3 Acceptance criteria: 100% expected results as defined for each strain on the panel.

Note: In the case of a positive result, retest that strain 96 times with no failures allowed to demonstrate a 95% upper confidence limit on the POD of 0.05 or lower.

Environmental Interference

1 Definition: Ability of the assay to detect target organism in the presence of nontarget organisms or environmental substances and to be free of cross-reaction from environmental organisms and substances (Appendix A).

2 Test conditions: Test pooled environmental panel organisms at 10 times AMDL in the presence or absence of Bacillus anthracis Ames at the AMDL. Test environmental substances as suspensions in the presence or absence of Bacillus anthracis Ames at the AMDL.

3 Acceptance criteria: 100% expected results for environmental organisms (i.e., no false negatives in the presence of Bacillus anthracis Ames, and no false positives in the absence of Bacillus anthracis Ames).

Note: In the case of an unexpected result, retest individual strains 96 times with no failures allowed to demonstrate an estimated 5% lower confidence limit on the POD of 0.95 or higher. Data from environmental substances are for informational purposes only.

Collaborative Validation Study

Reproducibility

1 Definition: Precision under conditions where independent test results are obtained with the same methods on equivalent test items in different laboratories with different operators using separate instruments.

2 Test conditions: Test Bacillus anthracis Ames spores at AMDL and near neighbor organism at 10 times AMDL on dust-loaded filters or in dust-loaded aerosol collection liquid. At least 12 replicates per material per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 Acceptance criteria: Must produce at least 10 valid data sets. Report standard deviation of reproducibility (sR).

POD at the AMDL Under Reproducibility Conditions (formerly termed System False-Negative Rate)

1 Definition: Rate of positive system results in a population of known positive test portions.

2 Test conditions: Test Bacillus anthracis Ames spores at AMDL on dust-loaded filters or in dust-loaded aerosol collection
**Table 1. Bacillus anthracis PCR method: Inclusivity panel**

<table>
<thead>
<tr>
<th>No.</th>
<th>Cluster</th>
<th>Genotype</th>
<th>Strain</th>
<th>MRI No.</th>
<th>Origin</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA1</td>
<td>A1a</td>
<td>7</td>
<td>Canadian bison</td>
<td>107448</td>
<td>Wood bison</td>
<td>pX01+, pX02+, VNTR genotype group A1a</td>
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<tr>
<td>BA2</td>
<td>A3a</td>
<td>45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>V770-NP-1R</td>
<td>107240</td>
<td>Vaccine (USA)</td>
<td>pX01+, pX02-, VNTR genotype group A3a</td>
</tr>
<tr>
<td>BA3</td>
<td>A2</td>
<td>29</td>
<td>PAK-1</td>
<td>107518</td>
<td>Sheep (Pakistan)</td>
<td>pX01+, pX02+, VNTR genotype group A2</td>
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<tr>
<td>BA4</td>
<td>A3a</td>
<td>51</td>
<td>BA1015</td>
<td>107446</td>
<td>Bovine (MD)</td>
<td>pX01+, pX02+, VNTR genotype group A3a</td>
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<tr>
<td>BA5</td>
<td>A3b</td>
<td>62</td>
<td>Ames</td>
<td>107517</td>
<td>Bovine (Texas)</td>
<td>pX01+, pX02+, VNTR genotype group A3b</td>
</tr>
<tr>
<td>BA6</td>
<td>A3c</td>
<td>67</td>
<td>K3</td>
<td>107497</td>
<td>South Africa</td>
<td>pX01+, pX02+, VNTR genotype group A3c</td>
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<tr>
<td>BA7</td>
<td>A3d</td>
<td>68</td>
<td>Ohio ACB</td>
<td>107339</td>
<td>Pig</td>
<td>pX01+, pX02+, VNTR genotype group A3d</td>
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<tr>
<td>BA8</td>
<td>A4</td>
<td>69</td>
<td>SK-102 (Pakistan)</td>
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<td>Imported wool (Pakistan)</td>
<td>pX01+, pX02+, VNTR genotype group A4</td>
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<tr>
<td>BA9</td>
<td>A4</td>
<td>77</td>
<td>Volum 1B</td>
<td>107539</td>
<td>USAMRIID&lt;sup&gt;a&lt;/sup&gt;</td>
<td>pX01+, pX02+, VNTR genotype group A4</td>
</tr>
<tr>
<td>BA10</td>
<td>B1</td>
<td>82</td>
<td>BA1035</td>
<td>107451</td>
<td>Human (South Africa)</td>
<td>pX01+, pX02+, VNTR genotype group B1</td>
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<tr>
<td>BA11</td>
<td>B2</td>
<td>80</td>
<td>RA3</td>
<td>107520</td>
<td>Bovine (France)</td>
<td>pX01+, pX02+, VNTR genotype group B2</td>
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<tr>
<td>BA12</td>
<td>C</td>
<td>Unk&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2002013094 (240)</td>
<td>124030</td>
<td>Louisiana</td>
<td>pX01+, pX02+, VNTR genotype group C</td>
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<tr>
<td>BA13</td>
<td>A1a</td>
<td>8</td>
<td>Pasteur</td>
<td>107171</td>
<td>USAMRIID</td>
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<tr>
<td>BA14</td>
<td>A3b</td>
<td>59, 61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Sterne</td>
<td>107453</td>
<td>USAMRIID</td>
<td>pX01+, pX02+, VNTR genotype group A3b</td>
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<tr>
<td>BA15</td>
<td>A1b</td>
<td>23</td>
<td>Turkey No. 32</td>
<td>107255</td>
<td>Human (Turkey)</td>
<td>pX01+, pX02+, VNTR genotype group A1b</td>
</tr>
</tbody>
</table>

