AOAC SMPR® 2016.012

Standard Method Performance Requirements (SMPRs®) for Detection and Identification of *Variola* Virus

Intended Use: Laboratory Use by Trained Technicians

1 Applicability

Detection of *Variola* virus DNA in collection buffers from aerosol collection devices for DoD applications.

Note: Method developers are advised to check the AOAC website for the most up to date version of this SMPR before initiating a validation.

2 Analytical Technique

Polymerase chain reaction (PCR) methods.

3 Definitions

Acceptable minimum detection level (AMDL).—Predetermined minimum level of an analyte, as specified by an expert committee that must be detected by the candidate method at a specified probability of detection (POD). The AMDL is dependent on the intended use. (Draft EN ISO/CD 16140-1: Microbiology of food and animal feeding stuffs—Method validation—Part 1: Terminology of method validation, vs 17-03-2011)

Exclusivity.—Study involving pure nontarget strains that are potentially cross-reactive that shall not be detected or enumerated by the tested method. (Draft EN ISO/CD 16140-1: Microbiology of food and animal feeding stuffs—Method validation—Part 1: Terminology of method validation, vs 17-03-2011)

Inclusivity.—Study involving pure target strains that shall be detected or enumerated by the alternative method. (Draft EN ISO/CD 16140-1: Microbiology of food and animal feeding stuffs—Method validation—Part 1: Terminology of method validation, vs 17-03-2011)

Maximum time-to-assay 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 [*Appendix H: Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods*, *Official Methods of Analysis of AOAC INTERNATIONAL*, 21st Ed., 2019].

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.

Variola virus.—A member of the genus *Orthopoxvirus* and the causative agent of smallpox.

4 System Suitability Tests and/or Analytical Quality Control

Controls listed in Table 1 shall be embedded in assays as appropriate. Manufacturer must provide written justification if controls are not embedded in the assay.

5 Validation Guidance

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

6 Method Performance Requirements

See Table 2.

7 Maximum Time-to-Assay Result

≤4 h.

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

ANNEX I
Inclusivity Panel

The inclusivity panel shall include:

(*1*) Sequences from at least two representative strains, one strain from each major clade of *Variola* virus [Li et. al. (October 2, 2007) *On the origin of smallpox: correlating Variola phylogenics with historical smallpox records, PNAS* **104**(40), 15787–15792]

(*2*) Any other strain with differences in the assay primer and/or probe target sequences based on bioinformatic analysis. *See* Annex III.

Table 1. Controls

Control	Description	Implementation
Positive	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	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	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 run

Table 2. Method performance requirements	
Parameter	Minimum performance requirement
Acceptable minimum detection level (AMDL)	50 000 copies/mL *Variola* virus target DNA in the candidate method sample collection buffer. Copies/mL refers to number of viral genomes or equivalent plasmid copies containing target viral gene or gene fragment.
Probability of detection at AMDL within sample collection buffer	≥0.95
Probability of detection at AMDL in an aerosol environmental matrix	≥0.95 (OMA Appendix O, Part 2)
Inclusivity panel purified DNA	All inclusivity strains (Annex I) must test positive at 2× the AMDL[a]
Exclusivity panel purified DNA	All exclusivity strains (Annex II and OMA Appendix O, Part 1) must test negative at 10× the AMDL[a]
System false-negative rate using spiked aerosol environmental matrix	≤5% (OMA Appendix O, Part 2)
System false-positive rate using aerosol environmental matrix	≤5% (OMA Appendix O, Part 2)

[a] 100% correct analyses are expected. All aberrations 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]. Some aberrations may be acceptable if the aberrations are investigated, and acceptable explanations can be determined and communicated to method users.

Note: The World Health Organization (WHO) restricts access to *Variola* virus genomic material; use of any genomic sequences greater than 500 bp requires written permission/approval from the WHO. Insertion of *Variola* virus DNA into other *Orthopoxviruses* is prohibited.

More details can be found at:

WHO Advisory Committee on *Variola* Virus Research: Report of the Seventeenth Meeting: Annex 5: WHO recommendations concerning the distribution, handling and synthesis of *Variola* virus DNA, http://apps.who.int/iris/bitstream/10665/205564/1/WHO_OHE_PED_2016.1_eng.pdf

WHO recommendations concerning the distribution, handling and synthesis of *Variola* virus DNA, http://www.who.int/csr/disease/smallpox/SummaryrecommendationsMay08.pdf

ANNEX II
Exclusivity Panel (Near Neighbor)

The exclusivity panel shall include:

Table 3. Core exclusivity panel		
Species	Strain	Commercial availability
Vaccinia	Elstree (Lister vaccine)	ATCC VR-1549
Cowpox	Brighton	ATCC VR-302
Ectromelia	Moscow	ATCC VR-1374
Monkeypox	V79-I-005	BEI NR-2324
Monkeypox	USA-2003	BEI NR-2500
Raccoonpox	Herman	ATCC VR-838
Skunkpox	SKPV-USA-1978-WA	ATCC VR-1830
Volepox	VPXV-USA-1985-CA	ATCC VR-1831
Camelpox	V78-I-2379	BEI NR-49736
Taterapox	V71-I-016	BEI NR-49737
Parapoxvirus Orf	Vaccine	Colorado Serum Co.

(*1*) All poxvirus strains listed in Table 3 (*Note*: *See* AOAC website for the most recent list.)

(*2*) Any additional strains determined through the bioinformatics analysis, performed in accordance with Annex III, with greater similarity to the assay's target region(s) than the strains listed in Table 3.

ANNEX III
Bioinformatics Analyses of Signature Sequences Underlying Variola Virus Assays

In silico screening will be performed on signature sequences (e.g., oligo primers) to demonstrate specificity to *Variola* virus and inclusivity across all sequenced *Variola* virus strains.

In silico results are suggestive of potential performance issues, so will guide necessary additions to the wet screening panels. *In silico* identification of potential cross-reactions (false positives) or nonverifications (false negatives) would require the affected strains be included in the exclusivity or inclusivity panels, respectively, if available.

A vendor-selected tool to carry out the bioinformatics evaluation should be able to predict hybridization events between signature components and a sequence in a database including available genomic sequence data, using public GenBank nt (http://www.ncbi.nlm.nih.gov/genbank/). The selected tool should be able to identify predicted hybridization events based on platform annealing temperatures, thus ensuring an accurate degree of allowed mismatch is incorporated in predictions. The program should detect possible amplicons from any selected database of sequence.

Potential tools for *in silico* screening of real-time PCR signatures include:

(*1*) http://sourceforge.net/projects/simulatepcr/files/?source=navbar. This program will find all possible amplicons and real-time fluorescing events from any selected database of sequence.

(*2*) NCBI tools

The vendor submission should include:

(*1*) Description of sequence databases used in the *in silico* analysis

(*2*) Description of conditions used for *in silico* analysis. Stringency of *in silico* analysis must match bench hybridization conditions.

(*3*) Description of tool used for bioinformatics evaluation. Data demonstrating the selected tool successfully predicts specificity that has been confirmed by wet-lab testing on designated isolates. These data can be generated retrospectively using published assays.

(*4*) List of additional strains to be added to the inclusivity (Annex I) or exclusivity (Annex II) panels based on the bioinformatics evaluation