Appendix C: Selected Pathogen Methods

SAM 2022 — 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 (QC) samples (Section 7.1.2). Also, if required, a modification or an appropriate replacement method may be warranted for a specific pathogen/sample type/environmental matrix or a combination thereof. Additionally, the SAM Pathogen primary and alternate points of contact should be consulted for additional guidance (Section 4.0, Points of Contact).

The fitness of a method for an intended use is related to site-specific data quality objectives (DQOs) for a particular environmental remediation activity. These selected pathogen methods have been assigned tiers (below) to indicate a level of method usability for the specific analyte and sample type. The assigned tiers pertain only to technical aspects of method usability, and do not pertain to aspects such as cost, equipment availability, and sample throughput. Assigned usability tiers are indicated next to each method or method combination throughout this appendix.

Tier I: The method was developed for the pathogen and sample type. The method has been evaluated by multiple laboratories, a detailed protocol has been developed, and suitable QC measures and checks are provided. (Examples: EPA Method 1623.1 [*Cryptosporidium* in water]; Standard Methods 9260 E [*Shigella* culture method].)

Tier II: The pathogen is the target of the method, and the method has been evaluated by one or more laboratories. The available data and/or information indicate that additional testing and/or modifications will likely be needed. (Example: Cunningham et al. 2010. [*Shigella* molecular method].)

Tier III: The pathogen is not the target of the method but the method is for the specific sample type and the pathogen is similar to the target of the method (i.e. vegetative bacteria, spore-forming bacteria, virus or protozoan). Data and expert opinion suggest, however, that the method(s) may be applicable with modifications. (Example: EPA *Yersinia pestis* protocol for *Chlamydophila psittaci* in water.)

***Notes*:**
Samples should not be stored indefinitely, and should be processed and analyzed as soon as possible upon receipt.

If viability determinations are needed (e.g., for post decontamination phase samples), a viability-based procedure (such as culture) should be used. Rapid analysis techniques (such as PCR, immunoassays) without culture are preferred for determination of the extent and magnitude of contamination (e.g., for site characterization phase samples). Please see Figure 7-1.

Column headings are defined in Section 7.0.

