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

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 of AOAC INTERNATIONAL, 2016, 20th Ed., Appendix I](http://www.eoma.aoac.org/app_i.pdf)).

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.

Approved by the AOAC Stakeholder Panel on Agent Detection Assays (SPADA). Final Version Date: September 1, 2016.

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

### Table 1. Method performance requirements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Minimum performance requirement</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AMDL</strong></td>
<td>2000 standardized cells of <em>Burkholderia pseudomallei</em> 1026b per mL liquid in the candidate method sample collection buffer</td>
</tr>
<tr>
<td>Probability of detection at AMDL within sample collection buffer</td>
<td>≥0.95</td>
</tr>
<tr>
<td>Probability of detection at AMDL in environmental matrix materials</td>
<td>≥0.95</td>
</tr>
<tr>
<td>System false-negative rate using spiked environmental matrix materials</td>
<td>≤5%</td>
</tr>
<tr>
<td>System false-positive rate using environmental matrix materials</td>
<td>≤5%</td>
</tr>
<tr>
<td>Inclusivity</td>
<td>All inclusivity strains (Table 3) must test positive at 2× the AMDL*</td>
</tr>
<tr>
<td>Exclusivity</td>
<td>All exclusivity strains (Table 4 and Annex I, Part 2) must test negative at 10× the AMDL*</td>
</tr>
</tbody>
</table>

* 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, 2016, 20th Ed., AOAC INTERNATIONAL, Rockville, MD, USA, Appendix I, http://www.eoma.aoac.org/app_i.pdf](http://www.eoma.aoac.org/app_i.pdf)).
matrix samples; Part 2—Environmental organisms study; and Part 3—Potential interferents applicable to Department of Defense applications (added in June 2015 for the Department of Defense project).

**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.

**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.

<table>
<thead>
<tr>
<th>Table 2. Controls</th>
<th>Description</th>
<th>Implementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>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.</td>
<td>Single use per sample (or sample set) run</td>
</tr>
<tr>
<td>Negative control</td>
<td>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.</td>
<td>Single use per sample (or sample set) run</td>
</tr>
<tr>
<td>Inhibition control</td>
<td>Designed to specifically address the impact of a sample or sample matrix on the assay’s ability to detect the target organism.</td>
<td>Single use per sample (or sample set) run</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3. Inclusivity panel</th>
<th>Species</th>
<th>Isolate</th>
<th>Available from</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. pseudomallei</td>
<td>MSHR668</td>
<td>BEI Resources</td>
<td></td>
<td>Clinical Australian isolate</td>
</tr>
<tr>
<td>B. pseudomallei</td>
<td>MSHR1655</td>
<td></td>
<td></td>
<td>Clinical Australian isolate DBPAO*</td>
</tr>
<tr>
<td>B. pseudomallei</td>
<td>K96243</td>
<td>BEI Resources</td>
<td></td>
<td>Clinical Thai isolate</td>
</tr>
<tr>
<td>B. pseudomallei</td>
<td>MSHR305</td>
<td>BEI Resources</td>
<td></td>
<td>Clinical Australian isolate</td>
</tr>
<tr>
<td>B. pseudomallei</td>
<td>1026b</td>
<td>BEI Resources</td>
<td></td>
<td>Clinical Thai isolate</td>
</tr>
<tr>
<td>B. pseudomallei</td>
<td>7894</td>
<td></td>
<td></td>
<td>DBPAO</td>
</tr>
<tr>
<td>B. pseudomallei</td>
<td>MSHR840</td>
<td></td>
<td></td>
<td>Clinical Australian isolate DBPAO</td>
</tr>
<tr>
<td>B. pseudomallei</td>
<td>576a</td>
<td>BEI Resources</td>
<td></td>
<td>Clinical Thai isolate</td>
</tr>
<tr>
<td>B. pseudomallei</td>
<td>HBPU10134a</td>
<td>BEI Resources</td>
<td></td>
<td>Clinical Thai isolate</td>
</tr>
<tr>
<td>B. pseudomallei</td>
<td>RF80</td>
<td></td>
<td></td>
<td>Environmental isolate from Thailand</td>
</tr>
</tbody>
</table>

* DBPAO = Defense Biological Products Assurance Office.
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 retested at 10 times the AMDL with and without the target gene or gene fragment 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
  - *Burkholderia cepacia* (proposed)
  - *Burkholderia thailandensis* (proposed)

- **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 gladioli*
  - *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*
  - *Mycobacterium smegmatis*
  - *Neisseria lactamica*
  - *Pseudomonas aeruginosa*
  - *Rhodobacter sphaeroides*
  - *Riemerella anatipestifer*
  - *Shewanella oneidensis*
  - *Staphylococcus aureus*
  - *Stenotrophomonas maltophilia*
  - *Streptococcus pneumoniae*
  - *Streptomyces coelicolor*
  - *Synechocystis*
  - *Vibrio cholerae*

