AOAC SMPR 2016.XXX; Version 6

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)
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).

Exclusivity
Study involving pure non-target 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)
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 I.

5. **System suitability tests and/or analytical quality control:**
The controls listed in Table II 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 (AOAC INTERNATIONAL Official Methods of Analysis, 2012, 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.

7. Maximum time-to-results: Within four hours.

Table I: Method Performance Requirements

Parameter	**Minimum Performance Requirement**
AMDL	2,000 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 III) must test positive at 2x the AMDL †
Exclusivity	All exclusivity strains (Table IV and Annex I; part 2) must test negative at 10x the AMDL†.
Notes: † 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. [1]	

[1] Official Methods of Analysis of AOAC INTERNATIONAL (2012) 19th Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, APPENDIX I; also on-line at http://www.eoma.aoac.org/app_i.pdf.

66 **TABLE II: Controls**

67

Control	Description	Implementation
Positive Control	This control is 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 (ie 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	This control is 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	This control is 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 III: Inclusivity Panel

Species	Isolate
B. pseudomallei	MSHR668
B. pseudomallei	MSHR1655
B. pseudomallei	K96243
B. pseudomallei	MSHR305
B. pseudomallei	1026b
B. pseudomallei	7894
B. pseudomallei	MSHR840
B. pseudomallei	576
B. pseudomallei	HBPUB10134a

Table IV: Exclusivity Panel (near-neighbor)

	Species	Isolate
1	B. mallei	Strain 6
2	B. mallei	NCTC10247
3	B. thailandensis	CDC3015869 (aka TXDOH)
4	B. thailandensis	H0587
5	B. thailandensis	Malaysia20 (aka Bp7046)
6	B. thailandensis	E1 (aka Bp7045)
7	B. humptydooensis (proposed)	MSMB43 (aka Bp5365)
8	B. humptydooensis (proposed)	MSMB1589 (aka Bp7270)
9	MSMB264	MSMB0265 (aka Bp7063)
10	B. oklahomensis	1974002358 (aka Bp0072)
11	B. oklahomensis-like	BDU8 (aka Bp7004)
12	MSMB175	TSV85 (aka Bp7000)
13	B. ubonensis	MSMB2036 (aka Bp7062)
14	B. ubonensis	MSMB1189 (aka Bp7434)
15	B. multivorans	AU1185 (aka Bp7344)
16	B. stagnalis	MSMB735 (aka Bp7657)
17	B. cepacia	MSMB1824 (aka Bp7307)
18	B. vietnamiensis	FL-2-3-30-S1-D0 (aka Bp7021)
19	B. vietnamiensis	AU1233 (aka Bp7345)

Note: Strains and species from item 3 to 19 can be used as an exclusivity panel for *B. mallei* assays.

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 re-tested at 10 times the AMDL.

Annex I: Environmental Factors For Validating Biological Threat Agent Detection Assays

[Adapted from the Environmental Factors Panel approved by SPADA on June 10, 2010.]

The Environmental Factors Studies supplement the biological threat agent near-neighbor exclusivity testing panel. There are three parts to Environmental Factors studies: part 1 - environmental matrix samples; part 2 - the environmental organisms study; and part 3 - the potential interferents applicable to Department of Defense applications.[2]

Part 1:

Environmental Matrix Samples - Aerosol Environmental Matrices

Method developers shall obtain environmental matrix samples that are representative and consistent with the collection method that is anticipated to ultimately be used in the field. This includes considerations that may be encountered when the collection system is deployed operationally such as collection medium, duration of collection, diversity of geographical areas that will be sampled, climatic/environmental conditions that may be encountered and seasonal changes in the regions of deployment.

Justifications for the selected conditions that were used to generate the environmental matrix and limitations of the validation based on those criteria must be documented.

- Method developers shall test the environmental matrix samples for interference using samples inoculated with a target biological threat agent sufficient to achieve 95% probability of detection.
- Cross-reactivity testing will include sufficient samples and replicates to ensure each environmental condition is adequately represented.

[2] Added in June 2015 for the Department of Defense project.

Part 2: Environmental Panel Organisms - This list is comprised of identified organisms from the environment.

Inclusion of all environmental panel organisms is not a requirement if a method developer provides appropriate justification that the intended use of the assay permits the exclusion of specific panel organisms. Justification for exclusion of any environmental panel organism(s) must be documented and submitted.

Organisms and cell lines may be tested as isolated DNA, or as pools of isolated DNA. Isolated DNA may be combined into pools of up to 10 panel organisms, with each panel organism represented at 10 times the AMDL, where possible. The combined DNA pools are tested in the presence (at 2 times the AMDL) and absence of the target gene or gene fragment. If an unexpected result occurs, each of the individual environmental organisms from a failed pool must be individually re-tested at 10 times the AMDL with and without the target gene or gene fragment at 2x the AMDL in the candidate method DNA elution buffer.