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Bacteria[2]										
Bacillus anthracis [Anthrax]	NA	Sample Processing	EPA *Bacillus anthracis* (BA) Protocol (EPA/600/R-17/213)	I	EPA BA Protocol (EPA/600/R-17/213)	I	Silvestri et al. 2016. J. of Microbiol. Methods. 130: 6-13	II	EPA BA Protocol (EPA/600/R-17/213)	I
	Culture	Analytical Technique	EPA BA Protocol (EPA/600/R-17/213)	I	EPA BA Protocol (EPA/600/R-17/213)	I	EPA BA Protocol (EPA/600/R-17/213)	I	EPA BA Protocol (EPA/600/R-17/213)	I
	Real-time PCR/ RV-PCR									
Brucella spp. (*B. abortus, B. melitensis, B. suis*) [Brucellosis]	NA	Sample Processing	EPA *Yersinia pestis* (YP) Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	III	EPA YP Protocol (EPA/600/R-16/109)	III
	Culture	Analytical Technique	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Brucella* species	I
	Real-time PCR	Analytical Technique	Hinić et al. 2008. J. Microbiol. Methods. 75(2): 375-378	II	Hinić et al. 2008. J. Microbiol. Methods. 75(2): 375-378	II	Hinić et al. 2008. J. Microbiol. Methods. 75(2): 375-378	II	Hinić et al. 2008. J. Microbiol. Methods. 75(2): 375-378	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Burkholderia mallei [Glanders] and *Burkholderia pseudomallei* [Melioidosis]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Hall et al. 2019. PLoS Negl. Trop. Dis. 13(9):e0007727	II	EPA YP Protocol (EPA/600/R-16/109)	III
	Culture	Analytical Technique	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*	I	ASM Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases: *Burkholderia mallei* and *B. pseudomallei*	I
	Real-time PCR	Analytical Technique	Tomaso et al. 2006. Clin. Chem. 52(2): 307-310 and Novak et al. 2006. J. Clin. Microbiol. 44(1): 85-90	II	Tomaso et al. 2006. Clin. Chem. 52(2): 307-310 and Novak et al. 2006. J. Clin. Microbiol. 44(1): 85-90	II	Tomaso et al. 2006. Clin. Chem. 52(2): 307-310 and Novak et al. 2006. J. Clin. Microbiol. 44(1): 85-90	II	Tomaso et al. 2006. Clin. Chem. 52(2): 307-310 and Novak et al. 2006. J. Clin. Microbiol. 44(1): 85-90	II
Campylobacter jejuni [Campylobacteriosis]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Hiett. 2017. Methods Mol. Biol. 1512:1-8	II	Hiett. 2017. Methods Mol. Biol. 1512:1-8	II
	Culture	Analytical Technique	ISO 17995	I	ISO 17995	I	ISO 17995	I	ISO 17995	I
	Real-time PCR	Analytical Technique	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II
Chlamydophila psittaci (formerly known as *Chlamydia psittaci*) [Psittacosis]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	III	EPA YP Protocol (EPA/600/R-16/109)	III
	Tissue culture PCR	Analytical Technique	Madico et al. 2000. J. Clin. Microbiol. 38(3): 1085-1093	II	Madico et al. 2000. J. Clin. Microbiol. 38(3): 1085-1093	II	Madico et al. 2000. J. Clin. Microbiol. 38(3): 1085-1093	II	Madico et al. 2000. J. Clin. Microbiol. 38(3): 1085-1093	II
Coxiella burnetii [Q-fever]	NA	Sample Processing	EPA BA Protocol (EPA/600/R-17/213)	III	Hodges et al. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose et al. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-17/213)	III	EPA Method 1682 (EPA-821-R-06-14)	III	EPA and CDC Joint Collection Protocol (Ultrafiltration [UF]) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	Raoult et al. 1991. Antimicrob. Agents Chemother. 35(10): 2070-2077	II	Raoult et al. 1991. Antimicrob. Agents Chemother. 35(10): 2070-2077	II	Raoult et al. 1991. Antimicrob. Agents Chemother. 35(10): 2070-2077	II	Raoult et al. 1991. Antimicrob. Agents Chemother. 35(10): 2070-2077	II