<sup>a</sup> MRI = MRI Global; USAMRIID = The United States Army Medical Research Institute For Infectious Diseases.

Approved by AOAC SPADA on April 24, 2007.

<sup>b</sup> Organism contains only seven of eight MLVA markers due to the lack of pX02. Genotypes listed are consistent with seven of the eight markers. (Note: Footnote applies to BA2 and BA14 genotype designations.)

<sup>c</sup> Unk = Unknown.

**Table 2. Bacillus anthracis PCR method: Exclusivity panel**

<table>
<thead>
<tr>
<th>No.</th>
<th>Species</th>
<th>Strain</th>
<th>Plasmid status</th>
</tr>
</thead>
<tbody>
<tr>
<td>BANN1</td>
<td>B. cereus</td>
<td>S2-8</td>
<td>pX01-, pX02-</td>
</tr>
<tr>
<td>BANN2</td>
<td>B. cereus</td>
<td>3A</td>
<td>pX01-, pX02-</td>
</tr>
<tr>
<td>BANN3</td>
<td>B. thuringiensis</td>
<td>HD1011</td>
<td>pX01-, pX02-</td>
</tr>
<tr>
<td>BANN4</td>
<td>B. thuringiensis</td>
<td>97-27</td>
<td>pX01-, pX02-</td>
</tr>
<tr>
<td>BANN5</td>
<td>B. thuringiensis</td>
<td>HD682</td>
<td>pX01-, pX02-</td>
</tr>
<tr>
<td>BANN6</td>
<td>B. cereus</td>
<td>E33L</td>
<td>pX01-, pX02-</td>
</tr>
<tr>
<td>BANN7</td>
<td>B. cereus</td>
<td>D17</td>
<td>pX01-, pX02-</td>
</tr>
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<td>BANN8</td>
<td>B. thuringiensis</td>
<td>HD571</td>
<td>pX01-, pX02-</td>
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<td>BANN9</td>
<td>B. cereus</td>
<td>Al Hakam</td>
<td>pX01-, pX02-</td>
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<td>BANN10</td>
<td>B. cereus</td>
<td>ATCC 4342</td>
<td>pX01-, pX02-</td>
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<td>BANN11</td>
<td>B. cereus</td>
<td>FM1</td>
<td>pX01-, pX02-</td>
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<tr>
<td>BANN12</td>
<td>B. cereus</td>
<td>G9241</td>
<td>pBCX01&lt;sup&gt;a&lt;/sup&gt;, pX02-</td>
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<tr>
<td>BANN13</td>
<td>B. cereus</td>
<td>03BB102</td>
<td>pX01+, capA+, capB+, capC&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BANN14</td>
<td>B. cereus</td>
<td>03BB108</td>
<td>pX01+, capA+, capB+, capC&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>BANN15</td>
<td>B. thuringiensis subsp. israelensis</td>
<td>HD 1002</td>
<td>pX01-, pX02-</td>
</tr>
<tr>
<td>BANN16</td>
<td>B. thuringiensis subsp. kurstaki</td>
<td>HD 1</td>
<td>pX01-, pX02-</td>
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<tr>
<td>BANN17</td>
<td>B. thuringiensis subsp. morrisoni</td>
<td>HD 600</td>
<td>pX01-, pX02-</td>
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<td>BANN18</td>
<td>B. coagulans</td>
<td>ATCC 7050</td>
<td>pX01-, pX02-</td>
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<td>BANN19</td>
<td>B. mycoides</td>
<td>ATCC 6482</td>
<td>pX01-, pX02-</td>
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<tr>
<td>BANN20</td>
<td>B. megaterium</td>
<td>ATCC 14581</td>
<td>pX01-, pX02-</td>
</tr>
</tbody>
</table>

<sup>a</sup> pBCX01 is pX01-like, but not identical.

<sup>b</sup> capA, B, and C are contained within the pX02 plasmid of Bacillus anthracis; however, only the capA, B, and C sequences are found in 03BB102 and 03BB108.

Approved by AOAC SPADA on December 12, 2007.
At least 12 replicates per matrix per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 Acceptance criteria: Data for target agent must demonstrate an estimated 5% lower confidence limit on the CPOD of 0.95 or higher, where CPOD is the probability of detection calculated from pooled valid collaborative data.

