AOAC SMPR 2010.003

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

No.	Cluster	Genotype	Strain	MRI No.[a]	Origin	Characteristics
BA1	A1a	7	Canadian bison	107448	Wood bison	pX01+, pX02+, VNTR genotype group A1a
BA2	A3a	45[b]	V770-NP-1R	107240	Vaccine (USA)	pX01+, pX02-, VNTR genotype group A3a
BA3	A2	29	PAK-1	107518	Sheep (Pakistan)	pX01+, pX02+, VNTR genotype group A2
BA4	A3a	51	BA1015	107446	Bovine (MD)	pX01+, pX02+, VNTR genotype group A3a
BA5	A3b	62	Ames	107517	Bovine (Texas)	pX01+, pX02+, VNTR genotype group A3b
BA6	A3c	67	K3	107497	South Africa	pX01+, pX02+, VNTR genotype group A3c
BA7	A3d	68	Ohio ACB	107339	Pig	pX01+, pX02+, VNTR genotype group A3d
BA8	A4	69	SK-102 (Pakistan)	107449	Imported wool (Pakistan)	pX01+, pX02+, VNTR genotype group A4
BA9	A4	77	Vollum 1B	107539	USAMRIID[a]	pX01+, pX02+, VNTR genotype group A4
BA10	B1	82	BA1035	107451	Human (South Africa)	pX01+, pX02+, VNTR genotype group B1
BA11	B2	80	RA3	107520	Bovine (France)	pX01+, pX02+, VNTR genotype group B2
BA12	C	Unk[c]	2002013094 (240)	124030	Louisiana	pX01+, pX02+, VNTR genotype group C
BA13	A1a	8	Pasteur	107171	USAMRIID	pX01-, pX02+, VNTR genotype group A1a
BA14	A3b	59, 61[b]	Sterne	107453	USAMRIID	pX01+, pX02-, VNTR genotype group A3b
BA15	A1b	23	Turkey No. 32	107255	Human (Turkey)	pX01+, pX02+, VNTR genotype group A1b

[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.

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

No.	Species	Strain	Plasmid status
BANN1	*B. cereus*	S2-8	pXO1-, pXO2-
BANN2	*B. cereus*	3A	pXO1-, pXO2-
BANN3	*B. thuringiensis*	HD1011	pXO1-, pXO2-
BANN4	*B. thuringiensis*	97-27	pXO1-, pXO2-
BANN5	*B. thuringiensis*	HD682	pXO1-, pXO2-
BANN6	*B. cereus*	E33L	pXO1-, pXO2-
BANN7	*B. cereus*	D17	pXO1-, pXO2-
BANN8	*B. thuringiensis*	HD571	pXO1-, pXO2-
BANN9	*B. cereus*	Al Hakam	pXO1-, pXO2-
BANN10	*B. cereus*	ATCC 4342	pXO1-, pXO2-
BANN11	*B. cereus*	FM1	pXO1-, pXO2-
BANN12	*B. cereus*	G9241	pBCXO1+[a], pXO2-
BANN13	*B. cereus*	03BB102	pXO1+, capA+, capB+, capC+[b]
BANN14	*B. cereus*	03BB108	pXO1+, capA+, capB+, capC+[b]
BANN15	*B. thuringiensis*	subsp. *israelensis* HD 1002	pXO1-, pXO2-
BANN16	*B. thuringiensis*	subsp. *kurstaki* HD 1	pXO1-, pXO2-
BANN17	*B. thuringiensis*	subsp. *morrisoni* HD 600	pXO1-, pXO2-
BANN18	*B. coagulans*	ATCC 7050	pXO1-, pXO2-
BANN19	*B. mycoides*	ATCC 6462	pXO1-, pXO2-
BANN20	*B. megaterium*	ATCC 14581	pXO1-, pXO2-

[a] pBCXO1 is pXO1-like, but not identical.

[b] capA, B, and C are contained within the pXO2 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.

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.

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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 Cultivatable Bacteria Identified as Being Present in Air and Soil

Acinetobacter lwoffii
Agrobacterium tumefaciens
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
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

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 fumigatis
Aureobasidium pullulans
Cladosporium cladosporioides
Cladosporium sphaerospermum
Epicoccum nigrum
Eurotium amstelodami
Mucor racemosus
Paecilomyces variotii
Penicillum 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 familiarus (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 coddling 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
$MgSO_4$
Boric acid
Powdered toothpaste
Popcorn salt
EDTA
ZEP
Rid-X

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.