate	107-49-3	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Tetramethylenedisulfotetramine	80-12-6	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50

Analyte	CAS RN	Method	Section
Thallium sulfate	10031-59-1	200.7 (EPA OW)	5.2.2
		200.8 (EPA OW)	5.2.3
		3031 (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		6010C (EPA SW-846)	5.2.28
		6020A (EPA SW-846)	5.2.29
		IO-3.1 (EPA ORD)	5.2.46
		IO-3.4 (EPA ORD)	5.2.47
		IO-3.5 (EPA ORD)	5.2.48
		9102 (NIOSH)	5.2.68
Thiodiglycol (TDG)	111-48-8	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
Thiofanox	39196-18-4	531.2 (EPA OW)	5.2.12
		3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.38
		8321B (EPA SW-846)	5.2.42
		5601 (NIOSH)	5.2.57
1,4-Thioxane	15980-15-1	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
Titanium tetrachloride	7550-45-0	3050B (EPA SW-846)	5.2.17
		6010C (EPA SW-846)	5.2.28
		6020A (EPA SW-846)	5.2.29
		9102 (NIOSH)	5.2.68
Triethanolamine (TEA)	102-71-6	3520C (EPA SW-846)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8290A Appendix A (EPA SW-846)	5.2.38
		8321B (EPA SW-846)	5.2.42
		TO-10A (EPA ORD)	5.2.50

Analyte	CAS RN	Method	Section
Trimethyl phosphite	121-45-9	3535A (EPA SW-846)	5.2.19
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
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)	5.2.19
		3570 (EPA SW-846)	5.2.22
		8290A Appendix A (EPA SW-846)	5.2.38
		8330B (EPA SW-846)	5.2.43
Vanadium pentoxide	1314-62-1	200.7 (EPA OW)	5.2.2
		200.8 (EPA OW)	5.2.3
		3031 (EPA SW-846)	5.2.16
		3050B (EPA SW-846)	5.2.17
		6010C (EPA SW-846)	5.2.28
		6020A (EPA SW-846)	5.2.29
		IO-3.1 (EPA ORD)	5.2.46
		IO-3.4 (EPA ORD)	5.2.47
		IO-3.5 (EPA ORD)	5.2.48
		9102 (NIOSH)	5.2.68
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)	5.2.18
		3535A (EPA SW-846)	5.2.19
		3541 (EPA SW-846)	5.2.20
		3545A (EPA SW-846)	5.2.21
		3570 (EPA SW-846)	5.2.22
		3580A (EPA SW-846)	5.2.24
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
VX [O-ethyl-S-(2-diisopropylaminoethyl)methyl-phosphonothiolate]	50782-69-9	3570 (EPA SW-846)	5.2.22
		3571 (EPA SW-846)	5.2.23
		8270D (EPA SW-846)	5.2.37
		8290A Appendix A (EPA SW-846)	5.2.38
		TO-10A (EPA ORD)	5.2.50
White phosphorus	12185-10-3	3570 (EPA SW-846)	5.2.22
		7580 (EPA SW-846)	5.2.34
		8290A Appendix A (EPA SW-846)	5.2.38
		7905 (NIOSH)	5.2.66
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)	5.2.50
BZ (Quinuclidinyl benzilate)	6581-06-2	8270D (EPA SW-846)	5.2.37
3-Chloro-1,2-propanediol	96-24-2	TO-15 (EPA ORD)	5.2.51
Chlorosarin Chlorosoman	1445-76-7 7040-57-5	TO-15 (EPA ORD)	5.2.51
Crimidine	535-89-7	8321B (EPA SW-846)	5.2.42

Analyte	CAS RN	Method	Section
Diisopropyl methylphosphonate (DIMP)	1445-75-6	8270D (EPA SW-846)	5.2.37
		TO-15 (EPA ORD)	5.2.51
Dimethylphosphoramidic acid	33876-51-6	8270D (EPA SW-846)	5.2.37
1,4-Dithiane	505-29-3	3585 (EPA SW-846)	5.2.25
		5030C (EPA SW-846)	5.2.26
		5035A (EPA SW-846)	5.2.27
		8260C (EPA SW-846)	5.2.36
EA2192 [Diisopropylaminoethyl methyl-thiophosphonate] Ethyl methylphosphonic acid (EMPA)	73207-98-4 1832-53-7	8270D (EPA SW-846)	5.2.37
Hydrogen fluoride	7664-39-3	7906 (NIOSH)	5.2.67
Isopropyl methylphosphonic acid (IMPA)	1832-54-8	8270D (EPA SW-846)	5.2.37
Mercury, Total	7439-97-6	7470A (EPA SW-846)	5.2.31
		7471B (EPA SW-846)	5.2.32
Methamidophos	10265-92-6	5600 (NIOSH)	5.2.56
Methoxymercuric acetate	151-38-2	7470A (EPA SW-846)	5.2.31
		7471B (EPA SW-846)	5.2.32
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3	TO-15 (EPA ORD)	5.2.51
Methylphosphonic acid (MPA)	993-13-5	8270D (EPA SW-846)	5.2.37
Perfluoroisobutylene (PFIB)	382-21-8	Journal of Chromatography A. 2005. 1098: 156–165	5.2.85
Pinacolyl methyl phosphonic acid (PMPA)	616-52-4	8270D (EPA SW-846)	5.2.37
Sarin Soman (GD)	107-44-8 96-64-0	TO-15 (EPA ORD)	5.2.51
1,4-Thioxane	15980-15-1	3585 (EPA SW-846)	5.2.25
		5030C (EPA SW-846)	5.2.26
		5035A (EPA SW-846)	5.2.27
		8260C (EPA SW-846)	5.2.36

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
NEMI	EPA, USGS	http://www.nemi.gov
U.S. EPA OW Methods	EPA OW	http://www.epa.gov/safewater/methods/sourcalt.html
U.S. EPA SW-846 Methods	EPA OSWER	http://www.epa.gov/epaoswer/hazwaste/test/main.htm
U.S. EPA ORD Methods	EPA ORD	http://www.epa.gov/ttnamti1/
U.S. EPA Air Toxics Methods	EPA OAR	http://www.epa.gov/ttn/amtic/airtox.html

Name	Publisher	Reference
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
European GESTIS database	HVBG	http://www.hvbg.de/e/bia/gestis/analytical_methods/index.html
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
Journal of Chromatography A*	Elsevier Science Publishers	http://www.elsevier.com/
Journal of Forensic Sciences*	ASTM International	http://www.astm.org

* Subscription and/or purchase required.

5.1.2 General Quality Control (QC) Guidance 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. In addition to the laboratories being capable of generating accurate and precise data during an emergency situation, they must be able to deliver results in a timely and efficienct manner. Therefore, laboratories must be prepared with calibrated instruments, the proper standards, standard analytical procedures, standard operating procedures, and qualified and trained technicians. 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 data quality objectives 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, most laboratories will not have access to analytical standards for calibration and QC. Use of these agents is strictly controlled by the DoD and access is limited. For information regarding purchase and distribution of ultradilute agents for laboratory use, please contact Terry Smith, EPA's Office of Emergency Management, at (202) 564-2908.

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 SAPs 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 (MS) 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 (MSD) 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; and
  - Method blanks.
- Demonstration of analytical method suitability for intended use:
  - Detection and quantitation limits;
  - Precision and recovery (verify measurement system has adequate accuracy); and
  - 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:
  - MS/MSDs (recovery and precision);
  - QC samples (system accuracy and sensitivity at levels of concern);
  - Surrogate spikes (where appropriate);
  - Continuing calibration verification; and
  - Method blanks.

QC tests should be consistent with Good Laboratory Practices and be run as frequently as necessary to ensure the reliability of analytical results. As with the identification of needed QC samples, the frequency of QC sampling should be established based on an evaluation of data quality objectives. 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 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 data quality objectives. 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 OSWER coordinator responsible for laboratory activities during the specific event to ensure QA/QC procedures are performed consistently across laboratories. OSWER is planning to develop QA/QC guidance for laboratory support. 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 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, guidance, 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:

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

Please note that the Electronic Code of Federal Regulations (e-CFR) is available at http://ecfr.gpoaccess.gov/.

5.2 Method Summaries

Summaries for the analytical methods listed in Appendix A are provided in Sections 5.2.1 through 5.2.86. These sections contain summary information only, 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. The full version of the method should be consulted prior to sample analysis.

5.2.1 EPA Method 8: Determination of Sulfuric Acid and Sulfur Dioxide Emissions from Stationary Sources

Analyte(s)	CAS RN
Sulfur Trioxide	7446-11-9

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Isokinetic extraction
Determinative Technique: Titrimetry

Method Developed for: Sulfuric acid, sulfur trioxide, and sulfur dioxide in air
Method Selected for: SAM lists this method for preparation and analysis of air samples.
Detection and Quantitation: Collaborative tests have shown that the minimum detectable limit of the method is 0.06 mg/m^3 for sulfuric acid.

Description of Method: A gas sample is extracted isokinetically. Sulfuric acid and sulfur dioxide are separated, and both fractions are measured separately by the barium-thorin titration method. Sulfur

trioxide is measured by the analysis of sulfuric acid. Possible interfering agents include fluorides, free ammonia, and dimethyl aniline.

Special Considerations: Possible interfering agents include fluorides, free ammonia, and dimethyl aniline.

Source: EPA Emission Measurement Center (EMC) of the Office of Air Quality Planning and Standards (OAQPS). "Method 8: Determination of Sulfuric Acid and Sulfur Dioxide Emissions from Stationary Sources." http://www.epa.gov/sam/pdfs/EPA-Method8.pdf

5.2.2 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	7803-55-6
Arsenic, Total	740-38-2
Arsenic trioxide	1327-53-3
Arsine	7784-42-1
Calcium arsenate	7778-44-1
2-Chlorovinylarsonous acid (CVAA)	85090-33-1
Lead arsenate	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]	40334-70-1
Lewisite Oxide	1306-02-1
Sodium arsenite	7784-46-5
Thallium sulfate	10031-59-1
Vanadium pentoxide	1314-62-1

Analysis Purpose: Sample preparation and analysis
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.
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 metals 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.

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://www.epa.gov/sam/pdfs/EPA-200.7.pdf

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

Analyte(s)	CAS RN
Ammonium metavanadate	7803-55-6
Arsenic, Total	740-38-2
Arsenic trioxide	1327-53-3
Arsine	7784-42-1
Calcium arsenate	7778-44-1
2-Chlorovinylarsonous acid (CVAA)	85090-33-1
Lead arsenate	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]	40334-70-1
Lewisite Oxide	1306-02-1
Sodium arsenite	7784-46-5
Thallium sulfate	10031-59-1
Vanadium pentoxide	1314-62-1

Analysis Purpose: Sample preparation and analysis
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.
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.

Source: EPA. 1994. "Method 200.8: Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry," Revision 5.4. http://www.epa.gov/sam/pdfs/EPA-200.8.pdf

5.2.4 EPA Method 245.2: Mercury (Automated Cold Vapor Technique)

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

Analysis Purpose: Sample preparation and analysis
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 drinking water samples.
Detection and Quantitation: Applicable concentration range is 0.2 to 20.0 µg/L.

Description of Method: This method will determine 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 tin sulfate or tin 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.

Source: EPA. 1974. "Method 245.2: Mercury (Automated Cold Vapor Technique)." http://www.epa.gov/sam/pdfs/EPA-245.2.pdf

5.2.5 EPA Method 252.2: Osmium (Atomic Absorption, Furnace Technique)

Analyte(s)	CAS RN
Osmium tetroxide	20816-12-0

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Direct aspiration
Determinative Technique: Graphite furnace atomic absorption spectrophotometry (GFAA)

Method Developed for: Osmium in drinking, surface, and saline waters, and domestic and industrial wastes
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid and drinking water samples.
Detection and Quantitation: Detection limit for osmium is 20 µg/L. The optimal applicable concentration range is 50 to 500 µg/L.

Description of Method: This method will determine osmium tetroxide as osmium. Method 252.2 is not a stand alone method in that sections of the method reference "Methods of Chemical Analysis of Water and Waste", EPA/600/4-79/020, March 1983 (MCAWW). Samples are prepared according to the "direct aspiration method" (See Section 9.1 of the Atomic Absorption Methods section of MCAWW) except that the addition of sulfuric acid is omitted in the final adjustment. If only dissolved osmium is determined, the sample is filtered before acidification with nitric acid. For total osmium, the sample is digested with nitric and hydrochloric acids and made up to volume. Samples are analyzed according to the "furnace procedure" (see Section 9.3 of the Atomic Absorption Methods section of MCAWW), using GFAA. A representative aliquot of sample is placed in the graphite tube in the furnace, evaporated to dryness, chaffed, and atomized. Radiation from an excited element is passed through the vapor containing ground state atoms of the element. The intensity of the transmitted radiation decreases in proportion to the amount of the ground state element in the vapor. A monochromator isolates the characteristic radiation from the hollow cathode lamp and a photosensitive device measures the attenuated transmitted radiation.

Source: EPA. 1978. "Method 252.2: Osmium (Atomic Absorption, Furnace Technique)."
http://www.epa.gov/sam/pdfs/EPA-252.2.pdf

5.2.6 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
Fluoroacetic acid and fluoroacetate salts	NA
Methyl fluoroacetate	453-18-9
Sodium azide (analyze for hydrazoic acid)	26628-22-8

Analysis Purpose: Sample preparation and/or analysis
Sample Preparation Technique: For fluoride, use direct injection. For fluoroacetic acid, fluoroacetate salts, and methyl fluoroacetate, use Analytical Letters, 1994, 27(14): 2703-2718 (solid and non-aqueous liquid/organic solid samples), NIOSH Method S301-1 (air samples), and EPA SW-846 Method 3570/8290A Appendix A (wipe samples). For sodium azide, use water extraction, filtration, and acidification steps from the Journal of Forensic Science, 1998. 43(1):200-202 (solid samples), filtration and acidification steps from this journal (aqueous liquid and drinking water samples), and the acidification step from the journal with EPA SW-846 Method 3580A (non-aqueous liquid/organic solid samples).
Determinative Technique: Ion chromatography (IC)

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, fluoroacetic acid, fluoroacetate salts, and methyl fluoroacetate. It also should be used for analysis of solid and non-aqueous liquid/organic solid, air, and/or wipe samples for fluoroacetic acid, fluoroacetate salts, methyl fluoroacetate, and sodium azide when appropriate sample preparation techniques have been applied.
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 non-aqueous liquid/organic solid, 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 nS noise/drift per minute of monitored response over the background conductivity.

Source: EPA. 1997. "Method 300.1: Determination of Inorganic Anions in Drinking Water by Ion Chromatography," Revision 1.0. http://www.epa.gov/sam/pdfs/EPA-300.1.pdf

5.2.7 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 analysis
Sample Preparation Technique: Reflux-distillation
Determinative Technique: 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.
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, such as aldehydes, nitrate-nitrite, oxidizing agents, thiocyanate, thiosulfate, and sulfide, are eliminated or reduced by distillation.

Source: EPA. 1993. "Method 335.4: Determination of Total Cyanide by Semi-Automated Colorimetry," Revision 1.0. http://www.epa.gov/sam/pdfs/EPA-335.4.pdf

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

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

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Distillation
Determinative Technique: 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.
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.

Source: EPA. 1993. "Method 350.1: Nitrogen, Ammonia (Colorimetric, Automated Phenate)," Revision 2.0. http://www.epa.gov/sam/pdfs/EPA-350.1.pdf

5.2.9 EPA Method 507: Determination of Nitrogen- and Phosphorus-Containing Pesticides in Water by Gas Chromatography with a Nitrogen-Phosphorus Detector

Analyte(s)	CAS RN
Organophosphate pesticides, NOS	NA

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Solvent extraction
Determinative Technique: Gas chromatography – Nitrogen-phosphorus detector (GC-NPD)

Method Developed for: Nitrogen- and phosphorus-containing pesticides in ground water and finished drinking water
Method Selected for: SAM lists this method for preparation and analysis of drinking water samples.
Detection and Quantitation: Estimated detection limits (EDLs) and MDLs differ depending on the specific pesticide.

Description of Method: A 1-L sample is extracted with methylene chloride by shaking in a separatory funnel or mechanical tumbling in a bottle. The methylene chloride extract is isolated, dried, and concentrated to a volume of 5 mL during a solvent exchange to methyl *tert*-butyl ether (MTBE). The concentrations of pesticides in the extract are measured using a capillary column gas chromatography (GC) system equipped with a nitrogen-phosphorus detector (NPD). Specific analytes targeted by Method 507 are listed in Section 1.1 of the method.

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

Source: EPA. 1995. "Method 507: Determination of Nitrogen- and Phosphorus-Containing Pesticides in Water by Gas Chromatography with a Nitrogen-Phosphorus Detector," Revision 2.1. http://www.epa.gov/sam/pdfs/EPA-507.pdf

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

Analyte(s)	CAS RN
Carbon disulfide	75-15-0
1,2-Dichloroethane	107-06-2

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Purge and trap
Determinative Technique: GC-MS

Method Developed for: Purgeable volatile organic compounds (VOCs) in surface water, ground water, and drinking water in any stage of treatment
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid and/or drinking water samples.
Detection and Quantitation: Detection levels for carbon disulfide and 1,2-dichloroethane in reagent water have been found to be 0.093 µg/L and 0.02 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 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 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.

Source: EPA. 1992. "Method 524.2: Measurement of Purgeable Organic Compounds in Water by Capillary Column Gas Chromatography/Mass Spectrometry," Revision 4.0. http://www.epa.gov/sam/pdfs/EPA-524.2.pdf

5.2.11 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
Dichlorvos	62-73-7
Disulfoton	298-04-4
Disulfoton sulfoxide	2497-07-6
Fenamiphos	22224-92-6
Mevinphos	7786-34-7

Analyte(s)	CAS RN
Semivolatile Organic Compounds, NOS	NA

Analysis Purpose: Sample preparation and analysis
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 drinking water samples.
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.

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://www.epa.gov/sam/pdfs/EPA-525.2.pdf

5.2.12 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
Thiofanox	39196-18-4

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Direct injection
Determinative Technique: High performance liquid chromatography (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.
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://www.epa.gov/sam/pdfs/EPA-531.2.pdf

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

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

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: LSE or SPE
Determinative Technique: HPLC

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.
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 an ultraviolet (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://www.epa.gov/sam/pdfs/EPA-549.2.pdf

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

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

Analysis Purpose: Sample preparation and analysis
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 drinking water samples.
Detection and Quantitation: The EDL using 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://www.epa.gov/sam/pdfs/EPA-551.1.pdf

5.2.15 EPA Method 614: The Determination of Organophosphorus Pesticides in Municipal and Industrial Wastewater

Analyte(s)	CAS RN
Organophosphate pesticides, NOS	NA

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Solvent extraction
Determinative Technique: Gas chromatography – Flame photometric detector (GC-FPD)

Method Developed for: Organophosphorus pesticides in municipal and industrial wastewater
Method Selected for: SAM lists this method for preparation and analysis of aqueous liquid samples.

Description of Method: This is a GC method applicable to the determination of organophosphate pesticides in industrial and municipal discharges using a GC with a phosphorus-specific flame photometric detector (FPD) or thermionic bead detector in the nitrogen mode. A measured volume of sample, approximately 1 L, is extracted with 15% methylene chloride in hexane using a separatory funnel. The extract is dried and concentrated to a volume of 10 mL or less. GC conditions are described for the separation and measurement of the compounds in the extract by flame photometric or thermionic bead GC. Specific analytes targeted by Method 614 are listed in Section 1.1 of the method.

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

Source: EPA. "Method 614: The Determination of Organophosphorus Pesticides in Municipal and Industrial Wastewater." http://www.epa.gov/sam/pdfs/EPA-614.pdf

5.2.16 EPA Method 3031 (SW-846): Acid Digestion of Oils for Metals Analysis by Atomic Absorption or ICP Spectrometry

Analyte(s)	CAS RN
Ammonium metavanadate	7803-55-6
Arsenic, Total	740-38-2
Arsenic trioxide	1327-53-3
Calcium arsenate	7778-44-1
2-Chlorovinylarsonous acid (CVAA)	85090-33-1
Lead arsenate	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]	40334-70-1
Lewisite Oxide	1306-02-1
Sodium arsenite	7784-46-5
Thallium sulfate	10031-59-1
Vanadium pentoxide	1314-62-1

Analysis Purpose: Sample preparation
Sample Preparation Technique: Acid digestion
Determinative Technique: EPA SW-846 Method 6010C or Method 6020A

Method Developed for: Metals in oils, oil sludges, tars, waxes, paints, paint sludges and other viscous petroleum products
Method Selected for: SAM lists this method for preparation of non-aqueous liquid/organic solid samples.

Description of Method: This method is used to prepare samples for the determination of arsenic trioxide, lewisite, lewisite degradation products, and sodium arsenite as total arsenic; thallium sulfate as total thallium; and ammonium metavanadate and vanadium pentoxide as total vanadium. A 0.5-g sample of oil, oil sludge, tar, wax, paint, or paint sludge is mixed with potassium permanganate and sulfuric acid. The mixture is then treated with nitric and hydrochloric acids, filtered, and diluted to volume. Excess manganese may be removed with ammonium hydroxide. Digestates are analyzed by Method 6020A or 6010C (SW-846).

Source: EPA. 1996. "Method 3031 (SW-846): Acid Digestion of Oils for Metals Analysis by Atomic Absorption or ICP Spectrometry," Revision 0. http://www.epa.gov/sam/pdfs/EPA-3031.pdf

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

Analyte(s)	CAS RN
Ammonium metavanadate	7803-55-6
Arsenic, Total	740-38-2
Arsenic trioxide	1327-53-3
Arsine	7784-42-1
Calcium arsenate	7778-44-1
2-Chlorovinylarsonous acid (CVAA)	85090-33-1
Lead arsenate	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]	40334-70-1
Lewisite Oxide	1306-02-1
Osmium tetroxide	20816-12-0
Sodium arsenite	7784-46-5
Thallium sulfate	10031-59-1
Titanium tetrachloride	7550-45-0
Vanadium pentoxide	1314-62-1

Analysis Purpose: Sample preparation
Sample Preparation Technique: Acid digestion
Determinative Technique: EPA SW-846 Method 6010C, Method 6020A, or Method 7010. 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.

Description of Method: This method is used to prepare samples for the determination of arsenic trioxide, arsine, lewisite, lewisite degradation products, 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.

Source: EPA. 1996. "Method 3050B (SW-846): Acid Digestion of Sediments, Sludges, and Soils," Revision 2. http://www.epa.gov/sam/pdfs/EPA-3050b.pdf

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

Analyte(s)	CAS RN
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
BZ (Quinuclidinyl benzilate)	6581-06-2
Chlorfenvinphos	470-90-6
3-Chloro-1,2-propanediol	96-24-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos	2921-88-2
Cyclohexyl sarin (GF)	329-99-7
Diesel Range Organics	NA
Diphacinone	82-66-6
N-Ethyldiethanolamine (EDEA)	139-87-7
Fenamiphos	22224-92-6
Fentanyl	437-38-7
Fluoroacetamide	640-19-7
Methyl hydrazine	60-34-4
N-Methyldiethanolamine (MDEA)	105-59-9
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
Parathion	56-38-2
Phenol	108-95-2
Phosphamidon	13171-21-6
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
Semivolatile Organic Compounds, NOS	NA
Tear gas (CS) [chlorobenzylidene malonitrile]	2698-41-1
Tetramethylenedisulfotetramine	80-12-6
Thiofanox	39196-18-4

Analyte(s)	CAS RN
Triethanolamine (TEA)	102-71-6
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: 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 fenamiphos and semivolatile organic compounds should be prepared and analyzed by EPA Method 525.2; drinking water samples for thiofanox should be prepared and analyzed by EPA Method 531.2; all other drinking water samples and all aqueous liquid samples should be prepared using this method (SW-846 Method 3520C).

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.

Source: EPA. 1996. "Method 3520C (SW-846): Continuous Liquid-Liquid Extraction," Revision 3. http://www.epa.gov/sam/pdfs/EPA-3520c.pdf

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

Analyte(s)	CAS RN
4-Aminopyridine	504-24-5
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
BZ (Quinuclidinyl benzilate)	6581-06-2
Chlorfenvinphos	470-90-6
3-Chloro-1,2-propanediol	96-24-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos	2921-88-2

Analyte(s)	CAS RN
Crimidine	535-89-7
Cyclohexyl sarin (GF)	329-99-7
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
1,4-Dithiane	505-29-3
EA2192 [Diisopropylaminoethyl methylthiolophosphonate]	73207-98-4
Ethyl methylphosphonic acid (EMPA)	1832-53-7
Ethyldichloroarsine (ED)	598-14-1
N-Ethyldiethanolamine (EDEA)	139-87-7
Fenamiphos	22224-92-6
Fentanyl	437-38-7
Fluoroacetamide	640-19-7
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
Methamidophos	10265-92-6
Methyl hydrazine	60-34-4
Methyl parathion	298-00-0
N-Methyldiethanolamine (MDEA)	105-59-9
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Methylphosphonic acid (MPA)	993-13-5
Mevinphos	7786-34-7
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

Analyte(s)	CAS RN
Nicotine compounds	54-11-5
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0
Parathion	56-38-2
Pentaerythritol tetranitrate (PETN)	78-11-5
Phencyclidine	77-10-1
Phenol	108-95-2
Phorate	298-02-2
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
Semivolatile Organic Compounds, NOS	NA
Soman (GD)	96-64-0
Strychnine	57-24-9
Tabun (GA)	77-81-6
Tear gas (CS) [chlorobenzylidene malonitrile]	2698-41-1
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Thiodiglycol (TDG)	111-48-8
Thiofanox	39196-18-4
1,4-Thioxane	15980-15-1
Triethanolamine (TEA)	102-71-6
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

Analysis Purpose: Sample preparation
Sample Preparation Technique: SPE

Determinative Technique: 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 dichlorvos, fenamiphos, mevinphos, and semivolatile organic compounds NOS should be prepared and analyzed by EPA Method 525.2; drinking water samples for polychlorinated biphenyls should be prepared and analyzed by EPA Method 508; drinking water samples for thiofanox should be prepared and analyzed by EPA Method 531.2; all other drinking water samples and all aqueous liquid samples should be prepared using this method (SW-846 Method 3535A).

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: Tetramethylenedisulfotetramine may require SPE extraction using acetone or methyl ethylketone.

Source: EPA. 1998. "Method 3535A (SW-846): Solid-Phase Extraction (SPE)," Revision 1. http://www.epa.gov/sam/pdfs/EPA-3535a.pdf

5.2.20 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
Chlorfenvinphos	470-90-6
3-Chloro-1,2-propanediol	96-24-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos	2921-88-2
Cyclohexyl sarin (GF)	329-99-7
Diesel Range Organics	NA
Diphacinone	82-66-6
Disulfoton	298-04-4
Disulfoton sulfoxide	2497-07-6

Analyte(s)	CAS RN
N-Ethyldiethanolamine (EDEA)	139-87-7
Fenamiphos	22224-92-6
Fentanyl	437-38-7
Fluoroacetamide	640-19-7
Methamidophos	10265-92-6
Methyl hydrazine	60-34-4
N-Methyldiethanolamine (MDEA)	105-59-9
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
Organophosphate pesticides, NOS	NA
Parathion	56-38-2
Phenol	108-95-2
Phosphamidon	13171-21-6
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
Semivolatile Organic Compounds, NOS	NA
Tear gas (CS) [chlorobenzylidene malonitrile]	2698-41-1
Tetramethylenedisulfotetramine	80-12-6
Thiofanox	39196-18-4
Triethanolamine (TEA)	102-71-6
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: Automated Soxhlet extraction (ASE)
Determinative Technique: 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.

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.

Source: EPA. 1994. "Method 3541 (SW-846): Automated Soxhlet Extraction," Revision 0. http://www.epa.gov/sam/pdfs/EPA-3541.pdf

5.2.21 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
Chlorfenvinphos	470-90-6
3-Chloro-1,2-propanediol	96-24-2
Chloropicrin	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos	2921-88-2
Crimidine	535-89-7
Cyclohexyl sarin (GF)	329-99-7
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 sulfoxide	2497-07-6
1,4-Dithiane	505-29-3

Analyte(s)	CAS RN
EA2192 [Diisopropylaminoethyl methylthiolophosphonate]	73207-98-4
Ethyl methylphosphonic acid (EMPA)	1832-53-7
Ethyldichloroarsine (ED)	598-14-1
N-Ethyldiethanolamine (EDEA)	139-87-7
Fenamiphos	22224-92-6
Fentanyl	437-38-7
Fluoroacetamide	640-19-7
Isopropyl methylphosphonic acid (IMPA)	1832-54-8
Methamidophos	10265-92-6
Methyl hydrazine	60-34-4
Methyl parathion	298-00-0
N-Methyldiethanolamine (MDEA)	105-59-9
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Methylphosphonic acid (MPA)	993-13-5
Mevinphos	7786-34-7
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
Organophosphate pesticides, NOS	NA
Parathion	56-38-2
Phencyclidine	77-10-1
Phenol	108-95-2
Phorate	298-02-2
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
Semivolatile Organic Compounds, NOS	NA
Soman (GD)	96-64-0

Analyte(s)	CAS RN
Strychnine	57-24-9
Tabun (GA)	77-81-6
Tear gas (CS) [chlorobenzylidene malonitrile]	2698-41-1
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Thiodiglycol (TDG)	111-48-8
Thiofanox	39196-18-4
1,4-Thioxane	15980-15-1
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

Analysis Purpose: Sample preparation
Sample Preparation Technique: PFE
Determinative Technique: 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.
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: Sodium sulfate can cause clogging, and air drying or palletized diatomaceous earth may be preferred.

Source: EPA. 1998. "Method 3545A (SW-846): Pressurized Fluid Extraction (PFE)," Revision 1. http://www.epa.gov/sam/pdfs/EPA-3545a.pdf

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

Analyte(s)	CAS RN
Acrylamide	79-06-1
Acrylonitrile	107-13-1
Aldicarb (Temik)	116-06-3
Aldicarb sulfone	1646-88-4
Aldicarb sulfoxide	1646-87-3
4-Aminopyridine	504-24-5
BZ (Quinuclidinyl benzilate)	6581-06-2
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
Carbofuran (Furadan)	1563-66-2
Chlorfenvinphos	470-90-6
3-Chloro-1,2-propanediol	96-24-2
Chloropicrin	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos	2921-88-2
Crimidine	535-89-7
Cyclohexyl sarin (GF)	329-99-7
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 sulfoxide	2497-07-6
1,4-Dithiane	505-29-3
EA2192 [Diisopropylaminoethyl methylthiolophosphonate]	73207-98-4
Ethylmethyl phosphonate (EMPA)	1832-53-7

Analyte(s)	CAS RN
N-Ethyldiethanolamine (EDEA)	139-87-7
Fenamiphos	22224-92-6
Fentanyl	437-38-7
Fluoroacetamide	640-19-7
Fluoroacetic acid and fluoroacetate salts	NA
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
Methamidophos	10265-92-6
Methomyl	16752-77-5
Methyl acrylonitrile	126-98-7
Methyl fluoroacetate	453-18-9
Methyl hydrazine	60-34-4
Methyl parathion	298-00-0
N-Methyldiethanolamine (MDEA)	105-59-9
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Methylphosphonic acid (MPA)	993-13-5
Mevinphos	7786-34-7
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
Mustard, sulfur / Mustard gas (HD)	505-60-2
Nicotine compounds	54-11-5
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0
Organophosphate pesticides, NOS	NA
Oxamyl	23135-22-0
Parathion	56-38-2
Pentaerythritol tetranitrate (PETN)	78-11-5

Analyte(s)	CAS RN
Phencyclidine	77-10-1
Phenol	108-95-2
Phorate	298-02-2
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
Sarin (GB)	107-44-8
Semivolatile Organic Compounds, NOS	NA
Soman (GD)	96-64-0
Strychnine	57-24-9
Tabun (GA)	77-81-6
Tear gas (CS) [chlorobenzylidene malonitrile]	2698-41-1
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Thiodiglycol (TDG)	111-48-8
Thiofanox	39196-18-4
1,4-Thioxane	15980-15-1
Triethanolamine (TEA)	102-71-6
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
VX [O-ethyl-S-(2-diisopropylaminoethyl)methyl-phosphonothiolate]	50782-69-9
White phosphorus	12185-10-3

Analysis Purpose: Sample preparation
Sample Preparation Technique: MSE

Determinative Technique: 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: 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 wipe samples.

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.

Source: EPA. 2002. "Method 3570 (SW-846): Microscale Solvent Extraction (MSE)," Revision 0. http://www.epa.gov/sam/pdfs/EPA-3570.pdf

5.2.23 EPA Method 3571 (SW-846): Extraction of Solid and Aqueous Samples for Chemical Agents

Analyte(s)	CAS RN
Mustard, sulfur / Mustard gas (HD)	505-60-2
Sarin (GB)	107-44-8
VX [O-ethyl-S-(2-diisopropylaminoethyl)methyl-phosphonothiolate]	50782-69-9

Analysis Purpose: Sample preparation
Sample Preparation Technique: Solvent microextraction
Determinative Technique: EPA SW-846 Method 8270D

Method Developed for: HD, GB, and VX in concrete, charcoal, wood, water, brine, ash, coral, sand, and soil
Method Selected for: SAM lists this method for preparation of solid, non-aqueous liquid/organic solid, aqueous liquid, and drinking water samples.

Description of Method: This method provides procedures for sample collection and extraction of the referenced compounds from solids and aqueous samples. A separate extract is required for each agent to be measured. Glacial acetic acid is added as a preservative to samples being assayed for GB and glacial acetic acid/sodium chloride is a preservative for samples assayed for HD. No preservative is added for VX. Samples are extracted with 10% isopropanol in dichloromethane by vortex mixing and filtered, if necessary. An optional water wash is included for VX that back-extracts the compound from heavy organics that could interfere with the assay. An optional column cleanup procedure is described to separate GB from heavy organics, if needed. Solvents are used to elute the extract first through the Carboprep90 column, then the silica column.