DNA in this list that already appear in the inclusivity or exclusivity panel do not need to be tested again as part of the environmental factors panel.

- **Potential bacterial biothreat agents**
  Bacillus anthracis Ames
  Yersinia pestis Colorado-92
  Francisella tularensis subsp. *tularensis* Schu-S4
  Burkholderia pseudomallei
  Burkholderia mallei
  Brucella melitensis

- **Cultivatable bacteria identified as being present in air soil or water**
  Acinetobacter lwoffii
  Agrobacterium tumefaciens
  Bacillus amyloliquefaciens
  Bacillus cohnii
  Bacillus psychrosaccharolyticus
  Bacillus benzoevorans
  Bacillus megaterium
  Bacillus horikoshii
  Bacillus macroides
  Bacteroides fragilis
  Burkholderia cepacia
  Burkholderia gladoli
  Burkholderia stabilis
  Burkholderia plantarii
  Chryseobacterium indologenes
  Clostridium sardiniense
  Clostridium perfringens
  Deinococcus radiodurans
  Delftia acidovorans
  Escherichia coli K12

Fusobacterium nucleatum
Lactobacillus plantarum
Legionella pneumophilas
Listeria monocytogenes
Moraxella nonliquefaciens
Mycobacterium smegmatis
Neisseria lactamica
Pseudomonas aeruginosa
Rhodobacter sphaeroides
Riemerella anatipestifer
Shewanella oneidensis
Staphylococcus aureus
Stenotophomonas maltophilia
Streptococcus pneumoniae
Streptomyces coelicolor
Synechocystis
Vibrio cholerae

- **Microbial eukaryotes**

<u>Freshwater amoebae</u>
Acanthamoeba castellanii
Naegleria fowleri

<u>Fungi</u>
Alternaria alternata
Aspergillus fumagatis
Aureobasidium pullulans
Cladosporium cladosporioides
Cladosporium sphaerospermum
Epicoccum nigrum
Eurotium amstelodami
Mucor racemosus
Paecilomyces variotii
Penicillum chrysogenum
Wallemia sebi

- **DNA from higher eukaryotes**

  Plant Pollen[3]

  Zea mays (corn)
  Pinus spp . (pine)
  Gossypium spp. (Cotton)

  Arthropods

  Aedes aegypti (ATCC /CCL-125(tm) mosquito cell line)
  Aedes albopictus (Mosquito C6/36 cell line)
  Dermatophagoides pteronyssinus (Dust mite -commercial source)
  Xenopsylla cheopis Flea (Rocky Mountain labs)
  Drosophilia cell line
  Musca domestica (housefly) ARS, USDA, Fargo, ND
  Gypsy moth cell lines LED652Y cell line (baculovirus)– Invitrogen
  Cockroach (commercial source)
  Tick (*Amblyomma* and *Dermacentor* tick species for *F. tularensis* detection assays)[4]

  Vertebrates

  Mus musculus (ATCC/HB-123) mouse
  Rattus norvegicus (ATCC/CRL-1896) rat
  Canis familiaris(ATCC/CCL-183) dog
  Felis catus (ATCC/CRL-8727) cat
  Homo sapiens (HeLa cell line ATCC/CCL-2) human
  Gallus gallus domesticus (Chicken)
  Capri hirca (Goat[5])

- **Biological insecticides** – Strains of *B. thuringiensis* present in commercially available insecticides have been extensively used in hoaxes and are likely to be harvested in air collectors. For these reasons, it should be used to assess the specificity of these threat assays.

  B. thuringiensis subsp*. israelensis*
  B. thuringiensis subsp*. kurstaki*
  B. thuringiensis subsp*. morrisoni*
  Serenade (Fungicide) *B. subtilis* (QST713)

  Viral agents have also been used for insect control. Two representative products are:

  Gypcheck for gypsy moths (*Lymanteria dispar* nuclear polyhedrosis virus)

  Cyd-X for coddling moths (Coddling moth granulosis virus)

[3] If pollen is unavailable, vegetative DNA is acceptable
[4] Added by SPADA on March 22, 2016.
[5] Added by SPADA on September 1, 2015.

Part 3: Potential Interferents Study

The Potential Interferents Study supplements the Environmental Factors Study, and is applicable to all biological threat agent detection assays for Department of Defense applications. Table 1a provides a list of potential interferents that are likely to be encountered in various Department of Defense applications.