	Real-time PCR	Analytical Technique	Panning et al. 2008. BMC Microbiol. 8:77	II	Panning et al. 2008. BMC Microbiol. 8:77	II	Panning et al. 2008. BMC Microbiol. 8:77	II	Panning et al. 2008. BMC Microbiol. 8:77	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Escherichia coli O157:H7	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1680 (EPA-821-R-14-009)	I	EPA *Eschericia coli* O157:H7 (EC) Protocol (EPA/600/R-10/056)	I
	Culture	Analytical Technique	EPA EC Protocol (EPA/600/R-10/056)	I	EPA EC Protocol (EPA/600/R-10/056)	I	EPA EC Protocol (EPA/600/R-10/056)	I	EPA EC Protocol (EPA/600/R-10/056)	I
	Real-time PCR	Analytical Technique	Sen et al. 2011. Environ. Sci. Technol. 45(7): 2250-2256	II	Sen et al. 2011. Environ. Sci. Technol. 45(7): 2250-2256	II	Sen et al. 2011. Environ. Sci. Technol. 45(7): 2250-2256	II	Sen et al. 2011. Environ. Sci. Technol. 45(7): 2250-2256	II
Francisella tularensis [Tularemia]	NA	Sample Processing	EPA *Francisella tularensis* (FT) Protocol (EPA/600/R-19/110)	I	EPA FT Protocol (EPA/600/R-19/110)	I	EPA Method 1682 (EPA-821-R-06-14)	III	EPA FT Protocol (EPA/600/R-19/110)	I
	Culture	Analytical Technique	EPA FT Protocol (EPA/600/R-19/110)	I	EPA FT Protocol (EPA/600/R-19/110)	I	EPA FT Protocol (EPA/600/R-19/110)	I	EPA FT Protocol (EPA/600/R-19/110)	I
	Real-time PCR/ RV-PCR	Analytical Technique	EPA FT Protocol (EPA/600/R-19/110)	I	EPA FT Protocol (EPA/600/R-19/110)	I	EPA FT Protocol (EPA/600/R-19/110)	I	EPA FT Protocol (EPA/600/R-19/110)	I
Legionella pneumophila [Legionellosis]	NA	Sample Processing	US DHHS. 2005. Procedures for the Recovery of *Legionella* from the Environment	I	Kozak et al. 2013. Identification of Legionella in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak et al. 2013. Identification of Legionella in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak et al. 2013. Identification of Legionella in the Environment. Methods Mol. Biol. 954: 3-25	I
	Culture	Analytical Technique	Kozak et al. 2013. Identification of Legionella in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak et al. 2013. Identification of Legionella in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak et al. 2013. Identification of Legionella in the Environment. Methods Mol. Biol. 954: 3-25	I	Kozak et al. 2013. Identification of Legionella in the Environment. Methods Mol. Biol. 954: 3-25	I
	Real-time PCR	Analytical Technique	ISO Method ISO/TS 12869:2019	I	ISO Method ISO/TS 12869:2019	I	ISO Method ISO/TS 12869:2019	I	ISO Method ISO/TS 12869:2019	I
Leptospira interrogans [Leptospirosis]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	III	Standard Method 9260 I: *Leptospira*	I
	Culture	Analytical Technique	Standard Method 9260 I: *Leptospira*	I	Standard Method 9260 I: *Leptospira*	I	Standard Method 9260 I: *Leptospira*	I	Standard Method 9260 I: *Leptospira*	I
	Real-time PCR	Analytical Technique	Palaniappan et al. 2005. Mol. Cell Probes. 19(2): 111-117	II	Palaniappan et al. 2005. Mol. Cell Probes. 19(2): 111-117	II	Palaniappan et al. 2005. Mol. Cell Probes. 19(2): 111-117	II	Palaniappan et al. 2005. Mol. Cell Probes. 19(2): 111-117	II
Listeria monocytogenes [Listeriosis]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	Iwu and Okoh. 2020. PLoS ONE. 15(2): e0228956.	II	Iwu and Okoh. 2020. PLoS ONE. 15(2): e0228956.	II
	Culture	Analytical Technique	Hitchins et al. 2017. Bacteriological Analytical Manual Online	I	Hitchins et al. 2017. Bacteriological Analytical Manual Online	I	Hitchins et al. 2017. Bacteriological Analytical Manual Online	I	Hitchins et al. 2017. Bacteriological Analytical Manual Online	I
	Real-time PCR	Analytical Technique	USDA, FSIS. 2021. Microbiology Laboratory Guidebook MLG 8.13	I	USDA, FSIS. 2021. Microbiology Laboratory Guidebook MLG 8.13	I	USDA, FSIS. 2021. Microbiology Laboratory Guidebook MLG 8.13	I	USDA, FSIS. 2021. Microbiology Laboratory Guidebook MLG 8.13	I

