AOAC SMPR® 2016.009

Standard Method Performance Requirements (SMPRs®) for DNA-Based Methods of Detecting *Brucella suis* in Field-Deployable, Department of Defense Aerosol Collection Devices

Intended Use: Field-Deployed Use for Analysis of Aerosol Collection Filters and/or Liquids

1 Applicability

Detection of *Brucella suis* in collection buffers from aerosol collection devices. Field-deployable assays are preferred.

2 Analytical Technique

Molecular detection of nucleic acid.

3 Definitions

Acceptable minimum detection level (AMDL).—Predetermined minimum level of an analyte, as specified by an expert committee which must be detected by the candidate method at a specified probability of detection (POD).

Exclusivity.—Study involving pure nontarget strains, which are potentially cross-reactive, that shall not be detected or enumerated by the candidate method.

Inclusivity.—Study involving pure target strains that shall be detected or enumerated by the candidate method.

Maximum time-to-result.—Maximum time to complete an analysis starting from the collection buffer to assay result.

Probability of detection (POD).—Proportion of positive analytical outcomes for a qualitative method for a given matrix at a specified analyte level or concentration with a ≥0.95 confidence interval.

System false-negative rate.—Proportion of test results that are negative contained within a population of known positives.

System false-positive rate.—Proportion of test results that are positive contained within a population of known negatives.

4 Method Performance Requirements

See Table 1.

5 System Suitability Tests and/or Analytical Quality Control

Controls listed in Table 2 shall be embedded in assays as appropriate. Manufacturer must provide written justification if controls are not embedded in the assay.

6 Validation Guidance

AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures (*Official Methods of Analysis of AOAC INTERNATIONAL*, 2019, 21st Ed., Appendix I).

Inclusivity and exclusivity panel organisms used for evaluation must be characterized and documented to truly be the species and strains they are purported to be.

If a specified inclusivity or exclusivity isolate is not commercially available in the United States at this time, use the GenBank accession number to test the genomic sequence with *in silico* analysis.

7 Maximum Time-to-Results

Within 4 h.

8 Guidance

Organisms may be tested as isolated DNA, or combined to form pooled isolated DNA. Isolated DNA may be combined into pools of up to 10 exclusivity panel organisms, with each panel organism represented at 10 times the AMDL. If an unexpected result occurs, each of the exclusivity organisms from a failed pool must be individually retested at 10 times the AMDL.

If the isolate is not commercially available in the United States at this time, use the GenBank accession number to test the genomic sequence with *in silico* analysis.

Environmental Panel Organisms

See Environmental Factors for Validating Biological Threat Agent Detection Assays [*Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., Appendix O].

Approved by the AOAC Stakeholder Panel on Agent Detection Assays (SPADA). Final Version Date: September 1, 2016. Revised: October 2018 to replace sections on Environmental Panel Organisms with reference to OMA Appendix O: Environmental Factors for Validating Biological Threat Agent Detection Assays

Table 1. Method performance requirements

Parameter	Minimum performance requirement
AMDL	2000 genomic equivalents of *Brucella suis* (biovar 1, type strain 1330) per mL liquid in the candidate method sample collection buffer
Probability of detection at AMDL within sample collection buffer	≥0.95
Probability of detection at AMDL in environmental matrix materials	≥0.95
System false-negative rate using spiked environmental matrix materials	≤5%
System false-positive rate using environmental matrix materials	≤5%
Inclusivity	All inclusivity strains (Table 3) must test positive at 2× the AMDL[a]
Exclusivity	All exclusivity strains (Table 4 and OMA Appendix O, Part 1) must test negative at 10× the AMDL[a]

[a] 100% correct analyses are expected. All discrepancies are to be retested following the *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Biological Threat Agent Methods and/or Procedures* [*Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA, Appendix I, http://www.eoma.aoac.org/app_i.pdf].

Table 2. Controls		
Control	Description	Implementation
Positive control	Designed to demonstrate an appropriate test response. The positive control should be included at a low but easily detectable concentration, and should monitor the performance of the entire assay. The purpose of using a low concentration of positive control is to demonstrate that the assay sensitivity is performing at a previously determined level of sensitivity. It is recommended that a technique (i.e., unique distinguishable signature) is used to confirm whether the positive control is the cause of a positive signal generated by a sample.	Single use per sample (or sample set) run
Negative control	Designed to demonstrate that the assay itself does not produce a detection in the absence of the target organism. The purpose of this control is to rule out causes of false positives, such as contamination in the assay or test.	Single use per sample (or sample set) run
Inhibition control	Designed to specifically address the impact of a sample or sample matrix on the assay's ability to detect the target organism.	Single use per sample (or sample set) run

Table 3. Inclusivity panel[a]					
No.	Strain designation	Biovar	ATCC/BEI/GB Accession No.	Available from	Comment
1	*B. suis* 1330	1	ATCC 23444 BEI NR-302	BEI Resources	Swine, USA
2	*B. suis Thomsen*	2	ATCC 23445 BEI NR-303	BEI Resources	Hare, Denmark
3	*B. suis* 686	3	ATCC 23446 BEI NR-304	BEI Resources	Swine, USA
4	*B. suis* 40	4	ATCC 23447 BEI NR-305	BEI Resources	Reindeer, Russia
5	*B. suis* 513	5	ACBK00000000[b]	GenBank	Mouse, Russia
6	*B. suis* S2	NA	ALOS00000000.1[b]	GenBank	Naturally attenuated vaccine strain used in China

[a] The *Brucella* Working Group recognizes that *B. suis* biovar 5 is difficult to distinguish from the other *B. suis* biovars. The working group concluded that *B. suis* biovar 5 should be included as a part of the *B. suis* inclusivity panel with caution that *B. suis* biovar 5 may be very difficult to differentiate from other *B. suis* biovars. However, the SMPR does not require candidate assays to differentiate biovars.

