Appendix D: Selected Biotoxin Methods

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SAM 2017 — Appendix D: Selected Biotoxin Methods

The fitness of a method for its intended use is related to data quality objectives (DQOs) for a particular environmental remediation activity. The tiers below have been assigned to the methods selected for each biotoxin/sample type pair to indicate a level of method usability for the specific biotoxin and sample type for which it has been selected. The assigned tiers reflect the conservative view for DQOs involving timely implementation of methods for analysis of a high number of samples (such that multiple laboratories are necessary), and appropriate quality control. The sample types indicated reflect respresentative examples and are not necessarily inclusive of all sample types that might be encountered by laboratories following a contamination incident. Assigned usability tiers are indicated next to each method or method combination throughout this appendix.

Tier I: The biotoxin and sample type are both targets of the method(s). Data are available for all aspects of method performance and QC measures supporting its use without modifications.

Tier II: The biotoxin is a target of the method, and the method has been evaluated by one or more laboratories. The sample type may or may not be a target of the method, and available data and/or information regarding sample preparation indicate that analyses of similar sample types were successful. However, additional testing and/or modifications may be needed.

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

***Notes*:**
The presence of disinfectants (e.g., chlorine) and/or preservatives added during water sample collection to slow degradation (e.g., pH adjustors, de-chlorinating agents) could possibly affect analytical results. When present, the impact of these agents on method performance should be evaluated if not previously determined.

Column headings are defined in Section 8.0.

Analyte(s)	CAS RN	Analysis Type	Analytical Technique	Aerosol (filter/cassette, liquid impinger)		Solid (soil, powder)		Particulate (swabs, wipes, dust socks)		Non-Drinking Water (surface water, waste water)		Drinking Water	
Abrin	Abrin (1393-62-0) Abrine (526-31-8)	Presumptive	Immunoassay (LFA)	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	II	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	II	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2014) 12(1): 49-62	II
		Presumptive (Abrine)	LC-MS-MS	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	I
		Confirmatory	Immunoassays (ELISA and ECL)	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II	Adapted from Journal of Food Protection (2008) 71(9): 1868-1874	II
		Biological Activity	Enzyme activity	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II	Adapted from Analytical Biochemistry (2008) 378(1): 87-89	II
Aflatoxins	B1 (27261-02-5) B2 (22040-96-6) G1 (1385-95-1) G2 (7241-98-7)	Presumptive and Confirmatory (B1, B2, G1, G2)	Immunoaffinity (column) purification / LC-FL (detection)	Adapted from 991.31 (AOAC)	II	Adapted from 991.31 (AOAC)	II	Adapted from 991.31 (AOAC)	II	Adapted from 991.31 (AOAC)	II	Adapted from 991.31 (AOAC)	II
α-Amanitin	23109-05-9	Presumptive	Immunoassay (ELISA)	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II
		Confirmatory	LC-MS-MS	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	II	EPA 600/R-13/022	I
Anatoxin-a	64285-06-9	Presumptive	Immunoassay (ELISA)	See summary in Section 8.2.4.1	III	See summary in Section 8.2.4.1	III	See summary in Section 8.2.4.1	III	See summary in Section 8.2.4.1	III	See summary in Section 8.2.4.1	III
		Confirmatory	LC-MS-MS	Method 545 (EPA)	II	Method 545 (EPA)	II	Method 545 (EPA)	II	Method 545 (EPA)	II	Method 545 (EPA)	I