Source: EPA. 2007. "Method 3571 (SW-846): Extraction of Solid and Aqueous Samples for Chemical Agents," Revision 0. http://www.epa.gov/sam/pdfs/EPA-3571.pdf

5.2.24 EPA Method 3580A (SW-846): Waste Dilution

Analyte(s)	CAS RN
Brodifacoum	56073-10-0
Bromadiolone	28772-56-7
BZ (Quinuclidinyl benzilate)	6581-06-2
Chlorfenvinphos	470-90-6
3-Chloro-1,2-propanediol	96-24-2
Chloropicrin	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos	2921-88-2
Crimidine	535-89-7
Cyclohexyl sarin (GF)	329-99-7
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 sulfoxide	2497-07-6
1,4-Dithiane	505-29-3
EA2192 [Diisopropylaminoethyl methylthiolophosphonate]	73207-98-4
Ethyl methylphosphonic acid (EMPA)	1832-53-7
Ethyldichloroarsine (ED)	598-14-1
N-Ethyldiethanolamine (EDEA)	139-87-7
Fenamiphos	22224-92-6
Fentanyl	437-38-7

Analyte(s)	CAS RN
Fluoroacetamide	640-19-7
Isopropyl methylphosphonic acid (IMPA)	1832-54-8
Methamidophos	10265-92-6
Methyl hydrazine	60-34-4
Methyl parathion	298-00-0
N-Methyldiethanolamine (MDEA)	105-59-9
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Methylphosphonic acid (MPA)	993-13-5
Mevinphos	7786-34-7
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
Organophosphate pesticides, NOS	NA
Parathion	56-38-2
Phencyclidine	77-10-1
Phenol	108-95-2
Phorate	298-02-2
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
Semivolatile Organic Compounds, NOS	NA
Sodium azide (analyze for hydrazoic acid)	26628-22-8
Soman (GD)	96-64-0
Strychnine	57-24-9
Tabun (GA)	77-81-6
Tear gas (CS) [chlorobenzylidene malonitrile]	2698-41-1
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Thiodiglycol (TDG)	111-48-8

Analyte(s)	CAS RN
Thiofanox	39196-18-4
1,4-Thioxane	15980-15-1
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

Analysis Purpose: Sample preparation
Sample Preparation Technique: Waste dilution
Determinative Technique: 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 non-aqueous waste samples
Method Selected for: SAM lists this method for preparation of non-aqueous liquid/organic solid samples.

Description of Method: This method describes solvent dilution of a non-aqueous waste sample prior to cleanup and/or analysis. One gram of sample is weighed into a capped tube and the sample is diluted to 10.0 mL with an appropriate solvent.

Special Considerations: The method is designed for wastes that may contain organic chemicals at a concentration greater than 20,000 mg/kg and that are soluble in the dilution solvent.

Source: EPA. 1992. "Method 3580A (SW-846): Waste Dilution," Revision 1.
http://www.epa.gov/sam/pdfs/EPA-3580a.pdf

5.2.25 EPA Method 3585 (SW-846): Waste Dilution for Volatile Organics

Analyte(s)	CAS RN
Allyl alcohol	107-18-6
Carbon disulfide	75-15-0
2-Chloroethanol	107-07-3
Cyanogen chloride	506-77-4
1,2-Dichloroethane	107-06-2
Ethylene oxide	75-21-8
Gasoline Range Organics	NA
Kerosene	64742-81-0

Analyte(s)	CAS RN
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-Dithiane	505-29-3
1,4-Thioxane	15980-15-1

Analysis Purpose: Sample preparation
Sample Preparation Technique: Waste dilution
Determinative Technique: 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 non-aqueous waste
Method Selected for: SAM lists this method for preparation of non-aqueous liquid/organic solid samples.

Description of Method: This method describes solvent dilution of a non-aqueous waste sample prior to direct injection analysis. It is designed for use in conjunction with GC or GC-MS analysis of wastes that may contain organic chemicals at a concentration greater than 1 mg/kg and that are soluble in the dilution solvent. Highly contaminated or highly complex samples may be diluted prior to analysis for volatiles using direct injection. One gram of sample is weighed into a capped tube or volumetric flask. The sample is diluted to 2.0 to 10.0 mL with *n*-hexadecane or other appropriate solvent. Diluted samples are injected into the GC or GC-MS for analysis.

Special Considerations: For use in analysis of wastes that may contain organic chemicals at a concentration greater than 1 mg/kg and that are soluble in the dilution solvent.

Source: EPA. 1996. "Method 3585 (SW-846): Waste Dilution for Volatile Organics," Revision 0. http://www.epa.gov/sam/pdfs/EPA-3585.pdf

5.2.26 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
Cyanogen chloride	506-77-4
1,2-Dichloroethane	107-06-2
Ethylene oxide	75-21-8
Gasoline Range Organics	NA
Kerosene	64742-81-0
Propylene oxide	75-56-9

Analyte(s)	CAS RN
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-Dithiane	505-29-3
1,4-Thioxane	15980-15-1

Analysis Purpose: Sample preparation
Sample Preparation Technique: Purge and trap
Determinative Technique: 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.

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.

Source: EPA. 2003. "Method 5030C (SW-846): Purge-and-Trap for Aqueous Samples, Revision 3. http://www.epa.gov/sam/pdfs/EPA-5030c.pdf

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

Analyte(s)	CAS RN
Allyl alcohol	107-18-6
Carbon disulfide	75-15-0
2-Chloroethanol	107-07-3
Cyanogen chloride	506-77-4
1,2-Dichloroethane	107-06-2
Ethylene oxide	75-21-8
Gasoline Range Organics	NA
Kerosene	64742-81-0
Propylene oxide	75-56-9

Analyte(s)	CAS RN
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-Dithiane	505-29-3
1,4-Thioxane	15980-15-1

Analysis Purpose: Sample preparation
Sample Preparation Technique: Purge and trap
Determinative Technique: 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.

Description of Method: This method describes a closed-system purge-and-trap process for analysis of VOCs in solid samples containing low levels (0.5 to 200 µg/kg) of VOCs. The method also provides specific procedures for preparation of samples containing high levels (>200 µg/kg) of VOCs. For low-level VOCs, a 5-g sample is collected into a vial that is placed into an autosampler device. Reagent water, surrogates, and internal standards are added automatically, and the vial is heated to 40°C. The volatiles are purged into an appropriate trap using an inert gas combined with sample agitation. When purging is complete, the trap is heated and backflushed with helium to desorb the trapped sample components into a GC for analysis. For high-level VOCs, samples are 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://www.epa.gov/sam/pdfs/EPA-5035a.pdf

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

Analyte(s)	CAS RN
Ammonium metavanadate	7803-55-6
Arsenic, Total	740-38-2
Arsenic trioxide	1327-53-3
Calcium arsenate	7778-44-1
2-Chlorovinylarsonous acid (CVAA)	85090-33-1
Lead arsenate	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]	40334-70-1

Analyte(s)	CAS RN
Lewisite Oxide	1306-02-1
Osmium tetroxide	20816-12-0
Sodium arsenite	7784-46-5
Thallium sulfate	10031-59-1
Titanium tetrachloride	7550-45-0
Vanadium pentoxide	1314-62-1

Analysis Purpose: Analysis
Sample Preparation Technique: EPA SW-846 Method 3050B (solid samples), Method 3031 (non-aqueous liquid/organic solid samples), and NIOSH Method 9102 (wipe samples)
Determinative Technique: ICP-AES

Method Developed for: Trace elements in solution
Method Selected for: SAM lists this method for analysis of solid, non-aqueous liquid/organic solid, and wipe samples. Osmium tetroxide and titanium tetrachloride are not of concern in non-aqueous liquid/organic solid samples.
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, 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 Methods 3050B), and non-aqueous liquid/organic solid samples (prepared using SW-846 Methods 3031) are analyzed by ICP-AES.

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

Source: EPA. 2000. "Method 6010C (SW-846): Inductively Coupled Plasma-Atomic Emission Spectrometry," Revision 3. http://www.epa.gov/sam/pdfs/EPA-6010c.pdf

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

Analyte(s)	CAS RN
Ammonium metavanadate	7803-55-6
Arsenic, Total	740-38-2
Arsenic trioxide	1327-53-3
Calcium arsenate	7778-44-1
2-Chlorovinylarsonous acid (CVAA)	85090-33-1
Lead arsenate	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]	541-25-3

Analyte(s)	CAS RN
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]	40334-70-1
Lewisite oxide	1306-02-1
Sodium arsenite	7784-46-5
Thallium sulfate	10031-59-1
Titanium tetrachloride	7550-45-0
Vanadium pentoxide	1314-62-1

Analysis Purpose: Analysis
Sample Preparation Technique: EPA SW-846 Method 3050B (solid samples), Method 3031 (non-aqueous liquid/organic solid samples), and NIOSH Method 9102 (wipe samples)
Determinative Technique: ICP-MS

Method Developed for: Elements in water samples and in waste extracts or digests
Method Selected for: SAM lists this method for analysis of solid, non-aqueous liquid/organic solid, and wipe samples. Titanium tetrachloride is not of concern in non-aqueous liquid/organic solid samples.
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, 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. Aqueous samples (prepared using SW-846 Method 5050), soil samples (prepared using SW-846 Methods 3050B or 5050), non-aqueous liquid/organic solid samples (prepared using SW-846 Methods 3050B or 3031), and air filter/particle samples (prepared using IO Method 3.5) 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.

Source: EPA. 1998. "Method 6020A (SW-846): Inductively Coupled Plasma-Mass Spectrometry," Revision 1. http://www.epa.gov/sam/pdfs/EPA-6020a.pdf

5.2.30 EPA Method 7010 (SW-846): Graphite Furnace Atomic Absorption Spectrophotometry

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

Analysis Purpose: Analysis
Sample Preparation Technique: EPA SW-846 Method 3050B (solid samples) and NIOSH Method 9102 (wipe samples)
Determinative Technique: GFAA

Method Developed for: Metals in environmental samples including, but not limited to, ground water, domestic and industrial wastes, extracts, soils, sludges, sediments, and similar wastes
Method Selected for: SAM lists this method for analysis of solid and wipe samples.
Detection and Quantitation: Detection limits vary with each sample type and instrument used. The analytical range may be extended by sample dilution.

Description of Method: This method determines arsine as arsenic in environmental samples. Soil samples (prepared using SW-846 Method 3050B) are analyzed by GFAA. A representative aliquot of the sample is placed in the graphite tube in the furnace, evaporated to dryness, charred, and atomized.

Source: EPA. 1998. "Method 7010 (SW-846): Graphite Furnace Atomic Absorption Spectrophotometry," Revision 0. http://www.epa.gov/sam/pdfs/EPA-7010.pdf

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

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

Analysis Purpose: Sample preparation and/or analysis
Sample Preparation Technique: Acid digestion (solid and aqueous liquid samples) and 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 use if problems occur when using EPA SW-846 Method 7473 for these analytes during preparation and analysis of aqueous liquid samples. (See Footnote 13 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://www.epa.gov/sam/pdfs/EPA-7470a.pdf

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

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

Analysis Purpose: Sample preparation and/or analysis
Sample Preparation Technique: Acid digestion (solid and aqueous liquid samples) and 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 13 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://www.epa.gov/sam/pdfs/EPA-7471b.pdf

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

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

Analysis Purpose: Sample preparation and/or analysis
Sample Preparation Technique: Thermal decomposition (solid and aqueous liquid samples) and NIOSH Method 9102 (wipe samples)
Determinative Technique: 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, aqueous liquid, and wipe samples.
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 and 7470A (EPA SW-846) for aqueous liquid samples.

Source: EPA. 1998. "Method 7473 (SW-846): Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry," Revision 0. http://www.epa.gov/sam/pdfs/EPA-7473.pdf

5.2.34 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 analysis
Sample Preparation Technique: Solvent extraction (solid, non-aqueous liquid/organic solid, aqueous liquid, and drinking water samples) and 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, non-aqueous liquid/organic solid, aqueous liquid, drinking water, and wipe samples.
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 (USAEC 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.

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 an NPD. Wet soil or sediment samples are analyzed by extracting a 40 g wet-weight aliquot of the sample with a mixture of 10.0 mL degassed reagent water and 10.0 mL isooctane. The extraction is performed in a glass jar on a platform shaker for 18 hours. A 1.0 µL aliquot of the extract is analyzed by GC-NPD, providing sensitivity on the order of 1 µg/kg.

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://www.epa.gov/sam/pdfs/EPA-7580.pdf

5.2.35 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: Analysis
Sample Preparation Technique: EPA SW-846 Method 3545A/3541 or Method 5035A (solid samples), Method 3580A or Method 3585 (non-aqueous liquid/organic 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.
Determinative Technique: Gas chromatograph – Flame ionization detector (GC-FID)

Method Developed for: Various nonhalogenated VOCs and semivolatile organic compounds in water samples
Method Selected for: SAM lists this method for analysis of solid, non-aqueous liquid/organic solid, aqueous liquid, drinking water, and wipe samples.
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://www.epa.gov/sam/pdfs/EPA-8015c.pdf

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

Analyte(s)	CAS RN
Allyl alcohol	107-18-6
Carbon disulfide	75-15-0
2-Chloroethanol	107-07-3
Cyanogen chloride	506-77-4
1,2-Dichloroethane	107-06-2
Ethylene oxide	75-21-8
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-Dithiane	505-29-3
1,4-Thioxane	15980-15-1

Analysis Purpose: Analysis
Sample Preparation Technique: EPA SW-846 Method 5035A (solid samples), Method 3585 (non-aqueous liquid/organic solid samples), and Method 5030C (aqueous liquid and drinking water samples)
Determinative Technique: GC-MS

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, 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, non-aqueous liquid/organic solid, aqueous liquid, and drinking water samples. For carbon disulfide and 1,2-dichloroethane only, EPA Method 524.2 (rather than 8260C) should be used for analysis of drinking water samples.
Detection and Quantitation: Using standard quadrupole instrumentation and the purge-and-trap technique, estimated quantitation limits 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, estimated quantitation limits (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://www.epa.gov/sam/pdfs/EPA-8260c.pdf

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

Analyte(s)	CAS RN
Chlorfenvinphos	470-90-6
3-Chloro-1,2-propanediol[1]	96-24-2
Chloropicrin[2]	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos	2921-88-2
Crimidine[3]	535-89-7
Cyclohexyl sarin (GF)	329-99-7
Dichlorvos	62-73-7
Dicrotophos	141-66-2

Analyte(s)	CAS RN
Dimethylphosphite	868-85-9
Disulfoton	298-04-4
Disulfoton sulfoxide	2497-07-6
1,4-Dithiane[4]	505-29-3
Ethyldichloroarsine (ED)	598-14-1
Fenamiphos	22224-92-6
Fluoroacetamide	640-19-7
Methamidophos	10265-92-6
Methyl hydrazine	60-34-4
Methyl parathion	298-00-0
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Mevinphos	7786-34-7
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
Mustard, sulfur / Mustard gas (HD)[5]	505-60-2
Nicotine compounds	54-11-5
Organophosphate pesticides, NOS	NA
Parathion	56-38-2
Phencyclidine	77-10-1
Phenol	108-95-2
Phorate	298-02-2
Phosphamidon	13171-21-6
R 33 (VR) [methylphosphonothioic acid, S-[2-(diethylamino)ethyl] O-2-methylpropyl ester]	159939-87-4
Sarin (GB)[5]	107-44-8
Semivolatile Organic Compounds, NOS	NA
Soman (GD)	96-64-0
Strychnine	57-24-9
Tabun (GA)	77-81-6
Tear gas (CS) [chlorobenzylidene malonitrile]	2698-41-1

Analyte(s)	CAS RN
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine[2,6]	80-12-6
Thiodiglycol (TDG)	111-48-8
1,4-Thioxane[4]	15980-15-1
Trimethyl phosphite[2]	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
VX [O-ethyl-S-(2-diisopropylaminoethyl)methyl-phosphonothiolate][5]	50782-69-9
The following analyte should be determined by this method **only** if liquid chromatography-mass spectrometry (LC-MS) [electrospray] procedures are not available to the laboratory. Sample preparation methods should remain the same.	
BZ (Quinuclidinyl benzilate)[1]	6581-06-2
Diisopropyl methylphosphonate (DIMP)	1445-75-6
Dimethylphosphoramidic acid[1]	33876-51-6
EA2192 [Diisopropylaminoethyl methylthiolophosphonate][1]	73207-98-4
Ethylmethyl phosphonate (EMPA)[1]	1832-53-7
Isopropyl methylphosphonic acid (IMPA)[1]	1832-54-8
Methylphosphonic acid (MPA)[1]	993-13-5
Pinacolyl methyl phosphonic acid (PMPA)[1]	616-52-4

[1] For this analyte, SW-846 Method 8270D must be modified to include a derivatization step.
[2] If proplems occur with analyses, lower the injection temperature.
[3] If problems occur when using this method, it is recommended that SW-846 Method 8321B be used. Sample preparation methods should remain the same.
[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, Method 3585 for non-aqueous liquid/organic solid samples, and Method 5030C for aqueous liquid and drinking water samples) be used.
[5] For this analyte, refer to EPA SW-846 Method 8271 for GC-MS conditions.
[6] This analyte may require selective ion monitoring (SIM) analyses.

Analysis Purpose: Analysis
Sample Preparation Technique: EPA SW-846 Method 3545A/3541 (solid samples), Method 3580A (non-aqueous liquid/organic solid samples), Method 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.
Determinative Technique: GC-MS

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, non-aqueous liquid/organic solid, aqueous liquid, drinking water, and/or wipe samples. *Please note*: drinking water samples for dichlorvos, disulfoton, disulfoton sulfoxide, fenamiphos, mevinphos, and semivolatile organic compounds NOS should be prepared and analyzed by EPA Method 525.2; aqueous liquid and drinking water samples for organophosphate pesticides NOS should be prepared and analyzed by EPA Methods 614 and 507, respectively; aqueous liquid and drinking water samples for chloropicrin should be prepared and analyzed by EPA Method 551.1; all other analyte/sample type combinations should be analyzed by this method (SW-846 8270D).
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. For the determination of 3-chloro-1,2-propanediol, dimethylphosphoramidic acid, EA2192, EMPA, IMPA, MPA, and pinacolyl methyl phosphonic acid, a derivatization step is required prior to injection into the GC-MS. The phosphonic acids require derivatization with a trimethylsilyl agent and 3-chloro-1,2-propanediol requires derivatization with a heptafluorobutyryl agent.

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://www.epa.gov/sam/pdfs/EPA-8270d.pdf

5.2.38 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
Carbofuran (Furadan)	1563-66-2

Analyte(s)	CAS RN
Chlorfenvinphos	470-90-6
3-Chloro-1,2-propanediol	96-24-2
Chloropicrin	76-06-2
Chlorosarin	1445-76-7
Chlorosoman	7040-57-5
Chlorpyrifos	2921-88-2
Crimidine	535-89-7
Cyclohexyl sarin (GF)	329-99-7
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 sulfoxide	2497-07-6
1,4-Dithiane	505-29-3
EA2192 [Diisopropylaminoethyl methylthiolophosphonate]	73207-98-4
Ethylmethyl phosphonate (EMPA)	1832-53-7
N-Ethyldiethanolamine (EDEA)	139-87-7
Fenamiphos	22224-92-6
Fentanyl	437-38-7
Fluoroacetamide	640-19-7
Fluoroacetic acid and fluoroacetate salts	NA
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

Analyte(s)	CAS RN
Methamidophos	10265-92-6
Methomyl	16752-77-5
Methyl acrylonitrile	126-98-7
Methyl fluoroacetate	453-18-9
Methyl hydrazine	60-34-4
Methyl parathion	298-00-0
N-Methyldiethanolamine (MDEA)	105-59-9
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3
Methylphosphonic acid (MPA)	993-13-5
Mevinphos	7786-34-7
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
Mustard, sulfur / Mustard gas (HD)	505-60-2
Nicotine compounds	54-11-5
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX)	2691-41-0
Organophosphate pesticides, NOS	NA
Oxamyl	23135-22-0
Parathion	56-38-2
Pentaerythritol tetranitrate (PETN)	78-11-5
Phencyclidine	77-10-1
Phenol	108-95-2
Phorate	298-02-2
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
Sarin (GB)	107-44-8
Semivolatile Organic Compounds, NOS	NA
Soman (GD)	96-64-0
Strychnine	57-24-9

Analyte(s)	CAS RN
Tabun (GA)	77-81-6
Tear gas (CS) [chlorobenzylidene malonitrile]	2698-41-1
Tetraethyl pyrophosphate	107-49-3
Tetramethylenedisulfotetramine	80-12-6
Thiodiglycol (TDG)	111-48-8
Thiofanox	39196-18-4
1,4-Thioxane	15980-15-1
Triethanolamine (TEA)	102-71-6
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
VX [O-ethyl-S-(2-diisopropylaminoethyl)methyl phosphonothiolate]	50782-69-9
White phosphorus	12185-10-3

Analysis Purpose: Sample preparation
Sample Preparation Technique: Solvent extraction
Determinative Technique: 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.

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

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

Source: 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://www.epa.gov/sam/pdfs/EPA-8290a.pdf

5.2.39 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 analysis
Sample Preparation Technique: Solvent extraction (solid, aqueous liquid, and drinking water samples) and 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, drinking water, and wipe samples.
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://www.epa.gov/sam/pdfs/EPA-8315a.pdf

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

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

Analysis Purpose: Sample preparation and/or analysis
Sample Preparation Technique: Direct injection (aqueous liquid and drinking water samples), water extraction (solid and non-aqueous liquid/organic solid samples), and 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, non-aqueous liquid/organic solid, aqueous liquid, drinking water, and wipe samples.
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 and non-aqueous liquid/organic solid samples should be water extracted prior to injection. Aqueous liquid and drinking water samples can be directly injected.

Source: EPA. 1994. "Method 8316 (SW-846): Acrylamide, Acrylonitrile and Acrolein by High Performance Liquid Chromatography (HPLC)," Revision 0. http://www.epa.gov/sam/pdfs/EPA-8316.pdf

5.2.41 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 analysis
Sample Preparation Technique: Solvent extraction (solid, non-aqueous liquid/organic solid, and aqueous liquid samples), and 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, non-aqueous liquid/organic solid, aqueous liquid, and wipe samples.
Detection and Quantitation: The estimated MDLs vary with each analyte and range from 1.7 to 9.4 µg/L for aqueous liquid 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.

Source: EPA. 2000. "Method 8318A (SW-846): N-Methylcarbamates by High Performance Liquid Chromatography (HPLC)," Revision 1. http://www.epa.gov/sam/pdfs/EPA-8318a.pdf

5.2.42 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)[1]	6581-06-2
Diisopropyl methylphosphonate (DIMP)[2]	1445-75-6
Dimethylphosphoramidic acid[1]	33876-51-6
Diphacinone	82-66-6
EA2192 [Diisopropylaminoethyl methylthiolophosphonate][1]	73207-98-4
Ethylmethyl phosphonate (EMPA)[1]	1832-53-7
N-Ethyldiethanolamine (EDEA)	139-87-7
Fentanyl	437-38-7
Isopropyl methylphosphonic acid (IMPA)[1]	1832-54-8
N-Methyldiethanolamine (MDEA)	105-59-9
Methylphosphonic acid (MPA)[1]	993-13-5
Pinacolyl methyl phosphonic acid (PMPA)[1]	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[3]	535-89-7

[1] LC-MS (electrospray) procedures are preferred for these analytes; however, if this technique is not available to the laboratory, GC-MS procedures using derivatization based on SW-846 Method 8270D may be used. Sample preparation methods should remain the same. Both electrospray LC-MS and GC-MS derivatization procedures are currently under development.

[2] If problems occur with the analysis of DIMP using EPA SW-846 Method 8321B, use SW-846 Method 8270D.

[3] This analyte needs to be determined using a wavelength of 230 nm.

Analysis Purpose: Analysis

Sample Preparation Technique: EPA SW-846 Method 3545A/3541 (solid samples), 3580A (non-aqueous liquid/organic solid samples), 3520C/3535A (aqueous liquid and drinking water samples), and Method 3570/8290A Appendix A (wipe samples). For thiofanox, EPA Method 531.2 (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.

Determinative Technique: HPLC

Method Developed for: Solvent-extractable nonvolatile compounds, including dyes, organophosphorus compounds, phenoxyacid herbicides, and carbamates in solid, water, aqueous, and non-aqueous samples
Method Selected for: SAM lists this method for analysis of solid, non-aqueous liquid/organic solid, aqueous liquid, drinking water, and wipe samples.

Description of Method: This method provides reversed-phase HPLC, thermospray (TSP) MS, and UV conditions for detection of the target analytes. Sample extracts can be analyzed by direct injection into the TSP or onto a LC-TSP interface. A gradient elution program is used to separate the compounds. Primary analysis may be performed by UV detection; however, positive results should be confirmed by TSP-MS. Quantitative analysis may be performed by either TSP-MS or UV detection, using either an external or internal standard approach. TSP-MS detection may be performed in either a negative ionization (discharge electrode) mode or a positive ionization mode, with a single quadrupole MS. The use of MS-MS techniques is an option. The analytical range and detection limits vary depending on the target analyte and instrument used.

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 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://www.epa.gov/sam/pdfs/EPA-8321b.pdf

5.2.43 EPA Method 8330B (SW-846): Nitroaromatics and Nitramines 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
Pentaerythritoltetranitrate (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 analysis
Sample Preparation Technique: Solvent extraction or direct injection (solid and non-aqueous liquid/organic solid samples), EPA SW-846 Method 3535A (aqueous liquid and drinking water samples), and EPA SW-846 Method 3570/8290A Appendix A (wipe samples)
Determinative Technique: HPLC

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, non-aqueous liquid/organic solid, aqueous liquid, drinking water, and wipe samples. Aqueous liquid and drinking water samples are prepared using Methods 3535A or 8330B prior to analysis.
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) using the appropriate sample preparation technique (solid phase extraction by 3535A or solvent extraction by 8330B) and, if necessary, sample cleanup procedures. Method 8330 provides a salting-out extraction procedure for low concentration (ppt or ng/L) of explosives residues in surface or ground water. 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://www.epa.gov/sam/pdfs/EPA-8330b.pdf

5.2.44 EPA ILM05.3 Cyanide: Analytical Methods for Total Cyanide Analysis

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

Analysis Purpose: Sample preparation and/or analysis
Sample Preparation Technique: Acid digestion followed by distillation
Determinative Technique: Spectrophotometry

Method Developed for: Metals in water, sediment, sludge, and soil
Method Selected for: SAM lists this method for preparation and/or analysis of solid, aqueous liquid, and wipe samples.
Detection and Quantitation: The method quantitation limits are 10 µg/L or 2.5 mg/kg

Description of Method: The method allows for either large volume (500-mL aqueous liquid samples or 1-g to 5-g solid samples mixed with 500 mL of reagent water) or medium volume (50-mL aqueous liquid samples or 1-g solid samples mixed with 50 mL of reagent water) sample preparation. Aqueous liquid samples are tested for sulfides and oxidizing agents prior to preparation. Sulfides are removed with cadmium carbonate or lead carbonate. Samples are treated with sulfuric acid and magnesium chloride and distilled into a sodium hydroxide solution. The solution is treated with color agents and the cyanide determined as an ion complex by visible spectrophotometry.

Special Considerations: Surfactants may interfere with the distillation procedure.

Source: EPA. "ILM05.3: Exhibit D – Part D: Analytical Methods for Total Cyanide Analysis." http://www.epa.gov/sam/pdfs/EPA-ILM05.3.pdf

5.2.45 EPA Region 7 RLAB Method 3135.2I: Cyanide, Total and Amenable in Aqueous and Solid Samples Automated Colorimetric with Manual Digestion

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

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Acid digestion followed by distillation

Determinative Technique: Spectrophotometry

Method Developed for: Cyanide in drinking, ground, and surface waters, domestic and industrial waste waters, sediments and solid waste
Method Selected for: SAM lists this method for preparation and analysis of solid, aqueous liquid, drinking water, and wipe samples.
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 Region 7. 2008. "RLAB Method 3135.2I: Cyanide, Total and Amenable in Aqueous and Soil Samples Automated Colorimetric with Manual Digestion." http://www.epa.gov/sam/pdfs/EPA-3135.2I.pdf

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

Analyte(s)	CAS RN
Ammonium metavanadate	7803-55-6
Arsenic, Total	740-38-2
Arsenic trioxide	1327-53-3
Calcium arsenate	7778-44-1
2-Chlorovinylarsonous acid (CVAA)	85090-33-1
Lead arsenate	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]	40334-70-1
Lewisite Oxide	1306-02-1
Osmium tetroxide	20816-12-0
Sodium arsenite	7784-46-5

Analyte(s)	CAS RN
Thallium sulfate	10031-59-1
Vanadium pentoxide	1314-62-1

Analysis Purpose: Sample preparation
Sample Preparation Technique: Acid extraction
Determinative Technique: 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.

Description of Method: This method supports determination of arsenic trioxide, lewisite compounds, lewisite oxide, CVAA, 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://www.epa.gov/sam/pdfs/EPA-IO-3.1.pdf

5.2.47 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	7803-55-6
Arsenic, Total	740-38-2
Arsenic trioxide	1327-53-3
Calcium arsenate	7778-44-1
2-Chlorovinylarsonous acid (CVAA)	85090-33-1
Lead arsenate	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]	40334-70-1
Lewisite Oxide	1306-02-1
Osmium tetroxide	20816-12-0
Sodium arsenite	7784-46-5
Thallium sulfate	10031-59-1
Vanadium pentoxide	1314-62-1

Analysis Purpose: Analysis
Sample Preparation Technique: EPA Method IO-3.1
Determinative Technique: ICP-AES

Method Developed for: Metals in ambient particulate matter
Method Selected for: SAM lists this method for analysis of air samples.

Description of Method: This method determines arsenic trioxide, lewisite, lewisite degradation products, 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. The extracts are analyzed by ICP-AES or ICP-MS (see Method IO-3.5 in Section 5.2.48). 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-3.4: 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." http://www.epa.gov/sam/pdfs/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." http://www.epa.gov/sam/pdfs/EPA-IO-2.1.pdf

5.2.48 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	7803-55-6
Arsenic, Total	740-38-2
Arsenic trioxide	1327-53-3
Calcium arsenate	7778-44-1
2-Chlorovinylarsonous acid (CVAA)	85090-33-1
Lead arsenate	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]	40334-70-1
Lewisite Oxide	1306-02-1
Sodium arsenite	7784-46-5
Thallium sulfate	10031-59-1
Vanadium pentoxide	1314-62-1

Analysis Purpose: Analysis
Sample Preparation Technique: EPA Method IO-3.1
Determinative Technique: ICP-MS

Method Developed for: Metals in ambient particulate matter
Method Selected for: SAM lists this method for analysis of air samples.

Description of Method: This method determines arsenic trioxide, lewisite, lewisite degradation products, 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 and the extracts analyzed by ICP-MS or ICP-AES (see Method IO-3.4 in Section 5.2.47). 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)." http://www.epa.gov/sam/pdfs/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." http://www.epa.gov/sam/pdfs/EPA-IO-2.1.pdf

5.2.49 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	151-38-2

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Acid digestion for particulate mercury
Determinative Technique: 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.
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/min. The traps are directly desorbed onto a second (analytical) trap. The mercury desorbed from the analytical trap is determined by Atomic Fluorescence Spectrometry. Particulate mercury is sampled on glass-fiber filters at a flow rate of 30 L/min. 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)." http://www.epa.gov/sam/pdfs/EPA-IO-5.pdf

5.2.50 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[1,2]	96-24-2
Chlorosarin[2]	1445-76-7
Chlorosoman[2]	7040-57-5
Chlorpyrifos	2921-88-2
Cyclohexyl sarin (GF)	329-99-7
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 [Diisopropylaminoethyl methylthiolophosphonate][1]	73207-98-4
Ethyl methylphosphonic acid (EMPA)[1]	1832-53-7
N-Ethyldiethanolamine (EDEA)	139-87-7
Fenamiphos	22224-92-6
Fentanyl	437-38-7
Fluoroacetamide	640-19-7
Isopropyl methylphosphonic acid (IMPA)[1]	1832-54-8
Methamidophos[3]	10265-92-6
Methyl parathion	298-00-0
N-Methyldiethanolamine (MDEA)	105-59-9

Analyte(s)	CAS RN
1-Methylethyl ester ethylphosphonofluoridic acid (GE)[2]	1189-87-3
Methylphosphonic acid (MPA)[1]	993-13-5
Mevinphos	7786-34-7
Mustard, nitrogen (HN-1) [bis(2-chloroethyl)ethylamine]	538-07-8
Mustard, nitrogen (HN-2) [2,2'-dichloro-N-methyldiethylamine N,N-bis(2-chloroethyl)methylamine]	51-75-2
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1
Mustard, sulfur / Mustard gas (HD)	505-60-2
Parathion	56-38-2
Phencyclidine	77-10-1
Phenol	108-95-2
Phorate	298-02-2
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
Sarin (GB)[2]	107-44-8
Semivolatile Organic Compounds, NOS	NA
Soman (GD)[2]	96-64-0
Tabun (GA)	77-81-6
Tear gas (CS) [chlorobenzylidene malonitrile]	2698-41-1
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
VX [O-ethyl-S-(2-diisopropylaminoethyl)methyl-phosphonothiolate]	50782-69-9

Analyte(s)	CAS RN
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.
[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, it is recommended that NIOSH Method 5600 be used.

Analysis Purpose: Sample preparation and analysis
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.
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.

Source: EPA. 1999. "Air Method, Toxic Organics-10A (TO-10A): Compendium of Methods for the Determination of Inorganic 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)." http://www.epa.gov/sam/pdfs/EPA-TO-10a.pdf

5.2.51 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

Analyte(s)	CAS RN
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
Sarin (GB)	107-44-8
Soman (GD)	96-64-0

Analysis Purpose: Sample preparation and analysis
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.
Detection and Quantitation: This method applies to ambient concentrations of VOCs above 0.5 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 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. For 3-chloro-1,2-propanediol, Method TO-15 must be modified to include a derivatization step.

