Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Bacillus anthracis* in Aerosol Collection Filters and/or Liquids

Intended Use: Laboratory use for analysis of aerosol collection filters and/or liquids

**Method Developer**  
and Independent Validation

**Probability of Detection at the Acceptable Minimum Detection Level**

1 **Definitions**  
Probability of detection (POD) is the proportion of positive analytical outcomes for a qualitative method for a given matrix at a given agent level or concentration. POD is concentration-dependent. The acceptable minimum detection level (AMDL) is the predetermined minimum level of a biological threat agent, which must be detected by the candidate method with an estimated 5% lower confidence limit on the POD of 0.95 or higher. The AMDL is dependent on the intended use.

2 **Test Conditions**  
AMDL is 20,000 standardized *Bacillus anthracis* Ames spores per filter; 2000 standardized spores per mL; 2000 genome equivalents per mL.

3 **Acceptance Criteria**  
No more than one failure in 96 replicates.

**Inclusivity**

1 **Definition**  
Strains or isolates or variants of the target agent(s) that the method can detect (Table 1).

2 **Test Conditions**  
Test inclusivity panel at AMDL.

3 **Acceptance Criteria**  
100% expected results as defined for each strain on the panel.  
*Note:* In the case of a negative result, retest that strain 96 times with no failures allowed to demonstrate an estimated 5% lower confidence limit on the POD of 0.95 or higher.

**Exclusivity**

1 **Definition**  
Nontarget agents, which are potentially cross-reactive, that are not detected by the method (Table 2).

2 **Test Conditions**  
Test exclusivity near neighbor panel at 10 times AMDL.

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.

**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.a</th>
<th>Origin</th>
<th>Characteristics</th>
</tr>
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<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>45b</td>
<td>V770-NP-1R</td>
<td>107240</td>
<td>Vaccine (USA)</td>
<td>pX01+, pX02-, VNTR genotype group A3a</td>
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<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>
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<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>
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<tr>
<td>BA8</td>
<td>A4</td>
<td>69</td>
<td>SK-102 (Pakistan)</td>
<td>107449</td>
<td>Imported wool (Pakistan)</td>
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<tr>
<td>BA9</td>
<td>A4</td>
<td>77</td>
<td>Volumn 1B</td>
<td>107539</td>
<td>USAMRIIDb</td>
<td>pX01+, pX02+, VNTR genotype group A4</td>
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<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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<td>BA12</td>
<td>C</td>
<td>Unk c</td>
<td>2002013094 (240)</td>
<td>124030</td>
<td>Louisiana</td>
<td>pX01+, pX02+, VNTR genotype group C</td>
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<td>BA13</td>
<td>A1a</td>
<td>8</td>
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<td>BA14</td>
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<td>59, 61a</td>
<td>Sterne</td>
<td>107453</td>
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<td>Turkey No. 32</td>
<td>107255</td>
<td>Human (Turkey)</td>
<td>pX01+, pX02+, VNTR genotype group A1b</td>
</tr>
</tbody>
</table>

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a MRI = MRI Global; USAMRIID = The United States Army Medical Research Institute For Infectious Diseases.  
Approved by AOAC SPADA on April 24, 2007.  
b 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.)  
c Unk = Unknown.
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 (Annex 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 ($s_R$).

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 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 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

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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.

ANNEX 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 Cultivable Bacteria Identified as Being Present in Air and Soil

*Acinetobacter lwofii*

*Agrobacterium tumefaciens*

*Bacillus cohnii*

*Bacillus psychrosaccharolyticus*

*Bacillus benzoearvans*

3 DNA Viruses

*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 fumigatus*

*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

**Avian**

Chicken

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 (*Coddling 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
Popcorn salt
EDTA
ZEP
Rid-X

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*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.*