AOAC SMPR® 2016.008

Standard Method Performance Requirements (SMPRs®) for DNA-Based Methods of Detecting *Yersinia pestis* in Field-Deployable, Department of Defense Aerosol Collection Devices

Intended Use: Field-Deployed Use for Analysis of Aerosol Collection Filters and/or Liquids

1 Applicability

Detection of *Yersinia pestis* in collection buffers from aerosol collection devices. Field-deployable assays are preferred.

2 Analytical Technique

Molecular detection of nucleic acid.

3 Definitions

Acceptable minimum detection level (AMDL).—The predetermined minimum level of an analyte, as specified by an expert committee which must be detected by the candidate method at a specified probability of detection (POD).

Environmental factors.—For the purposes of this SMPR: Any factor in the operating environment of an analytical method, whether abiotic or biotic, that might influence the results of the method.

Exclusivity.—Study involving pure nontarget strains, which are potentially cross-reactive, that shall not be detected or enumerated by the candidate method.

Inclusivity.—Study involving pure target strains that shall be detected or enumerated by the candidate method.

Interferents.—A . . . substance in analytical procedures . . . that, at a (the) given concentration, causes a systematic error in the analytical result (International Union of Pure and Applied Chemistry Analytical Chemistry Division Commission on Analytical Reactions and Reagents Definition and Classification of Interferences in Analytical Procedures Prepared for Publication by W.E. Van Der Linden, *Pure Appl. Chem*. **61**(1), 91–95(1989). Printed in Great Britain, 1989, IUPAC). Sometimes also known as interferants.

Maximum time-to-result.—Maximum time to complete an analysis starting from the collection buffer to assay result.

Probability of detection (POD).—Proportion of positive analytical outcomes for a qualitative method for a given matrix at a specified analyte level or concentration with a ≥0.95 confidence interval.

System false-negative rate.—Proportion of test results that are negative contained within a population of known positives.

System false-positive rate.—Proportion of test results that are positive contained within a population of known negatives.

4 Method Performance Requirements

See Table 1.

5 System Suitability Tests and/or Analytical Quality Control

Controls listed in Table 2 shall be embedded in assays as appropriate. Manufacturer must provide written justification if controls are not embedded in the assay.

6 Validation Guidance

Official Methods of Analysis (2019) 21st Ed., Appendix I: *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures*, AOAC INTERNATIONAL, Rockville, MD, USA.

Inclusivity and exclusivity panel organisms used for evaluation must be characterized and documented to truly be the species and strains they are purported to be.

7 Maximum Time-to-Results

Within 4 h.

8 Guidance on Combining DNA for Exclusivity Evaluation

Organisms may be tested as isolated DNA, or combined to form a pool of isolated DNA. Isolated DNA may be combined into pools of up to 10 exclusivity panel organisms, with each panel organism represented at 10 times the AMDL, where possible. If an unexpected result occurs, each of the exclusivity organisms from a failed pool must be individually retested at 10 times the AMDL.

Environmental Panel Organisms

See Environmental Factors for Validating Biological Threat Agent Detection Assays [*Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., Appendix O].

Approved by the AOAC Stakeholder Panel on Agent Detection Assays (SPADA). Final Version Date: March 22, 2016. Revised: October 2018 to replace sections on Environmental Panel Organisms with reference to OMA Appendix O: Environmental Factors for Validating Biological Threat Agent Detection Assays

Table 1. Method performance requirements

Parameter	Minimum performance requirement
AMDL	2000 standardized cells of *Yersinia pestis* strain CO92 per mL liquid in the candidate method sample collection buffer
Probability of detection at AMDL within sample collection buffer	≥0.95
Probability of detection at AMDL in environmental matrix materials	≥0.95
System false-negative rate using spiked environmental matrix materials	≤5%
System false-positive rate using environmental matrix materials	≤5%
Inclusivity	All inclusivity strains (Table 3) must test positive at 2x the AMDL[a]
Exclusivity	All exclusivity strains (Table 4 and OMA Appendix O, Part 1) must test negative at 10x the AMDL[a]

[a] 100% correct analyses are expected. All discrepancies are to be retested following the *AOAC Guidelines for Validation of Biological Threat Agent Methods and/or Procedures* [*Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA, Appendix I; http://www.eoma.aoac.org/app_i.pdf].