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)." http://www.epa.gov/sam/pdfs/EPA-TO-15.pdf

5.2.52 NIOSH Method 1612: Propylene Oxide

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

Analysis Purpose: Sample preparation and analysis
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.
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/min. One milliliter of carbon disulfide is added to the vial and allowed to sit for 30 minutes prior to analysis with occasional agitation. 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://www.epa.gov/sam/pdfs/NIOSH-1612.pdf

5.2.53 NIOSH Method 2016: Formaldehyde

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

Analysis Purpose: Sample preparation and analysis
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.
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/min. 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://www.epa.gov/sam/pdfs/NIOSH-2016.pdf

5.2.54 NIOSH Method 2513: Ethylene Chlorohydrin

Analyte(s)	CAS RN
2-Chloroethanol (ethylene chlorohydrin)	107-07-3

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Solvent desorption
Determinative Technique: GC-FID

Method Developed for: Ethylene chlorohydrin in air
Method Selected for: SAM lists this method for preparation and analysis of air samples.
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/min 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://www.epa.gov/sam/pdfs/NIOSH-2513.pdf

5.2.55 NIOSH Method 3510: Monomethylhydrazine

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

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Samples are collected into a bubbler containing hydrochloric acid.
Determinative Technique: Spectrophotometry

Method Developed for: Monomethylhydrazine in air
Method Selected for: SAM lists this method for preparation and analysis of air samples.
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/min. Samples are then mixed with phosphomolybdic acid solution and transferred to a large test tube for spectrophotometric analysis. Positive interferences that have been noted include 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://www.epa.gov/sam/pdfs/NIOSH-3510.pdf

5.2.56 NIOSH Method 5600: Organophosphorus Pesticides

Analyte(s)	CAS RN
Disulfoton	298-04-4
Disulfoton sulfoxide	2497-07-6
Organophosphate pesticides, NOS	NA
The following analyte should be determined by this method **only** if problems (e.g., insufficient recovery, interferences) occur when using Method TO-10A.	
Methamidophos	10265-92-6

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Solvent desorption
Determinative Technique: GC-FPD

Method Developed for: Organophosphorus pesticides in air
Method Selected for: SAM lists this method for preparation and analysis of air samples.
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 FPD. 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: 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://www.epa.gov/sam/pdfs/NIOSH-5600.pdf

5.2.57 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

Analyte(s)	CAS RN
Thiofanox	39196-18-4

Analysis Purpose: Sample preparation and analysis
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.
Detection and Quantitation: The detection limit for aldicarb is 1.2 µg per sample and 0.6 µg per sample for carbofuran, methyomyl, 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. 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 (betylated 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 GC column or by an MS.

Source: NIOSH. 1998. "Method 5601: Organonitrogen Pesticides," Issue 1.
http://www.epa.gov/sam/pdfs/NIOSH-5601.pdf

5.2.58 NIOSH Method 6001: Arsine

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

Analysis Purpose: Sample preparation and analysis
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.
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://www.epa.gov/sam/pdfs/NIOSH-6001.pdf

5.2.59 NIOSH Method 6002: Phosphine

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

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Solvent desorption with hot acidic permanganate solution
Determinative Technique: Spectrophotometry

Method Developed for: Phosphine in air
Method Selected for: SAM lists this method for preparation and analysis of air samples.
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://www.epa.gov/sam/pdfs/NIOSH-6002.pdf

5.2.60 NIOSH Method 6004: Sulfur Dioxide

Analyte(s)	CAS RN
Sulfur dioxide	7446-09-5

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Treated filter extracted with carbonate/bicarbonate solution
Determinative Technique: IC

Method Developed for: Sulfur dioxide in air
Method Selected for: SAM lists this method for preparation and analysis of air samples.
Detection and Quantitation: The working range of the method is 0.5 to 20 mg/m^3 for a 100-L sample.

Description of Method: In this method, sulfur dioxide is determined as sulfite plus sulfate. A volume of 40 to 200 L of air is drawn through a sodium carbonate-treated filter that is preceded by a 0.8 µm filter to remove particulates and sulfuric acid. The treated filter is extracted with a carbonate/bicarbonate solution and the extract analyzed by IC for sulfite and sulfate. The sulfur dioxide is present as sulfite on the filter; however, because sulfite oxidizes to sulfate, both ions must be determined and the results summed.

Special Considerations: The method is subject to interference from sulfur trioxide in dry conditions.

Source: NIOSH. 1994. "Method 6004: Sulfur Dioxide," Issue 2.
http://www.epa.gov/sam/pdfs/NIOSH-6004.pdf

5.2.61 NIOSH Method 6010: Hydrogen Cyanide

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

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Solvent desorption
Determinative Technique: Spectrophotometry

Method Developed for: Hydrogen cyanide in air
Method Selected for: SAM lists this method for preparation and analysis of air samples.
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 2 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.

Source: NIOSH. 1994. "Method 6010: Hydrogen Cyanide," Issue 2.
http://www.epa.gov/sam/pdfs/NIOSH-6010.pdf

5.2.62 NIOSH Method 6013: Hydrogen Sulfide

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

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Solvent extraction
Determinative Technique: IC

Method Developed for: Hydrogen sulfide in air
Method Selected for: SAM lists this method for preparation and analysis of air samples.
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 1.2 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://www.epa.gov/sam/pdfs/NIOSH-6013.pdf

5.2.63 NIOSH Method 6015: Ammonia

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

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Water extraction
Determinative Technique: Spectrophotometry

Method Developed for: Ammonia in air
Method Selected for: SAM lists this method for preparation and analysis of air samples.
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 silca 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://www.epa.gov/sam/pdfs/NIOSH-6015.pdf

5.2.64 NIOSH Method 6402: Phosphorus Trichloride

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

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Add reagent to samples in bubbler solution and heat
Determinative Technique: Spectrophotometry

Method Developed for: Phosphorus trichloride in air

Method Selected for: SAM lists this method for preparation and analysis of air samples.
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 to phosphoric acid and color agents are added. The solution is analyzed by visible spectrophotometry. Phosphorus (V) compounds do not interfere. The sample solutions are stable to oxidation by air during sampling.

Source: NIOSH. 1994. "Method 6402: Phosphorus Trichloride," Issue 2.
http://www.epa.gov/sam/pdfs/NIOSH-6402.pdf

5.2.65 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 analysis
Sample Preparation Technique: Solvent desorption
Determinative Technique: IC

Method Developed for: Inorganic acids in air
Method Selected for: SAM lists this method for preparation and analysis of air samples.
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://www.epa.gov/sam/pdfs/NIOSH-7903.pdf

5.2.66 NIOSH Method 7905: Phosphorus

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

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: GC solid sorbent tube and solvent extracted (desorbed)
Determinative Technique: GC-FPD or GC-NPD

Method Developed for: Phosphorus in air
Method Selected for: SAM lists this method for preparation and analysis of air samples.
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. Samples prepared by this method can be analyzed alternatively by GC-NPD. Five to 100 L of air is drawn through a GC solid sorbent tube, and the sorbent is extracted (desorbed) with xylene. Phosphorus is determined by GC-FPD or GC-NPD. 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://www.epa.gov/sam/pdfs/NIOSH-7905.pdf

5.2.67 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

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 11 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://www.epa.gov/sam/pdfs/NIOSH-7906.pdf

5.2.68 NIOSH Method 9102: Elements on Wipes

Analyte(s)	CAS RN
Ammonium metavanadate	7803-55-6
Arsenic, Total	740-38-2
Arsenic trioxide	1327-53-3
Arsine	7784-42-1
Calcium arsenate	7778-44-1
2-Chlorovinylarsonous acid (CVAA)	85090-33-1
Ethyldichloroarsine (ED)	598-14-1
Lead arsenate	7645-25-2
Lewisite 1 (L-1) [2-chlorovinyldichloroarsine]	541-25-3
Lewisite 2 (L-2) [bis(2-chlorovinyl)chloroarsine]	40334-69-8
Lewisite 3 (L-3) [tris(2-chlorovinyl)arsine]	40334-70-1

Analyte(s)	CAS RN
Lewisite Oxide	1306-02-1
Mercury, Total	7439-97-6
Methoxyethylmercuric acetate	151-38-2
Osmium tetroxide	20816-12-0
Sodium arsenite	7784-46-5
Thallium sulfate	10031-59-1
Titanium tetrachloride	7550-45-0
Vanadium pentoxide	1314-62-1

Analysis Purpose: Sample preparation
Sample Preparation Technique: Acid digestion
Determinative Technique: EPA SW-846 Methods 6010C, 6020A, 7010, 7473, and 8270D. 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.
Detection and Quantitation: The range for arsenic is 0.261–105 µg/wipe; for thallium 0.136–50.0 µg/wipe; for vanadium 0.0333–25.0 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.

Source: NIOSH. 2003. "Method 9102, Issue 1: Elements on Wipes."
http://www.epa.gov/sam/pdfs/NIOSH-9102.pdf

5.2.69 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: EPA Method 300.1 Rev 1.0

Method Developed for: Fluoroacetate anion in air
Method Selected for: SAM lists this method for preparation of air samples.

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 IC using electrolytic conductivity detection.

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://www.epa.gov/sam/pdfs/NIOSH-S301-1.pdf

5.2.70 OSHA Method 40: Methylamine

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

Analysis Purpose: Sample preparation and analysis
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.
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/min 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://www.epa.gov/sam/pdfs/OSHA-Method40.pdf

5.2.71 OSHA Method 54: Methyl Isocyanate (MIC)

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

Analysis Purpose: Sample preparation and analysis

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.

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. http://www.epa.gov/sam/pdfs/OSHA-Method54.pdf

5.2.72 OSHA Method 61: Phosgene

Analyte(s)	CAS RN
Perfluoroisobutylene (PFIB)	382-21-8
Phosgene	75-44-5

Analysis Purpose: Sample preparation and analysis
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.

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 PFIB and phosgene should be confirmed by either a secondary GC column or by an MS. If problems occur when using this method for determination of PFIB, it is recommended that a method based on the following journal article be used: Journal of Chromatography A. 1098: (2005) 156–165.

Source: OSHA. 1986. "Method 61: Phosgene." Method originally obtained from www.osha.gov, but is provided here for reference. http://www.epa.gov/sam/pdfs/OSHA-Method61.pdf

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

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

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Buffer desorption

Determinative Technique: IC

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.
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 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://www.epa.gov/sam/pdfs/OSHA-ID-211.pdf

5.2.74 OSHA Method ID216SG: Boron Trifluoride (BF_3)

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

Analysis Purpose: Sample preparation and analysis
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.
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://www.epa.gov/sam/pdfs/OSHA-ID216SG.pdf

5.2.75 OSHA Method PV2004: Acrylamide

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

Analysis Purpose: Sample preparation and analysis
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.
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://www.epa.gov/sam/pdfs/OSHA-PV2004.pdf

5.2.76 OSHA Method PV2103: Chloropicrin

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

Analysis Purpose: Sample preparation and analysis
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.
Detection and Quantitation: The detection limit is 0.01 ng, with a 1-µL injection volume. This is the smallest amount that could be detected under normal operating conditions. The working range is 33.2–1330 µg/m^3.

Description of Method: This method determines the concentration of chloropicrin in air by GC-ECD. Samples are collected by drawing a known volume of air through two 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://www.epa.gov/sam/pdfs/OSHA-PV2103.pdf

5.2.77 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 analysis
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.

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

5.2.78 ASTM Method D6480-99: 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 analysis
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.

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 (ED) and EDXA at a magnification from 15,000 to 20,000X.

Source: ASTM. 1999. "Method D6480-99: 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/DATABASE.CART/HISTORICAL/D6480-99.htm

5.2.79 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 analysis
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.
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

5.2.80 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: 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.

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/

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

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

Analysis Purpose: Analysis
Sample Preparation Technique: Standard Method 4500-NH_3 B
Determinative Technique: Spectrophotometry

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.
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/

5.2.82 Standard Method 4500-Cl G: DPD Colorimetric Method

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

Analysis Purpose: Sample preparation and/or analysis
Sample Preparation Technique: Water samples are buffered and colorimetric agent is added. Procedures in Analyst, 1999. 124: 1853–1857 are used for preparation of air samples.
Determinative Technique: 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.
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/

5.2.83 Literature Reference for Chlorine (Analyst, 1999. 124: 1853–1857)

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

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

Method Developed for: Active chlorine in air
Method Selected for: SAM lists this procedure for preparation of air samples.
Detection and Quantitation: Detection limit of 0.1 mg 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 mg of chlorine. At flow rates ranging from 31 to 294 mL/min, 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 spectophotometric analysis." Analyst. 124(12): 1853–1857. www.epa.gov/sam/pdfs/Analyst124_pg1853-1857.pdf

5.2.84 Literature Reference for Fluoroacetate salts (Analytical Letters, 1994. 27 (14): 2703–2718)

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

Analysis Purpose: Sample preparation
Sample Preparation Technique: Ultrasonic extraction
Determinative Technique: EPA Method 300.1, Revision 1.0

Method Developed for: Sodium fluoroacetate in soil
Method Selected for: SAM lists this procedure for preparation of solid and non-aqueous liquid/organic solid samples.

Description of Method: Sodium fluoroacetate is determined at sub-microgram per gram concentrations in small (~1 g) soil samples. Samples are ultrasonically extracted with water, filtered, and analyzed by Method 300.1.

Source: Tomkins, B.A. 1994. "Screening-Procedure for Sodium Fluoroacetate (Compound 1080) at Sub-Microgram/Gram Concentrations in Soils." Analytical Letters. 27(14): 2703–2718. http://www.informaworld.com/smpp/content~content=a747219004~db=all~order=page

5.2.85 Literature Reference for Perfluoroisobutylene (Journal of Chromatography A, 2005. 1098: 156–165)

Analyte(s)	CAS RN
Perfluoroisobutylene (PFIB)	382-21-8

Analysis Purpose: Sample preparation and analysis
Sample Preparation Technique: Thermal desorption
Determinative Technique: GC-FPD / GC-NPD / GC-MS

Method Developed for: Phosgene and PFIB in air
Method Selected for: SAM lists this method for use if problems occur when using OSHA Method 61 for the analysis of perfluoroisobutylene during preparation and analysis of air samples. (See Footnote 16 of Appendix A.)
Detection and Quantitation: Limits of detection for PFIB with 2-aminothiophenol (PFIB-ATP) and PFIB with 3,4-dimercaptotoluene (PFIB-DMT), using 10-L air samples (typical sampling volume) by GC-MS analyses were determined to be 2 and 19 ng/m^3, respectively.

Description of Method: This procedure is for the identification and measurement of phosgene and perfluoroisobutylene in air using GC-MS, GC-NPD, or GC-FPD. This procedure is to be used in the event that OSHA Method 61 is problematic for the determination of PFIB. Air samples are collected by drawing known volumes of air through sampling tubes containing Tenax® TA sorbent coated with 2-aminothiophenol (ATP) or 3,4-dimercaptotoluene (DMT). The derivatized samples are thermally desorbed and analyzed with one of the GC techniques. The 2-aminothiophenol derivative can be analyzed by either of the three techniques, whereas the 3,4-dimercaptotoluene is not suitable for analysis by GC-NPD or GC-FPD.

Special Considerations: If a determinative technique other than GC-MS is used, the presence of PFIB should be confirmed by either a secondary GC column or by an MS.

Source: Muir, B., Cooper, D.B., Carric, W.A., Timperley, C.M., Slater, B.J., and Quick, S. 2005. "Analysis of Chemical Warfare Agents III. Use of bis-Nucleophiles in the Trace Level Determination of Phosgene and Perfluoroisobutylene." Journal of Chromatography A. 1098: 156–165. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6TG8-4H87GFP-2&_user=10&_coverDate=12%2F09%2F2005&_rdoc=22&_fmt=high&_orig=browse&_srch=doc-info(%23toc%235248%232005%23989019998%23611985%23FLA%23display%23Volume)&_cdi=5248&_sort=d&_docanchor=&_ct=30&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=f94d098b299600e1c42bf46c6b6e71ac

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

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

Analysis Purpose: Sample preparation
Sample Preparation Technique: Water extraction, filtration, and/or acidification
Determinative Technique: 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, non-aqueous liquid/organic solid, aqueous liquid, and drinking water samples.
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. An acidification step may be required prior to using EPA SW-846 Method 3580A for preparation of non-aqueous liquid/organic solid 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://journalsip.astm.org/JOURNALS/FORENSIC/PAGES/2933.htm

Section 6.0: Selected Radiochemical Methods

A list of analytical methods to be used in analyzing environmental samples for radiochemical contaminants during homeland security events 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.
- **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.
- **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 radiological element or isotope. 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 radioisotope per unit of mass, volume, or area sampled.

6.1 General Guidance

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

For additional information on the properties of the radionuclides listed in Appendix B, 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 (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) Web sites provide some additional information pertaining to radionuclides of interest and selection of radiochemical methods.

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: 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.

Sections 6.2.1 through 6.2.27, below, provide summaries of the qualitative and confirmatory methods listed in Appendix B. Once a method has been identified in Appendix B, **Table 6-1** can be used to locate the method summary.

Table 6-1. Radiochemical Methods and Corresponding Text Section Numbers

Analyte / Analyte Class	CAS RN	Method	Section
Gross Alpha	NA	900.0 (EPA)	6.2.2
		FRMAC, Vol 2, pg. 33	6.2.16
Gross Beta	NA	AP1 (ORISE)	6.2.17
		7110 B (SM)	6.2.21
Gamma	NA	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.12
Americium-241	14596-10-2	Am-01-RC (HASL-300)	6.2.9
		Am-02-RC (HASL-300)	6.2.10
		Am-04-RC (HASL-300)	6.2.11
		Pu-12-RC (HASL-300)	6.2.14
		AP11 (ORISE)	6.2.18
		D3084 (ASTM)	6.2.19
Californium-252	13981-17-4	Am-01-RC (HASL-300)	6.2.9
		Am-04-RC (HASL-300)	6.2.11
		Pu-12-RC (HASL-300)	6.2.14
		AP11 (ORISE)	6.2.18
		D3084 (ASTM)	6.2.19

Analyte / Analyte Class	CAS RN	Method	Section
Cesium-137	10045-97-3	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.12
Cobalt-60	10198-40-0	7120 (SM)	6.2.22
Curium-244	13981-15-2	Am-01-RC (HASL-300)	6.2.9
		Am-04-RC (HASL-300)	6.2.11
		Pu-12-RC (HASL-300)	6.2.14
		AP11 (ORISE)	6.2.18
		D3084 (ASTM)	6.2.19
Europium-154	15585-10-1	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.12
		7120 (SM)	6.2.22
Iodine-131	10043-66-0	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.12
Iridium-192	14694-69-0	901.1 (EPA)	6.2.3
		Ga-01-R (HASL-300)	6.2.12
		7120 (SM)	6.2.22
Plutonium-238	13981-16-3	EMSL-33 (EPA)	6.2.8
		AP11 (ORISE)	6.2.18
Plutonium-239	15117-48-3	D3084 (ASTM)	6.2.19
Polonium-210	13981-52-7	Method 111 (EPA)	6.2.1
		Po-02-RC (HASL-300)	6.2.13
Radium-226	13982-63-3	903.0 (EPA)	6.2.4
		903.1 (EPA)	6.2.5
		EMSL-19 (EPA)	6.2.7
		D3084 (ASTM)	6.2.19
		7500-Ra B (SM)	6.2.23
		7500-Ra C (SM)	6.2.24
Ruthenium-103	13968-53-1	901.1 (EPA)	6.2.3
Ruthenium-106	13967-48-1	Ga-01-R (HASL-300)	6.2.12
Selenium-75	14265-71-5	7120 (SM)	6.2.22
Strontium-90	10098-97-2	Sr-03-RC (HASL-300)	6.2.15
		7500-Sr B (SM)	6.2.25
Uranium-234	13966-29-5	908.0 (EPA)	6.2.6
		EMSL-33 (EPA)	6.2.8
		AP11 (ORISE)	6.2.18
Uranium-235	15117-96-1	D3084 (ASTM)	6.2.19
Uranium-238	7440-61-1	D3972 (ASTM)	6.2.20
		7500-U B (SM)	6.2.26
		7500-U C (SM)	6.2.27

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 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
NEMI	U.S. EPA, USGS	http://www.nemi.gov
CFR Promulgated Test Methods (TM)	U.S. EPA, Technical Transfer Network (TTN) 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)	U.S. 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.
Radiochemical Analytical Procedures for Analysis of Environmental Samples, March 1979. EMSL-LV-0539-17	U.S. 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	U.S. Department of Energy (DOE), Environmental Measurements Laboratory (EML) / Now U.S. DHS	http://www.eml.st.dhs.gov/publications/procman.cfm 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	United States DOE National Nuclear Security Administration (NNSA)	http://www.nv.doe.gov/nationalsecurity/homelandsecurity/frmac/manuals.aspx
Oak Ridge Institute for Science and Education (ORISE) Laboratory Procedures Manual	ORISE Independent Environmental Assessment and Verification	http://orise.orau.gov/ieav/survey-projects/lab-manual.htm
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*	APHA, AWWA, and WEF	http://www.standardmethods.org

* Subscription and/or purchase required.

6.1.2 General QC Guidance 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 responding to emergency situations. 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 transmitted to decision makers.

The level or amount of QC needed often depends on the intended purpose of the data that are generated. Various levels of QC may be required if the data are generated during contaminant presence/absence qualitative determinations versus confirmatory analyses. The specific needs for data generation should be identified. QC requirements and data quality objectives 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 required 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.

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 SAPs developed to address specific analytes and sample types of concern. 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 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
- MS/MSD recoveries; and
- 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 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 health and safety plan for handling samples that may contain target 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 6.2 contain specific requirements, guidance, 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:

- OSHA - 29 CFR part 1910.1450. Occupational Exposure to Hazardous Chemicals in Laboratories. http://www.access.gpo.gov/nara/cfr/waisidx_06/29cfr1910a_06.html
- EPA - 40 CFR part 260. Hazardous Waste Management System: General. 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. http://www.access.gpo.gov/nara/cfr/waisidx_07/40cfr270_07.html

- NRC - 10 CFR part 20. Standards for Protection Against Radiation http://www.access.gpo.gov/nara/cfr/waisidx_00/10cfr20_00.html
- U.S. DOE. Order O 435.1: Radioactive Waste Management. July 1, 1999. Available at: www.directives.doe.gov/pdfs/doe/doetext/neword/435/o4351.html
- U.S. 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
- U.S. 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
- U.S. EPA. 1996. *Profile and Management Options for EPA Laboratory Generated Mixed Waste*. Office of Radiation and Indoor Air, Washington, DC
- EPA 402-R-96-015. August. Profile and Management Options for EPA Laboratory Generated Mixed Waste. Available at: http://epa.gov/radiation/docs/mixed-waste/402-r-96-015.pdf
- U.S. EPA. 2001. Changes to 40 CFR 266 (Storage, Treatment, Transportation, and Disposal of Mixed Waste), *Federal Register* 66:27217-27266, May 16
- U.S. EPA. 2002. *RCRA Orientation Manual*. OSWER, Washington, DC. EPA530-R-02-016. 259 pp. Available at: http://www.epa.gov/epaoswer/general/orientat/
- Waste Management in a Radioanalytical Laboratory, Chapter 17 MARLAP Manual, July 2004
- National Research Council. 1995. *Prudent Practices in the Laboratory; Handling and Disposal of Chemicals*, National Academy Press, Washington, DC
- National Council on Radiation Protection and Measurements (NCRP). 2002. *Risk-Based Classification of Radioactive and Hazardous Chemical Wastes*, 7910 Woodmont Avenue, Suite 400, Bethesda, MD 20814–3095
- U.S. Nuclear Regulatory Commission (NRC) / U.S. EPA. 1995. Low-Level Mixed Waste Storage Guidance, *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 6.2.27. 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. 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
Determinative 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.

Source: EPA EMC, prepared by the OAQPS. 2000. "Method 111: Determination of Polonium-210 Emissions from Stationary Sources." http://www.epa.gov/sam/pdfs/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
Determinative 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[2]. A traceable standards-based efficiency curve must be developed for each calibration nuclide (Th-230 and Sr-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).

[2] EPA lists standards for use when analyzing drinking water in the table at 40 CFR 141.25 (footnote 11).

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.

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://www.epa.gov/sam/pdfs/EPA-900.0.pdf

6.2.3 EPA Method 901.1: Gamma Emitting Radionuclides in Drinking Water

Analyte(s)	CAS RN
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
Ruthenium-103	13968-53-1
Ruthenium-106	13967-48-1
Selenium-75	14265-71-5

Analysis Purpose: Qualitative and confirmatory analysis
Determinative 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 drinking water samples.

Description of Method: This method is applicable for analysis of water samples that contain radionuclides that emit gamma photons with energies ranging from approximately 60 to 2000 keV. The method uses gamma spectroscopy for measurement of gamma photons emitted from radionuclides without separating them from the sample matrix. A homogeneous aliquot of water is placed into a standard geometry (normally a Marinelli beaker) for gamma counting, typically using a high purity germanium (HPGe) detector. Detectors such as Germanium (Lithium) (Ge(Li)) or thallium-activated sodium iodide (NaI(Tl)) 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 NaI(Tl) 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 dependent on 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://www.epa.gov/sam/pdfs/EPA-901.1.pdf

6.2.4 EPA Method 903.0: Alpha-Emitting Radium Isotopes in Drinking Water

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Qualitative determination
Determinative Technique: Alpha counting

Method Developed for: Total soluble alpha emitting radioisotopes of radium, namely radium-223, radium-224 and radium-226 in drinking water
Method Selected for: SAM lists this method for qualitative determination in drinking water samples.

Description of Method: This method covers measurement of the total soluble alpha emitting radioisotopes of radium, namely radium-223, radium-224 and radium-226 in drinking water. The method does not give an accurate measurement of radium-226 content in the sample when other alpha emitters are present. If radium-223 and radium-224 are present, the results can be used to provide a gross determination of radium-226. When the total radium alpha activity of a drinking water sample is greater than 5 pCi/L, use of Method 903.1 (Radium-226 in Drinking Water) is preferred. Radium in the water sample is collected by coprecipitation with barium and lead sulfate, and purified by re-precipitation from EDTA solution. Citric acid is added to ensure that complete interchange occurs before the first precipitation step. The final barium sulfate precipitate is alpha counted to determine the total disintegration rate of the radium isotopes. By making a correction for the ingrowth of radon and its alpha emitting progeny for the elapsed time after separation, one can determine radium activity in the sample. Presence of significant natural barium in the sample can result in a falsely high yield. 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 903.0: Alpha-Emitting Radium Isotopes in Drinking Water." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*, EPA/600/4/80/032. http://www.epa.gov/sam/pdfs/EPA-903.0.pdf

6.2.5 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
Determinative 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, a daughter product of radium-226. Radium-226 is concentrated and separated from the water sample by coprecipitation on barium sulfate. The precipitate is dissolved in 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://www.epa.gov/sam/pdfs/EPA-903.1.pdf

6.2.6 EPA Method 908.0: Uranium in Drinking Water – 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
Determinative Technique: Alpha counting

Method Developed for: Total uranium alpha particle activity in drinking water
Method Selected for: SAM lists this method for qualitative determination in drinking water samples.

Description of Method: This method measures total uranium alpha activity of a sample, without doing an isotopic uranium analysis. The sample is acidified with hydrochloric acid and boiled to eliminate carbonate and bicarbonate ions. Uranium is coprecipitated with ferric hydroxide and separated from the sample. The uranium is then separated from other radionuclides that were carried down with the ferric hydroxide by dissolving the hydroxide precipitate in hydrochloric acid, putting the solution through an anion exchange column, washing the column with hydrochloric acid, and finally eluting the uranium with hydrochloric acid. The uranium eluate is evaporated and the uranium chemical form is converted to nitrate. The residue is transferred to a stainless steel planchet, dried, flamed, and counted for alpha particle activity. Since uranium is a naturally occurring radionuclide, reagents must be checked for uranium contamination by analyzing a complete reagent blank by the same procedure as used for the samples. Based on a 1000- mL sample and 100-minute counting time in a single laboratory study, the minimum detectable level for this method is 1.0 pCi/L.

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. 1980. "Method 908.0: Uranium in Drinking Water – Radiochemical Method." *Prescribed Procedures for Measurement of Radioactivity in Drinking Water*, EPA/600/4/80/032. http://www.epa.gov/sam/pdfs/EPA-908.0.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
Determinative 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://www.epa.gov/sam/pdfs/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
Determinative 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 Sample*. http://www.epa.gov/sam/pdfs/EPA-EMSL-33.pdf

6.2.9 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
Determinative 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. 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 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, U.S. DOE (EML is currently part of the U.S. DHS). 1997. "HASL-300 Method Am-01-RC: Americium in Soil." *EML Procedures Manual*, HASL-300, 28th Edition.
http://www.epa.gov/sam/pdfs/EML-Am-01-RC.pdf

6.2.10 EML HASL-300 Method Am-02-RC: Americium-241 in Soil-Gamma Spectrometry

Analyte(s)	CAS RN
Americium-241	14596-10-2

Analysis Purpose: Qualitative determination
Determinative Technique: Gamma spectrometry

Method Developed for: Americium-241 in large volume soil samples
Method Selected for: SAM lists this method for qualitative determination in soil/sediment samples.

Description of Method: This method uses gamma spectrometry for determination of americium-241 in soil. Americium-241 decays with the emission of a gamma ray at 59.5 keV with a decay frequency (abundance or yield) of 35.9%. The sample is placed into an appropriately sized standard geometry (normally a Marinelli beaker) after drying and grinding the sample for homogenization. Gamma-ray attenuation corrections are required if the calibration source and the sample are in a different matrix or are of different densities. The LLD for 600 to 800 g of soil in a Marinelli beaker is 0.74 mBq for a 1000-minute count.

Source: EML, U.S. DOE (EML is currently part of the U.S. DHS). 1997. "HASL-300 Method Am-02-RC: Americium-241 in Soil-Gamma Spectrometry." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sam/pdfs/EML-Am-02-RC.pdf

6.2.11 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
Determinative 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, and air filters.

Description of Method: This method is specific to measurement of americium isotopes in samples that do not contain lanthanides, but also can be used for measurement of californium and curium. The method uses microprecipitation and determination by alpha spectrometry. Americium-243 is added to the sample to determine chemical yield. The sample is processed through separation steps using ion exchange resins. The eluate from the ion exchange column containing americium (and all other ions, except plutonium) is evaporated, redissolved, and loaded onto a 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, U.S. DOE (EML is currently part of the U.S. DHS). 1997. "HASL-300 Method Am-04-RC: Americium in QAP Water and Air Filters - Eichrom's TRU Resin." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sam/pdfs/EML-Am-04-RC.pdf

6.2.12 EML HASL-300 Method Ga-01-R: Gamma Radioassay

Analyte(s)	CAS RN
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
Ruthenium-103	13968-53-1
Ruthenium-106	13967-48-1
Selenium-75	14265-71-5

Analysis Purpose: Qualitative and confirmatory analysis or gross gamma determination
Determinative 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 aqueous/liquid, soil/sediment, surface wipes, and/or air filter samples.

Description of Method: This method uses gamma spectroscopy 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 HPGe 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 HPGe and GeLi) detectors and above 50 keV for NaI(Tl) detectors.

Source: EML, U.S. DOE (EML is currently part of the U.S. DHS). 1997. "HASL-300 Method Ga-01-R: Gamma Radioassay." *EML Procedures Manual*, HASL-300, 28th Edition.
http://www.epa.gov/sam/pdfs/EML-Ga-01-R.pdf

6.2.13 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
Determinative 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, and soil/sediment samples.

Description of Method: This method uses alpha spectrometry for determination of polonium in water, vegetation, soil, and air filter samples. Polonium equilibrated with Po-208 or Po-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 Pb-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, U.S. DOE (EML is currently part of the U.S. DHS). 1997. "HASL-300 Method Po-02-RC: Polonium in Water, Vegetation, Soil, and Air Filters." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sam/pdfs/EML-Po-02-RC.pdf

6.2.14 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
Determinative 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 Am-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, U.S. DOE (EML is currently part of the U.S. DHS). 1997. "HASL-300 Method Pu-12-RC: Plutonium and/or Americium in Soil or Sediments." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sam/pdfs/EML-Pu-12-RC.pdf

6.2.15 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
Determinative Technique: Beta counting by low-background gas flow proportional detector

Method Developed for: Strontium-90 in vegetation, water, air filters and soil
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of soil/sediment, surface wipe, and air filter samples.

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, U.S. DOE (EML is currently part of the U.S. DHS). 1997. "HASL-300 Method Sr-03-RC: Strontium-90 in Environmental Samples." *EML Procedures Manual*, HASL-300, 28th Edition. http://www.epa.gov/sam/pdfs/EML-Sr-03-RC.pdf

6.2.16 FRMAC Method Volume 2, Page 33: Gross Alpha and Beta in Air

Analysis Purpose: Gross alpha and gross beta determination
Determinative 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://www.epa.gov/sam/pdfs/FRMAC-Vol2-pg33.pdf

6.2.17 ORISE Method AP-1: Gross Alpha and Beta for Various Matrices

Analysis Purpose: Gross alpha and gross beta determination
Determinative 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 samples.

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 chemical 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 processed to provide homogeneity, 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 AP-1: Gross Alpha and Beta for Various Matrices." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program*. http://www.epa.gov/sam/pdfs/ORISE-AP-1.pdf

6.2.18 ORISE Method AP-11: 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
Determinative 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 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 AP-11: Sequential Determination of the Actinides in Environmental Samples Using Total Sample Dissolution and Extraction Chromatography." *Laboratory Procedures Manual for the Environmental Survey and Site Assessment Program*.
http://www.epa.gov/sam/pdfs/ORISE-AP-11.pdf

6.2.19 ASTM Method D3084: 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
Uranium-234	13966-29-5
Uranium-235	15117-96-1
Uranium-238	7440-61-1

Analysis Purpose: Qualitative determination
Determinative Technique: Alpha spectrometry

Method Developed for: Alpha particle spectra in water
Method Selected for: SAM lists this method for qualitative determination in drinking water, aqueous/liquid, soil and sediment, surface wipes, and/or air filter samples.

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. 1996. "Method D3084: Standard Practice for Alpha Spectrometry in Water." *Annual Book of ASTM Standards*, Vol. 11.02. http://www.astm.org/DATABASE.CART/HISTORICAL/D3084-96.htm

6.2.20 ASTM Method D3972: Standard Test Method for Isotopic Uranium in Water by Radiochemistry

Analyte(s)	CAS RN
Uranium-234	13966-29-5
Uranium-235	15117-96-1

Analyte(s)	CAS RN
Uranium-238	7440-61-1

Analysis Purpose: Confirmatory analysis
Determinative 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: Standard Test Method for Isotopic Uranium in Water by Radiochemistry." *Annual Book of ASTM Standards*, Vol. 11.02.
http://www.astm.org/Standards/D3972.htm

6.2.21 Standard Method 7110 B: Gross Alpha and Gross Beta Radioactivity (Total, Suspended, and Dissolved)

Analysis Purpose: Gross alpha and gross beta determination
Determinative 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/

6.2.22 Standard Method 7120: Gamma-Emitting Radionuclides

Analyte(s)	CAS RN
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
Determinative Technique: Gamma spectrometry

Method Developed for: Gamma emitting radionuclides in water
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of 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 standard containing a mixture of gamma energies from approximately 100 to 2000 keV is used for energy 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/

6.2.23 Standard Method 7500-Ra B: Radium: Precipitation Method

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Qualitative determination
Determinative 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/

6.2.24 Standard Method 7500-Ra C: Radium: Emanation Method

Analyte(s)	CAS RN
Radium-226	13982-63-3

Analysis Purpose: Confirmatory determination
Determinative 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/

6.2.25 Standard Method 7500-Sr B: Total Radioactive Strontium and Strontium-90: Precipitation Method

Analyte(s)	CAS RN
Strontium-90	10098-97-2

Analysis Purpose: Qualitative and confirmatory analysis
Determinative Technique: Beta counting by low-background gas flow proportional detector

Method Developed for: Strontium-90 or total radioactive strontium in drinking water or filtered raw water
Method Selected for: SAM lists this method for qualitative and confirmatory analysis of drinking water and aqueous/liquid samples.