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Non-typhoidal *Salmonella* (Not applicable to *S.* Typhi) [Salmonellosis]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	I	EPA Method 1200 (EPA 817-R-12-004)	I
	Culture	Analytical Technique	EPA Method 1682 (EPA-821-R-06-14) or EPA Method 1200 (EPA 817-R-12-004)	I	EPA Method 1682 (EPA-821-R-06-14) or EPA Method 1200 (EPA 817-R-12-004)	I	EPA Method 1682 (EPA-821-R-06-14) or EPA Method 1200 (EPA 817-R-12-004)	I	EPA Method 1682 (EPA-821-R-06-14) or EPA Method 1200 (EPA 817-R-12-004)	I
	Real-time PCR	Analytical Technique	Jyoti et al. 2011. Environ. Sci. Technol. 45(20): 8996-9002	II	Jyoti et al. 2011. Environ. Sci. Technol. 45(20): 8996-9002	II	Jyoti et al. 2011. Environ. Sci. Technol. 45(20): 8996-9002	II	Jyoti et al. 2011. Environ. Sci. Technol. 45(20): 8996-9002	II
Salmonella Typhi [Typhoid fever]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	I	EPA *Salmonella* Typhi (ST) Protocol (EPA 600/R-10/133)	I
	Culture	Analytical Technique	EPA ST Protocol (EPA 600/R-10/133)	I	EPA ST Protocol (EPA 600/R-10/133)	I	EPA ST Protocol (EPA 600/R-10/133)	I	EPA ST Protocol (EPA 600/R-10/133)	I
	Real-time PCR	Analytical Technique	CDC Laboratory Assay	I	CDC Laboratory Assay	I	CDC Laboratory Assay	I	CDC Laboratory Assay	I
Shigella spp. [Shigellosis]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	III	Standard Method 9260 E: *Shigella*	I
	Culture	Analytical Technique	Standard Method 9260 E: *Shigella*	I	Standard Method 9260 E: *Shigella*	I	Standard Method 9260 E: *Shigella*	I	Standard Method 9260 E: *Shigella*	I
	Real-time PCR	Analytical Technique	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II	Cunningham et al. 2010. J. Clin. Microbiol. 48(8): 2929-2933	II
Staphylococcus aureus	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	III	Li et al. 2015. Environ. Sci. Technol. 49: 14249-14256	II
	Culture	Analytical Technique	Standard Method 9213 B: *Staphylococcus aureus*	I	Standard Method 9213 B: *Staphylococcus aureus*	I	Standard Method 9213 B: *Staphylococcus aureus*	I	Standard Method 9213 B: *Staphylococcus aureus*	I
	Real-time PCR	Analytical Technique	Chiang et al. 2007. J. Food Prot. 70(12): 2855-2859	II	Chiang et al. 2007. J. Food Prot. 70(12): 2855-2859	II	Chiang et al. 2007. J. Food Prot. 70(12): 2855-2859	II	Chiang et al. 2007. J. Food Prot. 70(12): 2855-2859	II
Vibrio cholerae [Cholera]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	III	EPA YP Protocol (EPA/600/R-16/109)	III	EPA Method 1682 (EPA-821-R-06-14)	III	EPA *Vibrio cholerae* (VC) Protocol (EPA 600/R-10/139)	I
	Culture	Analytical Technique	EPA VC Protocol (EPA 600/R-10/139)	I	EPA VC Protocol (EPA 600/R-10/139)	I	EPA VC Protocol (EPA 600/R-10/139)	I	EPA VC Protocol (EPA 600/R-10/139)	I
	Real-time PCR	Analytical Technique	Blackstone et al. 2007. J. Microbiol. Methods. 68(2): 254-259	II	Blackstone et al. 2007. J. Microbiol. Methods. 68(2): 254-259	II	Blackstone et al. 2007. J. Microbiol. Methods. 68(2): 254-259	II	Blackstone et al. 2007. J. Microbiol. Methods. 68(2): 254-259	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Yersinia pestis [Plague]	NA	Sample Processing	EPA YP Protocol (EPA/600/R-16/109)	I	EPA YP Protocol (EPA/600/R-16/109)	I	EPA Method 1682 (EPA-821-R-06-14)	III	EPA YP Protocol (EPA/600/R-16/109)	I
	Culture Real-time PCR/ RV-PCR	Analytical Technique	EPA YP Protocol (EPA/600/R-16/109)	I	EPA YP Protocol (EPA/600/R-16/109)	I	EPA YP Protocol (EPA/600/R-16/109)	I	EPA YP Protocol (EPA/600/R-16/109)	I
Viruses[3]										
Adenoviruses: Enteric and non-enteric (A-F)	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977.	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67.	II	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	Boczek et al. 2016. J. Microbiol. Methods. 122: 43-49 or Green and Loewenstein. 2005. Curr. Protoc. Microbiol. 14C.1.1-14C.1.19	II	Boczek et al. 2016. J. Microbiol. Methods. 122: 43-49 or Green and Loewenstein. 2005. Curr. Protoc. Microbiol. 14C.1.1-14C.1.19	II	Boczek et al. 2016. J. Microbiol. Methods. 122: 43-49 or Green and Loewenstein. 2005. Curr. Protoc. Microbiol. 14C.1.1-14C.1.19	II	Boczek et al. 2016. J. Microbiol. Methods. 122: 43-49 or Green and Loewenstein. 2005. Curr. Protoc. Microbiol. 14C.1.1-14C.1.19	II