Analyte(s)	CAS RN	Analysis Type	Analytical Technique	Aerosol (filter/cassette, liquid impinger)		Solid (soil, powder)		Particulate (swabs, wipes, dust socks)		Non-Drinking Water (surface water, waste water)		Drinking Water	
Botulinum neurotoxins (Serotoypes A, B, C, D, E, F, and G)	Type A (93384-43-1) Type B (93384-44-2) Type C (93384-45-3) Type D (93384-46-4) Type E (93384-47-5) Type F (107231-15-2) Type G (107231-16-3)	Presumptive (Types A and B)	Immunoassay (LFA)	Adapted from EPA Environmental Technology Verification report	II	Adapted from EPA Environmental Technology Verification report	II	Adapted from EPA Environmental Technology Verification report	II	Adapted from EPA Environmental Technology Verification report	II	Adapted from EPA Environmental Technology Verification report	I
		Presumptive (Types A, B, D, E, F, and G)	Immunocapture Forster Resonance Energy Transfer (FRET)-based activity assay	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II	Adapted from Analytical Biochemistry (2011) 411(2): 200-209	II
		Presumptive (Types A-G)	Immunoassay (fluorescent bead-based)	See summary in Section 8.2.5.3	II	See summary in Section 8.2.5.3	II	See summary in Section 8.2.5.3	II	See summary in Section 8.2.5.3	II	See summary in Section 8.2.5.3	II
		Presumptive (Type A)	Immunoassay (ECL)	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II	Adapted from Journal of the Science of Food and Agriculture (2014) 94: 707-712	II
		Confirmatory (Types A-G)	LC-MS-MS (Types A, B, E and F) MALDI-TOF MS (Types A-G)	Adapted from J. Agric.Food Chem. 63(4): 1133-1141	II	Adapted from J. Agric.Food Chem. 63(4): 1133-1141	II	Adapted from J. Agric.Food Chem. 63(4): 1133-1141	II	Adapted from J. Agric.Food Chem. 63(4): 1133-1141	II	Adapted from J. Agric.Food Chem. 63(4): 1133-1141	II
		Biological Activity (Total)	Mouse Bioassay	APHA Press Compendium of Methods, Chapter 32	I	APHA Press Compendium of Methods, Chapter 32	I	APHA Press Compendium of Methods, Chapter 32	I	APHA Press Compendium of Methods, Chapter 32	I	APHA Press Compendium of Methods, Chapter 32	I
Brevetoxins	98112-41-5 (A form) 79580-28-2 (B form)	Presumptive (B forms)	Immunoassay (ELISA)	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II
		Confirmatory (A and B forms)	LC-MS	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II	Adapted from Toxicon (2015) 96: 82-88	II
Cylindrospermopsin	143545-90-8	Presumptive	Immunoassay (ELISA)	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II	Adapted from Environmental Sciences and Technology (2010) 44: 7361-7368	II
		Confirmatory	LC-MS-MS	Method 545 (EPA)	II	Method 545 (EPA)	II	Method 545 (EPA)	II	Method 545 (EPA)	II	Method 545 (EPA)	I
Diacetoxyscirpenol (DAS)	2270-40-8	Presumptive	Immunoassay (ELISA)	See summary in Section 8.2.8.1	III	See summary in Section 8.2.8.1	III	See summary in Section 8.2.8.1	III	See summary in Section 8.2.8.1	III	See summary in Section 8.2.8.1	III
		Confirmatory	LC-MS-MS	Adapted from Food Research International (2015) 72: 247-255	II	Adapted from Food Research International (2015) 72: 247-255	II	Adapted from Food Research International (2015) 72: 247-255	II	Adapted from Food Research International (2015) 72: 247-255	II	Adapted from Food Research International (2015) 72: 247-255	II

Analyte(s)	CAS RN	Analysis Type	Analytical Technique	Aerosol (filter/cassette, liquid impinger)		Solid (soil, powder)		Particulate (swabs, wipes, dust socks)		Non-Drinking Water (surface water, waste water)		Drinking Water	
Domoic acid (DA)	14277-97-5	Presumptive	Immunoassay (ELISA)	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II	Adapted from Journal of AOAC International (2007) 90(4): 1011-1027	II
		Presumptive	Immunoassay (ELISA)	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II	Adapted from Journal of Shellfish Research (2008) 27(5): 1301-1310	II
		Confirmatory	LC-UV	Adapted from Journal of the Mexican Chemical Society (2011) 55(2): 65-71	II	Adapted from Journal of the Mexican Chemical Society (2011) 55(2): 65-71	II	Adapted from Journal of the Mexican Chemical Society (2011) 55(2): 65-71	II	Adapted from Journal of the Mexican Chemical Society (2011) 55(2): 65-71	II	Adapted from Journal of the Mexican Chemical Society (2011) 55(2): 65-71	II
Microcystins	96180-79-9 (LA) 154037-70-4 (LF) 101043-37-2 (LR) 123304-10-9 (LY) 111755-37-4 (RR) 101064-48-6 (YR)	Presumptive (Total Adda-containing microcystins)	Immunoassay (ELISA)	Method 546 (EPA)	II	Method 546 (EPA)	II	Method 546 (EPA)	II	Method 546 (EPA)	I	Method 546 (EPA)	I
		Confirmatory (LA, LF, LR, LY, RR, YR)	LC-MS-MS	Method 544 (EPA)	III	Method 544 (EPA)	III	Method 544 (EPA)	III	Method 544 (EPA)	II	Method 544 (EPA)	I
Picrotoxin	124-87-8	Presumptive	TBD	TBD		TBD		TBD		TBD		TBD	
		Confirmatory	LC-UV	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II	Adapted from Journal of Pharmaceutical and Biomedical Analysis (1989) 7(3): 369-375	II
Ricin	Ricin (9009-86-3) Ricinine (5254-40-3)	Presumptive	Immunoassay (LFA)	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I	Adapted from Biosecurity and Bioterrorism: Biodefense Strategy, Practice, and Science (2013) 11(4): 237-250	I
		Presumptive	Immunoassay (ELISA)	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II
		Presumptive	Immunoassay (ECL)	Adapted from Journal of AOAC International (2008) 91(2): 376-382	II	Adapted from Journal of AOAC International (2008) 91(2): 376-382	II	Adapted from Journal of AOAC International (2008) 91(2): 376-382	II	Adapted from Journal of AOAC International (2008) 91(2): 376-382	II	Adapted from Journal of AOAC International (2008) 91(2): 376-382	II
		Presumptive (Ricinine)	LC-MS-MS	EPA 600/R-13/022 (EPA/CDC)	II	EPA 600/R-13/022 (EPA/CDC)	II	EPA 600/R-13/022 (EPA/CDC)	II	EPA 600/R-13/022 (EPA/CDC)	II	EPA 600/R-13/022 (EPA/CDC)	I
		Confirmatory	Immunocapture / LC-MS-MS	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from-Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	I
		Biological Activity	Immuncapture / MALDI-TOF MS	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	II	Adapted from Analytical Chemistry (2011) 83: 2897-2905	I

