AOAC SMPR® 2016.010

Standard Method Performance Requirements (SMPRs®) for DNA-Based Methods of Detecting *Burkholderia pseudomallei* 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 *Burkholderia pseudomallei* 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).

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.

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

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

If an isolate designated in the inclusivity or exclusivity panel is not commercially available in the United States at this time, use the genomic sequence for *in silico* analysis.

7 Maximum Time-to-Results

Within 4 h.

8 Guidance

Organisms may be tested as isolated DNA, or combined to form pooled 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. 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: September 1, 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 *Burkholderia pseudomallei* 1026b 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 2× the AMDL[a]
Exclusivity	All exclusivity strains (Table 4 and OMA Appendix O, Part 1) must test negative at 10× the AMDL[a]

[a] 100% correct analyses are expected. All discrepancies are to be retested following the *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., AOAC INTERNATIONAL, Rockville, MD, USA, Appendix I, http://www.eoma.aoac.org/app_i.pdf].

Table 2. Controls		
Control	Description	Implementation
Positive control	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. It is recommended that a technique (i.e., unique distinguishable signature) is used to confirm whether the positive control is the cause of a positive signal generated by a sample.	Single use per sample (or sample set) run
Negative control	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 control	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			
Species	Isolate	Available from	Comment
B. pseudomallei	MSHR668 BEI NR-9922	BEI Resources	Clinical Australian isolate
B. pseudomallei	MSHR1655		Clinical Australian isolate DBPAO[a]
B. pseudomallei	K96243 BEI NR-4073	BEI Resources	Clinical Thai isolate
B. pseudomallei	MSHR305 BEI NR-44225	BEI Resources	Clinical Australian isolate
B. pseudomallei	1026b BEI NR-9910 BEI NR-4074	BEI Resources	Clinical Thai isolate
B. pseudomallei	7894		DBPAO
B. pseudomallei	MSHR840		Clinical Australian isolate DBPAO
B. pseudomallei	576a BEI NR-9916	BEI Resources	Clinical Thai isolate
B. pseudomallei	HBPUB10134a BEI NR-44220	BEI Resources	Clinical Thai isolate
B. pseudomallei	RF80		Environmental isolate from Thailand

[a] DBPAO = Defense Biological Products Assurance Office.

Table 4. Exclusivity panel (near neighbor)[a]		
	Species	Isolate
1	*B. mallei*	Strain 6 NCTC 10248 BEI NR-36126
2	*B. mallei*	China 5 BEI NR-21
3	*B. thailandensis*	CDC3015869 (TXDOH)
4	*B. thailandensis*	H0587
5	*B. thailandensis*	Malaysia20
6	*B. thailandensis*	E1
7	*B. humptydooensis* (proposed)	MSMB43 ATCC BAA-2767
8	*B. humptydooensis* (proposed)	MSMB1589
9	*Burkholderia* species MSMB264	MSMB0265
10	*B. oklahomensis*	1974002358
11	*B. oklahomensis*-like	BDU8
12	*Burkholderia* species MSMB175	TSV85
13	*B. ubonensis*	MSMB2036
14	*B. ubonensis*	MSMB1189
15	*B. multivorans*	AU1185
16	*B. stagnalis*	MSMB735
17	*B. cepacia (B. cenocepacia)*	MSMB1824
18	*B. vietnamiensis*	FL-2-3-30-S1-D0
19	*B. vietnamiensis*	AU1233

[a] Strains and species from items 3 to 19 can be used as an exclusivity panel for *B. mallei* assays.