	Real-time PCR	Analytical Technique	Jothikumar et al. 2005. Appl. Environ. Microbiol. 71(6): 3131-3136	II	Jothikumar et al. 2005. Appl. Environ. Microbiol. 71(6): 3131-3136	II	Jothikumar et al. 2005. Appl. Environ. Microbiol. 71(6): 3131-3136	II	Jothikumar et al. 2005. Appl. Environ. Microbiol. 71(6): 3131-3136	II
Astroviruses	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Integrated Cell Culture Real-time reverse transcription-PCR	Analytical Technique	Grimm et al. 2004. Can. J. Microbiol. 50(4): 269-278	II	Grimm et al. 2004. Can. J. Microbiol. 50(4): 269-278	II	Grimm et al. 2004. Can. J. Microbiol. 50(4): 269-278	II	Grimm et al. 2004. Can. J. Microbiol. 50(4): 269-278	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Caliciviruses: Noroviruses	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Real-time reverse transcription-PCR	Analytical Technique	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I
Caliciviruses: Sapovirus	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	Parwani et al. 1991. Arch. Virol. 120(1-2): 115-122	II	Parwani et al. 1991. Arch. Virol. 120(1-2): 115-122	II	Parwani et al. 1991. Arch. Virol. 120(1-2): 115-122	II	Parwani et al. 1991. Arch. Virol. 120(1-2): 115-122	II
	Real-time reverse transcription-PCR	Analytical Technique	Oka et al. 2006. J. Med. Virol. 78(10): 1347-1353	II	Oka et al. 2006. J. Med. Virol. 78(10): 1347-1353	II	Oka et al. 2006. J. Med. Virol. 78(10): 1347-1353	II	Oka et al. 2006. J. Med. Virol. 78(10): 1347-1353	II
Coronaviruses: SARS-associated human coronavirus (SARS-CoV-2, SARS-CoV, and MERS-CoV)	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Shah et al. 2021. J. Virol. Methods. 297. 114251	II	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	Pagat et al. 2007. Applied Biosafety 12(2): 100-108	II	Pagat et al. 2007. Applied Biosafety 12(2): 100-108	II	Pagat et al. 2007. Applied Biosafety 12(2): 100-108	II	Pagat et al. 2007. Applied Biosafety 12(2): 100-108	II
	Real-time reverse transcription-PCR	Analytical Technique	McMinn et al. 2021 Sci. Total Environ. 774: 145727	II	McMinn et al. 2021 Sci. Total Environ. 774: 145727	II	McMinn et al. 2021 Sci. Total Environ. 774: 145727	II	McMinn et al. 2021 Sci. Total Environ. 774: 145727	II
	Rapid viability-reverse transcription-PCR	Analytical Technique	Shah et al. 2021. J. Virol. Methods. 297. 114251	II	Shah et al. 2021. J. Virol. Methods. 297. 114251	II	Shah et al. 2021. J. Virol. Methods. 297. 114251	II	Shah et al. 2021. J. Virol. Methods. 297. 114251	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Hepatitis E virus (HEV)	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	Zaki et al. 2009. Pathog. Dis. 56: 73-79	II	Zaki et al. 2009. Pathog. Dis. 56: 73-79	II	Zaki et al. 2009. Pathog. Dis. 56: 73-79	II	Zaki et al. 2009. Pathog. Dis. 56: 73-79	II
	Real-time reverse transcription-PCR	Analytical Technique	Jothikumar et al. 2006. J. Virol. Methods. 131(1): 65-71	II	Jothikumar et al. 2006. J. Virol. Methods. 131(1): 65-71	II	Jothikumar et al. 2006. J. Virol. Methods. 131(1): 65-71	II	Jothikumar et al. 2006. J. Virol. Methods. 131(1): 65-71	II
Influenza H5N1 virus	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	II	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67.	III	EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	Krauss et al. 2012. Influeza Virus Isolation. Methods Mol. Biol. 865: 11-24	II	Krauss et al. 2012. Influeza Virus Isolation. Methods Mol. Biol. 865: 11-24	II	Krauss et al. 2012. Influeza Virus Isolation. Methods Mol. Biol. 865: 11-24	II	Krauss et al. 2012. Influeza Virus Isolation. Methods Mol. Biol. 865: 11-24	II
	Real-time reverse transcription-PCR	Analytical Technique	Ng et al. 2005. Emerg. Infect. Dis. 11(8): 1303-1305	II	Ng et al. 2005. Emerg. Infect. Dis. 11(8): 1303-1305	II	Ng et al. 2005. Emerg. Infect. Dis. 11(8): 1303-1305	II	Ng et al. 2005. Emerg. Infect. Dis. 11(8): 1303-1305	II
Picornaviruses: Enteroviruses	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I	EPA Method 1615 (EPA/600/R-10/181)	I
	Reverse transcription-PCR									