Table 2. Controls		
Control	Description	Implementation
Positive	Designed to demonstrate an appropriate test response. The positive control should be included at a low but easily detectable concentration, and should monitor the performance of the entire assay. The purpose of using a low concentration of positive control is to demonstrate that the assay sensitivity is performing at a previously determined level of sensitivity.	Single use per sample (or sample set) run
Negative	Designed to demonstrate that the assay itself does not produce detection in the absence of the target organism. The purpose of this control is to rule out causes of false positives, such as contamination in the assay or test.	Single use per sample (or sample set) run
Inhibition	Designed to specifically address the impact of a sample or sample matrix on the assay's ability to detect the target organism.	Single use per sample (or sample set) run

Table 3. Inclusivity panel				
No.	Strain	Achtman genotype	Comment	Availability[a]
1	CO92	1.ORI.c	Well-studied example of epidemic strain of pestis, recent isolate	CDC, USAMRIID
2	KIM	2.Med	Well-studied strain in academic circles, virulence data extensive	CDC, USAMRIID
3	Antiqua	1.Ant b	Ancient strain near root of tree	CDC, USAMRIID
4	Pestoides B	0.PE1		CDC, USAMRIID
5	Pestoides F	0.PE2.a	pPst negative, old strain in terms of phylogeny	CDC, USAMRIID
6	Pestoides G	0.PE2.b	pPst negative	CDC, USAMRIID
7	Angola	0.PE3	A "pestoides" in everything except name	CDC, USAMRIID
8	Nairobi	1.Ant a		CDC, USAMRIID
9	Harbin35	2 Ant	Rumored to be used or resulted from infection during experiments by Japanese BW Unit 731	CDC, USAMRIID
10	PBM19	1.ORI.a		CDC, USAMRIID
11	Java9	1.ORI	pFra negative	CDC, USAMRIID
12	A1122	1.ORI.a	Well-characterized U.S. isolate that is pgm- and pCD-; also has 2X large pPst plasmid	CDC, USAMRIID
13	Nicholisk 41	2.ANT		CDC, USAMRIID
14	Shasta	1.ORI	YE0387; Shasta (20 Oct 54); Shasta; human case; USA: Ca; 1960 6LY; UCC YERS074	CDC, USAMRIID
15	Dodson	1.ORI	Dodson (Aug 70); human case: male age 4.5 years; USA: Arizona (Tuba City); 27 Jun 67; UCC YERS073	CDC, USAMRIID
16	El Dorado			
Note on plasmid nomenclature: pMT1 = pFRA; pPCP1 = pPST = pPLA; pCD1 = pYB = pCAD				
[a] CDC = Centers for Disease Control and Prevention; USAMRIID = U.S. Army Medical Research Institute of Infectious Diseases.				

Table 4. Exclusivity panel (near-neighbor)					
	Species	Strain		Comment	Availability[a]
YPNN1	*Yersinia ruckeri*	YERS063			USAMRIID
YPNN2	*Yersinia rohdei*	YERS062			USAMRIID
YPNN3	*Yersinia pseudotuberculosis*	PB1/+	1	Sequenced	WRAIR
YPNN4	*Yersinia pseudotuberculosis*	IP32953	1	Sequenced	WRAIR
YPNN5	*Yersinia pseudotuberculosis*	YPIII	3	Sequenced	WRAIR
YPNN6	*Yersinia pseudotuberculosis*	Pa3606	1b		WRAIR
YPNN7	*Yersinia pseudotuberculosis*	IB	1b		WRAIR
YPNN8	*Yersinia pseudotuberculosis*	EP2/+	1		WRAIR
YPNN9	*Yersinia pseudotuberculosis*	MD67	1		WRAIR
YPNN10	*Yersinia pseudotuberculosis*	1	1a		WRAIR
YPNN11	*Yersinia enterocolitica*	WA	O:8		WRAIR
YPNN12	*Yersinia enterocolitica*	8081	O:8	Sequenced	WRAIR
YPNN13	*Yersinia enterocolitica*	2516-87	O:9		WRAIR
YPNN14	*Yersinia kirstensenii*	Y231		Nonpathogenic	WRAIR
YPNN15	*Yersinia frederiksenii*	Y225		Nonpathogenic	WRAIR
YPNN16	*Yersinia intermedia*	Y228		Nonpathogenic	WRAIR
YPNN17	*Yersinia aldovae*	670-83		Nonpathogenic	WRAIR

[a] USAMRIID = U.S. Army Medical Research Institute of Infectious Diseases; WRAIR = Walter Reed Army Institute of Research.