[b] Available in the whole genome database at GenBank.

Table 4. Exclusivity panel[a,b]					
No.	Strain designation	Biovar	ATCC/BEI/Accession No.	Available from	Comment
1	*B. abortus* S19	1	NR-10134	NVSL	S19 vaccine strain, smooth
2	*B. abortus* RB51	1	BEI NR-2552	NVSL BEI Resources	RB51 vaccine strain, rough
3	*B. abortus* 86/8/59	2	ATCC 23449 BEI NR-231	BEI Resources	Bovine, England
4	*B. abortus* 12	3	ATCC 17385 BEI NR-229	BEI Resources	
5	*B. abortus Tulya*	3	ATCC 23450 BEI NR-232	BEI Resources	Human, Uganda
6	*B. abortus* 292 (39/94)	4	ATCC 23451 BEI NR-233	BEI Resources	Bovine, England
7	*B. abortus* B3196	5	ATCC 23452 BEI NR-234	BEI Resources	Bovine, England
8	*B. abortus* 870	6	ATCC 23453 BEI NR-261	BEI Resources	Bovine, Africa
9	*B. abortus* 63/75	7	ATCC 23454 BEI NR-262	BEI Resources	Bovine, Africa
10	*B. abortus* C68	9	ATCC 23455 BEI NR-263	BEI Resources	Bovine, England
11	*B. abortus* 544	1	ATCC 23448 BEI NR-69	BEI Resources	Bovine, England
12	*B. melitensis* 16M	1	ATCC 23456 BEI NR-256	BEI Resources	Goat, USA
13	*B. melitensis* 63/9	2	ATCC 23457 CP007789 CP007788 BEI NR-257	Not commercially available in the U.S. at this time	Goat, Turkey
14	*B. melitensis Ether*	3	ATCC 23458 BEI NR-258	BEI Resources	Goat, Italy
15	*B. melitensis* bv. 1 str. Rev. 1	1	ACEG00000000	Not commercially available in the U.S. at this time	Elberg origin, *B. melitensis* vaccine strain
16	*B. canis* RM-666	NA	ATCC 23365 NR-683	ATCC	Dog
17	*B. neotomae* 5K33	NA	ATCC 23459 BEI NR-684	ATCC BEI Resources	Desert Wood Rat
18	*B. ovis* 63-390	NA	ATCC 25840 BEI NR-682	ATCC BEI Resources	Ram, Australia
19	*B. ceti* B1/94	NA	AZBH02000000	Not commercially available in the U.S. at this time	Porpoise, Scotland
20	*B. pinnipedialis* B2/94	NA	ACBN00000000	Not commercially available in the U.S. at this time	Seal, Scotland
21	*Brucella* spp. 83/13	NA	ACBQ00000000	Not commercially available in the U.S. at this time	Rat, Australia
22	*B. inopinata* BO1	NA	ADEZ00000000	Not commercially available in the U.S. at this time	Human, Oregon
23	*Brucella* sp. BO2	NA	ADFA00000000	Not commercially available in the U.S. at this time	Human, Australia
24	*B. papionis* F8/08-60(T)	NA	ACXD00000000	Not commercially available in the U.S. at this time	Novel Brucella associated with primates (NVSL 07-0026)
26	*B. microti* CCM 4915	NA	CP001578 CP001579	Not commercially available in the U.S. at this time	Vvole, Czech Republic
27	*B. vulpis*	NA	LN997863-LN997864	Not commercially available in the U.S. at this time	Red fox, Austria
28	*Agrobacterium tumefaciens*	NA	ATCC 4452	ATCC	

Table 4. ***(continued)***

No.	Strain designation	Biovar	ATCC/BEI/Accession No.	Available from	Comment
29	*Ochrobactrum anthropi*	NA	ATCC 49188	ATCC	
30	*Ochrobactrum intermedium* LMG 3301	NA	SAMN02472089		

[a] The *Brucella* Working Group is aware that *B. canis* can infect humans, causing approximately 100 cases of human brucellosis annually. The working group is also aware of the close relationship between *B. suis* and *B. canis.* In fact, the taxonomic classification of all *Brucella* spp. has undergone debate during the last few decades, with some scientists proposing that all *Brucella* spp. should be reclassified as *B. melitensis* on the basis of results of DNA-DNA hybridization, and that the current species should be reclassified as biovars. However, the classic taxonomic scheme for the *Brucella* spp. and existing biovars was reapproved in 2003 [Osterman, B., & Moriyon, I. (2006) International Committee on Systematics of Prokaryotes: Subcommittee on the Taxonomy of *Brucella*, *Int. J. Syst. Evol. Microbiol.* **56**, 1173–1175] on the basis of host specificity, phenotypic characteristics, varying virulence, and genotyping data. For these reasons as well as directions from DoD to focus on *B. suis,* the working group determined to develop this SMPR for the specific detection of *B. suis*.

[b] The *Brucella* Working Group is aware of Russian vaccines using *B. abortus* SR82 and *B. abortus* 7579, and other strains may also be in use. These vaccine strains were not available at the time this SMPR was adopted. Consequently the working group decided not to include these vaccine strains in the exclusivity panel.