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Picornaviruses: Hepatitis A virus (HAV)	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Integrated Cell Culture	Analytical Technique	Hyeon et al. 2011. J. Food Prot. 74(10):1756-1761	II	Hyeon et al. 2011. J. Food Prot. 74(10):1756-1761	II	Hyeon et al. 2011. J. Food Prot. 74(10):1756-1761	II	Hyeon et al. 2011. J. Food Prot. 74(10):1756-1761	II
	Real-time Reverse Transcription-PCR									
Reoviruses: Rotavirus (Group A)	NA	Sample Processing	Raynor et al. 2021. PLoS ONE. 16(1): e0244977	III	Park et al. 2015. Appl. Environ. Microbiol. 81(17): 5987-5992	III	Staggemeier et al. 2015. J Virol. Methods. 213: 65-67	III	EPA Method 1642 (EPA 820-R-18-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Tissue Culture	Analytical Technique	EPA Method 1615 (EPA/600/R-10/181)	III	EPA Method 1615 (EPA/600/R-10/181)	III	EPA Method 1615 (EPA/600/R-10/181)	III	EPA Method 1615 (EPA/600/R-10/181)	III
	Real-time reverse transcription-PCR	Analytical Technique	Jothikumar et al. 2009. J. Virol. Methods. 155(2): 126-131	II	Jothikumar et al. 2009. J. Virol. Methods. 155(2): 126-131	II	Jothikumar et al. 2009. J. Virol. Methods. 155(2): 126-131	II	Jothikumar et al. 2009. J. Virol. Methods. 155(2): 126-131	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Protozoa										
Cryptosporidium spp. [Cryptosporidiosis]	NA	Sample Processing	EPA BA Protocol (EPA/600/R-17/213)	III	Hodges et al. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose et al. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-17/213)	III	Zopp et al. 2016. Agric. Environ. Lett. 1:160031	II	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	I/I/III
	Cell Culture Immunofluorescence Procedure	Analytical Technique	Bukhari et al. 2007. Can. J. Microbiol. 53(5): 656-663	II	Bukhari et al. 2007. Can. J. Microbiol. 53(5): 656-663	II	Bukhari et al. 2007. Can. J. Microbiol. 53(5): 656-663	II	Bukhari et al. 2007. Can. J. Microbiol. 53(5): 656-663	II
	IMS/FA	Analytical Technique	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1622 (EPA 815-R-05-001) or EPA Method 1623.1 (EPA 816-R-12-001)	I
	Real-time PCR	Analytical Technique	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185 and Jiang et al. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141	II	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185 and Jiang et al. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141	II	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185 and Jiang et al. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141	II	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185 and Jiang et al. 2005. Appl. Environ. Microbiol. 71(3): 1135-1141	II
Entamoeba histolytica	NA	Sample Processing	EPA BA Protocol (EPA/600/R-17/213)	III	Hodges et al. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose et al. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-17/213)	III	Ogbolu et al. 2011. Afr. J. Med. med. Sci. 40: 85-87	II	EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	III
	Cell Culture	Analytical Technique	Stringert. 1972. J Parasitol. 58(2): 306-310	II	Stringert. 1972. J Parasitol. 58(2): 306-310	II	Stringert. 1972. J Parasitol. 58(2): 306-310	II	Stringert. 1972. J Parasitol. 58(2): 306-310	II
