AOAC SMPR® 2015.011

Standard Method Performance Requirements (SMPRs®) for Detection of *Coxiella burnetii*

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

1 Applicability

Specific detection of *Coxiella burnetii* 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).—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).

Coxiella burnetii.—Naturally obligate intracellular bacterial pathogen of the *Legionellales* family.

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

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

Maximum time-to-result.—Maximum time to complete an analysis starting from the test portion preparation 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.

Table 1. Method performance requirements

Parameter	Minimum performance requirement
AMDL	2000 genomic equivalents/mL of *Coxiella burnetii* target DNA in the candidate method sample collection buffer
Probability of detection at AMDL within sample collection buffer using Nine Mile RSA439 isolate, Clone 4	≥0.95
Probability of detection at AMDL in environmental matrix materials using Nine Mile RSA439 isolate, Clone 4	≥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 all environmental organisms (section 1.2 of OMA Appendix O) 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].

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

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

Inclusivity and exclusivity panel members 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.

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: September 1, 2015. 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 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 run

Table 3. Inclusivity panel

Phylogenetic group	Isolate (example)
Group 1	Nine Mile RSA493 Nine Mile RSA439
Group 2	Henzerling
Group 3	Idaho Goat
Group 4	K
Group 5	G
Group 6	Dugway

Table 4. Exclusivity panel (near-neighbor)

Species	Strain
Legionella pneumophila	Philadelphia 1
Legionella pneumophila	Wadsworth 1
Legionella pneumophila	Sg6
Legionella longbeachae	ATCC No. 33462
Rickettsiella spp.	If obtainable