Description of Method: A known amount of inactive strontium ions, in the form of strontium nitrate, is added as a "carrier." The carrier, alkaline earths, and rare earths are precipitated as the carbonate to concentrate the radiostrontium. The carrier, along with the radionuclides of strontium, is separated from other radioactive elements and inactive sample solids by precipitation as strontium nitrate using fuming nitric acid solution. The carrier and radionuclides of strontium are precipitated as strontium carbonate, which is dried, weighed to determine recovery of carrier, and measured for radioactivity. The activity of the final precipitate is due to radioactive strontium only, because all other radioactive elements have been removed. Because it is impossible to separate the isotopes of strontium-89 and strontium-90 by any chemical procedure, the amount of strontium-90 is determined by separating and measuring the activity of yttrium-90, its daughter product. This method involves beta counting by a gas-flow internal proportional counter or thin end-window low-background proportional counter. A correction is applied to compensate for loss of carriers and activity during the various purification steps.

Source: APHA, AWWA, and WEF. 2005. "Method 7500-Sr B: Total Radioactive Strontium and Strontium-90: Precipitation Method." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

6.2.26 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
Determinative 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, and 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/

6.2.27 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
Determinative 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/

Section 7.0: Selected Pathogen Methods

A list of the most appropriate methods currently available for use in analyzing environmental samples for pathogens is provided in Appendix C. This list represents an initial effort towards the goal of providing standardized analytical procedures. These methods should be used to support remediation activities (site assessment through clearance) following a homeland security event. The purpose of this section is to provide summary information regarding the procedures listed. Methods are listed for each pathogen that may need to be measured and analyzed following an 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 standardized methods for pathogens are not currently available. Future steps include the development and validation of standardized methods. The literature references will be replaced as standardized, validated protocols become available.

Pathogens that are categorized as Biosafety Level 4 (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 this document. All other pathogens are to be handled using BSL-2 or BSL-3 containment and practices (as appropriate) using SAM procedures. If known, the BSL classification for each pathogen is provided in the method summaries in Sections 7.2.2 through 7.2.42). Pathogens that are considered to be solely of agricultural concern (i.e., animal and plant pathogens) are not currently included. Such pathogens may be considered for possible inclusion in future document revisions. Some of the pathogens addressed by this document are commonly found in the environment, and the methods listed in Appendix C assume that analyses will be used to evaluate contamination levels that are above background levels. If possible, an investigation of initial background levels, as well as appropriate controls for background levels, should be performed. It is anticipated that additional site- or event-specific information also will be required to support the analytical results.

Selection of methods from Appendix C should be based on specific data and information needs, including whether there is a need to determine either the presence of a pathogen, the viability of a pathogen, or both. Although culture-based methods have been selected for many of the pathogens, molecular techniques such as PCR will likely be used for viruses because of the difficulty and time required to propagate these agents in host cell cultures. Prior to the start of site decontamination, viability may not be an issue and rapid mass screening techniques, such as PCR, may be more appropriate. After decontamination, viability may be required to evaluate the efficacy of decontamination procedures; thus, a technique that determines viability should be chosen. In such cases, methods that combine rapid sample processing and viability determination, such as culture confirmed by PCR, should be considered for processing large numbers of samples in a timely manner. Viability procedures are listed for each pathogen where available. Commercially available spore strips also may be used as general indicators that a decontamination process (e.g., fumigation) has successfully been applied. Spore strips, however, cannot replace negative-culture results as an indicator of decontamination efficacy.

Users of this document should be aware that analysis of environmental samples poses specific problems, and it is likely that a single analytical procedure will not be applicable to all sample types. The methods listed in this document attempt to address a wide range of environmental samples (e.g., drinking water, soil), each with specific physical and biological properties (e.g., pH, inhibitory substances, background microorganisms). Within each sample type, a high level of variability may also exist. For example, soils sampled in one geographical area may have qualities affecting analysis that are not present in soils from other areas. Sample preparatory techniques, such as immunomagnetic separation (IMS), may be useful for reducing the impact of these variables. Analyzing representative quantities of environmental samples also presents a problem and can result in reduced analytical sensitivity. In these cases, sample concentration techniques, such as membrane filtration (MF) and ultrafiltration (UF), may be useful. In addition to removing background microorganisms and inhibitory substances, IMS may also be useful for

concentrating sample aliquots to ensure that the full sample volume can be assayed. This may allow methods such as PCR to be more sensitive.

Appendix C includes the following information:

- **Pathogen(s).** A specific causative agent (e.g., viruses, bacteria) of disease.
- **Viability.** Ability to grow and/or reproduce.
- **Analytical technique.** An analytical instrument or procedure used to determine the identity, quantity, and/or viability of a pathogen.
- **Analytical method.** The unique identifier or number assigned to an analytical method by the method publisher.
- **Solid (soil, powder).** The recommended method/procedure for the pathogen of interest in solid samples such as soil and powders.
- **Particulate (swabs, wipes, filters).** The recommended method/procedure to measure the pathogen of interest in particulate sample collection devices such as swabs, wipes and high efficiency particulate air (HEPA) filters.
- **Liquid/water (filter, grab).** The recommended method/procedure for the pathogen of interest in liquid or aqueous samples that have been concentrated or grab samples.
- **Drinking water (filter, grab).** The recommended method/procedure for the pathogen of interest in potable water samples that have been concentrated or grab samples.
- **Aerosol (growth media, filter, liquid).** The recommended method/procedure for the pathogen of interest in air sample collectors such as growth media, filters, or liquid.

Some of the methods in Appendix C include multiple analytical techniques by inference. The analytical technique listed in Appendix C 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 test listed as "culture" may 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 potential for use of multiple cell culture media for primary isolation, allowance for selection of a defined subset of a larger number of biochemical tests for biochemical testing, or use of alternative devices for sample concentration. The method may provide guidance as to which options should be used under particular circumstances, or this may be left to the discretion of the laboratory.

7.1 General Guidance

This section provides a general overview of how to identify the appropriate pathogen method(s) for a given pathogen as well as recommendations for QC procedures.

For additional information on the properties of the pathogens listed in Appendix C, 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 Web site (http://www.bt.cdc.gov/) and the FDA Center for Food Safety and Applied Nutrition (CFSAN) "Bad Bug Book" (http://www.cfsan.fda.gov/~mow/intro.html). 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 method that is to be used on an environmental sample, locate the pathogen in Appendix C: 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 (solid, particulate, liquid/drinking water or aerosol).

Sections 7.2.1 through 7.2.42 below provide summaries of the analytical methods listed in Appendix C. Once a method has been identified in Appendix C, **Table 7-1** can be used to locate the method summary.

Table 7-1. Pathogen Methods and Corresponding Text Section Numbers

Pathogen	Method	Section
Bacteria [Disease]		
Bacillus anthracis [Anthrax]	LRN	7.2.1
	CDC Basic Diagnostic Testing Protocols for Level A Laboratories for the Presumptive Identification of *Bacillus anthracis*	7.2.7
Brucella spp. [Brucellosis]	LRN	7.2.1
	ASM Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism: *Brucella* species	7.2.21
Burkholderia mallei [Glanders]	LRN	7.2.1
Burkholderia pseudomallei [Melioidosis]	ASM Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism: *Burkholderia mallei* and *Burkholderia pseudomallei*	7.2.22
Campylobacter jejuni [Campylobacteriosis]	SM 9260 G	7.2.18
	Molecular and Cellular Probes. 2006. 20: 269–279	7.2.24
Chlamydophila psittaci [Psittacosis]	Journal of Clinical Microbiology. 2000. 38: 1085–1093	7.2.25
Coxiella burnetii [Q-fever]	LRN	7.2.1
Escherichia coli O157:H7	SM 9260 F	7.2.17
	Applied and Environmental Microbiology. 2003. 69(10): 6327–6333	7.2.26
Francisella tularensis [Tularemia]	LRN	7.2.1
	CDC, ASM, APHL Basic Protocols for Level A Laboratories for the Presumptive Identification of *Francisella tularensis*	7.2.8
Leptospira spp. [Leptospirosis]	SM 9260 I	7.2.20
Listeria monocytogenes [Listeriosis]	USDA Laboratory Guidebook MLG 8A.03	7.2.12
	FDA/Bacteriological Analytical Manual Chapter 10, 2003	7.2.13
Non-typhoidal *Salmonella* [Salmonellosis] (Method not applicable for *Salmonella* Typhi)	Method 1682	7.2.6
	Journal of Applied Microbiology. 2007. 102(2): 516–530	7.2.27
Salmonella Typhi [Typhoid fever]	CDC Laboratory Assay: *S.* Typhi	7.2.9
	SM 9260 B	7.2.15

Pathogen	Method	Section
Shigella spp. [Shigellosis]	CDC Laboratory Assay: *Shigella*	7.2.10
	SM 9260 E	7.2.16
Staphylococcus aureus	SM 9213 B	7.2.14
Vibrio cholerae O1 and O139 [Cholera]	CDC Laboratory Assay: *V. cholerae*	7.2.11
	SM 9260 H	7.2.19
Yersinia pestis [Plague]	LRN	7.2.1
	ASM Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism: *Yersinia pestis*	7.2.23
Viruses		
Adenoviruses: A-F	Applied and Environmental Microbiology. 2005. 71(6): 3131–3136	7.2.28
Astroviruses	Canadian Journal of Microbiology. 2004. 50: 269–278	7.2.29
Caliciviruses: Noroviruses	Journal of Clinical Microbiology. 2004. 42(10): 4679–4685	7.2.30
Caliciviruses: Sapoviruses	Journal of Medical Virology, 2006. 78 (10): 1347–1353	7.2.31
Coronaviruses: SARS	Journal of Virological Methods. 2004. 122: 29–36	7.2.32
Hepatitis E Virus	Journal of Virological Methods. 2006. 131(1): 65–71	7.2.33
Influenza H5N1 Virus	Emerging Infectious Diseases. 2005. 11(8): 1303–1305	7.2.34
Picornaviruses: Enteroviruses	USEPA Manual of Methods for Virology EPA/600/4–84/013, 2001	7.2.2
	Applied and Environmental Microbiology. 2003. 69(6): 3158–3164	7.2.35
Picornaviruses: Hepatitis A Virus Reoviruses: Rotaviruses	Applied and Environmental Microbiology. 2003. 69(6): 3158–3164	7.2.35
Protozoa		
Cryptosporidium spp. [Cryptosporidiosis]	Method 1622	7.2.4
	Method 1623	7.2.5
	Applied and Environmental Microbiology. 1999. 65(9): 3936–3941	7.2.36
	Applied and Environmental Microbiology. 2007. 73(13): 4218–4225	7.2.37
Entamoeba histolytica	Journal of Parasitology. 1972. 58(2): 306–310	7.2.38
	Journal of Clinical Microbiology. 2005. 43(11): 5491–5497	7.2.39
Giardia spp. [Giardiasis]	Method 1623	7.2.5
	Transactions of the Royal Society of Tropical Medicine and Hygiene. 1983. 77(4): 487–488	7.2.40
Toxoplasma gondii [Toxoplasmosis]	Emerging Infectious Diseases. 2006. 12(2): 326–329	7.2.41
	Applied and Environmental Microbiology. 2004. 70(7): 4035–4039	7.2.42

Pathogen	Method	Section
Helminths		
Baylisascaris procyonis [Raccoon roundworm fever]	EPA/625/R92/013	7.2.3

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, VCSBs, and journal articles. Methods are listed in numerical order under each publisher. For additional information regarding sample preparation and analysis procedures available through consensus standards organizations, please use the contact information provided in **Table 7-2**.

Table 7-2. Sources of Pathogen Methods

Name	Publisher	Reference
NEMI	U.S. EPA, USGS	http://www.nemi.gov
U.S. EPA Microbiology Methods	U.S. EPA	http://www.epa.gov/microbes/
Information Collection Requirements Rule (ICR) Microbial Laboratory Manual	U.S. EPA ORD	http://www.epa.gov/nerlcwww/icrmicro.pdf
USEPA Manual of Methods for Virology	U.S. EPA	http://www.epa.gov/nerlcwww/about.htm
Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage and Sludge	U.S. EPA, National Risk Management Research Laboratory (NRMRL)	http://www.epa.gov/nrmrl/pubs/625r92013/625r92013.htm
CDC Laboratory Assays	HHS, CDC	eija.trees@cdc.hhs.gov
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	U.S. FDA, CFSAN	http://www.cfsan.fda.gov/~ebam/bam-toc.html
OSHA Methods	OSHA	http://www.osha.gov
NIOSH Methods	NIOSH	http://www.cdc.gov/niosh/nmam/
Standard Methods for the Examination of Water and Wastewater, 21st Edition, 2005*	APHA, AWWA, and WEF	http://www.standardmethods.org
*Annual Book of ASTM Standards**	ASTM International	http://www.astm.org
Applied and Environmental Microbiology (AEM)*	American Society for Microbiology (ASM)	http://aem.asm.org/
Journal of Clinical Microbiology*	ASM	http://jcm.asm.org/
Clinical Microbiology Procedures Handbook, 2nd Edition, 2004*	ASM	http://estore.asm.org/viewItemDetails.asp?ItemID=323
Molecular and Cellular Probes*	Elsevier	http://www.elsevier.com
Canadian Journal of Microbiology*	NRC Research Press	http://pubs.nrc-cnrc.gc.ca/
Journal of Medical Virology*	Wiley InterScience	http://www3.interscience.wiley.com/cgi-bin/home

Name	Publisher	Reference
Journal of Virological Methods*	Elsevier	http://www.elsevier.com
Emerging Infectious Diseases	CDC	http://www.cdc.gov/ncidod/EID/
Journal of Parasitology*	American Society of Parasitologists	http://www.bioone.org
Transactions of the Royal Society of Tropical Medicine and Hygiene*	The Royal Society of Tropical Medicine and Hygiene	http://www.rstmh.org/
Diagnostic Procedures in Veterinary Bacteriology and Mycology	Academic Press	http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1481267
Sentinel Level Clinical Microbiology Laboratory Guidelines	ASM	http://www.asm.org/Policy/index.asp?bid=6342
Journal of Applied Microbiology*	Blackwell Publishing	http://www.blackwellpublishing.com/journal.asp?ref=1364-5072&site=1

* Subscription and/or purchase required. ASM does not require a subscription or purchase 6 months after the publication date.

7.1.2 General QC Guidance 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 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; and (5) properly document analytical QC procedures and corrective actions.

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 should be based on those needs, and should be 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.

Ongoing analysis of control samples to ensure the continued reliability of the analytical results should also be performed. At a minimum, the following QC analyses should be conducted on an ongoing basis:

- Media and reagent sterility checks;
- Positive and negative controls;
- Method blanks;
- Reference matrix spikes to evaluate initial and ongoing method/analyst performance, if available;
- Matrix spikes to evaluate method performance in the sample type of interest;
- MSD and/or sample replicates to assess method precision; and
- Instrument calibration checks and temperature controls.

QC procedures should be performed as frequently as necessary to ensure the reliability of analytical results.

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 OSWER coordinator responsible for laboratory activities during the specific event to ensure QA/QC procedures are performed consistently across laboratories. OSWER is planning to develop QA/QC guidance for laboratory support. EPA program offices will be responsible for ensuring that the QA/QC practices are implemented.

7.1.3 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 CBR contaminants, and laboratory staff should be trained in and implement the safety procedures included in the plan. 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 in such a manner as to enhance dispersion or virulence characteristics. These conditions may warrant special handling for samples arising from intentional contamination incidents. A laboratory must be made aware of these potential circumstances, and 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 Section 7.2 contain specific requirements, guidance, 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 may cause serious or potentially lethal disease and also have the potential for aerosol transmission. BSLs are provided in the method summaries in Section 7.2 whenever available. It is important to note, however, that 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:

- Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition, found at http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm
- "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, found at http://www.cdc.gov/mmwr/pdf/rr/rr5119.pdf.
- Microbiology Biosafety for Level A Laboratories, found at http://www.bt.cdc.gov/documents/PPTResponse/table3bbiosafety.pdf
- Select Agent Rules and Regulations (42 CFR part 73 and 9 CFR part 121) found at http://www.cdc.gov/od/sap/pdfs/42_cfr_73_final_rule.pdf and http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=/ecfrbrowse/Title09/9cfr121_main_02.tpl

The following methods provide information regarding waste management:

- EPA – Hazardous Waste Management (40 CFR parts 260) and EPA Administered Permit Programs (40 CFR part 270), found at http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?sid=cac9da30cd241fa70d461e4a917eb75e&c=ecfr&tpl=/ecfrbrowse/Title40/40tab_02.tpl

Other resources that can be consulted for additional information include the following:

- OSHA – Hazardous Waste Operations and Emergency Response (29 CFR part 1910.120) found 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) found at http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=10106
- OSHA – Respiratory Protection (29 CFR part 1910.134) found 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) http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?sid=585c275ee19254ba07625d8c92fe925f&c=ecfr&tpl=/ecfrbrowse/Title49/49cfrv2_02.tpl

7.2 Method Summaries

Summaries of the analytical methods listed in Appendix C are provided in Sections 7.2.1 through 7.2.42. Each summary contains a table identifying the pathogen(s) and sample type to which the method applies, a brief description of the method, performance data (if available), and a link to, or source for, obtaining a full version of the method.

7.2.1 Laboratory Response Network (LRN)

The LRN was created in accordance with Presidential 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 4–6 hours) presumptive detection of biothreat agents and emerging infectious disease agents. These rapid presumptive assays are part of sample type/analyte 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.

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. Additional LRN comparable assays (e.g., PCR) also are being developed or acquired that may be used in place of LRN assays. Except for *Coxiella burnetii*, culture methods are available for all of these pathogens as Sentinel Laboratory Guidelines (http://www.asm.org/Policy/index.asp?bid=6342).

The agents identified below and listed in Appendix C are included in the HHS/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/OD/ohs/biosfty/bmbl5/bmbl5toc.htm).

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)
Centers for Disease Control and Prevention (CDC)
1600 Clifton Road NE, Mailstop C-18
Atlanta, GA 30333
Telephone: (404) 639-2790
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
Web site: www.aphl.org
E-mail: info@aphl.org

7.2.2 USEPA Manual of Methods for Virology, EPA/600/4-84/013, April 2001

Pathogen(s)	Agent Category	BSL
Picornaviruses: Enteroviruses	Viruses	Not specified

Analysis Purpose: Detection and viability
Sample Preparation: Samples should be prepared according to the procedures described in the USEPA Manual of Methods for Virology (EPA/600/4-84/013, April 2001).
Analytical Technique: Tissue culture with serum neutralization

Method Developed for: Enteroviruses in water
Method Selected for: SAM lists this manual for detection and viability assessment in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This manual describes procedures for determining the infectivity of enteroviruses, including a neutralization test used to identify these viruses. The test uses reference-typing sera directed against isolated waterborne viruses, and consists of simultaneously inoculating virus and antiserum into a microtiter plate, incubating the virus-antibody mixture for 2 hours, adding a suspension of host cells to the mixture, incubating the host cells-virus-antibody mixture for 3 days, and then examining the cells daily for 5 more days for the presence/absence of a cytopathic effect (CPE). The test uses the Lim Benyesh-Melnick (LB-M) antiserum pools, which consist of 61 equine antisera, including LB-M antiserum pools A–H for the identification of 41 enteroviruses. Chapters 7 and 14 in this manual describe procedures for the collection and preparation of virus samples. Sample preparation procedures described include concentration and processing of waterborne viruses by positively charged 1MDS cartridge filters and flocculation. These general procedures can be used for many viruses and may be adapted for analysis of particulate, liquid, water, and aerosol samples.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Special Considerations: This manual also describes procedures for preparation of samples for adenovirus, astrovirus, norovirus, sapovirus, coronavirus – severe acute respiratory syndrome (SARS), hepatitis E virus, influenza H5N1 virus, picornaviruses (enterovirus and hepatitis A virus), and reovirus (rotavirus).

Source: EPA. 2001. "Chapter 12 – Identification of Enteroviruses." *USEPA Manual of Methods for Virology*, EPA/600/4-84/013. http://www.epa.gov/sam/pdfs/EPA-600-4-84-013.pdf

7.2.3 USEPA Environmental Regulations and Technology, Control of Pathogens and Vector Attraction in Sewage Sludge EPA/625/R-92/013, July 2003: *Baylisascaris procyonis*

Pathogen(s) [Disease]	Agent Category	BSL
Baylisascaris procyonis [Raccoon roundworm fever]	Helminths	2

Analysis Purpose: Detection and viability
Sample Preparation: Samples should be prepared according to the procedures in EPA/625/R-92/013.
Analytical Technique: Microscopy and embryonation of eggs

Method Developed for: *Baylisascaris procyonis* in wastewater, sludge, and compost samples
Method Selected for: SAM lists these protocols for detection and viability assessment of *Baylisascaris procyonis* in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than wastewater, sludge, and compost. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: The protocol describes procedures for analysis of solid and wastewater samples and may be adapted to analysis of particulate, liquid, water, and aerosol 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 ova, 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 *Ascaris* eggs are fully embryonated. The concentrate is then microscopically examined for the categories of *Ascaris* ova on a counting chamber.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Source: EPA. 2003. "Appendix I: Test Method for Detecting, Enumerating, and Determining the Viability of Ascaris Ova in Sludge." *USEPA Environmental Regulations and Technology: Control of Pathogens and Vector Attractions in Sewage Sludge*, EPA/625/R-92/013.
http://www.epa.gov/sam/pdfs/EPA-625-R-92-013.pdf

7.2.4 EPA Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA

Pathogen(s) [Disease]	Agent Category	BSL
Cryptosporidium spp. [Cryptosporidiosis]	Protozoa	2

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Samples should be prepared according to procedures in EPA Method 1622.
Analytical Technique: IMS and fluorescence assay (FA) microscopy

Method Developed for: *Cryptosporidium* in surface water
Method Selected for: SAM lists this method for detection in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than surface water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This method describes procedures for analysis of drinking water samples and may be adapted for analysis of solid and particulate samples. A water sample is filtered and the oocysts and extraneous materials are retained on the filter. Materials on the filter are eluted, the eluate is 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 of the oocysts.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, MS/MSD, and blank.

Source: EPA. 2005. "Method 1622: *Cryptosporidium* in Water by Filtration/IMS/FA."
http://www.epa.gov/sam/pdfs/EPA-1622.pdf

7.2.5 EPA Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA

Pathogen(s) [Disease]	Agent Category	BSL
Cryptosporidium spp. [Cryptosporidiosis]	Protozoa	2
Giardia spp. [Giardiasis]	Protozoa	2

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Samples should be prepared according to procedures in EPA Method 1623.
Analytical Technique: IMS and FA microscopy

Method Developed for: *Cryptosporidium* and *Giardia* in surface water
Method Selected for: SAM lists this method for the detection of *Cryptosporidium* spp. in solid, particulate, liquid, and water samples and *Giardia* spp. in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than surface water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This method describes procedures for analysis of water samples and may be adapted for assessment of solid, particulate, and liquid samples. A water sample is filtered and the oocysts and cysts and extraneous materials are retained on the filter. Materials on the filter are eluted, the eluate is centrifuged to pellet the oocysts and cysts, and the supernatant fluid is aspirated. A solution containing anti-*Cryptosporidium* or 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. This method is not intended to determine viability of the parasites.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, MS/MSD, and blank.

Source: EPA. 2001. "Method 1623: *Cryptosporidium* and *Giardia* in Water by Filtration/IMS/FA." http://www.epa.gov/sam/pdfs/EPA-1623.pdf

7.2.6 EPA Method 1682: *Salmonella* spp.

Pathogen(s) [Disease]	Agent Category	BSL
Non-typhoidal *Salmonella* [Salmonellosis] (Method not applicable to *S.* Typhi)	Bacteria	2

Analysis Purpose: Detection and viability
Sample Preparation: Solid samples should be prepared according to EPA Method 1680; particulate samples should be prepared according to "Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004. Emerging Infectious Diseases. 10(6):1023–1029 (www.epa.gov/sam/pdfs/EID-10(6)-pgs1023-1029.pdf) and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied and Environmental Microbiology. 72(6): 4429–4430 (www.epa.gov/sam/pdfs/AEM-72(6)-pgs4429-4430.pdf).

Analytical Technique: Culture and immunoassay

Method Developed for: Non-typhoidal *Salmonella* in biosolids
Method Selected for: SAM lists this method for detection and viability assessment in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than biosolids. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This method describes procedures for analysis of solid samples (biosolids) and may be adapted for assessment of water, liquid, particulate, and aerosol samples. Prepared samples are inoculated into tubes of tryptic soy broth (TSB) and incubated for 24 hours. Positive (turbid) tubes are spotted onto plates of modified semisolid Rappaport-Vassiliadis (MSRV) medium and incubated at 42°C for 16 to 18 hours. The MSRV medium uses novobiocin and malachite green to inhibit non-*Salmonella* species, while allowing most *Salmonella* species to grow. Presumptive colonies are isolated on xylose lysine deoxycholate (XLD) agar and confirmed using lysine iron agar (LIA), triple sugar iron (TSI) agar, and urea broth, followed by serological typing using polyvalent O antisera.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, matrix spike, and blank.

Special Considerations: This method will not detect *Salmonella* Typhi. MSRV and the elevated incubation temperature (42°C) are inhibitory for *S.* Typhi.

Source: EPA. 2006. "Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by MSRV Medium." http://www.epa.gov/sam/pdfs/EPA-1682.pdf

7.2.7 CDC, ASM, APHL: Basic Diagnostic Testing Protocols for Level A Laboratories for the Presumptive Identification of *Bacillus anthracis*

Pathogen(s) [Disease]	Agent Category	BSL
Bacillus anthracis [Anthrax]	Bacteria	3

Analysis Purpose: Detection and viability
Sample Preparation: Solid samples should be prepared according to EPA Method 1680; particulate samples should be prepared according to "Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004. Emerging Infectious Diseases. 10(6): 1023–1029 (www.epa.gov/sam/pdfs/EID-10(6)-pgs1023-1029.pdf) and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied and Environmental Microbiology. 72(6): 4429–4430 (www.epa.gov/sam/pdfs/AEM-72(6)-pgs4429-4430.pdf).
Analytical Technique: Culture

Method Developed for: *B. anthracis* in clinical samples
Method Selected for: SAM lists this protocol for detection and viability assessment in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This method describes procedures for analysis of clinical samples and may be adapted for assessment of solid, particulate, and liquid samples. Samples are plated directly on sheep blood agar (SBA) or first enriched in a nutrient broth (e.g., TSB). Incubation is for at least 3 days at 35°C–37°C. *B. anthracis* forms 2–5 mm, flat or slightly convex colonies with irregular edges on SBA,

with no ß-hemolysis. Presumptive identification is made by culture examination, microscopy, motility testing, and biochemical testing. *B. anthracis* is a non-motile, Gram-positive rod with central to sub-terminal spores, and is catalase-negative. Cultures (isolates) that cannot be ruled out as *B. anthracis* based on the characteristics noted above may be referred to an appropriate reference laboratory for confirmation.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Special Considerations: *B. 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, http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm).

Source: CDC, ASM, and APHL. 2002. "Basic Diagnostic Testing Protocols for Level A Laboratories for the Presumptive Identification of *Bacillus anthracis*." http://www.epa.gov/sam/pdfs/CDC-Anthrax.pdf

7.2.8 CDC, ASM, APHL: Basic Protocols for Level A Laboratories for the Presumptive Identification of *Francisella tularensis*

Pathogen(s) [Disease]	Agent Category	BSL
Francisella tularensis [Tularemia]	Bacteria	3

Analysis Purpose: Detection and viability
Sample Preparation: Solid samples should be prepared according to EPA Method 1680; particulate samples should be prepared according to "Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004. Emerging Infectious Diseases. 10(6): 1023–1029 (www.epa.gov/sam/pdfs/EID-10(6)-pgs1023-1029.pdf) and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied and Environmental Microbiology. 72(6): 4429–4430 (www.epa.gov/sam/pdfs/AEM-72(6)-pgs4429-4430.pdf).
Analytical Technique: Culture

Method Developed for: *F. tularensis* in clinical samples
Method Selected for: SAM lists this protocol for detection and viability assessment in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This method describes procedures for analysis of clinical samples and may be adapted for assessment of solid, particulate, and liquid samples. Samples are plated directly on a cysteine-supplemented media such as chocolate agar (CA), Thayer-Martin (TM) agar, or buffered charcoal yeast extract (BCYE) agar. After incubation at 35°C–37°C for at least 48 hours, *F. tularensis* forms small, gray-white to opaque colonies. Presumptive identification is made by culture examination, microscopy, motility testing, and biochemical testing. *F. tularensis* is a minute, pleomorphic, faint-straining, Gram-negative coccobacillus and is weakly catalase-positive, oxidase-negative, ß-lactamase-positive, and urease-negative. Cultures (isolates) that cannot be ruled out as *F. tularensis* based on the characteristics noted above may be referred to an appropriate reference laboratory for confirmation.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Special Considerations: *F. 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 CDC's BMBL, 5th Edition, http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm).

Source: CDC, ASM, and APHL. 2001. "Basic Protocols for Level A Laboratories for the Presumptive Identification of *Francisella tularensis*." http://www.epa.gov/sam/pdfs/CDC-Ftularemia.pdf

7.2.9 CDC Laboratory Assay: "Triplex PCR for Detection of *S.* Typhi Using SmartCycler®"

Pathogen(s) [Disease]	Agent Category	BSL
Salmonella Typhi [Typhoid fever]	Bacteria	2/3 (aerosol release)

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Solid samples should be prepared according to "Quantification of Bias Related to the Extraction of DNA Directly from Soils," Frostegard, A., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F., Jeannin, P., Nesme, X., and Simonet, P. 1999. Applied and Environmental Microbiology. 65(12): 5409–5420 (www.epa.gov/sam/pdfs/AEM_65(12)-pgs5409-5420.pdf).
Analytical Technique: Real-time PCR

Method Developed for: *S.* Typhi from cultures or isolates
Method Selected for: SAM lists these procedures for detection in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of isolates and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. The assay uses real-time PCR for identification of *S.* Typhi. Cell lysate templates are prepared by suspending a portion of a colony in 300 µL of distilled water and boiling for 10 minutes. After centrifugation at 4,500 rpm for 2 minutes, 1 µL of the supernatant is used in the PCR reaction. Alternatively, deoxyribonucleic acid (DNA) may be purified using a commercially available kit or automated DNA extraction system. PCR is performed on a SmartCycler® using primers and probes designed for the Vi capsular gene (*viaB*), the H antigen gene (*fliC-d*), and the tyvelose epimerase gene (*tyv*). This assay is also available for the LightCycler® platform as three single target PCRs.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: HHS, CDC, Laboratory Assay. "Triplex PCR for Detection of *S.* Typhi Using SmartCycler®." Contact: Dr. Eija Trees, CDC, email: eija.trees@cdc.hhs.gov.

7.2.10 CDC Laboratory Assay: "Detection of Diarrheagenic *Eshcerichia* coli and *Shigella* Using LightCycler®"

Pathogen(s) [Disease]	Agent Category	BSL
Shigella spp. [Shigellosis]	Bacteria	2

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Solid samples should be prepared according to "Quantification of Bias Related to the Extraction of DNA Directly from Soils," Frostegard, A., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F., Jeannin, P., Nesme, X., and Simonet, P. 1999. Applied and Environmental Microbiology. 65(12): 5409–5420 (www.epa.gov/sam/pdfs/AEM-65(12)-pgs5409-5420.pdf).
Analytical Technique: Real-time PCR

Method Developed for: *Shigella* from cultures or isolates
Method Selected for: SAM lists these procedures for detection in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of isolates and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. The assay uses real-time PCR for identification of *Shigella*. Cell lysate templates are prepared by suspending a portion of a colony in 300 µL of distilled water and boiling for 10 minutes. After centrifugation at 4,500 rpm for 2 minutes, 1 µL of the supernatant is used in the PCR reaction. Alternatively, DNA may be purified using a commercially available kit or automated DNA extraction system. PCR is performed on a LightCycler® using primers and probes designed for the *ipa*H-plasmid, which encodes the invasive plasmid antigen H. This gene can be found on both the chromosome and a plasmid for *Shigella* spp. An alternative multiplex PCR is described targeting both *ipa*H and Stx_1 (Shiga toxin) genes using TaqMan® chemistry and SmartCycler®.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: HHS, CDC, Laboratory Assay. "Detection of Diarrheagenic *Eshcerichia coli* and *Shigella* Using LightCycler®." Contact: Dr. Eija Trees, CDC, email: eija.trees@cdc.hhs.gov.

7.2.11 CDC Laboratory Assay: "TaqMan Assays for Detection of *V. cholerae ctx*A, O1 *rfb*, and O139 *rfb*."

Pathogen(s) [Disease]	Agent Category	BSL
Vibrio cholerae [Cholera]	Bacteria	2

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Solid samples should be prepared according to "Quantification of Bias Related to the Extraction of DNA Directly from Soils," Frostegard, A., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F., Jeannin, P., Nesme, X., and Simonet, P. 1999. Applied and Environmental Microbiology. 65(12): 5409–5420 (www.epa.gov/sam/pdfs/AEM-65(12)-pgs5409-5420.pdf).
Analytical Technique: Real-time PCR

Method Developed for: *V. cholerae* O1 and O139 from cultures or isolates
Method Selected for: SAM lists these procedures for detection in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of isolates and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. The assay uses real-time PCR for identification of *V. cholerae*. Cell lysate templates are prepared by suspending a portion of a colony in 300 µL of 0.01M Tris-EDTA (TE) buffer and boiling for 10 minutes. After centrifugation at 4,500 rpm

for 2 minutes, 1 µL of the supernatant is used in the PCR reaction. Alternatively, DNA may be purified using a commercially available kit or automated DNA extraction system. PCR is performed on a LightCycler® as single target assays or on a SmartCycler® as multiplex PCR using primers and probes designed for the cholera toxin *ctx* gene, the O1 antigen O1*rfb* gene, and the O139 antigen O139 *rfb* gene.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: HHS, CDC, Laboratory Assay. "TaqMan Assays for Detection of *V. cholerae ctx*A, O1 *rfb*, and O139 *rfb*." Contact: Dr. Eija Trees, CDC, email: eija.trees@cdc.hhs.gov.