POD in the Absence of Analyte Under Reproducibility Conditions (formerly termed System False-Positive Rate)

1 Definition: Rate of positive system results in a population of known negative test portions.

2 Test conditions: Test near neighbor organism at 10 times AMDL on dust-loaded filters or in dust-loaded aerosol collection liquid. At least 12 replicates per matrix per collaborator with 12 collaborators (four collaborators at each of three test sites).

3 Acceptance criteria: Data for near neighbor must demonstrate a 95% upper confidence limit on the CPOD of 0.05 or lower, where CPOD is the probability of detection calculated from pooled valid collaborative data.

Acknowledgments

All or part of this work was funded by the Department of Homeland Security Science and Technology Directorate, award HSHQDC-08-C-00012.

AOAC SPADA approved PCR SMPRs as amended on January 22, 2009. PCR SMPRs (version 4) were revised on May 12, 2009 to reflect OMB proposal and to correct retest statistics. The final version as shown here was approved by SPADA on June 2, 2010 and contained revision to OMB requirement of 10 valid data sets for qualitative methods in the collaborative study.

Appendix A: Environmental Factors Panel

Organisms

1 Other biothreat agents

Yersinia pestis Colorado-92
Francisella tularensis subsp. tularensis Schu-S4
Burkholderia pseudomallei
Coxiella burnetii Nine Mile Phase I
Brucella melitensis
Ricinus communis (use ricin plant leaves as source of DNA)
Clostridium botulinum Type A

2 Cultivatable bacteria identified as being present in air and soil

Acinetobacter lwofii
Agrobacterium tumefaciens
Bacillus cohnii
Bacillus psychrosaccharolyticus
Bacillus benzoevorans
Bacillus megaterium
Bacillus horikoshii
Bacillus macrides
Bacteroides fragilis
Burkholderia cepacia
Burkholderia gladoli
Burkholderia stabilis
Burkholderia plantarii
Chryseobacterium indologenes
Clostridium sardiniense
Clostridium perfringens
Deinococcus radiodurans
Delfia acidavorans
Escherichia coli K12
Fusobacterium nucleatum
Lactobacillus plantarum
Moraxella nonliquefaciens
Mycobacterium smegmatis
Neisseria lactamica
Pseudomonas aeruginosa
Rhodobacter sphaeroides
Riemerella anatipestifer
Shewanella oneidensis
Staphylococcus aureus
Stenotrophomonas maltophilia
Streptococcus pneumoniae
Streptomyces coelicolor
Synechocystis
Vibrio cholerae
Legionella pneumophila
Listeria monocytogenes
Vaccinia virus (pox)
Adenovirus vaccine
Herpes simplex or CMV (whichever is available)
4 Microbial eukaryotes

Freshwater amoebae:
- Acanthamoeba castellanii
- Naegleria fowleri

Fungi:
- Alternaria alternata
- Aspergillus fumigatis
- Aureobasidium pullulans
- Cladosporium cladosporioides
- Cladosporium sphaerospermum
- Epicoccum nigrum
- Eurotium amstelodami
- Mucor racemosus
- Paecilomyces variotii
- Penicillium chrysogenum
- Saccharomyces cerevisiae
- Wallemia sebi

5 DNA from higher eukaryotes

Plants:
- Zea mays (corn)
- Pollen from Pinus spp. (pine)
- Cotton (use leaves from cotton plant as source of DNA)

Arthropods:
- Aedes aegypti (ATCC/CCL-125) mosquito cell line
- Aedes albopictus (C6/36) mosquito
- Dust mite (commercial source)
- Flea (Rocky Mountain labs)
- Drosophila cell line
- Musca domestica (housefly; ARS, USDA, Fargo, ND)
- Gypsy moth cell lines LED652Y cell line (baculovirus; Invitrogen)
- Cockroach (commercial source)
- Tick (Amblyomma)

Mammals:
- 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) human

6 Biological insecticides

- B. thuringiensis subsp. israelensis
- B. thuringiensis subsp. kurstaki
- B. thuringiensis subsp. morrisoni
- Gypcheck for gypsy moths (Lymanteria dispar nuclear polyhedrosis virus)
- Cyd-X for codling moths (Codling moth granulosis virus)

Substances

1 Soils
- Sandy
- Loam
- Clay
- Subsoil
- Silt

2 Dust

3 Powders and chemicals

- Bacillus thuringiensis powders (e.g., Dipel)
- Powdered milk
- Powdered infant formula (Fe fortified)
- Powdered infant formula (low Fe formulation)
- Powdered coffee creamer
- Powdered sugar
- Talcum powder
- Wheat flour
- Baking soda
- Chalk dust
- Brewer’s yeast
- Dry wall dust
- Cornstarch
- Baking powder
- GABA (Gama aminobutyric acid)
- L-Glutamic acid
- Kaolin
- Chitin
- Chitosan
- MgSO₄
- Boric acid
- Powdered toothpaste
The Environmental Factors Panel was originally approved in parts. SPADA approved the environmental organisms panel on December 13, 2007, and revised it on September 17, 2008. The soils were approved on January 22, 2009. The powders and chemicals were originally approved by SPADA on December 13, 2007, and revised on January 22, 2009. The entire Environmental Factors Panel was approved in final form as presented here on June 2, 2010.