- **Microbial eukaryotes**
  - Freshwater amoebae:  
    - *Acanthamoeba castellanii*
    - *Naegleria fowleri*
  - Fungi:  
    - *Alternaria alternata*
    - *Aspergillus fumagatis*
    - *Aureobasidium pullulans*
    - *Cladosporium cladosporioides*
    - *Cladosporium sphaerospermum*
    - *Epicoccum nigrum*
    - *Eurotium amstelodami*
    - *Mucor racemosus*
    - *Paecilomyces variotii*
    - *Penicillium chrysogenum*
    - *Wallenia sebi*

- **DNA from higher eukaryotes**
  Plant pollen (if pollen is unavailable, vegetative DNA is acceptable):
Zea mays (corn)
Pinus spp. (pine)
Gossypium spp. (cotton)

Arthropods:
Aedes aegypti (ATCC/CL-125™ 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, USA)
Gypsy moth cell lines
[LED652Y cell line (baculovirus); Invitrogen]
Tick (Amblyomma and Dermacentor tick species for F. tularensis detection assays; added by SPADA on March 22, 2016)

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 hircu (goat; added by SPADA on September 1, 2015)

• 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. morrisonii
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)

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 5 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 be inoculated at 2 times the AMDL (or acceptable minimum identification level; 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 µg/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 5 is offered for guidance and there are no mandatory minimum requirements for the number of potential interferents to be tested.
<table>
<thead>
<tr>
<th>Group 1: Petroleum-based</th>
<th>Compound</th>
<th>Potential theaters of operation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>JP-8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Airfield</td>
</tr>
<tr>
<td></td>
<td>JP-5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Naval</td>
</tr>
<tr>
<td></td>
<td>Diesel/gasoline mixture</td>
<td>Ground</td>
</tr>
<tr>
<td></td>
<td>Fog oil (standard grade fuel No. 2)</td>
<td>Naval, ground</td>
</tr>
<tr>
<td></td>
<td>Burning rubber&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Ground, airfield</td>
</tr>
<tr>
<td>Group 2: Exhaust</td>
<td>Gasoline exhaust</td>
<td>Ground</td>
</tr>
<tr>
<td></td>
<td>Jet exhaust</td>
<td>Naval, airfield</td>
</tr>
<tr>
<td></td>
<td>Diesel exhaust</td>
<td>Ground</td>
</tr>
<tr>
<td>Group 3: Obscurants</td>
<td>Terephthalic acid&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Ground</td>
</tr>
<tr>
<td></td>
<td>Zinc chloride smoke&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Ground</td>
</tr>
<tr>
<td></td>
<td>Solvent yellow 33&lt;sup&gt;f&lt;/sup&gt;</td>
<td>Ground</td>
</tr>
<tr>
<td>Group 4: Environmental</td>
<td>Burning vegetation</td>
<td>Ground, airfield</td>
</tr>
<tr>
<td></td>
<td>Road dust</td>
<td>Ground</td>
</tr>
<tr>
<td></td>
<td>Sea water (sea spray)</td>
<td>Naval</td>
</tr>
<tr>
<td>Group 5: Chemicals</td>
<td>Brake fluid&lt;sup&gt;g&lt;/sup&gt;</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td>Brake dust&lt;sup&gt;h&lt;/sup&gt;</td>
<td>Ground</td>
</tr>
<tr>
<td></td>
<td>Cleaning solvent, MIL-L-63460&lt;sup&gt;i&lt;/sup&gt;</td>
<td>All</td>
</tr>
<tr>
<td></td>
<td>Explosive residues: high explosives&lt;sup&gt;j&lt;/sup&gt; and artillery propellant&lt;sup&gt;k&lt;/sup&gt;</td>
<td>All</td>
</tr>
</tbody>
</table>

<sup>a</sup> JP-8: Air Force formulation jet fuel.
<sup>b</sup> 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.
<sup>c</sup> Burning rubber (tire smoke): Gaseous C1-C5 hydrocarbons: methane, ethane, isopropene, butadiene, propane. Polycyclic aromatic hydrocarbons (58–6800 ng/m<sup>3</sup>): parabenzo(a)pyrene, polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF). Metals (0.7–8 mg/m<sup>3</sup>): zinc, lead, cadmium.
<sup>d</sup> Terephthalic acid: Used in the AN/M83 hand grenade currently used by U.S. military.
<sup>e</sup> Zinc chloride smoke: Also known as “HC smoke.” Used in the M8 grenade and still used in 155 mm artillery shells. HC smoke is composed of 45% hexachloroethane, 45% zinc oxide, and 10% aluminum.
<sup>f</sup> Solvent yellow 33: IUPAC name: 2-(2-quinolyl)-1,3-indandione. A new formulation being develop for the M18 grenade.
<sup>g</sup> 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 and requires less maintenance.
<sup>h</sup> 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.
<sup>j</sup> High explosives: The M795 155 mm projectile is the U.S. Army/Marine Corp’s current standard projectile containing 10.8 kg TNT. The M795 projectile replaced the M107 rocket that contained Composition B which is a 60/40 mixture of RDX/TNT. RDX is cycloheximethylene trinitramine. Suggestion: test RDX/TNT together.
<sup>k</sup> 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.