7.2.12 USDA Laboratory Guidebook: "FSIS Procedure for the Use of a *Listeria monocytogenes* Polymerase Chain Reaction (PCR) Screening Test." MLG 8A.03. 2007.

Pathogen(s) [Disease]	Agent Category	BSL
Listeria monocytogenes [Listeriosis]	Bacteria	2

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Solid samples should be prepared according to "Quantification of Bias Related to the Extraction of DNA Directly from Soils," Frostegard, A., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F., Jeannin, P., Nesme, X., and Simonet, P. 1999. Applied and Environmental Microbiology. 65(12): 5409–5420 (www.epa.gov/sam/pdfs/AEM-65(12)-pgs5409-5420.pdf).
Analytical Technique: Real-time PCR

Method Developed for: *L. monocytogenes* in food samples
Method Selected for: SAM lists these procedures for detection in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of food samples and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. The method uses real-time PCR for identification of *L. moncytogenes*. Samples are homogenized in modified University of Vermont (UVM) broth and incubated at 30.0°C ± 2.0°C for 8–24 hours. PCR is performed with the BAX® System. Additional enrichment may be required at 35.0°C ± 2.0°C for 18–24 hours, using morpholinepropanesulfonic acid (MOPS) buffered *Listeria* enrichment broth (BLEB). LODs were determined to be better than 1 colony forming unit (CFU)/g in a 25 g meat or poultry sample, approximately 4.5 CFU/g in a 25 g pasteurized liquid whole egg blend sample, and 1.0×10^{-2} CFU/mL in a 500 mL brine sample.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: USDA, FSIS. 2007. "FSIS Procedure for the Use of a *Listeria monocytogenes* Polymerase Chain Reaction (PCR) Screening Test." *Laboratory Guidebook MLG 8A.03*.
http://www.epa.gov/sam/pdfs/USDA-MLG-8A.03.pdf

7.2.13 U.S. FDA Bacteriological Analytical Manual, Chapter 10, 2003: *Listeria monocytogenes*

Pathogen(s) [Disease]	Agent Category	BSL
Listeria monocytogenes [Listeriosis]	Bacteria	2

Analysis Purpose: Detection and viability
Sample Preparation: Solid samples should be prepared according to EPA Method 1680; particulate samples should be prepared according to "Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004. Emerging Infectious Diseases. 10(6): 1023–1029 (www.epa.gov/sam/pdfs/EID-10(6)-pgs1023-1029.pdf) and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied and Environmental Microbiology. 72(6): 4429–4430 (www.epa.gov/sam/pdfs/AEM-72(6)-pgs4429-4430.pdf).
Analytical Technique: Culture and immunoassay

Method Developed for: *Listeria monocytogenes* in food
Method Selected for: SAM lists this manual for detection and viability assessment in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of food samples and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. Prepared samples are incubated for 4 hours in BLEB without selective agents. Cycloheximide is added and incubation continued. At 24 and 48 hours, BLEB cultures are streaked onto esculin-containing selective isolation agar (i.e., Oxford Medium [OXA]), and incubated for an additional 24 to 48 hours. An *L. monocytogenes-L. ivanovii* differential selective agar, such as Biosynth Chromogen Medium (BCM), is streaked at 48 hours. Presumptive *Listeria* colonies are black with a black halo on esculin-containing media and blue on BCM plates. Isolated colonies are streaked onto Trypticase™ soy agar with yeast extract (TSAye), incubated for 24 to 48 hours, and examined for morphological and biochemical characteristics. *L. monocytogenes* is a rod-shaped Gram-positive, motile bacterium. It is catalase-positive, ramnose-positive, and mannitol- and xylose-negative. Purified isolates may be rapidly identified using commercially available biochemical typing kits. Confirmation is performed with commercially available sera.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Source: U.S. FDA, CFSAN. 2003. "Chapter 10 – Detection and Enumeration of *Listeria monocytogenes* in Foods." *Bacteriological Analytical Manual Online.* http://www.epa.gov/sam/pdfs/FDA-BAM-Chap10.pdf

7.2.14 Standard Method 9213 B: *Staphylococcus aureus*

Pathogen(s)	Agent Category	BSL
Staphylococcus aureus	Bacteria	2

Analysis Purpose: Detection and viability
Sample Preparation: Solid samples should be prepared according to EPA Method 1680; particulate samples should be prepared according to "Swab Materials and *Bacillus anthracis* Spore Recovery from

Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004. Emerging Infectious Diseases. 10(6): 1023–1029 (www.epa.gov/sam/pdfs/EID-10(6)-pgs1023-1029.pdf) and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied and Environmental Microbiology. 72(6): 4429–4430 (www.epa.gov/sam/pdfs/AEM-72(6)-pgs4429-4430.pdf).
Analytical Technique: Culture

Method Developed for: *Staphylococcus aureus* in water
Method Selected for: SAM lists this method for detection and viability assessment in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of water samples and may be adapted for assessment of solid, liquid, particulate, and aerosol samples. Prepared samples are inoculated into tubes of M-staphylococcus broth and incubated for 24 hours. Positive (turbid) tubes are streaked onto plates of Baird-Parker agar and incubated for 48 hours. Presumptive *S. aureus* colonies are tested for mannitol fermentation by the addition of a drop of bromthymol blue, a pH indicator. Isolated colonies are examined for morphological and biochemical characteristics. *S. aureus* is a Gram-positive coccus. Biochemical characterizations include catalase-positive, coagulase-positive, fermentation of mannitol, and anaerobic fermentation of glucose.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Source: 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 Standard Method 9260 B: General Qualitative Isolation and Identification Procedures for *Salmonella*

Pathogen(s) [Disease]	Agent Category	BSL
Salmonella Typhi [Typhoid fever]	Bacteria	2/3 (aerosol release)

Analysis Purpose: Detection and viability
Sample Preparation: Solid samples should be prepared according to EPA Method 1680; particulate samples should be prepared according to "Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004. Emerging Infectious Diseases. 10(6): 1023–1029 (www.epa.gov/sam/pdfs/EID-10(6)-pgs1023-1029.pdf) and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied and Environmental Microbiology. 72(6): 4429–4430 (www.epa.gov/sam/pdfs/AEM-72(6)-pgs4429-4430.pdf).
Analytical Technique: Culture and immunoassay

Method Developed for: *Salmonella* Typhi in water
Method Selected for: SAM lists this method for detection and viability assessment in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This method describes procedures for analysis of water samples and may be adapted for assessment of solid, particulate, and liquid samples. Concentrated samples are enriched in either selenite cystine, selenite-F, or tetrathionate broths and incubated at 35°C to 37°C for up to 5 days. An aliquot from each turbid tube is streaked onto bismuth sulfite (BS) plates and incubated at 35°C to 37°C for 24–48 hours. Presumptive positive colonies are then subjected to biochemical characterization. Confirmation is through serological testing using polyvalent O and Vi antiserum.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Special Considerations: This method is not preferred for non-typhoidal *Salmonella*.

Source: APHA, AWWA, and WEF. 2005. "Method 9260 B: General Qualitative Isolation and Identification Procedures for *Salmonella*." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

7.2.16 Standard Method 9260 E: *Shigella*

Pathogen(s) [Disease]	Agent Category	BSL
Shigella spp. [Shigellosis]	Bacteria	2

Analysis Purpose: Detection and viability
Sample Preparation: Solid samples should be prepared according to EPA Method 1680; particulate samples should be prepared according to "Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004. Emerging Infectious Diseases. 10(6): 1023–1029 (www.epa.gov/sam/pdfs/EID-10(6)-pgs1023-1029.pdf) and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied and Environmental Microbiology. 72(6): 4429–4430 (www.epa.gov/sam/pdfs/AEM-72(6)-pgs4429-4430.pdf)
Analytical Technique: Culture and immunoassay

Method Developed for: *Shigella* spp. in water and solids
Method Selected for: SAM lists this method for detection and viability assessment in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This method describes procedures for analysis of water samples and may be adapted for assessment of solid, particulate, and liquid samples. This method contains two options for sample concentration: MF (liquid samples) and centrifugation (liquid and solid samples) for analyses. Both options include inoculation of an enrichment medium (Selenite F broth). Isolation of the target pathogen is achieved by plating onto XLD and/or MacConkey agar. Biochemical identification consists of inoculating TSI and LIA slants. Confirmation is performed by slide agglutination tests using polyvalent antisera.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Source: APHA, AWWA, and WEF. 2005. "Method 9260 E: *Shigella*." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

7.2.17 Standard Method 9260 F: Pathogenic *Escherichia coli*

Pathogen(s)	Agent Category	BSL
Escherichia coli O157:H7	Bacteria	2

Analysis Purpose: Detection and viability
Sample Preparation: Solid samples should be prepared according to EPA Method 1680; particulate samples should be prepared according to "Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004. Emerging Infectious Diseases. 10(6): 1023–1029 (www.epa.gov/sam/pdfs/EID-10(6)-pgs1023-1029.pdf) and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied and Environmental Microbiology. 72(6): 4429–4430 (www.epa.gov/sam/pdfs/AEM-72(6)-pgs4429-4430.pdf).
Analytical Technique: Culture and immunoassay

Method Developed for: *Escherichia coli* O157:H7 in water and apple juice
Method Selected for: SAM lists this method for detection and viability assessment in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This method describes procedures for analysis of water and apple juice samples and may be adapted for assessment of solid, particulate, and liquid samples. The method allows for two options, one being a modification of SM 9221B followed by plating and biochemical identification. The second option, modification of a food method, allows for the analysis of large sample volumes. A 200-mL water sample is centrifuged; the pellet is resuspended in *E. coli* enrichment broth (EEB) and incubated for 6 hours. Tellurite Cefixime Sorbitol MacConkey (TC-SMAC) plates are inoculated with the enriched EEB culture, and incubated for up to 24 hours. Colorless colonies on TC-SMAC are tested for indole production. Presumptive positive colonies are then subjected to biochemical characterization. Confirmation is through serological testing.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Source: APHA, AWWA, and WEF. 2005. "Method 9260 F: Pathogenic *Escherichia coli*." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

7.2.18 Standard Method 9260 G: *Campylobacter jejuni*

Pathogen(s) [Disease]	Agent Category	BSL
Campylobacter jejuni [Campylobacteriosis]	Bacteria	2

Analysis Purpose: Detection and viability
Sample Preparation: Solid samples should be prepared according to EPA Method 1680; particulate samples should be prepared according to "Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004. Emerging Infectious Diseases. 10(6): 1023–1029 (www.epa.gov/sam/pdfs/EID-10(6)-pgs1023-1029.pdf) and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied

and Environmental Microbiology. 72(6): 4429–4430 (www.epa.gov/sam/pdfs/AEM-72(6)-pgs4429-4430.pdf).
Analytical Technique: Culture and immunoassay

Method Developed for: *Campylobacter jejuni* in water
Method Selected for: SAM lists this method for detection and viability assessment in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This method describes procedures for analysis of water samples and may be adapted for assessment of solid, particulate, and liquid samples. Water samples (1 to several liter volumes) are filtered using a cellulose nitrate membrane filter. Filters are placed on Skirrow's medium and incubated for 24 hours at 42°C under microaerophilic conditions. Alternatively, samples are enriched in *Campylobacter* broth supplemented with antibiotics and lysed horse blood under microaerophilic conditions at 37°C for 4 hours, then at 42°C for 24–48 hours prior to streaking on Skirrow's medium. Identification is made by culture examination, microscopy, motility testing, and biochemical testing. Biochemical tests include oxidase, catalase, nitrite and nitrate reduction, hydrogen sulfide production, and hippurate hydrolysis. Confirmation is performed using commercially available rapid serological test kits.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Special Considerations: Skirrow's and other selective media containing antibiotics (trimethoprim, vancomycin, polymixin) may prevent the growth of injured organisms.

Source: APHA, AWWA, and WEF. 2005. "Method 9260 G: *Campylobacter jejuni*." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

7.2.19 Standard Method 9260 H: *Vibrio cholerae*

Pathogen(s) [Disease]	Agent Category	BSL
Vibrio cholerae [Cholera]	Bacteria	2

Analysis Purpose: Detection and viability
Sample Preparation: Solid samples should be prepared according to EPA Method 1680; particulate samples should be prepared according to "Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004. Emerging Infectious Diseases. 10(6): 1023–1029 (www.epa.gov/sam/pdfs/EID-10(6)-pgs1023-1029.pdf) and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied and Environmental Microbiology. 72(6): 4429–4430 (www.epa.gov/sam/pdfs/AEM-72(6)-pgs4429-4430.pdf).
Analytical Technique: Culture and immunoassay

Method Developed for: *Vibrio cholerae* in water
Method Selected for: SAM lists this method for detection and viability assessment in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This method describes procedures for analysis of water samples and may be adapted for assessment of solid, particulate, and liquid samples. Samples are enriched in alkaline peptone

broth and incubated for up to 8 hours. Thiosulfate-citrate-bile salts-sucrose (TCBS) agar plates are inoculated with the incubated broth and incubated for 24 hours. Yellow sucrose-fermenting colonies are presumptive for *V. cholerae* and are plated on tryptic soy agar with 0.5% sodium chloride. Presumptive positive colonies are subjected to biochemical characterization. Confirmation is performed using slide agglutination assays for serological identification.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Source: APHA, AWWA, and WEF. 2005. "Method 9260 H: *Vibrio cholerae*." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

7.2.20 Standard Method 9260 I: *Leptospira*

Pathogen(s) [Disease]	Agent Category	BSL
Leptospira interrogans [Leptospirosis]	Bacteria	2

Analysis Purpose: Detection and viability
Sample Preparation: Solid samples should be prepared according to EPA Method 1680; particulate samples should be prepared according to "Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004 Emerging Infectious Diseases. 10(6): 1023–1029 (www.epa.gov/sam/pdfs/EID-10(6)-pgs1023-1029.pdf) and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied and Environmental Microbiology. 72(6): 4429–4430 (www.epa.gov/sam/pdfs/AEM-72(6)-pgs4429-4430.pdf).
Analytical Technique: Culture and immunoassay

Method Developed for: *Leptospira interrogans* in water
Method Selected for: SAM lists this method for detection and viability assessment in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This method describes procedures for analysis of water samples and may be adapted for assessment of solid, particulate, and liquid samples. Filter samples through a 0.22-µm filter, retaining the filtrate as inoculum. If the sample is turbid, a succession of filters of decreasing pore size may be used prior to the 0.22-µm filter. A tube of *Leptospira* Medium Base (Ellinghausen-McCullough Johnson Harris formulation [EMJH], supplemented with bovine serum albumin [BSA] and Tween®), is inoculated with the sample and incubated at 30°C for up to 6 weeks. Alternatively, the sample is added directly to the media, incubated overnight, passed through a 0.22-µm membrane filter, and incubation continued for up to 6 weeks. Cultures are examined by darkfield microscopy for motile leptospires. Confirmation is performed by microscopic agglutination test using reference antisera.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Source: APHA, AWWA, and WEF. 2005. "Method 9260 I: *Leptospira*." *Standard Methods for the Examination of Water and Wastewater*. 21st Edition. http://www.standardmethods.org/

7.2.21 ASM Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism: *Brucella* species

Pathogen(s) [Disease]	Agent Category	BSL
Brucella spp. [Burcellosis]	Bacteria	3

Analysis Purpose: Detection and viability
Sample Preparation: Solid samples should be prepared according to EPA Method 1680; particulate samples should be prepared according to "Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004. Emerging Infectious Diseases. 10(6): 1023–1029 (www.epa.gov/sam/pdfs/EID-10(6)-pgs1023-1029.pdf) and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied and Environmental Microbiology. 72(6): 4429–4430 (www.epa.gov/sam/pdfs/AEM-72(6)-pgs4429-4430.pdf).
Analytical Technique: Culture

Method Developed for: *Brucella* spp. in clinical samples
Method Selected for: SAM lists these guidelines for detection and viability assessment in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This method describes procedures for analysis of clinical samples and may be adapted for assessment of solid, particulate, and liquid samples. Samples are plated directly on CA or SBA and incubated at 35°C (5–10% carbon dioxide) for up to 7 days. Colonies are punctuate (minute), non-pigmented, and non-hemolytic at 48 hours. Presumptive identification is made by culture examination, microscopy, and biochemical testing. *Brucella* spp. are small, non-motile, Gram-negative coccobacilli and are catalase-, oxidase-, and urease-positive. Cultures (isolates) that cannot be ruled out as *Brucella* spp. based on the characteristics noted above may be referred to an appropriate reference laboratory for confirmation. Confirmation is performed using specific antiserum.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

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 CDC's BMBL, 5th Edition, http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm).

Source: ASM. 2004. "Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism: *Brucella* species." http://www.epa.gov/sam/pdfs/ASM-Brucella.pdf

7.2.22 ASM Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism: *Burkholderia mallei* and *Burkholderia pseudomallei*

Pathogen(s) [Disease]	Agent Category	BSL
Burkholderia mallei [Glanders]	Bacteria	3
Burkholderia pseudomallei [Melioidosis]	Bacteria	3

Analysis Purpose: Detection and viability

Sample Preparation: Solid samples should be prepared according to EPA Method 1680; particulate samples should be prepared according to "Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004. Emerging Infectious Diseases. 10(6): 1023–1029 (www.epa.gov/sam/pdfs/EID-10(6)-pgs1023-1029.pdf) and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied and Environmental Microbiology. 72(6): 4429–4430 (www.epa.gov/sam/pdfs/AEM-72(6)-pgs4429-4430.pdf).
Analytical Technique: Culture

Method Developed for: *B. mallei and B. pseudomallei* in clinical samples
Method Selected for: SAM lists these guidelines for detection and viability assessment in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This method describes procedures for analysis of clinical samples and may be adapted for assessment of solid, particulate, and liquid samples. Samples are plated directly on SBA and incubated at 35°C–37°C. At 48 hours, *B. mallei* forms gray, translucent colonies and *B. pseudomallei* forms small, smooth, creamy colonies with a musty odor that gradually change to dry, wrinkled colonies. Presumptive identification is made by culture examination, microscopy, motility testing, and biochemical testing. *B. mallei* are small, non-motile, Gram-negative coccobacilli or small rods and are oxidase-variable, catalase-positive, colistin-resistant, indole-negative, nitrate reductase-positive, and arginine dihydrolase-positive. *B. pseudomallei* are small, motile, Gram-negative rods and are oxidase-positive, catalase-positive, colistin-resistant, indole-negative, nitrate reductase-positive, and arginine dihydrolase-positive. Cultures (isolates) that cannot be ruled out as *B. mallei* or *B. pseudomallei* based on the characteristics noted above may be referred to an appropriate reference laboratory for confirmation.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Special Considerations: *B. mallei and B. 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 CDC's BMBL, 5th Edition, http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm).

Source: ASM. 2008. "Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism: *Burkholderia mallei* and *Burkholderia pseudomallei*." http://www.epa.gov/sam/pdfs/ASM-Bmallei-Bpseudomallei.pdf

7.2.23 ASM Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism: *Yersinia pestis*

Pathogen(s) [Disease]	Agent Category	BSL
Yersinia pestis [Plague]	Bacteria	3

Analysis Purpose: Detection and viability
Sample Preparation: Solid samples should be prepared according to EPA Method 1680; particulate samples should be prepared according to "Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004. Emerging Infectious Diseases. 10(6): 1023–1029 (www.epa.gov/sam/pdfs/EID-10(6)-pgs1023-1029.pdf) and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied

and Environmental Microbiology. 72(6): 4429–4430 (www.epa.gov/sam/pdfs/AEM-72(6)-pgs4429-4430.pdf).
Analytical Technique: Culture

Method Developed for: *Y. pestis* in clinical samples
Method Selected for: SAM lists these guidelines for detection and viability assessment in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: This method describes procedures for analysis of clinical samples and may be adapted for assessment of solid, particulate, and liquid samples. Samples are either plated directly on SBA or first enriched in a nutrient broth (e.g., TSB) prior to plating. Incubation is for at least 3 days at 28°C–30°C. *Y. pestis* produces 1.0–2.0 mm, gray-white to opaque colonies on SBA at 24 hours, with a "fried egg" appearance with longer incubation. There is little to no hemolysis on SBA. In broth, *Y. pestis* grows in flocculent clumps. Presumptive identification is made by culture examination, microscopy, and biochemical testing. *Y. pestis* is a bi-polar staining, Gram-negative rod and is oxidase-negative, catalase-positive, urease-negative, and indole-negative. Cultures (isolates) that cannot be ruled out as *Y. pestis* based on the characteristics noted above are referred to an appropriate reference laboratory for confirmation. Cultures (isolates) that cannot be ruled out as *Y. pestis* based on the characteristics noted above may be referred to an appropriate reference laboratory for confirmation.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Special Considerations: *Y. 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 CDC's BMBL, 5th Edition, http://www.cdc.gov/OD/ohs/biosfty/bmbl5/bmbl5toc.htm).

Source: ASM. 2005. "Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism: *Yersinia pestis*." http://www.epa.gov/sam/pdfs/ASM-Ypestis.pdf

7.2.24 Literature Reference for *Campylobacter jejuni* (Molecular and Cellular Probes. 2006. 20: 269–279)

Pathogen(s) [Disease]	Agent Category	BSL
Campylobacter jejuni [Campylobacteriosis]	Bacteria	2

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Solid samples should be prepared according to "Quantification of Bias Related to the Extraction of DNA Directly from Soils," Frostegard, A., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F., Jeannin, P., Nesme, X., and Simonet, P. 1999. Applied and Environmental Microbiology. 65(12): 5409–5420 (www.epa.gov/sam/pdfs/AEM-65(12)-pgs5409-5420.pdf).
Analytical Technique: Real-time PCR

Method Developed for: *Campylobacter jejuni* in clinical samples
Method Selected for: SAM lists these procedures for detection in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of clinical samples and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. The method uses real-time quantitative PCR (qPCR) for identification of *C. jejuni* that can be used in conjuction with either the ABI

Prism® 7700 or 7900 HT Thermal Cyclers. A high through-put method using guanidinium thiocyanate (GTC) and glass beads is used for extraction of DNA. *C. jejuni* uses the CJ F primer, CJ R primer, and CJ probe for real-time quantitative PCR. Results are evaluated against standard curves made with a 10-fold dilution series of *C. jejuni* NCTC 11168 DNA in a background of cecum (intestinal) DNA.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: Skanseng, B., Kaldhusdal, M., and Rudi, K. 2006. "Comparison of Chicken Gut Colonization by the Pathogens *Campylobacter jejuni* and *Clostridium perfringens* by the Real-time Quantitative PCR." Molecular and Cellular Probes. 20: 269–279. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6WNC-4JDN6C7-1&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_version=1&_urlVersion=0&_userid=10&md5=dcdea60d7ab66cd57b8ffef7d59ebd39

7.2.25 Literature Reference for *Chlamydophila psittaci* (Journal of Clinical Microbiology. 2000. 38(3): 1085–1093)

Pathogen(s) [Disease]	Agent Category	BSL
Chlamydophila psittaci [Psittacosis] (formerly known as *Chlamydia psittaci*)	Bacteria	2/3 (aerosols and large volumes)

Analysis Purpose: Detection and viability
Sample Preparation: Solid samples should be prepared according to "Quantification of Bias Related to the Extraction of DNA Directly from Soils," Frostegard, A., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F., Jeannin, P., Nesme, X., and Simonet, P. 1999. Applied and Environmental Microbiology. 65(12): 5409–5420 (www.epa.gov/sam/pdfs/AEM-65(12)-pgs5409-5420.pdf).
Analytical Technique: Tissue culture and PCR

Method Developed for: *Chlamydophila psittaci* in clinical samples
Method Selected for: SAM lists these procedures for detection and viability assessment in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of clinical samples and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. The method uses touchdown enzyme time release (TETR)-PCR for detection and identification of *Chlamydophila psittaci*. DNA is extracted from chlamydia cultures (Buffalo Green Monkey Kidney [BGMK] cells), minced clinical tissues, and respiratory samples by mixing with a chelating resin, heating first to 56°C for 15–30 minutes, then 100°C for 8–10 minutes. Primer sets specific for *C. psittaci* are designed based on the DNA sequences of the 16S ribosomal ribonucleic acid (rRNA) and 16S-23S rRNA genes (CPS 100/101). PCR is conducted on a Perkin-Elmer 480 with products separated by electrophoresis in 12% polyacrylamide gels with Tris-borate-EDTA buffer and visualized with ethidium bromide. *Chlamydophila psittaci* samples are to be handled with BSL-3 containment and practices.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on*

Environmental Samples document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: 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://www.epa.gov/sam/pdfs/JCM-38(3)-pgs1085-1093.pdf

7.2.26 Literature Reference for *Escherichia coli* O157:H7 (Applied and Environmental Microbiology. 2003. 69(10): 6327–6333)

Pathogen(s)	Agent Category	BSL
Escherichia coli O157:H7	Bacteria	2

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Solid samples should be prepared according to "Quantification of Bias Related to the Extraction of DNA Directly from Soils," Frostegard, A., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F., Jeannin, P., Nesme, X., and Simonet, P. 1999. Applied and Environmental Microbiology. 65(12): 5409–5420 (www.epa.gov/sam/pdfs/AEM-65(12)-pgs5409-5420.pdf).
Analytical Technique: Real-time PCR

Method Developed for: *E. coli* O157:H7 in cultures or isolates
Method Selected for: SAM lists these procedures for detection in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of isolates and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. The method uses multiplex real-time PCR performed on a Smart Cycler® with fluorogenic probes for identification of cultured *E. coli* O157:H7 isolates. Sample template (0.5 µL) is added to a total reaction volume of 25 µL. Primers and probes were designed to target the *stx1* and *stx2* (Shiga toxin) genes and the single-nucleotide polymorphism at position 93 of the *uidA* (ß-glucoronidase) gene, using 6-carboxy-X-rhodamine (ROX), 6-caraboxyfluorescein (FAM), and 6-carboxy-4,7,2',7'-tetrachlorofluorescein (TET) as respective fluorogens. The method was tested with 138 isolates, including 52 *E. coli* O157:H7 and two E. *coli* O157:H7$^-$ organisms. Specificity was 100% for all three target genes. Sensitivities were 98.6%, 100%, and 100% for *stx1*, *stx2*, and *uidA* O157:H7$^-$ targets, respectively. This assay did not detect two strains of the closely related *E. coli* O55:H7/H$^-$ serotype.

At a minimum, the following quality control checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR quality control checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document, www.epa.gov/nerlcwww/qa_qc_pcr10_04.pdf, or consult the point of contact identified in Section 4.

Source: Jinneman, K.C., Yoshitomi, K.J., and Weagant, S.D. 2003. "Multiplex Real-Time PCR Method to Identify Shiga Toxin Genes *stx1* and *stx2* and *Escherichia coli* O157:H7/H$^-$ Serotype." Applied and Environmental Microbiology. 69(10): 6327–6333. http://www.epa.gov/sam/pdfs/AEM-69(10)-pgs6327-6333.pdf.

7.2.27 Literature Reference for Non-Typhoidal *Salmonella* (Journal of Applied Microbiology. 2007. 102(2): 516–530)

Pathogen(s) [Disease]	Agent Category	BSL
Non-typhoidal *Salmonella* [Salmonellosis] (Method not applicable to *S.* Typhi)	Bacteria	2

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Solid samples should be prepared according to "Quantification of Bias Related to the Extraction of DNA Directly from Soils," Frostegard, A., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F., Jeannin, P., Nesme, X., and Simonet, P. 1999. Applied and Environmental Microbiology. 65(12): 5409–5420 (www.epa.gov/sam/pdfs/AEM-65(12)-pgs5409-5420.pdf).
Analytical Technique: Real-time PCR

Method Developed for: Non-typhoidal *Salmonella* from cultures or isolates
Method Selected for: SAM lists these procedures for detection in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of isolates and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. The method uses real-time PCR for identification of *Salmonella* spp. PCR templates are prepared from 1.0 mL of overnight bacterial cultures propagated overnight in TSB at 37.0°C. Cells are pelleted, washed twice with physiological saline, resuspended in molecular grade water, and boiled for 10 minutes. PCR is performed on a SmartCycler® II System using primers and probes designed for the *stn* gene. The protocol has been evaluated against 353 isolates, including 255 *S. enterica* representing 158 serotypes, 14 *S. bongori* representing 12 serotypes, and 84 non-*Salmonella* representing 56 species from 31 genera. The PCR method had 100% inclusivity, 96.4% exclusivity, and a level of detection of 3 CFUs per reaction for cultured *Salmonella* spp.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: Moore, M.M., and Feist, M.D. 2007. "Real-time PCR Method for *Salmonella* spp. Targeting the stn Gene." Journal of Applied Microbiology. 102(2): 516–530.
http://www3.interscience.wiley.com/journal/118490299/abstract

7.2.28 Literature Reference for Adenoviruses (Applied and Environmental Microbiology. 2005. 71(6): 3131–3136)

Pathogen(s)	Agent Category	BSL
Adenoviruses: Enteric and non-enteric (A-F)	Viruses	2

Analysis Purpose: Detection and viability
Sample Preparation: Samples should be prepared according to the procedures described in the USEPA Manual of Methods for Virology (EPA/600/4-84/013, April 2001).
Analytical Technique: Tissue culture and real-time PCR

Method Developed for: Human adenoviruses (HAdV) in cell culture lysate samples
Method Selected for: SAM lists these procedures for detection and viability assessment in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of cell culture lysates and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. The detection procedure uses a broadly reactive fluorogenic 5' nuclease (TaqMan®) quantitative real-time PCR assay for the detection of all six species (A–F) of HAdV on a R.A.P.I.D.® PCR system. Sensitive detection and discrimination of adenovirus F species (Adenovirus 40 [AdV40] and Adenovirus 41 [AdV41]) can be achieved by using a real-time fluorescence resonance energy transfer (FRET)-based PCR assay.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Special Considerations: For the viability assessment of adenovirus 40 and 41, given that they can be difficult to grow in culture, cell lines such as G293 (Journal of Medical Virology, 11(3): 215–231) or CaCo-2 (Journal of Medical Virology. 1994. 44(3): 310–315) may be considered when these viruses are suspected to be present. As detection of adenovirus in environmental samples can be difficult, additional methods such as described in *Effect of Adenovirus Resistance on UV Disinfection Experiments: A Report on the State of Adenovirus Science* (J. AWWA. 2006. 98(6):93–106) also may be useful.

