AOAC SMPR® 2016.007

Standard Method Performance Requirements (SMPRs®) for Detection of *Francisella tularensis* in Aerosol Collection Devices

Intended Use: Laboratory or Field Use by Department of Defense Trained Operators

1 Applicability

Detection of *Francisella tularensis* 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).—The 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.

Table 1. Method performance requirements

Parameter	Minimum performance requirement
AMDL	2000 standardized cells 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 a 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.	UCC[a] ID	Genus and species	Strain	Characteristics
1	FRAN001	*Francisella tularensis*	subsp. *tularensis*	Type A2 (Type strain)
2	FRAN004	*Francisella tularensis*	subsp. *holarctica* (LVS)	Type B (Russian)
3	FRAN012	*Francisella tularensis*	subsp. *holarctica*	Type B (United States)
4	FRAN016	*Francisella tularensis*	subsp. *tularensis* (SCHU S4)	Type A1 (United States)
5	FRAN024	*Francisella tularemia*	subsp. *holarctica* JAP (Cincinnati)	Type B (Japanese)
6	FRAN025	*Francisella tularensis*	subsp. *tularensis* (VT68)	Type A1 (United States)
7	FRAN029	*Francisella tularensis*	subsp. *holarctica* (425)	Type B (United States)
8	FRAN031	*Francisella tularensis*	subsp. *tularensis* (Scherm)	Type A1 (United States)
9	FRAN072	*Francisella tularensis*	subsp. *tularensis* (WY96)	Type A2 (United States)
10	N/A	*Francisella tularensis*	subsp. *mediasiatica*	

[a] UCC = Department of Defense Unified Culture Collection; components available through Biodefense and Emerging Infections Research Resources Repository.

Table 4. Exclusivity panel (near-neighbor)		
No.	Species	Strain
1	*Francisella philomiragia*	Jensen O#319L ATCC 25015
2	*Francisella philomiragia*	Jensen O#319-029 ATCC 25016
3	*Francisella philomiragia*	Jensen O#319-036 ATCC 25017
4	*Francisella philomiragia*	Jensen O#319-067 ATCC 25018
5	*Francisella philomiragia*	D7533, GA012794
6	*Francisella philomiragia*	E9923, GA012801
7	*Francisella novicida*	D9876, GA993548
8	*Francisella novicida*	F6168, GA993549
9	*Francisella novicida*	U112, GA993550
10	*Francisella hispaniensis*	DSM 22475

5 System Suitability Tests and/or Analytical Quality Control

The 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

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

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.

In silico analysis.—In silico screening shall be performed on signature sequences (e.g., oligo primers/probes/amplicons) to predict specificity and inclusivity across available sequenced *Francisella* strains. In silico results are suggestive of potential performance issues. Basic Local Alignment Search Tool (BLAST) (or a comparable tool) should be used to examine potential hybridization events between signature components and available *Francisella* genomic sequence data in GenBank®. Results of in silico analyses shall be included in method/assay performance evaluation reports.

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