Analyte(s)	CAS RN	Analysis Type	Analytical Technique	Aerosol (filter/cassette, liquid impinger)		Solid (soil, powder)		Particulate (swabs, wipes, dust socks)		Non-Drinking Water (surface water, waste water)		Drinking Water	
Saxitoxins	35523-89-8 (STX) 64296-20-4 (NEO) 58911-04-9 (dcSTX) 68683-58-9 (dcNEOSTX) 143084-69-9 (doSTX) 77462-64-7 (GTX 1 - 6) 122075-86-9 (dcGTX 1 - 4)	Presumptive (Total)	Receptor Binding Assay	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II
		Presumptive (Total)	Immunoassay (ELISA)	Adapted from Toxicon (2009) 54: 313-320	II	Adapted from Toxicon (2009) 54: 313-320	II	Adapted from Toxicon (2009) 54: 313-320	II	Adapted from Harmful Algae (2016) 56: 77-90	I	Adapted from Harmful Algae (2016) 56: 77-90	I
		Confirmatory (STXs and GTXs)	LC-MS-MS	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II	Adapted from Journal of Chromatography A (2015) 1387: 1-12	II
Shiga and Shiga-like Toxins	Stx (75757-64-1)	Presumptive (Stx, Stx-1 and Stx-2)	Immunoassay (ELISA)	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	II	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	II	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	II	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	II	Adapted from Austin Immunology (2016) 1(2): 1007:1-7	II
		Confirmatory (Stx, Stx-1 and Stx-2)	LC-MS-MS	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II	Adapted from Analytical Chemistry (2014) 86: 4698-4706	II
Staphylococcal enterotoxins	37337-57-8 (SEA) 39424-53-8 (SEB) 39424-54-9 (SEC) 12788-99-7 (SED) 39424-55-0 (SEE)	Presumptive (SEA - SEE)	Enzyme Immunoassay (ELFA)	2007.06 (AOAC)	II	2007.06 (AOAC)	II	2007.06 (AOAC)	II	2007.06 (AOAC)	II	2007.06 (AOAC)	II
		Presumptive (SEB)	Immunoassay (ECL)	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III	Adapted from Journal of AOAC International (2014) 97(3): 862-867	III
		Confirmatory (SEA - SEE)	Immunoassay (ELISA)	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II	Adapted from Letters in Applied Microbiology (2011) 52: 468-474	II
T-2 Mycotoxin	21259-20-1 (T-2) 26934-87-2 (HT-2)	Presumptive (T-2)	Immunoassay (ELISA)	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II	Adapted from Journal of Food Protection (2005) 68(6): 1294-1301	II
		Confirmatory (T-2 and HT-2)	LC-MS	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II	Adapted from Rapid Communications in Mass Spectrometry (2006) 20(9): 1422-1428	II
Tetrodotoxin	9014-39-5	Presumptive	Receptor Binding Assay	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II	Method 2011.27 (AOAC)	II
		Confirmatory	LC-MS-MS	Adapted from Toxicon (2016) 119: 64-71	II	Adapted from Toxicon (2016) 119: 64-71	II	Adapted from Toxicon (2016) 119: 64-71	II	Adapted from Toxicon (2016) 119: 64-71	II	Adapted from Toxicon (2016) 119: 64-71	II