Source: Jothikumar, N., Cromeans, T.L., Hill, V.R., Lu, X., Sobsey, M., 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://www.epa.gov/sam/pdfs/AEM-71(6)-pgs3131-3136.pdf

7.2.29 Literature Reference for Astroviruses (Canadian Journal of Microbiology. 2004. 50: 269–278)

Pathogen(s)	Agent Category	BSL
Astroviruses	Viruses	Not specified

Analysis Purpose: Detection and viability
Sample Preparation: Samples should be prepared according to the procedures described in the USEPA Manual of Methods for Virology (EPA/600/4-84/013, April 2001).
Analytical Technique: Integrated cell culture/real-time reverse transcription-PCR

Method Developed for: Astroviruses in clinical samples
Method Selected for: SAM lists these procedures for detection and viability assessment in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of clinical samples and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. The method detects eight astrovirus serotypes. The method is a reverse transcription-PCR procedure optimized for use in a real-time PCR assay using an ABI Prism® 7000 and can be integrated with sample-cell culture (CaCo-2 cells) to enhance sensitivity. Water samples are collected by filtration (1MDS filter), and viruses are eluted

using a beef extract solution (1.5%, pH 9.5). Viruses are concentrated using Celite® adsorption (pH 4.0), filtration, and Celite®-elution with sodium phosphate (0.15 M, pH 9.0), followed by further concentration and processing to remove inhibitors (ultracentrifugation, solvent extraction, and molecular weight [MW]-exclusion filtration). Concentrated samples are analyzed directly or indirectly (following cell culture) by a two-step reverse transcription-PCR (i.e., reverse transcription followed by PCR) assay using astrovirus-specific primer sets. Detection of amplicons is by gel electrophoresis with subsequent confirmation by hybridization (dot-blot) using digoxigenin-labeled internal (nested) probes or by real-time detection using fluorogenic probes.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: 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://pubs.nrc-cnrc.gc.ca/rp-ps/articleOptions.jsp?jcode=cjm&ftl=w04-012&lang=eng

7.2.30 Literature Reference for Noroviruses (Journal of Clinical Microbiology. 2004. 42(10): 4679–4685)

Pathogen(s)	Agent Category	BSL
Caliciviruses: Noroviruses	Viruses	Not specified

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Samples should be prepared according to procedures described in the USEPA Manual of Methods for Virology (EPA/600/4-84/013, April 2001).
Analytical Technique: Real-time reverse transcription-PCR

Method Developed for: Noroviruses in clinical samples
Method Selected for: SAM lists these procedures for detection in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of clinical samples and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. This method is an assay for the detection and quantitation of norovirus using LightCycler® real-time reverse transcription-PCR technology. Viral ribonucleic acid (RNA) is extracted using either a commercial kit or a silica-based method. For Norovirus G-1, primers based on the capsid gene sequence are used, and for Norovirus G-II, primers based on the polymerase gene sequence are used. A SYBR® Green I system is used in the reaction for visualization. External standard curves for the quantification of norovirus are established using RNA transcripts from strains S5 and S19, corresponding to G-I/4 and G-II/12, respectively.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: Pang, X., Lee, B., Chui, L., Preiksaitis, J.K., and Monroe, S.S. 2004. "Evaluation and Validation of Real-Time Reverse Transcription-PCR Assay Using the LightCycler System for Detection

and Quantitation of Norovirus." Journal of Clinical Microbiology. 42(10): 4679–4685. http://www.epa.gov/sam/pdfs/JCM-42(10)-pgs4679-4685.pdf

7.2.31 Literature Reference for Sapoviruses (Journal of Medical Virology. 2006. 78(10): 1347–1353)

Pathogen(s)	Agent Category	BSL
Caliciviruses: Sapoviruses	Viruses	Not specified

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Samples should be prepared according to procedures described in the USEPA Manual of Methods for Virology (EPA/600/4-84/013, April 2001).
Analytical Technique: Real-time reverse transcription-PCR

Method Developed for: Sapoviruses in clinical samples
Method Selected for: SAM lists these procedures for detection in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of clinical samples and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. The method is a TaqMan®-based real-time reverse transcriptase PCR assay that uses an ABI 7500 system. The assay has the ability to detect four of the five distinct sapovirus (SaV) genogroups (GI–GV). Sets of primers, based on the multiple alignment of 27 gene sequences for the polymerase-capsid junction in open reading frame 1 (ORF1), are used to detect human SaV GI, GII, GIV, and GV sequences in a single tube. Sensitivity using control plasmids range from 2.5×10^1 to 2.5×10^7 copies per tube. No cross-reactivity is observed against other enteric viruses, including norovirus (NoV), rotavirus, astrovirus, and adenovirus.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: 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

7.2.32 Literature Reference for Coronaviruses (SARS) (Journal of Virological Methods. 2004. 122: 29–36)

Pathogen(s)	Agent Category	BSL
Coronaviruses: SARS-associated human coronavirus	Viruses	2/3 (propagation)

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Samples should be prepared according to procedures described in the USEPA Manual of Methods for Virology (EPA/600/4-84/013, April 2001).
Analytical Technique: Reverse transcription-PCR

Method Developed for: SARS-associated human coronavirus in clinical samples
Method Selected for: SAM lists these procedures for detection in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of clinical samples and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. This method uses a conventional single-tube reverse transcription-PCR procedure conducted on a Stratagene Robocycler® and based on consensus primer sequences targeting conserved regions of coronavirus genome sequences. 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). Species identification is provided by sequencing the amplicon, although rapid screening can be performed by restriction enzyme analysis.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: 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: 29–36. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6T96-3W7XC6H-2&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_version=1&_urlVersion=0&_userid=10&md5=b711c55f4966e87bffb977bffa49f8db

7.2.33 Literature Reference for Hepatitis E Virus (Journal of Virological Methods. 2006. 131(1): 65–71)

Pathogen(s)	Agent Category	BSL
Hepatitis E virus (HEV)	Viruses	2

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Samples should be prepared according to procedures described in the USEPA Manual of Methods for Virology (EPA/600/4-84/013, April 2001).
Analytical Technique: Real-time reverse transcription-PCR

Method Developed for: Hepatitis E virus (HEV) in spiked water samples
Method Selected for: SAM lists these procedures for detection in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of spiked water samples and may be adapted for assessment of solid, particulate, aerosol, and liquid samples. The method uses a TaqMan® real-time reverse transcriptase-PCR assay using the R.A.P.I.D.® PCR systems to detect and quantitate all four major HEV genotypes that may be present in clinical and environmental samples. Primers and probes are based on the multiple sequence alignments of 27 gene sequences for the ORF3 region. Thirteen HEV isolates representing genotypes 1–4 were used to assess assay specificity, all thirteen isolates were positive.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: 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, Vol. 131(1): 65–71.
http://cat.inist.fr/?aModele=afficheN&cpsidt=17367357

7.2.34 Literature Reference for Influenza H5N1 (Emerging Infectious Diseases. 2005. 11(8): 1303–1305)

Pathogen(s)	Agent Category	BSL
Influenza H5N1 virus	Viruses	3

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Samples should be prepared according to procedures described in the USEPA Manual of Methods for Virology (EPA/600/4-84/013, April 2001).
Analytical Technique: Real-time reverse transcription-PCR

Method Developed for: Influenza H5N1 virus in clinical samples
Method Selected for: SAM lists these procedures for detection in solid, particulate, aerosol, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of clinical samples and may be adapted for assessment of solid, particulate, aerosol, liquid, and water samples. This is a two-step, real-time reverse transcriptase-PCR multiplex assay. It employs a mixture of two sets of primers and dual-labeled fluorescent probes that specifically target two different regions of the HA gene of H5N1. Viral ribonucleic acid (RNA) is extracted using a commercial viral RNA extraction kit and reverse transcribed with random hexamers. Five microliters of the complementary (cDNA) is used for PCR which is conducted on a LightCycler®. At the end of each annealing step, the fluorescent signal of each reaction is measured at a wavelength of 530 nm with the fluorimeter. The assay is specific for the H5 subtype. Influenza H5N1 virus samples are to be handled with BSL-3 containment and practices.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: 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.epa.gov/sam/pdfs/EID-11(8)-pgs1303-1305.pdf

7.2.35 Literature Reference for Enteric Viruses (Applied and Environmental Microbiology. 2003. 69(6): 3158–3164)

Pathogen(s)	Agent Category	BSL
Picornaviruses: Enteroviruses	Viruses	Not specified
Picornaviruses: Hepatitis A virus (HAV)	Viruses	2
Reoviruses: Rotaviruses (Group A)	Viruses	Not specified

Analysis Purpose: Detection of Enteroviruses and HAV; Detection and viability of Rotoviruses
Sample Preparation: Samples should be prepared according to procedures described in the USEPA Manual of Methods for Virology (EPA/600/4-84/013, April 2001).
Analytical Technique: Reverse transcription-PCR for detection and tissue culture for viability

Method Developed for: Enteroviruses, HAV, and Group A Rotaviruses in water
Method Selected for: SAM lists these procedures for detection of enterovirus and HAV in solid, particulate, aerosol, liquid, and water samples, but the procedures are not suitable for determining viability of these pathogens. These procedures should also be used for detection and viability assessment of rotavirus (Group A) in solid, particulate, aerosol, liquid, and water samples, although rotavirus is not likely to be viable in aerosol samples. Further research is needed to develop and standardize the procedures for environmental sample types other than water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of water samples and may be adapted for assessment of solid, particulate, aerosol, and liquid samples. The method is used to detect human enteric viruses (enteroviruses, HAV, rotavirus) in ground water samples. It is a multiplex reverse-transcription PCR procedure optimized for the simultaneous detection of enteroviruses, HAV, reoviruses, and rotaviruses. Water samples are collected by filtration and viruses are eluted using a beef extract solution (1.5%, pH 9.5). Viruses are concentrated using Celite® adsorption (pH 4.0), filtration, and Celite®-elution with sodium phosphate (0.15 M, pH 9.0), followed by further concentration and processing to remove inhibitors (ultracentrifugation, solvent extraction, and MW-exclusion filtration). Concentrated samples are analyzed by a two-step multiplex reverse transcription-PCR using virus-specific primer sets. Detection of amplicons is by gel electrophoresis with subsequent confirmation by hybridization (dot-blot) using digoxigenin-labeled internal (nested) probes.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: Fout, G.S., Martinson, B.C., Moyer, M.W.N., and Dahling, D.R. 2003. "A Multiplex Reverse Transcription-PCR Method for Detection of Human Enteric Viruses in Groundwater." Applied and Environmental Microbiology. 69(6): 3158–3164. http://www.epa.gov/sam/pdfs/AEM-69(6)-pgs3158-3164.pdf

7.2.36 Literature Reference for *Cryptosporidium* spp. (Applied and Environmental Microbiology. 1999. 65(9): 3936–3941)

Pathogen(s) [Disease]	Agent Category	BSL
Cryptosporidium spp. [Cryptosporidiosis]	Protozoa	2

Analysis Purpose: Detection and viability
Sample Preparation: Samples should be prepared according to the procedures in Applied and Environmental Microbiology. 1999. 65(9): 3939–3941.
Analytical Technique: Tissue culture

Method Developed for: *Cryptosporidium* in animal samples
Method Selected for: SAM lists these procedures for detection and viability assessment in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of animal samples and may be adapted for assessment of solid, particulate, liquid, and water samples. A cell culture infectivity assay capable of detecting infectious oocysts is used to quantify viable oocysts through sporozoite invasion and clustering of foci. Oocysts diluted in a standard 5- or 10-fold multiple dilution format are inoculated unto human ileocecal adenocarcinoma (HCT-8) cell monolayers. After incubation for 48 hours, anti-sporozoite polyclonal antibody is used to detect sporozoite invasion, and microscopy is used to confirm replication (life stages present). Levels of infection and clustering are used to determine the most probable number (MPN) of infectious oocysts in the stock suspension. For oocysts less than 30 days of age, the correlation between the initial oocyst inoculum and the MPN calculation is 0.9726. The relationship between the oocyst inoculum and the MPN diverge as the oocysts age. The 50% infective dose (ID50) in the cell culture system is approximately 10 oocysts.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Source: Slifko, T.R., Huffman, D.E., and Rose, J.B. 1999. "A Most-Probable-Number Assay for Enumeration of Infectious *Cryptosporidium parvum* Oocysts." Applied and Environmental Microbiology. 65(9): 3936–3941. http://www.epa.gov/sam/pdfs/AEM-65(9)-pgs3936-3941.pdf

7.2.37 Literature Reference for *Cryptosporidium* spp. (Applied and Environmental Microbiology. 2007. 73(13): 4218–4225)

Pathogen(s)	Agent Category	BSL
Cryptosporidium spp. [Cryptosporidiosis]	Protozoa	2

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Solid samples should be prepared according to "Quantification of Bias Related to the Extraction of DNA Directly from Soils," Frostegard, A., Courtois, S., Ramisse, V., Clerc, S., Bernillion, D., Le Gall, F., Jeannin, P., Nesme, X., and Simonet, P. 1999. Applied and Environmental Microbiology. 65(12): 5409–5420 (www.epa.gov/sam/pdfs/AEM-65(12)-pgs5409-5420.pdf).
Analytical Technique: Real-time PCR

Method Developed for: *Cryptosporidium* spp. in drinking water samples
Method Selected for: SAM lists this protocol for detection and viability assessment in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than drinking water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of drinking water samples and may be adapted for assessment of solid, particulate, aerosol, and liquid samples. The method uses real-time PCR for identification of *Cryptosporidium* spp. *C. parvum* oocysts are seeded into 100-L water samples at an

average concentration of 590,000 ± 84,000 oocysts. One hundred liters of the seeded drinking water is concentrated by hollow-fiber UF, with the filter eluted with a surfactant solution that is added to the retentate. A portion of the combined retentate is further concentrated with a 0.2 µm filter and one quarter of the filter used for PCR analysis. DNA for PCR analyses is recovered by bead beating, lysis of cells with a guanidine thiocyanate based buffer, and recovery and concentration of nucleic acids using spin columns. Real-time PCR is performed on an iCycler iQ4 detection system using primer sequences and a TaqMan® probe specific for *Cryptosporidium* spp. Samples from 8 sites were examined, with an average recovery efficiency of between 81% and 98%, with a cross-site average and standard deviation of 88% and 10% respectively.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Special Considerations: This method is only for genus-specific determination of *Cryptosporidium* and will not determine if the microorganism is infectious to humans.

Source: Hill, V.R., Kahler, A.M., Jothikumar, N., Johnson, T.B., Hahn, D., and Cromeans, T.L. 2007. "Multistate Evaluation of an Ultrafiltration-Based Procedure for Simultaneous Recovery of Enteric Microbes in 100-Liter Tap Water Samples." Applied and Environmental Microbiology. 73(13): 4218–4225. http://www.epa.gov/sam/pdfs/AEM-73(13)-pgs4218-4225.pdf

7.2.38 Literature Reference for *Entamoeba histolytica* (Journal of Parasitology. 1972. 58(2): 306–310)

Pathogen(s)	Agent Category	BSL
Entamoeba histolytica	Protozoa	2

Analysis Purpose: Detection and viability
Sample Preparation: Samples should be prepared according to the procedures in Journal of Parasitology. 1972. 58(2): 306–310.
Analytical Technique: Culture

Method Developed for: *Entamoeba histolytica* in clinical samples
Method Selected for: SAM lists these procedures for detection and viability assessment in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of clinical samples and may be adapted for assessment of solid, particulate, liquid, and water samples. *Entamoeba histolytica* cysts are placed in a modified TP-S-1 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 before using this protocol: positive control, negative control, and blank.

Source: 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.epa.gov/sam/pdfs/JP-58(2)-pgs306-310.pdf

7.2.39 Literature Reference for *Entamoeba histolytica* (Journal of Clinical Microbiology. 2005. 43(11): 5491–5497)

Pathogen(s)	Agent Category	BSL
Entamoeba histolytica	Protozoa	2

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Samples should be prepared according to the procedures in Journal of Clinical Microbiology. 2005. 43(11): 5491–5497.
Analytical Technique: Real-time PCR

Method Developed for: *Entamoeba histolytica* in clinical (fecal and liver abscess) samples
Method Selected for: SAM lists these procedures for detection in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of clinical samples and may be adapted for assessment of solid, particulate, liquid, and water samples. The method is a real-time PCR assay that targets the 18S rRNA gene sequence of *E. histolytica*. DNA is extracted using cell disruption and a commercial DNA extraction kit, with a second commercial kit used to remove potential PCR inhibitors. TaqMan® real-time PCR is conducted on a GeneAmp® 9700 Thermal Cycler the purified product. The LOD is 1 cell per mL of sample (SD ± 4). The method differentiates between *E. histolytica* and *E. dispar*.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: Qvarnstrom, Y., James, C., Xayavong, M., Holloway, B.P., Visvesvara, G.S., Sriram, R., and da Silva, A.J. 2005. "Comparison of Real-time PCR Protocols for Differential Laboratory Diagnosis of Amebiasis." Journal of Clinical Microbiology. 43(11): 5491–5497. http://www.epa.gov/sam/pdfs/JCM-43(11)-pgs5491-5497.pdf

7.2.40 Literature Reference for *Giardia spp.* (Transactions of the Royal Society of Tropical Medicine and Hygiene. 1983. 77(4): 487–488)

Pathogen(s) [Disease]	Agent Category	BSL
Giardia spp. [Giardiasis]	Protozoa	2

Analysis Purpose: Detection and viability
Analytical Technique: Culture

Method Developed for: *Giardia* in cell culture samples
Method Selected for: SAM lists these procedures for detection and viability assessment in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of cell culture samples and may be adapted for assessment of solid, particulate, liquid, and water samples. TYI-S-33 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.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Source: 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?_ob=ArticleURL&_udi=B75GP-4C0DS6C-W2&_user=10&_coverDate=12%2F31%2F1983&_alid=785177083&_rdoc=1&_fmt=high&_orig=search&_cdi=13100&_sort=d&_docanchor=&view=c&_ct=1&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=d2703ab0d4bca7099b48784586249bb5

7.2.41 Literature Reference for *Toxoplasma gondii* (Emerging Infectious Diseases. 2006. 12(2): 326–329)

Pathogen(s) [Disease]	Agent Category	BSL
Toxoplasma gondii [Toxoplasmosis]	Protozoa	2

Analysis Purpose: Detection and viability
Sample Preparation: Samples should be prepared according to the procedures in Emerging Infectious Diseases 12(2): 326–329.
Analytical Technique: Animal infectivity

Method Developed for: *Toxoplasma gondii* in water
Method Selected for: SAM lists these procedures for detection and viability assessment in solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of water samples and may be adapted for assessment of solid, particulate, liquid, and water samples. Water samples are filtered through fluoropore membrane filters and concentrated by centrifugation. The filters can be assayed by any of three methods. The first method involves performing a bioassay in *T. gondii*-seronegative chickens. Serum samples are tested by enzyme-linked immunosorbent assay (ELISA) and/or modified agglutination test until seroconversion, with the organs from seropositive animals examined microscopically for *T. gondii*. Mice are injected with brain and heart tissue of seropositive chickens, with parasites found in the lungs of mice being confirmatory for *T. gondii*. The second method is a similar bioassay with pigs and cats. For the third assay, DNA is extracted from the fluoropore membranes for PCR identification of isolates.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank.

Source: de Moura, L., Bahia-Oliveira, L.M.G., Wada, M.Y., Jones, J.L., Tuboi, S.H., Carmo, E.H., Ramalho, W.M., Camargo, N.J., Trevisan, R., Graca, R.M.T., da Silva, A.J., Moura, I., Dubey, J.P., and Garrett, D.O. 2006. "Waterborne Toxoplasmosis, Brazil, from Field to Gene." Emerging Infectious Diseases. 12(2): 326–329. http://www.epa.gov/sam/pdfs/EID-12(2)-pgs326-329.pdf

7.2.42 Literature Reference for *Toxoplasma gondii* (Applied and Environmental Microbiology. 2004. 70(7): 4035–4039)

Pathogen(s) [Disease]	Agent Category	BSL
Toxoplasma gondii [Toxoplasmosis]	Protozoa	2

Analysis Purpose: Detection, not suitable for viability
Sample Preparation: Samples should be prepared according to the procedures in Applied and Environmental Microbiology 70(7): 4035–4039.
Analytical Technique: Real-time PCR

Method Developed for: *Toxoplasma gondii* in water
Method Selected for: SAM lists these procedures for detection of in solid, particulate, liquid and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than water. See Appendix C or Table 7-1 for additional methods that should be used for this pathogen.

Description of Method: Procedures are described for analysis of water samples and may be adapted for assessment of solid, particulate, and liquid samples. The method uses a fluorogenic 5' nuclease (TaqMan®) real-time PCR assay for the detection of *T. gondii* oocyst DNA using gene-specific (B1 gene) primers and probe. The assay uses an iCycler Real-Time PCR Detection System. Water samples (10 to 100 L) are filtered to concentrate oocysts. Filters are eluted and recovered oocysts are further purified and concentrated by differential flotation and centrifugation. Final sample pellets are split and subjected to PCR detection and mouse bioassay. In experimental seeding assays, a parasite density of 10 oocysts/L is detectable in 100% of the cases, and a density of 1 oocyst/L is observable in 60% of the cases.

At a minimum, the following QC checks should be performed and evaluated before using this protocol: positive control, negative control, and blank. PCR QC checks should be performed according to *EPA Draft Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples* document www.epa.gov/sam/pdfs/EPA-QA-QC_PCR_Oct2004.pdf or consult the point of contact identified in Section 4.

Source: Villena, I., Aubert, D., Gomis, P., Ferte, H., Inglard, J-C., Denise-Bisiaux, H., Dondon, J-M., Pisano, E., Ortis, N., and Pinon, J.M. 2004. "Evaluation of a Strategy for *Toxoplasma gondii* Oocyst Detection in Water." Applied and Environmental Microbiology. 70(7): 4035–4039. http://www.epa.gov/sam/pdfs/AEM-70(7)-pgs4035-4039.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 homeland security event. 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 and development and validation 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 restoration 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.
- **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.
- **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 HEPA filters.
- **Liquid/drinking water.** The recommended method/procedure to measure the analyte of interest in liquid and drinking water samples.

Some of the biotoxins addressed in this document are commonly found in the environment, and the methods listed in Appendix D assume that analysis will be used to evaluate contamination levels that are above those of background. If possible, an investigation of initial background levels, as well as controls for background levels, should be performed.

Procedures listed in Appendix D for protein biotoxins are intended to address presumptive, confirmatory, and biological activity determinations. Because the confirmatory procedures listed for the small molecule biotoxins involve a determination of intact compound structure (an indication of biological activity capability), only presumptive and confirmatory methods are listed for these biotoxins. In terms of this document, presumptive methods, or methods that support a reasonable basis for accurate results, should be used in situations that require a large number of samples to be processed. Most of the presumptive methods listed in Appendix D use immunoassay techniques and are designed for large scale sample processing. The confirmatory method, or the method that corroborates the presumptive results, should be

used on the smaller subset of samples for which presumptive analysis indicates the presence of the biotoxin. Several techniques are listed in Appendix D as confirmatory and generally are more time consuming and expensive. The use of these terms in this document is not intended to redefine LRN usage of these terms. The terms presumptive and confirmatory as used by the LRN are described in Section 8.2.1. If it is necessary to determine the biological activity of a toxin, either an assay (for proteins) or a technique such as HPLC that determines whether the structure of the biotoxin is intact and likely to be biologically active (for small molecules) may be used. Biological activity analysis should be applied on an as-needed basis following analysis with the confirmatory technique.

Numerous analytical techniques using a variety of instrumentation (e.g., HPLC-MS, HPLC-FL, immunoassay [ELISA], immunoassay [lateral flow device (LFD)], etc.) have been cited in Appendix D. It is expected that a reduced number of these analytical techniques and instrumentation will be necessary after method verification and validation. In addition, it is recognized that new reports detailing advances in biotoxin analysis appear in the literature frequently. Accordingly, the individual techniques and methods listed in Appendix D are to be regarded as a starting point; after thoughtful consideration of current technologies at the time of remediation and consultation with the authority in charge of the remediation activity, these techniques and methods can be modified as necessary for analysis of a particular sample.

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 Guidance

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, 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:

- A U.S. Army Medical Research Institute of Infectious Diseases' document at 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.
- CDC regulations – Select Agents and Toxins, 42 CFR part 73, found at http://www.cdc.gov/od/sap/pdfs/42_cfr_73_final_rule.pdf
- The CDC has additional information regarding select agent toxins at the following Web site: http://www.cdc.gov/od/sap/sap/toxinamt.htm
- Syracuse Research Corporation's Physprop and Chemfate, part of the Environmental Fate Database supported by EPA. See http://www.syrres.com/esc/databases.htm.
- INCHEM at http://www.inchem.org/ contains both chemical and toxicity information.
- The RTECS database can be accessed via the NIOSH Web site at http://www.cdc.gov/niosh/rtecs/vz72d288.html#JWIDAW for toxicity information.
- EPA's IRIS: http://www.epa.gov/iris/ contains toxicity information.

- The Forensic Science and Communications Journal published by the Laboratory Division of the FBI. See http://www.fbi.gov/hq/lab/fsc/current/backissu.htm.

Additional research on biotoxin contaminants is ongoing within EPA.

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: 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 (solid, particulate, liquid/drinking water, or aerosol) corresponding to that particular analyte.

Sections 8.2.1 through 8.2.31 below provide summaries of the analytical methods listed in Appendix D. Once a method has been identified in Appendix D, **Table 8-1** can be used to locate the method summary.

Table 8-1. Biotoxin Methods and Corresponding Text Section Numbers

Biotoxin	Method	Section
Proteins		
Abrin	119th AOAC Annual Meeting & Exposition, 2005, p.613	8.2.7
	Pharmacology & Toxicology. 2001. 88(5): 255–260	8.2.8
	Analytical Biochemistry. 2008. 378: 87–89	8.2.9
Botulinum neurotoxins	LRN	8.2.1
	U.S. FDA, Bacteriological Analytical Manual Online, January 2001, Chapter 17, *Clostridium botulinum*	8.2.2
	Lateral Flow Immunoassay Kits	8.2.31
Ricin	LRN	8.2.1
	Analytical Biochemistry. 2008. 378: 87–89	8.2.9
	Journal of AOAC International. 2008. 91(2): 376–382	8.2.23
	Journal of Analytical Toxicology. 2005. 29: 149–155	8.2.24
	Lateral Flow Immunoassay Kits	8.2.31
Shiga and Shiga-like toxins (Stx, Stx-1, Stx-2)	U.S. FDA, Bacteriological Analytical Manual Online, January 2001, Appendix 1, Rapid Methods for Detecting Foodborne Pathogens	8.2.3
	Pharmacology & Toxicology. 2001. 88(5): 255–260	8.2.8
	Journal of Clinical Microbiology. 2007. 45(10): 3377–3380	8.2.26
Staphylococcal enterotoxins (SEB)	LRN	8.2.1
	AOAC Official Method 993.06	8.2.5
Staphylococcal enterotoxins (SEA, SEC)	AOAC Official Method 993.06	8.2.5
Small Molecules		
Aflatoxin (Type B1)	AOAC Official Method 991.31	8.2.4
	AOAC Official Method 994.08	8.2.6
α-Amanitin	Journal of Food Protection. 2005. 68(6): 1294–1301	8.2.12
	Journal of Chromatography B. 1991. 563(2): 299–311	8.2.13
Anatoxin-a	Biomedical Chromatography. 1996. 10: 46–47	8.2.14

Biotoxin	Method	Section
Brevetoxins (B form)	Environmental Health Perspectives. 2002. 110(2): 179–185	8.2.15
	Toxicon. 2004. 43(4): 455–465	8.2.16
α-Conotoxin	Biochemical Journal. 1997. 328: 245–250	8.2.10
	Journal of Medicinal Chemistry. 2004. 47(5): 1234–1241	8.2.11
Cylindrospermopsin	FEMS Microbiology Letters. 2002. 216: 159-164	8.2.17
	ELISA Kits for Cylindrospermopsin	8.2.29
Diacetoxyscirpenol (DAS)	International Journal of Food Microbiology. 1988. 6(1): 9–17	8.2.18
	Rapid Communications in Mass Spectrometry. 2006. 20(9): 1422–1428	8.2.19
Microcystins (Principal isoforms: LA, LR, YR, RR, LW)	Journal of AOAC International. 2001. 84(4): 1035–1044	8.2.20
	Analyst. 1994. 119(7): 1525–1530	8.2.21
Picrotoxin	Journal of Pharmaceutical and Biomedical Analysis. 1989. 7(3): 369–375	8.2.22
Saxitoxins (Principal isoforms STX, NEOSTX, GTX, dcGTX, dcSTX)	Journal of AOAC International. 1995. 78: 528–532	8.2.25
	ELISA Kits for Saxitoxin	8.2.30
T-2 Mycotoxin	Journal of Food Protection. 2005. 68(6): 1294–1301	8.2.12
	Rapid Communications in Mass Spectrometry. 2006. 20(9): 1422–1428	8.2.19
Tetrodotoxin	Journal of Clinical Laboratory Analysis. 1992. 6: 65–72	8.2.27
	Analytical Biochemistry. 2001. 290: 10-17	8.2.28

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, VCSBs, and journal articles. Methods are listed in numerical order under each publisher. 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 8-2**.

Table 8-2. Sources of Biotoxin Methods

Name	Publisher	Reference
U.S. FDA, Bacteriological Analytical Manual Online	U.S. FDA	http://www.cfsan.fda.gov/~ebam/bam-toc.html
Official Methods of Analysis of AOAC International*	AOAC International	http://www.aoac.org
NEMI	U.S. EPA, 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	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/

Name	Publisher	Reference
Environmental Health Perspectives*	National Institute of Environmental Health Sciences	http://www.niehs.nih.gov/
Toxicon*	Elsevier Science Publishers	http://www.elsevier.com/
Federation of European Microbiological Societies (FEMS) Microbiology Letters*	Blackwell Publishing	http://www.blackwellpublishing.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	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/

* Subscription and/or purchase required.

8.1.2 General QC Guidance 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. Ensuring data quality also requires that laboratory results are properly evaluated and the results of the data quality evaluation are 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 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, duplicates) 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; and
- 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

- 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
  - 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 QA/QC procedures prior to sample analysis. These contacts will consult with the EPA OSWER coordinator responsible for laboratory activities during the specific event to ensure QA/QC procedures are performed consistently across laboratories. OSWER is planning to develop QA/QC guidance for laboratory support. 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 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, guidance, 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 (http://www.absa.org/resriskgroup.html)
- *Biological Safety: Principles and Practices*, 4th Ed. ASM.Press (http://estore.asm.org/);
- CDC – 42 CFR part 72. Interstate Shipment of Etiological 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.

- EPA – 40 CFR part 270. EPA Administered Permit Programs: The Hazardous Waste Permit Program.
- OSHA – 29 CFR part 1910.1450. Occupational Exposure to Hazardous Chemicals in Laboratories.
- OSHA – 29 CFR part 1910.120. Hazardous Waste Operations and Emergency Response.
- USDA – 9 CFR part 121. Possession, Use, and Transfer of Select Agents and Toxins.

Please note that the e-CFR is available at http://ecfr.gpoaccess.gov/.

8.2 Method Summaries

Summaries for the analytical methods listed in Appendix D are provided in Sections 8.2.1 through 8.2.31. 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 table identifying the biotoxin(s) and sample type to which the method applies, a brief description of the method, performance data (if available), and a link to or source for obtaining a full version of the method.

8.2.1 Laboratory Response Network (LRN)

The agents and sample types identified below and listed in Appendix D are included in the HHS/USDA select agent list and should be analyzed in accordance with the appropriate LRN protocols.

The LRN was created in accordance with Presidential 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.

It is important to note that, in some cases, the procedures may not be fully developed or validated for each environmental sample type/analyte combination listed in Appendix D, nor are all LRN member laboratories necessarily capable of analyzing all of the sample type/analyte combinations.

Analyte(s)	Sample Type	CAS RN / Description
Botulinum neurotoxins (Serotypes A, B, E, F)	Solid, Particulate, Liquid/Water	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

Analyte(s)	Sample Type	CAS RN / Description
Ricin	Solid, Particulate, Liquid/Water	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
Staphylococcal enterotoxin B (SEB)	Solid, Particulate, Liquid/Water	39424-53-8 (SEB) / Monomeric protein of almost 28 kDa

Please note: Not all methods have been verified for the biotoxin/sample type combination listed in Appendix D. Please refer to the agent-specific method to identify the biotoxin/sample type combinations that have been validated. Any questions regarding information discussed in this section should be referred to the appropriate contact(s) listed in Section 4.

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)
Centers for Disease Control and Prevention (CDC)
1600 Clifton Road NE, Mailstop C-18
Atlanta, GA 30333
Telephone: (404) 639-2790
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 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

8.2.2 U.S. FDA, Bacteriological Analytical Manual Online, Chapter 17, 2001: Botulinum Neurotoxins

Analyte(s)	Agent Category	Description
Botulinum neurotoxins (Serotypes A, B, E, F)	Protein	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

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 manual for confirmation and biological activity assessment in aerosol samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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 form of toxin.

Source: U.S. FDA, CFSAN. 2001. "Chapter 17 – *Clostridium botulinum*."*Bacteriological Analytical Manual Online.* http://www.epa.gov/sam/pdfs/FDA-BAM-Chap17.pdf

8.2.3 U.S. FDA, Bacteriological Analytical Manual Online, Appendix 1, 2001: Rapid Methods for Detecting Foodborne Pathogens

Analyte(s)	Agent Category	CAS RN / Description
Shiga and Shiga-like toxins (Stx, Stx-1, Stx-2)	Protein	75757-64-1 (Stx) / Protein composed of one ~32 kDa A chain and five 7.7 kDa B chains

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 develop and standardize the procedures for environmental samples. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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: U.S. FDA, CFSAN. 2001. "Rapid Methods for Detecting Foodborne Pathogens." *Bacteriological Analytical Manual Online*. http://www.epa.gov/sam/pdfs/FDA-BAM-Appendix1.pdf

8.2.4 AOAC Official Method 991.31: Aflatoxins in Corn, Raw Peanuts, and Peanut Butter

Analyte(s)	Agent Category	CAS RN
Aflatoxin (Type B1)	Small Molecule	27261-02-5

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay

Method Developed for: Aflatoxins (Type B1) in corn, raw peanuts, and peanut butter
Method Selected for: SAM lists this method for presumptive analysis in aerosol, solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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 mAb 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.

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/

8.2.5 AOAC Official Method 993.06: Staphylococcal Enterotoxins in Selected Foods

Analyte(s)	Agent Category	CAS RN / Description
Staphylococcal enterotoxins (SEB, SEA, SEC)	Protein	39424-53-8 (SEB) / Monomeric protein of ~ 28 kDa; 37337-57-8 (SEA) & 39424-54-9 (SEC) / Monomeric proteins of ~ 27–27.5 kDa

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay

Method Developed for: Staphylococcal enterotoxins in selected foods
Method Selected for: SAM lists this method for presumptive analysis 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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

Description of Method: This method is an enzyme immunoassay (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 991.06: Staphylococcal Enterotoxins in Selected Foods." *Official Methods of Analysis of AOAC International*. 16th Edition, 4th Revision; Vol. I. http://www.aoac.org/

8.2.6 AOAC Official Method 994.08: Aflatoxin in Corn, Almonds, Brazil Nuts, Peanuts, and Pistachio Nuts

Analyte(s)	Agent Category	CAS RN
Aflatoxin (Type B1)	Small Molecule	27261-02-5

Analysis Purpose: Confirmatory
Analytical Technique: HPLC-FL

Method Developed for: Aflatoxins in corn, almonds, brazil nuts, peanuts, and pistachio nuts
Method Selected for: SAM lists this method for confirmatory analysis in aerosol, solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

Description of Method: This method is for the identification of aflatoxins in agricultural products. Samples are extracted using an acetonitrile-water (9 + 1) solution. Sample extracts are then run through a multifunctional cleanup column. The purified extract and standards are derivatized with trifluoroacetic acid, and then analyzed using a HPLC system with a FL detector. Specific aflatoxins can be identified by their retention time and quantified using standard curves. Method performance was characterized using various commodities (e.g., corn) at aflatoxin levels over a range of 5–30 ng/g. This method was originally designed for the analysis of aflatoxins (B1, B2 [CAS RN 22040-96-6], G1 [CAS RN 1385-95-1], and G2 [CAS RN 7241-98-7]) in commodities where cleanup was necessary to remove other food components, such as fats and proteins; the cleanup procedure may not be necessary for water analyses. Coupling the procedures, or a modification of the procedures, included in this method with an immunoassay and/or biological activity test (where available) will provide more information regarding specificity and toxicity.

Source: AOAC International. 1998. "Method 994.08: Aflatoxin in Corn, Almonds, Brazil Nuts, Peanuts, and Pistachio Nuts." *Official Methods of Analysis of AOAC International*. 16th Edition, 4th Revision; Vol. II. http://www.aoac.org/

8.2.7 Literature Reference for Abrin (119th AOAC Annual Meeting & Exposition, 2005, p. 613)

Analyte(s)	Agent Category	CAS RN / Description
Abrin	Protein	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 the crude preparations

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay

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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

Description of Method: Procedures are described for using mouse mAbs and rabbit derived polyclonal antibodies prepared against a mixture of abrin isozymes for three separate ELISA and electrochemiluminescence (ECL)-based assays in food products. The three assays vary by use of antibody combination: (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 dissolved into food products ranges from 0.1 to 0.5 ng/mL, using the ECL assay. The LOD for abrin dissolved into food products for the ELISA assays range between 1 and 4 ng/mL depending on the assay configuration. In all cases, the LODs are considerably 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.