Method developers and evaluators shall determine the most appropriate potential interferents for their application. Interferents shall be spiked at a final test concentration of 1 μg/ml directly into the sample collection buffer. Sample collection buffers spiked with potential interferents shall by inoculated at 2 times the AMDL (or AMIL) with one of the target biological threat agents.

Spiked / inoculated sample collection buffers shall be tested using the procedure specified by the candidate method. A candidate method that fails at the 1 microgram per ml level may be reevaluated at lower concentrations until the inhibition level is determined.

It is expected that all samples are correctly identified as positive.

Table 5a: Potential Interferents

Compounds		Potential Theaters of Operation
group 1: petroleum-based	JP-8[1]	airfield
	JP-5[2]	naval
	diesel/gasoline mixture	ground
	fog oil (standard grade fuel number 2)	naval, ground
	burning rubber[3]	ground, airfield
group 2: exhaust	gasoline exhaust	ground
	jet exhaust	naval, airfield
	diesel exhaust	ground
group 3: obscurants	terephthalic acid[4]	ground
	zinc chloride smoke[5]	ground
	solvent yellow 33[6]	ground
group 4: environmental	burning vegetation	ground, airfield
	road dust	ground
	sea water (sea spray)	naval
group 5: chemicals	brake fluid[7]	all
	brake dust[8]	ground
	cleaning solvent, *MIL-L-63460*[9]	all
	explosive residues a) high explosives[10] b) artillery propellant[11]	all

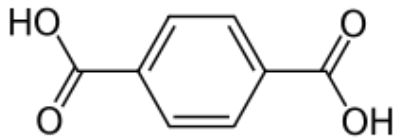
Table 1a is offered for guidance and there are no mandatory minimum requirements for the number of potential interferents to be tested.

[1] **JP-8**. Air Force formulation jet fuel.

[2] **JP-5**. A yellow kerosene-based jet fuel with a lower flash point developed for use in aircraft stationed aboard aircraft carriers, where the risk from fire is particularly great. JP-5 is a complex mixture of hydrocarbons, containing alkanes, naphthenes, and aromatic hydrocarbons.

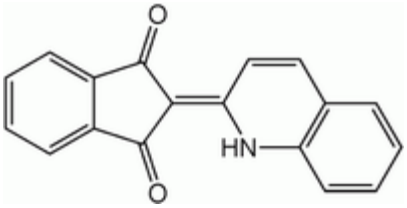
[3] **Burning rubber** (tire smoke). Gaseous C1-C5 hydrocarbons: methane; ethane; isopropene; butadiene; propane. Polycyclic aromatic hydrocarbons (58-6800 ng/m^3): parabenzo(a)pyrene; polychlorinated dibenzo-p-dioxins (PCDD); polychlorinated dibenzofurans (PCDF). Metals (0.7 - 8 mg/m^3): zinc; lead; cadmium.

[4] **Terephthalic acid.** Used in the AN/M83 hand grenade currently used by US military.



[5] **Zinc chloride smoke**. Also known as "zinc chloride smoke" and "HC smoke". Was used in the M8 grenade and still used in 155mm artillery shells. HC smoke is composed of 45% hexachloroethane, 45% zinc oxide, and 10% aluminum.

[6] **Solvent yellow 33** [IUPAC name: 2-(2-quinolyl)-1,3-indandione] is a new formulation being develop for the M18 grenade.



[7] **Brake fluid**. DOT 4 is the most common brake fluid, primarily composed of glycol and borate esters. DOT 5 is silicone-based brake fluid. The main difference is that DOT 4 is hydroscopic whereas DOT 5 is hydrophobic. DOT 5 is often used in military vehicles because it is more stable over time requires less maintenance

[8] **Brake dust**. Fe particles caused by abrasion of the cast iron brake rotor by the pad and secondly fibers from the semi metallic elements of the brake pad. The remainder of the dust residue is carbon content within the brake pad.

[9] **MIL-L-63460**, "Military Specification, Lubricant, Cleaner and Preservative for Weapons and Weapons Systems"; trade name **"**Break-Free CLP**".** Hyperlink: Midway USA.

[10] **High explosives**. The M795 155mm projectile is the US Army / Marine Corp's current standard projectile containing 10.8 kg of TNT. The M795 projectile replaced the M107 projectile that contained Composition B which is a 60/40 mixture of RDX/TNT. RDX is cyclotrimethylene trinitramine. Suggestion: test RDX/TNT together.

[11] **Artillery propellant**. Modern gun propellants are divided into three classes: single-base propellants which are mainly or entirely nitrocellulose based, double-base propellants composed of a combination of nitrocellulose and nitroglycerin, and triple base composed of a combination of nitrocellulose and nitroglycerin and nitroguanidine. Suggestion: test total nitrocellulose/ nitroglycerin nitroguanidine together.