

Standard Method Performance Requirements for Polymerase Chain Reaction (PCR) Methods for Detection of *Francisella tularensis* 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 *Francisella tularensis* subsp. *tularensis* Schu-S4 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).

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 *Francisella tularensis* subsp. *tularensis* Schu-S4 at the AMDL. Test environmental substances as suspensions in the presence or absence of *Francisella tularensis* subsp. *tularensis* Schu-S4 at the AMDL.

3 Acceptance Criteria

100% expected results for environmental organisms (i.e., no false negatives in the presence of *Francisella tularensis* subsp. *tularensis* Schu-S4, and no false positives in the absence of *Francisella tularensis* subsp. *tularensis* Schu-S4).

Table 1. *Francisella tularensis* PCR method: Inclusivity panel

No.	UCC ^a ID	Genus and species	Strain	Characteristics
FT1	FRAN001	<i>Francisella tularensis</i>	subsp. <i>tularensis</i>	Type A2 (Type strain)
FT2	FRAN004	<i>Francisella tularensis</i>	subsp. <i>holartica</i> (LVS)	Type B (Russian)
FT3	FRAN012	<i>Francisella tularensis</i>	subsp. <i>holartica</i>	Type B (United States)
FT4	FRAN016	<i>Francisella tularensis</i>	subsp. <i>tularensis</i> (SCHU S4)	Type A1 (United States)
FT5	FRAN024	<i>Francisella tularensis</i>	subsp. <i>holartica</i> JAP (Cincinnati)	Type B (Japanese)
FT6	FRAN025	<i>Francisella holartica</i>	subsp. <i>tularensis</i> (VT68)	Type B (United States)
FT7	FRAN029	<i>Francisella tularensis</i>	subsp. <i>holartica</i> (425)	Type B (United States)
FT8	FRAN031	<i>Francisella tularensis</i>	subsp. <i>tularensis</i> (Scherm)	Type A1 (United States)
FT9	FRAN072	<i>Francisella tularensis</i>	subsp. <i>tularensis</i> (WY96)	Type A2 (United States)

^a UCC = Department of Defense Unified Culture Collection; components available through Biodefense and Emerging Infections Research Resources Repository.

Table 2. *Francisella tularensis* PCR method: Exclusivity panel

No.	Species	Strain
FTNN1	<i>Francisella philomiragia</i>	Jensen O#319L ATCC 25015
FTNN2	<i>Francisella philomiragia</i>	Jensen O#319-029 ATCC 25016
FTNN3	<i>Francisella philomiragia</i>	Jensen O#319-036 ATCC 25017
FTNN4	<i>Francisella philomiragia</i>	Jensen O#319-067 ATCC 25018
FTNN5	<i>Francisella philomiragia</i>	D7533, GA012794
FTNN6	<i>Francisella philomiragia</i>	E9923, GA012801
FTNN7	<i>Francisella novicida</i>	D9876, GA993548
FTNN8	<i>Francisella novicida</i>	F6168, GA993549
FTNN9	<i>Francisella novicida</i>	U112, GA993550
FTNN10	<i>Wolbachia persica</i>	(Johns Hopkins)

Approved by AOAC SPADA on January 22, 2009.

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 *Francisella tularensis* subsp. *tularensis* Schu-S4 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 *Francisella tularensis* subsp. *tularensis* Schu-S4 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
Yersinia pestis Colorado-92
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 lwoffii
Agrobacterium tumefaciens
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
Moraxella nonliquefaciens
Mycobacterium smegmatis
Neisseria lactamica
Pseudomonas aeruginosa
Rhodobacter sphaeroides
Riemerella anatipetifer
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 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 (*Lymantria 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
MgSO₄
Boric acid
Powdered toothpaste
Popcorn salt

EDTA
Rid-X
ZEP

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.