Source: Garber, E.A., Aldrich, J.L., Wang, J., Brewer, V.A., O'Brien, T.W., and Sigal, G. 2005. "Detection of Abrin in Foods Using ELISA and Electrochemiluminescence (ECL) Technologies." 119th AOAC Annual Meeting & Exposition. p. 613.
http://www.accessdata.fda.gov/scripts/oc/scienceforum/sf2006/search/preview.cfm?abstract_id=848&backto=author

8.2.8 Literature Reference for Abrin and Shiga and Shiga-like Toxins (Pharmacology Toxicology. 2001. 88(5): 255–260)

Analyte(s)	Agent Category	CAS RN / Description
Abrin	Protein	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 the crude preparations
Shiga and Shiga-like Toxins (Stx, Stx-1, Stx-2)	Protein	75757-64-1 (Stx) / Protein composed of one ~32 kDa A chain and five 7.7 kDa B chains

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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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 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

8.2.9 Literature Reference for Abrin and Ricin (Analytical Biochemistry. 2008. 378(1): 87-89)

Analyte(s)	Agent Category	CAS RN / Description
Abrin	Protein	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 the crude preparations
Ricin	Protein	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

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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

Description of Method: This *in vitro* assay is an RNA N-glycosidase enzyme activity assay for the detection of purified abrin and ricin toxins (Types I and II) and 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 are 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. 2006. "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?_ob=ArticleURL&_udi=B6W9V-4S2F5PT-3&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_version=1&_urlVersion=0&_userid=10&md5=b70d0d8cd9eb15819cab1064f80c2acf

8.2.10 Literature Reference for α-Conotoxin (Biochemical Journal. 1997. 328: 245–250)

Analyte(s)	Agent Category	CAS RN
α-Conotoxin	Small Molecule	156467-85-5

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay

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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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: 245–250. http://www.epa.gov/sam/pdfs/BJ-328-pgs245-250.pdf

8.2.11 Literature Reference for α-Conotoxin (Journal of Medicinal Chemistry. 2004. 47(5): 1234–1241)

Analyte(s)	Agent Category	CAS RN
α-Conotoxin	Small Molecule	156467-85-5

Analysis Purpose: Confirmatory
Analytical Technique: High performance liquid chromatography – Mass spectrometer (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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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% trifluoroacetic acid (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

8.2.12 Literature Reference for α-Amanitin, T-2 Mycotoxin (Journal of Food Protection. 2005. 68(6): 1294–1301)

Analyte(s)	Agent Category	CAS RN / Description
α-Amanitin	Small Molecule	23109-05-9
T-2 Mycotoxin	Small Molecule	21259-20-1

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay

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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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., 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

8.2.13 Literature Reference for α-Amanitin (Journal of Chromatography B. 1991. 563(2): 299–311)

Analyte(s)	Agent Category	CAS RN
α-Amanitin	Small Molecule	23109-05-9

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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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

8.2.14 Literature Reference for Anatoxin-a (Biomedical Chromatography. 1996. 10: 46–47)

Analyte(s)	Agent Category	CAS RN
Anatoxin-a	Small Molecule	64285-06-9

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 develop and standardize the procedures for environmental sample types other than water. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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% 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: 46–47. http://www3.interscience.wiley.com/journal/18562/abstract

8.2.15 Literature Reference for Brevetoxins (Environmental Health Perspectives. 2002. 110(2): 179–185)

Analyte(s)	Agent Category	CAS RN
Brevetoxins (B form)	Small Molecule	79580-28-2

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay

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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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.epa.gov/sam/pdfs/EHP-110(2)-pgs179-185.pdf

8.2.16 Literature Reference for Brevetoxins (Toxicon. 2004. 43(4): 455–465)

Analyte(s)	Agent Category	CAS RN
Brevetoxins (B form)	Small Molecule	79580-28-2

Analysis Purpose: Confirmatory
Analytical Technique: High performance liquid chromatography tandem mass spectrometers (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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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 electrospray ionization (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

8.2.17 Literature Reference for Cylindrospermopsin (FEMS Microbiology Letters. 2002. 216(2): 159–164)

Analyte(s)	Agent Category	CAS RN
Cylindrospermopsin	Small Molecule	143545-90-8

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 develop and standardize the procedures for environmental sample types other than water. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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

8.2.18 Literature Reference for Diacetoxyscirpenol (DAS) (International Journal of Food Microbiology. 1988. 6(1): 9–17)

Analyte(s)	Agent Category	CAS RN
Diacetoxyscirpenol (DAS)	Small Molecule	2270-40-8

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay

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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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?_ob=ArticleURL&_udi=B6T7K-476FFT7-59&_user=10&_coverDate=02%2F29%2F1988&_rdoc=3&_fmt=high&_orig=browse&_srch=doc-info(%23toc%235061%231988%23999939998%23360572%23FLP%23display%23Volume)&_cdi=5061&_sort=d&_docanchor=&_ct=13&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=e0c1340f546d83de674c18de27f4f7d8

8.2.19 Literature Reference for Diacetoxyscirpenol (DAS) and T-2 Mycotoxin (Rapid Communications in Mass Spectrometry. 2006. 20(9): 1422–1428)

Analyte(s)	Agent Category	CAS RN
Diacetoxyscirpenol (DAS)	Small Molecule	2270-40-8
T-2 Mycotoxin	Small Molecule	21259-20-1

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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

Description of Method: A LC/APCI-MS procedure based on time-of-flight mass spectrometry (TOFMS), 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

8.2.20 Literature Reference for Microcystins (Journal of AOAC International. 2001. 84(4): 1035–1044)

Analyte(s)	Agent Category	CAS RN
Microcystins (Principal isoforms: LA, LR, LW, RR, YR)	Small Molecule	96180-79-9 (LA) 101043-37-2 (LR) 157622-02-1 (LW) 111755-37-4 (RR) 101064-48-6 (YR)

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay/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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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 microcystin 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

8.2.21 Literature Reference for Microcystins (Analyst. 1994. 119(7): 1525–1530)

Analyte(s)	Agent Category	CAS RN
Microcystins (Principal isoforms: LA, LR, LW, RR, YR)	Small Molecule	96180-79-9 (LA) 101043-37-2 (LR) 157622-02-1 (LW) 111755-37-4 (RR) 101064-48-6 (YR)

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 develop and standardize the procedures for environmental sample types other than water. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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

8.2.22 Literature Reference for Picrotoxin (Journal of Pharmaceutical & Biomedical Analysis. 1989. 7(3): 369–375)

Analyte(s)	Agent Category	CAS RN
Picrotoxin	Small Molecule	124-87-8

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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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?_ob=ArticleURL&_udi=B6TGX-44KG7R3-1K&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_acct=C000050221&_version=1&_urlVersion=0&_userid=10&md5=de051acb377a75cf755435f7d23dfdf7

8.2.23 Literature Reference for Ricin (Journal of AOAC International. 2008. 91(2): 376–382)

Analyte(s)	Agent Category	CAS RN / Description
Ricin	Protein	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

Analysis Purpose: Confirmatory
Analytical Technique: Immunoassay

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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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://www.atypon-link.com/AOAC/doi/abs/10.5555/jaoi.91.2.376

8.2.24 Literature Reference for Ricin by Ricinine detection (Journal of Analytical Toxicology. 2005. 29(3): 149–155)

Analyte(s)	Agent Category	CAS RN / Description
Ricinine (Ricin marker)	Small Molecule	5254-40-3 (ricinine) / small molecule, ricin marker

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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

Description of Method: Procedures are described for sample extraction by SPE, isocratic HPLC, followed by 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 limit of detection 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.

Source: Johnson, R.C., Lemire, S.W., Woolfitt, 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://www.jatox.com/abstracts/2005/April/149-johnson.html

8.2.25 Literature Reference for Saxitoxin (Journal of AOAC International. 1995. 78(2): 528–532)

Analyte(s)	Agent Category	CAS RN
Saxitoxins Principal isoforms: Saxitoxin (STX) Neosaxitoxin (NEOSTX) Gonyautoxin (GTX) Decarbamoylgonyautoxin (dcGTX) Decarbamoylsaxitoxin (dcSTX)	Small Molecule	35523-89-8 (STX) 64296-20-4 (NEOSTX) 77462-64-7 (GTX) None given (dcGTX) 58911-04-9 (dcSTX)

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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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.

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

8.2.26 Literature Reference for Shiga and Shiga-like Toxin (Journal of Clinical Microbiology. 2007. 45(10): 3377–3380)

Analyte(s)	Agent Category	CAS RN / Description
Shiga and Shiga-like Toxins (Stx, Stx-1, Stx-2)	Protein	75757-64-1 (Stx) / Protein composed of one ~32 kDa A chain and five 7.7 kDa B chains

Analysis Purpose: Presumptive
Analytical Technique: Optical immunoassay

Method Developed for: Shiga toxin in foods
Method Selected for: SAM lists these procedures for presumptive analysis in aerosol, solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

Description of Method: Procedures are described for a rapid optical immunoassay for the detection of Stx-1 and Stx-2 using a commercially available kit. Fecal samples (742 specimens) are assayed for Shiga toxins with and without enrichment of the specimens in broth. Duplicate assays are applied using either the rapid optical immunoassay or a commercially available ELISA kit. Samples producing positive results by immunoassay are confirmed by Vero cell cytotoxicity assay. Sensitivities of 96.8% are achieved for direct stool sample assays.

Source: Teel, L.D., Daly, J.A., Jerris, R.C., Maul, D., Svanas, G., O'Brien, A.D., and Park, C.H. 2007. "Rapid Detection of Shiga Toxin-Producing *Escherichia coli* by Optical Immunoassay." Journal of Clinical Microbiology. 45(10): 3377–3380. www.epa.gov/sam/pdfs/JCM-45(10)-pgs3377-3380.pdf

8.2.27 Literature Reference for Tetrodotoxin (Journal of Clinical Laboratory Analysis. 1992. 6(2): 65–72)

Analyte(s)	Agent Category	CAS RN
Tetrodotoxin	Small Molecule	9014-39-5

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay

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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

Description of Method: Procedures are described for a competitive inhibition enzyme immunoassay (CIEIA) for tetrodotoxin (TTX) 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

8.2.28 Literature Reference for Tetrodotoxin (Analytical Biochemistry. 2001. 290(1): 10–17)

Analyte(s)	Agent Category	CAS RN
Tetrodotoxin	Small Molecule	9014-39-5

Analysis Purpose: Confirmatory
Analytical Technique: LC/ESI-MS

Method Developed for: Tetrodotoxin 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 develop and standardize the procedures for environmental sample types. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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 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?_ob=ArticleURL&_udi=B6W9V-45BC729-BP&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_version=1&_urlVersion=0&_userid=10&md5=c01fdd21e0d9f104431816fd41869f1e

8.2.29 ELISA Kits for Cylindrospermopsin

Analyte(s)	Agent Category	CAS RN
Cylindrospermopsin	Small Molecule	143545-90-8

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay

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 develop and standardize the procedures for environmental sample types other than water. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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.
http://infotrek.er.usgs.gov/pls/apex/f?p=119:38:7526698938332159::::P38_METHOD_ID:9507

8.2.30 ELISA Kits for Saxitoxins

Analyte(s)	Agent Category	CAS RN
Saxitoxins Principal isoforms: Saxitoxin (STX) Neosaxitoxin (NEOSTX) Gonyautoxin (GTX) Decarbamoylgonyautoxin (dcGTX) Decarbamoylsaxitoxin (dcSTX)	Small Molecule	35523-89-8 (STX) 64296-20-4 (NEOSTX) 77462-64-7 (GTX) None given (dcGTX) 58911-04-9 (dcSTX)

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay

Method Developed for: STX in water and solid samples (e.g., shellfish)
Method Selected for: SAM lists these procedures for confirmatory analysis in aerosol, solid, particulate, liquid, and water samples. Further research is needed to develop and standardize the procedures for environmental sample types other than water. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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: This kit is not intended for other types of saxitoxin. 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) of these other types of saxitoxin may produce false positive responses.

Source: NEMI. 2006.
http://infotrek.er.usgs.gov/pls/apex/f?p=119:38:8989971104293493:::P38_METHOD_ID:9512

8.2.31 Lateral Flow Immunoassay Kits

Analyte(s)	Agent Category	CAS RN / Description
Botulinum neurotoxins (Types A, B)	Protein	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
Ricin	Protein	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

Analysis Purpose: Presumptive
Analytical Technique: Immunoassay

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 develop and standardize the procedures for environmental sample types other than water. See Appendix D or Table 8-1 for additional methods that should be used for this analyte.

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 lateral flow immunochromatographic device 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.

These two lateral flow immunoassay kits have been evaluated by the U.S. EPA ETV Program (http://www.epa.gov/sam/pdfs/ETV-BADD091904.pdf and http://www.epa.gov/sam/pdfs/ETV-BioThreat092104.pdf) for the detection of botulinum neurotoxins Types A and B and ricin. Information regarding the evaluation of test strips can be accessed at these sites.

Special Considerations: Crude preparations of ricin may also contain agglutinins that are unique to castor beans and that can cross-react in the immunoassays. Immunoassays with botulinum toxins may produce variable results with uncomplexed form of toxin.

Source: ETV. 2006. http://www.epa.gov/etv/

Section 9.0: Conclusions

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 a homeland security event.

The primary objective of this document is not necessarily to identify the "best" analytical methods, but rather to identify appropriate methods that represent a balance between providing existing, documented, determinative 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. This is a living guidance document for use by EPA and EPA-contracted laboratories tasked with analysis of environmental samples following a homeland security event. Recommended methods are subject to change based on procedure testing and advances in technology.

Any questions concerning the information in this document should be directed to the appropriate point(s) of contact listed in Section 4.

Appendix A: Selected Chemical Methods

Appendix A: Selected Chemical Methods

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples	Non-aqueous Liquid/Organic Solid Samples[1]	Aqueous Liquid Samples	Drinking Water Samples	Air Samples	Wipes
Acrylamide	79-06-1	HPLC	Sample Prep	Water extraction	Water extraction	8316 (EPA SW-846)	8316 (EPA SW-846)	PV2004 (OSHA)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8316 (EPA SW-846)	8316 (EPA SW-846)				8316 (EPA SW-846)
Acrylonitrile	107-13-1	HPLC	Sample Prep	Water extraction	Water extraction	8316 (EPA SW-846)	8316 (EPA SW-846)	PV2004 (OSHA)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8316 (EPA SW-846)	8316 (EPA SW-846)				8316 (EPA SW-846)
Aldicarb (Temik)	116-06-3	HPLC	Sample Prep	8318A (EPA SW-846)	8318A (EPA SW-846)	8318A (EPA SW-846)	531.2 (EPA OW)	5601 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative						8318A (EPA SW-846)
Aldicarb sulfone	1646-88-4	HPLC	Sample Prep	8318A (EPA SW-846)	8318A (EPA SW-846)	8318A (EPA SW-846)	531.2 (EPA OW)	5601 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative						8318A (EPA SW-846)
Aldicarb sulfoxide	1646-87-3	HPLC	Sample Prep	8318A (EPA SW-846)	8318A (EPA SW-846)	8318A (EPA SW-846)	531.2 (EPA OW)	5601 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative						8318A (EPA SW-846)
Allyl alcohol	107-18-6	GC-MS	Sample Prep	5035A (EPA SW-846)	3585 (EPA SW-846)	5030C (EPA SW-846)	5030C (EPA SW-846)	TO-15[2] (EPA ORD)	Not of concern
			Determinative	8260C (EPA SW-846)	8260C (EPA SW-846)	8260C (EPA SW-846)	8260C (EPA SW-846)		
4-Aminopyridine	504-24-5	HPLC	Sample Prep	8330B (EPA SW-846)	8330B (EPA SW-846)	3535A/8330B (EPA SW-846)	3535A/8330B (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative			8330B (EPA SW-846)	8330B (EPA SW-846)		8330B (EPA SW-846)
Ammonia	7664-41-7	Spectrophotometry	Sample Prep	Not of concern	Not of concern	4500- NH_3 B (SM)	350.1 (EPA OW)	6015 (NIOSH)	Not of concern
			Determinative			4500- NH_3 G (SM)			
Ammonium metavanadate (analyze for total vanadium)	7803-55-6	ICP-MS / ICP-AES	Sample Prep	3050B (EPA SW-846)	3031 (EPA SW-846)	200.7/200.8 (EPA OW)	200.7/200.8 (EPA OW)	IO-3.1 (EPA ORD)	9102 (NIOSH)
			Determinative	6010C/6020A (EPA SW-846)	6010C/6020A (EPA SW-846)			IO-3.4/IO-3.5 (EPA ORD)	6010C/6020A (EPA SW-846)
Arsenic, Total	7440-38-2	ICP-MS / ICP-AES	Sample Prep	3050B (EPA SW-846)	3031 (EPA SW-846)	200.7/200.8 (EPA OW)	200.7/200.8 (EPA OW)	IO-3.1 (EPA ORD)	9102 (NIOSH)
			Determinative	6010C/6020A (EPA SW-846)	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	Non-aqueous Liquid/Organic Solid Samples[1]	Aqueous Liquid Samples	Drinking Water Samples	Air Samples	Wipes
Arsenic trioxide (analyze for total arsenic)	1327-53-3	ICP-MS / ICP-AES	Sample Prep	3050B (EPA SW-846)	3031 (EPA SW-846)	200.7/200.8 (EPA OW)	200.7/200.8 (EPA OW)	IO-3.1 (EPA ORD)	9102 (NIOSH)
			Determinative	6010C/6020A (EPA SW-846)	6010C/6020A (EPA SW-846)			IO-3.4/IO-3.5 (EPA ORD)	6010C/6020A (EPA SW-846)
Arsine	7784-42-1	GFAA	Sample Prep	3050B (EPA SW-846)	Not of concern	200.7/200.8 (EPA OW)	200.7/200.8 (EPA OW)	6001 (NIOSH)	9102 (NIOSH)
			Determinative	7010 (EPA SW-846)					7010 (EPA SW-846)
Asbestos	1332-21-4	TEM	Sample Prep	D5755-03 (soft surfaces-microvac) (ASTM)	Not of concern	Not of concern	Not of concern	10312:1995 (ISO)	D6480-99 (hard surfaces-wipes) (ASTM)
			Determinative						
Boron trifluoride	7637-07-2	ISE	Sample Prep	Not of concern	Not of concern	Not of concern	Not of concern	ID216SG (OSHA)	Not of concern
			Determinative						
Brodifacoum	56073-10-0	HPLC	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)		8321B (EPA SW-846)
Bromadiolone	28772-56-7	HPLC	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)		8321B (EPA SW-846)
BZ (Quinuclidinyl benzilate)	6581-06-2	HPLC	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A[4] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)		8321B[3] (EPA SW-846)
Calcium arsenate (analyze as total arsenic)	7778-44-1	ICP-MS / ICP-AES	Sample Prep	3050B (EPA SW-846)	3031 (EPA SW-846)	200.7/200.8 (EPA OW)	200.7/200.8 (EPA OW)	IO-3.1 (EPA ORD)	9102 (NIOSH)
			Determinative	6010C/6020A (EPA SW-846)	6010C/6020A (EPA SW-846)			IO-3.4/IO-3.5 (EPA ORD)	6010C/6020A (EPA SW-846)
Carbofuran (Furadan)	1563-66-2	HPLC	Sample Prep	8318A (EPA SW-846)	8318A (EPA SW-846)	8318A (EPA SW-846)	531.2 (EPA OW)	5601 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative						8318A (EPA SW-846)
Carbon disulfide	75-15-0	GC-MS	Sample Prep	5035A (EPA SW-846)	3585 (EPA SW-846)	5030C (EPA SW-846)	524.2 (EPA OW)	TO-15 (EPA ORD)	Not of concern
			Determinative	8260C (EPA SW-846)	8260C (EPA SW-846)	8260C (EPA SW-846)			
Chlorfenvinphos	470-90-6	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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	Non-aqueous Liquid/Organic Solid Samples[1]	Aqueous Liquid Samples	Drinking Water Samples	Air Samples	Wipes
Chlorine	7782-50-5	Spectrophotometry	Sample Prep	Not of concern	Not of concern	4500-Cl G (SM)	4500-Cl G (SM)	Analyst, 124(12): 1853-1857	Not of concern
			Determinative					4500-Cl G (SM)	
2-Chloroethanol	107-07-3	GC-MS / GC-FID	Sample Prep	5035A (EPA SW-846)	3585 (EPA SW-846)	5030C (EPA SW-846)	5030C (EPA SW-846)	2513 (NIOSH)	Not of concern
			Determinative	8260C (EPA SW-846)	8260C (EPA SW-846)	8260C (EPA SW-846)	8260C (EPA SW-846)		
3-Chloro-1,2-propanediol	96-24-2	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A[6] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D[5] (EPA SW-846)	8270D[5] (EPA SW-846)	8270D[5] (EPA SW-846)	8270D[5] (EPA SW-846)		8270D[5] (EPA SW-846)
Chloropicrin	76-06-2	GC-MS / GC-ECD	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	551.1 (EPA OW)	551.1 (EPA OW)	PV2103 (OSHA)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D[7] (EPA SW-846)	8270D[7] (EPA SW-846)				8270D[7] (EPA SW-846)
Chlorosarin	1445-76-7	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A[6] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A[6] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
2-Chlorovinylarsonous acid (CVAA) (degradation product of Lewsite)	85090-33-1	ICP-MS / ICP-AES	Sample Prep	3050B (EPA SW-846)	3031 (EPA SW-846)	200.7/200.8 (EPA OW)	200.7/200.8 (EPA OW)	IO-3.1 (EPA ORD)	9102 (NIOSH)
			Determinative	6010C/6020A (EPA SW-846)	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	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
Crimidine	535-89-7	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D[8] (EPA SW-846)	8270D[8] (EPA SW-846)	8270D[8] (EPA SW-846)	8270D[8] (EPA SW-846)		8270D[8] (EPA SW-846)
Cyanide, Amenable to chlorination	NA	Spectrophotometry	Sample Prep	3135.2I (EPA RLAB)	Not of concern	3135.2I (EPA RLAB)	3135.2I (EPA RLAB)	Not of concern	3135.2I (EPA RLAB)
			Determinative						
Cyanide, Total	57-12-5	Spectrophotometry	Sample Prep	ILM05.3 CN (EPA CLP)	Not of concern	ILM05.3 CN (EPA CLP)	335.4 (EPA OW)	6010 (NIOSH)	ILM05.3 CN (EPA CLP)
			Determinative						

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples	Non-aqueous Liquid/Organic Solid Samples[1]	Aqueous Liquid Samples	Drinking Water Samples	Air Samples	Wipes
Cyanogen chloride	506-77-4	GC-MS	Sample Prep	5035A (EPA SW-846)	3585 (EPA SW-846)	5030C (EPA SW-846)	5030C (EPA SW-846)	TO-15 (EPA ORD)	Not of concern
			Determinative	8260C (EPA SW-846)	8260C (EPA SW-846)	8260C (EPA SW-846)	8260C (EPA SW-846)		
Cyclohexyl sarin (GF)	329-99-7	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
1,2-Dichloroethane (degradation product of HD)	107-06-2	GC-MS	Sample Prep	5035A (EPA SW-846)	3585 (EPA SW-846)	5030C (EPA SW-846)	524.2 (EPA OW)	TO-15 (EPA ORD)	Not of concern
			Determinative	8260C (EPA SW-846)	8260C (EPA SW-846)	8260C (EPA SW-846)			
Dichlorvos	62-73-7	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	525.2 (EPA OW)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)			8270D (EPA SW-846)
Dicrotophos	141-66-2	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative	8015C (EPA SW-846)	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	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A[6] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B[9] (EPA SW-846)	8321B[9] (EPA SW-846)	8321B[9] (EPA SW-846)	8321B[9] (EPA SW-846)		8321B[9] (EPA SW-846)
Dimethylphosphite	868-85-9	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
Dimethylphosphoramidic acid (degradation product of GA)	33876-51-6	HPLC	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A[4] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)		8321B[3] (EPA SW-846)
Diphacinone	82-66-6	HPLC	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)		8321B (EPA SW-846)
Disulfoton	298-04-4	GC-MS / GC-FPD	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	525.2 (EPA OW)	525.2 (EPA OW)	5600 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)				8270D (EPA SW-846)

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples	Non-aqueous Liquid/Organic Solid Samples[1]	Aqueous Liquid Samples	Drinking Water Samples	Air Samples	Wipes
Disulfoton sulfoxide	2497-07-6	GC-MS / GC-FPD	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	525.2 (EPA OW)	525.2 (EPA OW)	5600 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)				8270D (EPA SW-846)
1,4-Dithiane (degradation product of HD)	505-29-3	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D[10] (EPA SW-846)	8270D[10] (EPA SW-846)	8270D[10] (EPA SW-846)	8270D[10] (EPA SW-846)		8270D[10] (EPA SW-846)
EA2192 [Diisopropylaminoethyl methylthiolophosphonate] (hydrolysis product of VX)	73207-98-4	HPLC	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A[4] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)		8321B[3] (EPA SW-846)
Ethyl methylphosphonic acid (EMPA) (degradation product of VX)	1832-53-7	HPLC	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A[4] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)		8321B[3] (EPA SW-846)
Ethyldichloroarsine (ED)	598-14-1	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-15 (EPA ORD)	9102 (NIOSH)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
N-Ethyldiethanolamine (EDEA) (degradation product of HN-1)	139-87-7	HPLC	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)		8321B (EPA SW-846)
Ethylene oxide	75-21-8	GC-MS	Sample Prep	5035A (EPA SW-846)	3585 (EPA SW-846)	5030C (EPA SW-846)	5030C (EPA SW-846)	TO-15 (EPA ORD)	Not of concern
			Determinative	8260C (EPA SW-846)	8260C (EPA SW-846)	8260C (EPA SW-846)	8260C (EPA SW-846)		
Fenamiphos	22224-92-6	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	525.2 (EPA OW)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)			8270D (EPA SW-846)
Fentanyl	437-38-7	HPLC	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)		8321B (EPA SW-846)
Fluoride	16984-48-8	IC	Sample Prep	Not of concern	Not of concern	300.1, Rev 1.0 (EPA OW)	300.1, Rev 1.0 (EPA OW)	Not of concern	Not of concern
			Determinative						
Fluoroacetamide	640-19-7	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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	Non-aqueous Liquid/Organic Solid Samples[1]	Aqueous Liquid Samples	Drinking Water Samples	Air Samples	Wipes
Fluoroacetic acid and fluoroacetate salts (analyze for fluoroacetate ion)	NA	IC	Sample Prep	Analytical Letters, 27(14): 2703-2718	Analytical Letters, 27(14): 2703-2718	300.1, Rev 1.0 (EPA OW)	300.1, Rev 1.0 (EPA OW)	S301-1 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative	300.1, Rev 1.0 (EPA OW)	300.1, Rev 1.0 (EPA OW)			300.1, Rev 1.0 (EPA OW)	300.1, Rev 1.0 (EPA OW)
Formaldehyde	50-00-0	HPLC	Sample Prep	8315A (EPA SW-846)	Not of concern	8315A (EPA SW-846)	8315A (EPA SW-846)	2016 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative						8315A (EPA SW-846)
Gasoline Range Organics	NA	GC-FID	Sample Prep	5035A (EPA SW-846)	3585 (EPA SW-846)	5030C (EPA SW-846)	5030C (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative	8015C (EPA SW-846)	8015C (EPA SW-846)	8015C (EPA SW-846)	8015C (EPA SW-846)		8015C (EPA SW-846)
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	121-82-4	HPLC	Sample Prep	8330B (EPA SW-846)	8330B (EPA SW-846)	3535A/8330B (EPA SW-846)	3535A/8330B (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative			8330B (EPA SW-846)	8330B (EPA SW-846)		8330B (EPA SW-846)
Hexamethylenetriperoxidediamine (HMTD)	283-66-9	HPLC	Sample Prep	8330B (EPA SW-846)	8330B (EPA SW-846)	3535A/8330B (EPA SW-846)	3535A/8330B (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative			8330B (EPA SW-846)	8330B (EPA SW-846)		8330B (EPA SW-846)
Hydrogen bromide	10035-10-6	IC	Sample Prep	Not of concern	Not of concern	Not of concern	Not of concern	7903 (NIOSH)	Not of concern
			Determinative						
Hydrogen chloride	7647-01-0	IC	Sample Prep	Not of concern	Not of concern	Not of concern	Not of concern	7903 (NIOSH)	Not of concern
			Determinative						
Hydrogen cyanide	74-90-8	Spectrophotometry	Sample Prep	Not of concern	Not of concern	Not of concern	Not of concern	6010 (NIOSH)	Not of concern
			Determinative						
Hydrogen fluoride	7664-39-3	IC	Sample Prep	Not of concern	Not of concern	Not of concern	Not of concern	7903[11] (NIOSH)	Not of concern
			Determinative						
Hydrogen sulfide	7783-06-4	IC	Sample Prep	Not of concern	Not of concern	Not of concern	Not of concern	6013 (NIOSH)	Not of concern
			Determinative						
Isopropyl methylphosphonic acid (IMPA) (degradation product of GB)	1832-54-8	HPLC	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A[4] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)		8321B[3] (EPA SW-846)

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples	Non-aqueous Liquid/Organic Solid Samples[1]	Aqueous Liquid Samples	Drinking Water Samples	Air Samples	Wipes
Kerosene	64742-81-0	GC-FID	Sample Prep	5035A (EPA SW-846)	3585 (EPA SW-846)	5030C (EPA SW-846)	5030C (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative	8015C (EPA SW-846)	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-MS / ICP-AES	Sample Prep	3050B (EPA SW-846)	3031 (EPA SW-846)	200.7/200.8 (EPA OW)	200.7/200.8 (EPA OW)	IO-3.1 (EPA ORD)	9102 (NIOSH)
			Determinative	6010C/6020A (EPA SW-846)	6010C/6020A (EPA SW-846)			IO-3.4/IO-3.5 (EPA ORD)	6010C/6020A (EPA SW-846)
Lewisite 1 (L-1)[12] [2-chlorovinyldichloroarsine] (analyze for total arsenic)	541-25-3	ICP-MS / ICP-AES	Sample Prep	3050B (EPA SW-846)	3031 (EPA SW-846)	200.7/200.8 (EPA OW)	200.7/200.8 (EPA OW)	IO-3.1 (EPA ORD)	9102 (NIOSH)
			Determinative	6010C/6020A (EPA SW-846)	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 for total arsenic)	40334-69-8	ICP-MS / ICP-AES	Sample Prep	3050B (EPA SW-846)	3031 (EPA SW-846)	200.7/200.8 (EPA OW)	200.7/200.8 (EPA OW)	IO-3.1 (EPA ORD)	9102 (NIOSH)
			Determinative	6010C/6020A (EPA SW-846)	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 for total arsenic)	40334-70-1	ICP-MS / ICP-AES	Sample Prep	3050B (EPA SW-846)	3031 (EPA SW-846)	200.7/200.8 (EPA OW)	200.7/200.8 (EPA OW)	IO-3.1 (EPA ORD)	9102 (NIOSH)
			Determinative	6010C/6020A (EPA SW-846)	6010C/6020A (EPA SW-846)			IO-3.4/IO-3.5 (EPA ORD)	6010C/6020A (EPA SW-846)
Lewisite oxide (degradation product of Lewisite)	1306-02-1	ICP-MS / ICP-AES	Sample Prep	3050B (EPA SW-846)	3031 (EPA SW-846)	200.7/200.8 (EPA OW)	200.7/200.8 (EPA OW)	IO-3.1 (EPA ORD)	9102 (NIOSH)
			Determinative	6010C/6020A (EPA SW-846)	6010C/6020A (EPA SW-846)			IO-3.4/IO-3.5 (EPA ORD)	6010C/6020A (EPA SW-846)
Mercury, Total	7439-97-6	Spectrophotometry / CVAA / CVAFS	Sample Prep	7473[13] (EPA SW-846)	Not of concern	7473[13] (EPA SW-846)	245.2 (EPA OW)	IO-5 (EPA ORD)	9102 (NIOSH)
			Determinative						7473[13] (EPA SW-846)
Methamidophos	10265-92-6	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A[14] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
Methomyl	16752-77-5	HPLC	Sample Prep	8318A (EPA SW-846)	8318A (EPA SW-846)	8318A (EPA SW-846)	531.2 (EPA OW)	5601 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative						8318A (EPA SW-846)
Methoxyethylmercuric acetate (analyze for total mercury)	151-38-2	Spectrophotometry / CVAA / CVAFS	Sample Prep	7473[13] (EPA SW-846)	Not of concern	7473[13] (EPA SW-846)	245.2 (EPA OW)	IO-5 (EPA ORD)	9102 (NIOSH)
			Determinative						7473[13] (EPA SW-846)
Methyl acrylonitrile	126-98-7	HPLC	Sample Prep	Water extraction	Water extraction	8316 (EPA SW-846)	8316 (EPA SW-846)	PV2004 (OSHA)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8316 (EPA SW-846)	8316 (EPA SW-846)				8316 (EPA SW-846)

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples	Non-aqueous Liquid/Organic Solid Samples[1]	Aqueous Liquid Samples	Drinking Water Samples	Air Samples	Wipes
Methyl fluoroacetate (analyze for fluoroacetate ion)	453-18-9	IC	Sample Prep	Analytical Letters, 27(14): 2703-2718	Analytical Letters, 27(14): 2703-2718	300.1, Rev 1.0 (EPA OW)	300.1, Rev 1.0 (EPA OW)	S301-1 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative	300.1, Rev 1.0 (EPA OW)	300.1, Rev 1.0 (EPA OW)			300.1, Rev 1.0 (EPA OW)	300.1, Rev 1.0 (EPA OW)
Methyl hydrazine	60-34-4	GC-MS / Spectrophotometry	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	3510 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
Methyl isocyanate	624-83-9	HPLC	Sample Prep	Not of concern	Not of concern	Not of concern	Not of concern	OSHA 54	Not of concern
			Determinative						
Methyl parathion	298-00-0	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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	Not of concern	Not of concern	Not of concern	OSHA 40	Not of concern
			Determinative						
N-Methyldiethanolamine (MDEA) (degradation product of HN-2)	105-59-9	HPLC	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)		8321B (EPA SW-846)
1-Methylethyl ester ethylphosphonofluoridic acid (GE)	1189-87-3	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A[6] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A[4] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)		8321B[3] (EPA SW-846)
Mevinphos	7786-34-7	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	525.2 (EPA OW)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			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)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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	Non-aqueous Liquid/Organic Solid Samples[1]	Aqueous Liquid Samples	Drinking Water Samples	Air Samples	Wipes
Mustard, nitrogen (HN-3) [tris(2-chloroethyl)amine]	555-77-1	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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	3571 (EPA SW-846)	3571 (EPA SW-846)	3571 (EPA SW-846)	3571 (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D[15] (EPA SW-846)	8270D[15] (EPA SW-846)	8270D[15] (EPA SW-846)	8270D[15] (EPA SW-846)		8270D[15] (EPA SW-846)
Nicotine compounds (analyze as nicotine)	54-11-5	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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)	8330B (EPA SW-846)	3535A/8330B (EPA SW-846)	3535A/8330B (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative			8330B (EPA SW-846)	8330B (EPA SW-846)		8330B (EPA SW-846)
Organophosphate pesticides, NOS	NA	GC-MS / GC-NPD / GC-FPD	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	614 (EPA OW)	507 (EPA OW)	5600 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)				8270D (EPA SW-846)
Osmium tetroxide (analyze for total osmium)	20816-12-0	ICP-AES / GFAA	Sample Prep	3050B (EPA SW-846)	Not of concern	252.2 (EPA OW)	252.2 (EPA OW)	IO-3.1 (EPA ORD)	9102 (NIOSH)
			Determinative	6010C (EPA SW-846)				IO-3.4 (EPA ORD)	6010C (EPA SW-846)
Oxamyl	23135-22-0	HPLC	Sample Prep	8318A (EPA SW-846)	8318A (EPA SW-846)	8318A (EPA SW-846)	531.2 (EPA OW)	5601 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative						8318A (EPA SW-846)
Paraquat	4685-14-7	HPLC	Sample Prep	Not of concern	Not of concern	549.2 (EPA OW)	549.2 (EPA OW)	Not of concern	Not of concern
			Determinative						
Parathion	56-38-2	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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)	8330B (EPA SW-846)	3535A/8330B (EPA SW-846)	3535A/8330B (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative			8330B (EPA SW-846)	8330B (EPA SW-846)		8330B (EPA SW-846)
Perfluoroisobutylene (PFIB)	382-21-8	GC-MS / GC-NPD	Sample Prep	Not of concern	Not of concern	Not of concern	Not of concern	OSHA 61[16]	Not of concern
			Determinative						