	Real-time PCR	Analytical Technique	Mejia et al. 2013. Am. J. Trop. Med. Hyg. 88(6): 1041-1047	II	Mejia et al. 2013. Am. J. Trop. Med. Hyg. 88(6): 1041-1047	II	Mejia et al. 2013. Am. J. Trop. Med. Hyg. 88(6): 1041-1047	II	Mejia et al. 2013. Am. J. Trop. Med. Hyg. 88(6): 1041-1047	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Giardia spp. [Giardiasis]	NA	Sample Processing	EPA BA Protocol (EPA/600/R-17/213)	III	Hodges et al. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose et al. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-17/213)	III	Liang and Keeley. 2011. Appl. Environ. Microbiol. 77(18): 6476-6485	III	EPA Method 1623.1 (EPA 816-R-12-001) or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	I/III
	Cell Culture	Analytical Technique	Keister. 1983. T. Roy. Soc. Trop. Med. H. 77(4): 487-488	II	Keister. 1983. T. Roy. Soc. Trop. Med. H. 77(4): 487-488	II	Keister. 1983. T. Roy. Soc. Trop. Med. H. 77(4): 487-488	II	Keister. 1983. T. Roy. Soc. Trop. Med. H. 77(4): 487-488	II
	IMS/FA	Analytical Technique	EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1623.1 (EPA 816-R-12-001)	I	EPA Method 1623.1 (EPA 816-R-12-001)	I
	Real-time PCR	Analytical Technique	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185	II	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185	II	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185	II	Guy et al. 2003. Appl. Environ. Microbiol. 69(9): 5178-5185	II
Naegleria fowleri [Naegleriasis]	NA	Sample Processing	Not of concern[4]		Hodges et al. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose et al. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-17/213)	III	Mull et al. 2013. J. Parasitol. Res. 2013: 1-8	II	Cope et al. 2015. Clin. Infect. Dis. 60(8): e36-42 or EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001)	II/III
	Cell Culture	Analytical Technique	Not of concern[4]		Standard Method 9750: *Naegleria fowleri*	I	Standard Method 9750: *Naegleria fowleri*	I	Standard Method 9750: *Naegleria fowleri*	I
	Real-time PCR	Analytical Technique	Not of concern[4]		Mull et al. 2013. J. Parasitol. Res. 2013: 1-8	II	Mull et al. 2013. J. Parasitol. Res. 2013: 1-8	II	Mull et al. 2013. J. Parasitol. Res. 2013: 1-8	II
Toxoplasma gondii [Toxoplasmosis]	NA	Sample Processing	Lass et al. 2020. Parasitol. 1-11	II	Hodges et al. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose et al. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-17/213)	III	Escotte-Binet et al. 2019. Vet. Parasitol. 274: 108904	II	Villegas et al. 2010. J. Microbiol. Methods. 81(3): 219-225 or EPA Method 1623.1 (EPA 816-R-12-001)	II/III
	Cell Culture	Analytical Technique	Villegas et al. 2010. J. Microbiol. Methods. 81(3): 219-225	II	Villegas et al. 2010. J. Microbiol. Methods. 81(3): 219-225	II	Villegas et al. 2010. J. Microbiol. Methods. 81(3): 219-225	II	Villegas et al. 2010. J. Microbiol. Methods. 81(3): 219-225	II
	Real-time PCR	Analytical Technique	Yang et al. 2009. Appl. Environ. Microbiology. 75(11): 3477-3483	II	Yang et al. 2009. Appl. Environ. Microbiology. 75(11): 3477-3483	II	Yang et al. 2009. Appl. Environ. Microbiology. 75(11): 3477-3483	II	Yang et al. 2009. Appl. Environ. Microbiology. 75(11): 3477-3483	II

