## AOAC SMPR 2010.002

## Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Yersinia pestis* 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 *Yersinia pestis* CO-92 cells per filter; 2000 standardized cells 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).

**Table 1. *Yersinia pestis* PCR method: Inclusivity panel**

| No. | Strain | Biovar | Achtman genotype | Comment | Availability[a] |
|---|---|---|---|---|---|
| YP1 | CO92 | O | 1.ORI.c | Well-studied example of epidemic strain of pestis, recent isolate | CDC, WRAIR, USAMRIID |
| YP2 | KIM | M | 2.Med | Well-studied strain in academic circles, virulence data extensive | CDC, WRAIR, USAMRIID |
| YP3 | Antiqua | A | 1.Ant b | Ancient strain near root of tree | CDC, WRAIR, USAMRIID |
| YP4 | Pestoides B | M | 0.PE1 | | CDC, WRAIR, USAMRIID |
| YP5 | Pestoides F | A | 0.PE2.a | pPst negative, old strain in terms of phylogeny | CDC, WRAIR, USAMRIID |
| YP6 | Pestoides G | A | 0.PE2.b | pPst negative | CDC, WRAIR, USAMRIID |
| YP7 | Angola | A | 0.PE3 | A “pestoides” in everything except name | CDC, WRAIR, USAMRIID |
| YP8 | Nairobi | A | 1.Ant a | | CDC, WRAIR, USAMRIID |
| YP9 | Harbin35 | ? | 2 Ant | Rumored to be used or resulted from infection during experiments by Japanese BW Unit 731 | CDC, WRAIR, USAMRIID |
| YP10 | PBM19 | O | 1.ORI.a | | CDC, WRAIR, USAMRIID |
| YP11 | Java9 | O | 1.ORI | pFra negative | CDC, WRAIR, USAMRIID |
| YP12 | A1122 | O | 1.ORI.a | Well-characterized U.S. isolate that is pgm- and pCD-; also has 2X large pPst plasmid | CDC, WRAIR, USAMRIID |
| YP13 | Nicholisk 41 | M | 2.ANT | | CDC, WRAIR, USAMRIID |
| YP14 | Shasta | | 1.ORI | YE0387; SHASTA (20 OCT 54); SHASTA; HUMAN CASE; USA: CA; 1960 6LY; UCC YERS074 | CDC, USAMRIID |
| YP15 | Dodson | | 1.ORI | DODSON (AUG 70); HUMAN CASE: Male age 4.5 years; USA: Arizona (Tuba City); 27 JUN 67; UCC YERS073 | CDC, USAMRIID |
| YP16 | El Dorado | | | | |

[a] CDC = Centers for Disease Control and Prevention; WRAIR = Walter Reed Army Institute of Research; USAMRIID = The United States Army Medical Research Institute for Infectious Diseases.

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**Table 2. *Yersinia pestis* PCR method: Exclusivity panel**

| No. | Species | Strain | Serotype | Comment | Availability[a] |
|---|---|---|---|---|---|
| YPNN1 | *Yersinia ruckeri* | YERS063 | | | USAMRIID |
| YPNN2 | *Yersinia rohdei* | YERS062 | | | USAMRIID |
| YPNN3 | *Yersinia pseudotuberculosis* | PB1/+ | 1 | Sequenced | WRAIR |
| YPNN4 | *Yersinia pseudotuberculosis* | IP32953 | 1 | Sequenced | WRAIR |
| YPNN5 | *Yersinia pseudotuberculosis* | YPIII | 3 | Sequenced | WRAIR |
| YPNN6 | *Yersinia pseudotuberculosis* | Pa3606 | 1b | | WRAIR |
| YPNN7 | *Yersinia pseudotuberculosis* | IB | 1b | | WRAIR |
| YPNN8 | *Yersinia pseudotuberculosis* | EP2/+ | 1 | | WRAIR |
| YPNN9 | *Yersinia pseudotuberculosis* | MD67 | 1 | | WRAIR |
| YPNN10 | *Yersinia pseudotuberculosis* | 1 | 1a | | WRAIR |
| YPNN11 | *Yersinia enterocolitica* | WA | O:8 | | WRAIR |
| YPNN12 | *Yersinia enterocolitica* | 8081 | O:8 | Sequenced | WRAIR |
| YPNN13 | *Yersinia enterocolitica* | 2516-87 | O:9 | | WRAIR |
| YPNN14 | *Yersinia kirstensenii* | Y231 | | Nonpathogenic | WRAIR |
| YPNN15 | *Yersinia frederiksenii* | Y225 | | Nonpathogenic | WRAIR |
| YPNN16 | *Yersinia intermedia* | Y228 | | Nonpathogenic | WRAIR |
| YPNN17 | *Yersinia aldovae* | 670-83 | | Nonpathogenic | WRAIR |

[a] WRAIR = Walter Reed Army Institute of Research; USAMRIID = The United States Army Medical Research Institute for Infectious Diseases.
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*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.

***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 *Yersinia pestis* CO-92 at the AMDL. Test environmental substances as suspensions in the presence or absence of *Yersinia pestis* CO-92 at the AMDL.

*3 Acceptance Criteria*

100% expected results for environmental organisms (i.e., no false negatives in the presence of *Yersinia pestis* CO-92, and no false positives in the absence of *Yersinia pestis* CO-92).

*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 *Yersinia pestis* CO-92 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 reproducibililty ($s_R$).

***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 *Yersinia pestis* CO-92 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*

*Bacillus anthracis* Ames
*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.*