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples	Non-aqueous Liquid/Organic Solid Samples[1]	Aqueous Liquid Samples	Drinking Water Samples	Air Samples	Wipes
Phencyclidine	77-10-1	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
Phenol	108-95-2	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
Phorate	298-02-2	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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	Not of concern	Not of concern	Not of concern	OSHA 61	Not of concern
			Determinative						
Phosphamidon	13171-21-6	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
Phosphine	7803-51-2	Spectrophotometry	Sample Prep	Not of concern	Not of concern	Not of concern	Not of concern	6002 (NIOSH)	Not of concern
			Determinative						
Phosphorus trichloride	7719-12-2	Spectrophotometry	Sample Prep	Not of concern	Not of concern	Not of concern	Not of concern	6402 (NIOSH)	Not of concern
			Determinative						
Pinacolyl methyl phosphonic acid (PMPA) (degradation product of GD)	616-52-4	HPLC	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A[4] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)	8321B[3] (EPA SW-846)		8321B[3] (EPA SW-846)
Propylene oxide	75-56-9	GC-MS / GC-FID	Sample Prep	5035A (EPA SW-846)	3585 (EPA SW-846)	5030C (EPA SW-846)	5030C (EPA SW-846)	1612 (NIOSH)	Not of concern
			Determinative	8260C (EPA SW-846)	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)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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	3571 (EPA SW-846)	3571 (EPA SW-846)	3571 (EPA SW-846)	3571 (EPA SW-846)	TO-10A[6] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D[15] (EPA SW-846)	8270D[15] (EPA SW-846)	8270D[15] (EPA SW-846)	8270D[15] (EPA SW-846)		8270D[15] (EPA SW-846)

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples	Non-aqueous Liquid/Organic Solid Samples[1]	Aqueous Liquid Samples	Drinking Water Samples	Air Samples	Wipes
Semivolatile Organic Compounds, NOS	NA	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	525.2 (EPA OW)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)			8270D (EPA SW-846)
Sodium arsenite (analyze for total arsenic)	7784-46-5	ICP-MS / ICP-AES	Sample Prep	3050B (EPA SW-846)	3031 (EPA SW-846)	200.7/200.8 (EPA OW)	200.7/200.8 (EPA OW)	IO-3.1 (EPA ORD)	9102 (NIOSH)
			Determinative	6010C/6020A (EPA SW-846)	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	Sample Prep	J. of Forensic Sciences, 43(1): 200-202[17]	3580A[17] (EPA SW-846)	J. of Forensic Sciences, 43(1): 200-202[17]	J. of Forensic Sciences, 43(1): 200-202[17]	ID-211 (OSHA)	ID-211 (OSHA)
			Determinative	300.1, Rev 1.0[18] (EPA OW)	300.1, Rev 1.0[18] (EPA OW)	300.1, Rev 1.0[18] (EPA OW)	300.1, Rev 1.0[18] (EPA OW)		
Soman (GD)	96-64-0	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A[6] (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
Strychnine	57-24-9	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
Sulfur dioxide	7446-09-5	IC	Sample Prep	Not of concern	Not of concern	Not of concern	Not of concern	6004 (NIOSH)	Not of concern
			Determinative						
Sulfur trioxide	7446-11-9	Titrimetry	Sample Prep	Not of concern	Not of concern	Not of concern	Not of concern	Method 8 (EPA OAQPS)	Not of concern
			Determinative						
Tabun (GA)	77-81-6	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
Tear gas (CS) [chlorobenzylidene malonitrile]	2698-41-1	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
Tetraethyl pyrophosphate	107-49-3	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
Tetramethylenedisulfotetramine	80-12-6	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D[7] (EPA SW-846)	8270D[7] (EPA SW-846)	8270D[7] (EPA SW-846)	8270D[7] (EPA SW-846)		8270D[7] (EPA SW-846)

Analyte(s)	CAS RN	Determinative Technique	Method Type	Solid Samples	Non-aqueous Liquid/Organic Solid Samples[1]	Aqueous Liquid Samples	Drinking Water Samples	Air Samples	Wipes
Thallium sulfate (analyze for total thallium)	10031-59-1	ICP-MS / ICP-AES	Sample Prep	3050B (EPA SW-846)	3031 (EPA SW-846)	200.7/200.8 (EPA OW)	200.7/200.8 (EPA OW)	IO-3.1 (EPA ORD)	9102 (NIOSH)
			Determinative	6010C/6020A (EPA SW-846)	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	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
Thiofanox	39196-18-4	HPLC	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	531.2 (EPA OW)	5601 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)			8321B (EPA SW-846)
1,4-Thioxane (degradation product of HD)	15980-15-1	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D[10] (EPA SW-846)	8270D[10] (EPA SW-846)	8270D[10] (EPA SW-846)	8270D[10] (EPA SW-846)		8270D[10] (EPA SW-846)
Titanium tetrachloride (analyze for total titanium)	7550-45-0	ICP-MS / ICP-AES	Sample Prep	3050B (EPA SW-846)	Not of concern	Not of concern	Not of concern	Not of concern	9102 (NIOSH)
			Determinative	6010C/6020A (EPA SW-846)					6010C/6020A (EPA SW-846)
Triethanolamine (TEA) (degradation product of HN-3)	102-71-6	HPLC	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)	8321B (EPA SW-846)		8321B (EPA SW-846)
Trimethyl phosphite	121-45-9	GC-MS	Sample Prep	3545A (EPA SW-846)	3580A (EPA SW-846)	3535A (EPA SW-846)	3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D[7] (EPA SW-846)	8270D[7] (EPA SW-846)	8270D[7] (EPA SW-846)	8270D[7] (EPA SW-846)		8270D[7] (EPA SW-846)
1,3,5-Trinitrobenzene (1,3,5-TNB)	99-35-4	HPLC	Sample Prep	8330B (EPA SW-846)	8330B (EPA SW-846)	3535A/8330B (EPA SW-846)	3535A/8330B (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			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)	8330B (EPA SW-846)	3535A/8330B (EPA SW-846)	3535A/8330B (EPA SW-846)	Not of concern	3570/8290A Appendix A (EPA SW-846)
			Determinative			8330B (EPA SW-846)	8330B (EPA SW-846)		8330B (EPA SW-846)
Vanadium pentoxide (analyze for total vanadium)	1314-62-1	ICP-MS / ICP-AES	Sample Prep	3050B (EPA SW-846)	3031 (EPA SW-846)	200.7/200.8 (EPA OW)	200.7/200.8 (EPA OW)	IO-3.1 (EPA ORD)	9102 (NIOSH)
			Determinative	6010C/6020A (EPA SW-846)	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)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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	Non-aqueous Liquid/Organic Solid Samples[1]	Aqueous Liquid Samples	Drinking Water Samples	Air Samples	Wipes
VG [phosphonothioic acid, S-(2-(diethylamino)ethyl) O,O-diethyl ester]	78-53-5	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)	8270D (EPA SW-846)		8270D (EPA SW-846)
VM [phosphonothioic acid, methyl-, S-(2-(diethylamino)ethyl) O-ethyl ester]	21770-86-5	GC-MS	Sample Prep	3541/3545A (EPA SW-846)	3580A (EPA SW-846)	3520C/3535A (EPA SW-846)	3520C/3535A (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D (EPA SW-846)	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	3571 (EPA SW-846)	3571 (EPA SW-846)	3571 (EPA SW-846)	3571 (EPA SW-846)	TO-10A (EPA ORD)	3570/8290A Appendix A (EPA SW-846)
			Determinative	8270D[15] (EPA SW-846)	8270D[15] (EPA SW-846)	8270D[15] (EPA SW-846)	8270D[15] (EPA SW-846)		8270D[15] (EPA SW-846)
White phosphorus	12185-10-3	GC-NPD / GC-FPD	Sample Prep	7580 (EPA SW-846)	7580 (EPA SW-846)	7580 (EPA SW-846)	7580 (EPA SW-846)	7905 (NIOSH)	3570/8290A Appendix A (EPA SW-846)
			Determinative						7580 (EPA SW-846)

Footnotes

[1] An organic solid sample is a solid that completely dissolves in an organic solvent and leaves no solid residue.

[2] If problems occur when using this method, it is recommended that TO-10A be used.

[3] LC-MS (electrospray) procedures are preferred for these analytes; however, if this technique is not available to the laboratory, GC-MS procedures using derivatization based on SW-846 Method 8270D may be used. Sample preparation methods should remain the same. Both electrospray LC-MS and GC-MS derivatization procedures are currently under development.

[4] 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.

[5] For this analyte, SW-846 Method 8270D must be modified to include a derivatization step.

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

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

[8] If problems occur when using this method, it is recommended that SW-846 Method 8321B be used as the determinative method. Sample preparation methods should remain the same.

[9] If problems occur with the analysis of DIMP using EPA SW-846 Method 8321B, use SW-846 Method 8270D.

[10] If problems occur when using this method, it is recommended that SW-846 Method 8260C and appropriate corresponding sample preparation procedures (i.e., 5035A for solid samples, 3585 for non-aqueous liquid/organic solid samples, and 5030C for aqueous liquid and drinking water samples) be used.

[11] If problems occur when using this method, it is recommended that NIOSH Method 7906 be used.

[12] Laboratory testing is currently under way for speciation of Lewisite 1 using GC-MS techniques.

[13] If equipment is not available, use CVAA Methods 7471B (EPA SW-846) for solid samples and 7470A (EPA SW-846) for aqueous liquid samples.

[14] If problems occur when using this method, it is recommended that NIOSH Method 5600 be used.

[15] For this analyte, refer to EPA SW-846 Method 8271 for GC-MS conditions.

[16] If problems occur when using this method, it is recommended that a method based on the following journal article be used: J. Chrom. A, 1098: (2005) 156-165.

[17] 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. The acidification step from the journal should be used with EPA SW-846 Method 3580A for preparation of non-aqueous liquid/organic solid samples.

[18] If analyses are problematic, refer to column manufacturer for alternate conditions

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Appendix B: Selected Radiochemical Methods

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
Gross Alpha	Alpha/Beta counting	900.0 (EPA)	7110 B (SM)	AP1 (ORISE)	FRMAC, Vol 2, pg. 33	FRMAC, Vol 2, pg. 33
Gross Beta	Alpha/Beta counting	900.0 (EPA)	7110 B (SM)	AP1 (ORISE)	FRMAC, Vol 2, pg. 33	FRMAC, Vol 2, pg. 33
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)

Analyte(s)	CAS RN	Determinative Technique	Drinking Water Samples		Aqueous and Liquid Phase Samples		Soil and Sediment Samples		Surface Wipes		Air Filters	
			Qualitative Determination[1]	Confirmatory	Qualitative Determination[1]	Confirmatory	Qualitative Determination[1]	Confirmatory	Qualitative Determination[1]	Confirmatory	Qualitative Determination[1]	Confirmatory
Americium-241[2]	14596-10-2	Alpha/Gamma spectrometry	D3084 (ASTM)	Am-04-RC (HASL-300)	D3084 (ASTM)	Am-04-RC (HASL-300)	Am-02-RC (HASL-300)	Am-01-RC[3] (HASL-300)	D3084 (ASTM)	Am-04-RC (HASL-300)	D3084 (ASTM)	Am-04-RC (HASL-300)
Californium-252[2]	13981-17-4	Alpha spectrometry	D3084 (ASTM)	Am-04-RC (HASL-300)	D3084 (ASTM)	Am-04-RC (HASL-300)	D3084 (ASTM)	Am-01-RC[3] (HASL-300)	D3084 (ASTM)	Am-04-RC (HASL-300)	D3084 (ASTM)	Am-04-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)
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)
Curium-244[2]	13981-15-2	Alpha spectrometry	D3084 (ASTM)	Am-04-RC (HASL-300)	D3084 (ASTM)	Am-04-RC (HASL-300)	D3084 (ASTM)	Am-01-RC[3] (HASL-300)	D3084 (ASTM)	Am-04-RC (HASL-300)	D3084 (ASTM)	Am-04-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)
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 (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)
Plutonium-238[2]	13981-16-3	Alpha spectrometry	D3084 (ASTM)	EMSL-33 (EPA)	D3084 (ASTM)	EMSL-33 (EPA)	D3084 (ASTM)	EMSL-33 (EPA)	D3084 (ASTM)	EMSL-33 (EPA)	D3084 (ASTM)	EMSL-33 (EPA)
Plutonium-239[2]	15117-48-3	Alpha spectrometry	D3084 (ASTM)	EMSL-33 (EPA)	D3084 (ASTM)	EMSL-33 (EPA)	D3084 (ASTM)	EMSL-33 (EPA)	D3084 (ASTM)	EMSL-33 (EPA)	D3084 (ASTM)	EMSL-33 (EPA)

Analyte(s)	CAS RN	Determinative Technique	Drinking Water Samples		Aqueous and Liquid Phase Samples		Soil and Sediment Samples		Surface Wipes		Air Filters	
			Qualitative Determination[1]	Confirmatory	Qualitative Determination[1]	Confirmatory	Qualitative Determination[1]	Confirmatory	Qualitative Determination[1]	Confirmatory	Qualitative Determination[1]	Confirmatory
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)
Radium-226	13982-63-3	Alpha counting / spectrometry	903.0 (EPA)	903.1 (EPA)	7500-Ra B (SM)	7500-Ra C (SM)	D3084 (ASTM)	EMSL-19 (EPA)	D3084 (ASTM)	EMSL-19 (EPA)	D3084 (ASTM)	EMSL-19 (EPA)
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)
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)
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)
Strontium-90	10098-97-2	Beta counting by low-background gas flow proportional detector	7500-Sr B (SM)	7500-Sr B (SM)	7500-Sr B (SM)	7500-Sr B (SM)	Sr-03-RC (HASL-300)	Sr-03-RC (HASL-300)	Sr-03-RC (HASL-300)	Sr-03-RC (HASL-300)	Sr-03-RC (HASL-300)	Sr-03-RC (HASL-300)
Uranium-234[2]	13966-29-5	Alpha counting / spectrometry	908.0[4] (EPA)	D3972 (ASTM)	7500-U B[4] (SM)	7500-U C (SM)	D3084 (ASTM)	EMSL-33 (EPA)	D3084 (ASTM)	EMSL-33 (EPA)	D3084 (ASTM)	EMSL-33 (EPA)
Uranium-235[2]	15117-96-1	Alpha counting / spectrometry	908.0[4] (EPA)	D3972 (ASTM)	7500-U B[4] (SM)	7500-U C (SM)	D3084 (ASTM)	EMSL-33 (EPA)	D3084 (ASTM)	EMSL-33 (EPA)	D3084 (ASTM)	EMSL-33 (EPA)
Uranium-238[2]	7440-61-1	Alpha counting / spectrometry	908.0[4] (EPA)	D3972 (ASTM)	7500-U B[4] (SM)	7500-U C (SM)	D3084 (ASTM)	EMSL-33 (EPA)	D3084 (ASTM)	EMSL-33 (EPA)	D3084 (ASTM)	EMSL-33 (EPA)

[1] 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.

[2] 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.

[3] In cases where only small sample volumes (≤100 g) are available, use HASL-300 Method Pu-12-RC.

[4] This method was developed for measurement of total uranium and does not distinguish between uranium isotopes.

Appendix C: Selected Pathogen Methods

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 a replacement of a sample preparation 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., evaluation of the efficacy of disinfection), a viability-based procedure (such as culture) should be used. Rapid analysis techniques (such as PCR, some immunoassays) are preferred for estimating the extent of contamination and should be used in conjunction with culture for confirmation of identification. In cases where a method is listed as "requires modification of analytical method," the analytical method should be used as a starting point and modified as necessary for analysis of a particular sample type.

Pathogen(s) [Disease]	Analytical Technique	Analytical Method	Solid[1] (soil, powder)	Particulate[2] (swabs, wipes, HEPA)	Liquid/Water (filter, grab)	Drinking Water (filter, grab)	Aerosol (growth media, filter, liquid)
Bacteria							
Bacillus anthracis [Anthrax]	Culture	Basic Diagnostic Testing Protocols for Level A Laboratories for the Presumptive Identification of *Bacillus anthracis* CDC/ASM/APHL					
	Real-time PCR/ Immunoassay	LRN comparable assays					
Brucella spp. (*B. abortus, B. melitensis, B. suis*) [Brucellosis]	Culture	Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism: *Brucella* species ASM					
	Real-time PCR/ Immunoassay	LRN comparable assays					
Burkholderia mallei [Glanders]	Culture	Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism: *Burkholderia mallei* and *B. pseudomallei* ASM					
	Real-time PCR/ Immunoassay	LRN comparable assays					
Burkholderia pseudomallei [Melioidosis]	Culture	Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism: *Burkholderia mallei* and *B. pseudomallei* ASM					
	Real-time PCR/ Immunoassay	LRN comparable assays					
Campylobacter jejuni [Campylobacteriosis]	Culture	SM 9260 G	Requires modification of analytical method	Requires modification of analytical method	SM 9260 G	SM 9260 G	Unlikely to be viable
	Immunoassay	SM 9260 G	Requires modification of analytical method	Requires modification of analytical method	SM 9260 G	SM 9260 G	Unlikely to be viable
	Real-time PCR	Molecular and Cellular Probes 20: 269-279	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method

Pathogen(s) [Disease]	Analytical Technique	Analytical Method	Solid[1] (soil, powder)	Particulate[2] (swabs, wipes, HEPA)	Liquid/Water (filter, grab)	Drinking Water (filter, grab)	Aerosol (growth media, filter, liquid)
Chlamydophila psittaci (formerly known as *Chlamydia psittaci*) [Psittacosis]	Tissue culture	Journal of Clinical Microbiology 38(3): 1085-1093	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method
	PCR	Journal of Clinical Microbiology 38(3): 1085-1093	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method
Coxiella burnetii [Q-fever]	Culture	If analysis of this pathogen is required, contact the LRN (404-639-2790) for information on the LRN laboratory capable of receiving and processing the sample. In some cases, methods that apply to environmental samples may not be fully developed or validated.					
	Real-time PCR/ Immunoassay	LRN comparable assays					
Escherichia coli O157:H7	Culture	SM 9260 F	Requires modification of analytical method	Requires modification of analytical method	SM 9260 F	SM 9260 F	Unlikely to be viable
	Immunoassay	SM 9260 F	Requires modification of analytical method	Requires modification of analytical method	SM 9260 F	SM 9260 F	Unlikely to be viable
	Real-time PCR	Applied & Environmental Microbiology 69(10): 6327-6333	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method
Francisella tularensis [Tularemia]	Culture	Basic Protocols for Level A Laboratories for the Presumptive Identification of *Francisella tularensis* CDC/ASM/APHL					
	Real-time PCR/ Immunoassay	LRN comparable assays					
Leptospira (*L. interrogans* Serovars Icteroheamorrhagiae, Autralis, Balum, Bataviae, Bejro, Pomona) [Leptospirosis]	Culture	SM 9260 I	SM 9260 I	Requires modification of analytical method	SM 9260 I	SM 9260 I	Unlikely to be viable
	Immunoassay	SM 9260 I	SM 9260 I	Requires modification of analytical method	SM 9260 I	SM 9260 I	Unlikely to be viable
Listeria monocytogenes [Listeriosis]	Culture	FDA/Bacteriological Analytical Manual Chapter 10, 2003	FDA/Bacteriological Analytical Manual Chapter 10, 2003	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Unlikely to be viable
	Immunoassay	FDA/Bacteriological Analytical Manual Chapter 10, 2003	FDA/Bacteriological Analytical Manual Chapter 10, 2003	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Unlikely to be viable
	Real-time PCR	USDA Laboratory Guidebook MLG 8A.03	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method

Pathogen(s) [Disease]	Analytical Technique	Analytical Method	Solid[1] (soil, powder)	Particulate[2] (swabs, wipes, HEPA)	Liquid/Water (filter, grab)	Drinking Water (filter, grab)	Aerosol (growth media, filter, liquid)
Non-typhoidal *Salmonella* (Not applicable to *S.* Typhi) [Salmonellosis]	Culture	EPA Method 1682	EPA Method 1682	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Unlikely to be viable
	Immunoassay	EPA Method 1682	EPA Method 1682	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Unlikely to be viable
	Real-time PCR	Journal of Applied Microbiology 102(2): 516–530	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method
Salmonella Typhi [Typhoid fever]	Culture	SM 9260 B	Requires modification of analytical method	Requires modification of analytical method	SM 9260 B	SM 9260 B	Unlikely to be viable
	Immunoassay	SM 9260 B	Requires modification of analytical method	Requires modification of analytical method	SM 9260 B	SM 9260 B	Unlikely to be viable
	Real-time PCR	CDC Laboratory Assay: *S.* Typhi	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method
Shigella spp. [Shigellosis]	Culture	SM 9260 E	Requires modification of analytical method	Requires modification of analytical method	SM 9260 E	SM 9260 E	Unlikely to be viable
	Immunoassay	SM 9260 E	Requires modification of analytical method	Requires modification of analytical method	SM 9260 E	SM 9260 E	Unlikely to be viable
	Real-time PCR	CDC Laboratory Assay: *Shigella*	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method
Staphylococcus aureus	Culture	SM 9213 B	Requires modification of analytical method	Requires modification of analytical method	SM 9213 B	SM 9213 B	Requires modification of analytical method
Vibrio cholerae O1 and O139 [Cholera]	Culture	SM 9260 H	Requires modification of analytical method	Requires modification of analytical method	SM 9260 H	SM 9260 H	Unlikely to be viable
	Immunoassay	SM 9260 H	Requires modification of analytical method	Requires modification of analytical method	SM 9260 H	SM 9260 H	Unlikely to be viable
	Real-time PCR	CDC Laboratory Assay: *V. cholerae*	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method

Pathogen(s) [Disease]	Analytical Technique	Analytical Method	Solid[1] (soil, powder)	Particulate[2] (swabs, wipes, HEPA)	Liquid/Water (filter, grab)	Drinking Water (filter, grab)	Aerosol (growth media, filter, liquid)
Yersinia pestis [Plague]	Culture	Sentinel Laboratory Guidelines for Suspected Agents of Bioterrorism: *Yersinia pestis* ASM					
	Real-time PCR/ Immunoassay	LRN comparable assays					
Viruses							
Adenoviruses: Enteric and non-enteric (A-F)	Tissue culture[3]	Applied & Environmental Microbiology 71(6): 3131-3136	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]
	Real-time PCR	Applied & Environmental Microbiology 71(6): 3131-3136	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]
Astroviruses	Integrated Cell Culture/Reverse transcription-PCR	Canadian Journal of Microbiology 50: 269-278	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]
	Real-time reverse transcription-PCR	Canadian Journal of Microbiology 50: 269-278	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]
Caliciviruses: Noroviruses	Real-time reverse transcription-PCR	Journal of Clinical Microbiology 42(10): 4679-4685	Journal of Clinical Microbiology Vol. 42(10): 4679-4685[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]
Caliciviruses: Sapovirus	Real-time reverse transcription-PCR	Journal of Medical Virology 78(10): 1347-1353	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]
Coronaviruses: SARS-associated human coronavirus	Reverse transcription-PCR	Journal of Virological Methods 122: 29-36	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]
Hepatitis E virus (HEV)	Real-time reverse transcription-PCR	Journal of Virological Methods 131(1): 65-71	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]
Influenza H5N1 virus	Real-time reverse transcription-PCR	Emerging Infectious Diseases 11(8): 1303-1305	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Requires modification of analytical method[4]

Pathogen(s) [Disease]	Analytical Technique	Analytical Method	Solid[1] (soil, powder)	Particulate[2] (swabs, wipes, HEPA)	Liquid/Water (filter, grab)	Drinking Water (filter, grab)	Aerosol (growth media, filter, liquid)
Picornaviruses: Enteroviruses	Tissue culture	USEPA Manual of Methods for Virology EPA/600/4-84/013, April 2001	Requires modification of analytical method[4]	Requires modification of analytical method[4]	USEPA Manual of Methods for Virology EPA/600/4-84/013, April 2001	USEPA Manual of Methods for Virology EPA/600/4-84/013, April 2001	Requires modification of analytical method[4]
	Reverse transcription-PCR	Applied & Environmental Microbiology 69(6): 3158-3164	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Applied & Environmental Microbiology 69(6): 3158-3164[4]	Applied & Environmental Microbiology 69(6): 3158-3164[4]	Requires modification of analytical method[4]
Picornaviruses: Hepatitis A virus (HAV)	Reverse transcription-PCR	Applied & Environmental Microbiology 69(6): 3158-3164	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Applied & Environmental Microbiology 69(6): 3158-3164[4]	Applied & Environmental Microbiology 69(6): 3158-3164[4]	Requires modification of analytical method[4]
Reoviruses: Rotavirus (Group A)	Tissue culture	Applied & Environmental Microbiology 69(6): 3158-3164	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Applied & Environmental Microbiology 69(6): 3158-3164[4]	Applied & Environmental Microbiology 69(6): 3158-3164[4]	Requires modification of analytical method[4]
	Reverse transcription-PCR	Applied & Environmental Microbiology 69(6): 3158-3164	Requires modification of analytical method[4]	Requires modification of analytical method[4]	Applied & Environmental Microbiology 69(6): 3158-3164[4]	Applied & Environmental Microbiology 69(6): 3158-3164[4]	Requires modification of analytical method[4]
Protozoa							
Cryptosporidium spp. [Cryptosporidiosis]	Tissue culture	Applied & Environmental Microbiology 65(9): 3936-3941	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Unlikely to be found
	IMS/FA	EPA Method 1622 and/or Method 1623	Requires modification of analytical method	Requires modification of analytical method	EPA Method 1622 and/or Method 1623	EPA Method 1622 and/or Method 1623	Unlikely to be found
	Real-time PCR	Applied & Environmental Microbiology 73(13): 4218-4225	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Applied & Environmental Microbiology 73(13): 4218-4225	Unlikely to be found
Entamoeba histolytica	Culture	Journal of Parasitology 58(2): 306-310	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Unlikely to be found
	Real-time PCR	Journal of Clinical Microbiology 43(11): 5491-5497	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Unlikely to be found

Pathogen(s) [Disease]	Analytical Technique	Analytical Method	Solid[1] (soil, powder)	Particulate[2] (swabs, wipes, HEPA)	Liquid/Water (filter, grab)	Drinking Water (filter, grab)	Aerosol (growth media, filter, liquid)
Giardia spp. [Giardiasis]	Culture	Trans. R. Soc. Trop. Med. Hyg. 77(4): 487-488	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Requires modification of analytical method	Unlikely to be found
	IMS/FA	EPA Method 1623	Requires modification of analytical method	Requires modification of analytical method	EPA Method 1623	EPA Method 1623	Unlikely to be found
Toxoplasma gondii [Toxoplasmosis]	Animal infectivity	Emerging Infectious Diseases 12(2): 326-329	Requires modification of analytical method	Requires modification of analytical method	Emerging Infectious Diseases 12(2): 326-329	Emerging Infectious Diseases 12(2): 326-329	Unlikely to be found
	Real-time PCR	Applied & Environmental Microbiology 70(7): 4035-4039	Requires modification of analytical method	Requires modification of analytical method	Applied & Environmental Microbiology 70(7): 4035-4039	Applied & Environmental Microbiology 70(7): 4035-4039	Unlikely to be found
Helminths							
Baylisascaris procyonis [Raccoon roundworm infection]	Embryonation of eggs and microscopy	EPA/625/R-92/013	EPA/625/R-92/013	Requires modification of analytical method	EPA/625/R-92/013	Requires modification of analytical method	Unlikely to be found
General Remediation Efficacy							
Biological indicator (spore) strips	Culture	Manufacturers' Instructions					

[1] Solid samples (except those containing viruses) should be (1) prepared for culture according to EPA Method 1680, Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using Lauryl Tryptose Broth (LTB) and EC Medium, and (2) prepared for PCR according to "Quantification of Bias Related to the Extraction of DNA Directly from Soils," Frostegard, A., Courtois, S., Ramisse, V., Clerc, S., Bernillon, D., Le Gall, F., Jeannin, P., Nesme, X., and Simonet, P. 1999. Applied and Environmental Microbiology. 65(12): 5409-5420.

[2] Particulate samples (except those containing viruses) should be prepared for culture according to "Swab Materials and *Bacillus anthracis* Spore Recovery from Nonporous Surfaces," Rose, L., Jensen, B., Peterson, A., Banerjee, S.N., and Arduino, M.J. 2004. Emerging Infectious Diseases. 10(6): 1023-1029 and "Evaluation of a Macrofoam Swab Protocol for the Recovery of *Bacillus anthracis* Spores from a Steel Surface," Hodges, L.R., Rose, L.J., Peterson, A., Nobel-Wang, J., and Arduino, M.J. 2006. Applied and Environmental Microbiology. 72(6): 4429-4430.

[3] Given that adenovirus 40 and 41 can be difficult to grow in culture, additional cell lines such as G293 (Journal of Medical Virology. 1983. 11(3): 215-231) or Caco-2 (Journal of Medical Virology. 1994. 44(3): 310-315) may be considered when these viruses are suspected to be present.

[4] Samples should be prepared according to procedures found in USEPA Manual of Methods for Virology EPA/600/4-84/013, April 2001.

Appendix D: Selected Biotoxin Methods

Appendix D: Selected Biotoxin Methods

Analyte(s)	CAS RN / Description	Analysis Type	Analytical Technique	Aerosol (filter/cassette, liquid impinger)	Solid (soil, powder)	Particulate (swabs, wipes, filters)	Liquid/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 the crude preparations	Presumptive	Immunoassay[1]	Adapted from 119th AOAC Annual Meeting & Exposition, 2005, p. 613	Adapted from 119th AOAC Annual Meeting & Exposition, 2005, p. 613	Adapted from 119th AOAC Annual Meeting & Exposition, 2005, p. 613	Adapted from 119th AOAC Annual Meeting & Exposition, 2005, p. 613
		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
		Biological Activity	Enzyme activity[2]	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	Presumptive	Immunoassay[3]	Adapted from lateral flow immunassay kits	**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 8.2.1.		
		Confirmatory	Immunoassay[3] (ELISA)	Adapted from FDA Bacteriological Analytical Manual, Chapter 17			
		Biological Activity	Mouse Bioassay	Adapted from FDA Bacteriological Analytical Manual, Chapter 17			
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[1]	Adapted from lateral flow immunassay kits	**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 8.2.1.		
		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
		Confirmatory	Immunoassay	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[2]	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	Optical immunoassay	Adapted from Journal of Clinical Microbiology 45(10): 3377–3380	Adapted from Journal of Clinical Microbiology 45(10): 3377–3380	Adapted from Journal of Clinical Microbiology 45(10): 3377–3380	Adapted from Journal of Clinical Microbiology 45(10): 3377–3380
		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
		Biological Activity	Ribosome inactivation assay[2]	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

Analyte(s)	CAS RN / Description	Analysis Type	Analytical Technique	Aerosol (filter/cassette, liquid impinger)	Solid (soil, powder)	Particulate (swabs, wipes, filters)	Liquid/Drinking Water
Staphylococcal enterotoxins (SEB)	39424-53-8 (SEB) / Monomeric protein of ~ 28 kDa	Presumptive	Immunoassay	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 8.2.1.		
		Confirmatory	TBD	TBD	TBD	TBD	TBD
		Biological Activity	TBD	TBD	TBD	TBD	TBD
Staphylococcal enterotoxins (SEA, SEC)	37337-57-8 (SEA) 39424-54-9 (SEC) / Monomeric proteins of ~ 27–27.5 kDa	Presumptive	Immunoassay	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
		Biological Activity	TBD	TBD	TBD	TBD	TBD
Small Molecule							
Aflatoxin (Type B1)	27261-02-5	Presumptive	Immunoassay	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 994.08 (AOAC)	Adapted from 994.08 (AOAC)	Adapted from 994.08 (AOAC)	Adapted from 994.08 (AOAC)
α-Amanitin	23109-05-9	Presumptive	Immunoassay	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
Anatoxin-a	64285-06-9	Presumptive	TBD	TBD	TBD	TBD	TBD
		Confirmatory	HPLC-FL (precolumn derivatization)	Adapted from Biomedical Chromatography B 10: 46-47	Adapted from Biomedical Chromatography B 10: 46-47	Adapted from Biomedical Chromatography B 10: 46-47	Adapted from Biomedical Chromatography B 10: 46-47
Brevetoxins (B form)	79580-28-2	Presumptive	Immunoassay	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

Analyte(s)	CAS RN / Description	Analysis Type	Analytical Technique	Aerosol (filter/cassette, liquid impinger)	Solid (soil, powder)	Particulate (swabs, wipes, filters)	Liquid/Drinking Water
α-Conotoxin	156467-85-5	Presumptive	Immunoassay	Adapted from Biochemical Journal 328: 245-250	Adapted from Biochemical Journal 328: 245-250	Adapted from Biochemical Journal 328: 245-250	Adapted from Biochemical Journal 328: 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
Cylindrospermopsin	143545-90-8	Presumptive	Immunoassay	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
Diacetoxyscirpenol (DAS)	2270-40-8	Presumptive	Immunoassay	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
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/ 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
		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
Picrotoxin	124-87-8	Presumptive	Immunoassay	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
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	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
T-2 Mycotoxin	21259-20-1	Presumptive	Immunoassay	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

Analyte(s)	CAS RN / Description	Analysis Type	Analytical Technique	Aerosol (filter/cassette, liquid impinger)	Solid (soil, powder)	Particulate (swabs, wipes, filters)	Liquid/Drinking Water
Tetrodotoxin	9014-39-5	Presumptive	Immunoassay	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

[1] 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.

[2] 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.

[3] Immunoassays may produce variable results with uncomplexed form of toxin.

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