Pathogen(s) [Disease]	Analytical Technique	Method Type	Analytical Method							
			Air (air filters, impingers, impactor media and collection fluid)		Surfaces (swabs, wipes, Sponge-Sticks and filter cassettes)		Soil		Water (surface water, drinking water, wastewater and post decontamination wastewater)[1]	
Helminths										
Baylisascaris procyonis [Raccoon roundworm infection]	NA	Sample Processing	EPA BA Protocol (EPA/600/R-17/213)	III	Hodges et al. 2010. J. Microbiol. Methods. 81(2): 141-146 or Rose et al. 2011. Appl. Environ. Microbiol. 77(23): 8355-8359 or EPA BA Protocol (EPA/600/R-17/213)	III	Kazacos. 1983. AM. J. Vet. Res. Vol 44. No. 5: 896-900	II	EPA and CDC Joint Collection Protocol (UF) (EPA 600/R-21/280) and EPA Method 1642 (Filter Processing) (EPA 820-R-18-001) or Gatcombe et al. 2010. Parasitol. Res. 106: 499-504	III/II
	Real-time PCR	Analytical Technique	Gatcombe et al. 2010. Parasitol. Res. 106: 499-504	II	Gatcombe et al. 2010. Parasitol. Res. 106: 499-504	II	Gatcombe et al. 2010. Parasitol. Res. 106: 499-504	II	Gatcombe et al. 2010. Parasitol. Res. 106: 499-504	II
	Embryonation of Eggs and Microscopy	Analytical Technique	Control of Pathogens and Vector Attraction in Sewage Sludge (EPA/625/R-92/013)	II	Control of Pathogens and Vector Attraction in Sewage Sludge (EPA/625/R-92/013)	II	Control of Pathogens and Vector Attraction in Sewage Sludge (EPA/625/R-92/013)	II	Control of Pathogens and Vector Attraction in Sewage Sludge (EPA/625/R-92/013)	II

Footnotes

[1] A neutralizing agent (e.g., sodium thiosulfate) should be added to water samples that may have disinfectant residuals prior to sample processing and analysis. Additional sample processing may be required for wastewater samples to remove solids (see CDC's webpage for additional information on processing wastewater samples for viruses: https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/testing-methods.html).

[2] If the water sample processing method for bacterial analyses does not address large volume water samples, please refer to the EPA YP Protocol (EPA/600/R-16/109) for ultrafiltration of large volume water samples.

[3] Water samples should be processed according to Method 1642 for small volume water samples (e.g., 2 L) or the EPA and CDC Joint Collection Protocol (UF) and Method 1642 (filter processing) for volumes ≥ 10 L.

[4] *Naegleria fowleri* has not been shown to spread via water vapor or aerosol droplets (see CDC's webpage on *Naegleria fowleri* at https://www.cdc.gov/parasites/naegleria/infection-sources.html).