AOAC SMPR® 2015.012

Standard Method Performance Requirements (SMPRs®) for Identification of Venezuelan Equine Encephalitis Virus (VEEV)

Intended Use: Laboratory or Field Use by Department of Defense Trained Operators

1 Applicability

Identification of VEEV in liquid samples from aerosol collectors. The preferential method would be a field-deployable assay.

2 Analytical Technique

Molecular methods of detecting target-specific viral component(s).

3 Definitions

Acceptable minimum identification level (AMIL).—Predetermined minimum level of an analyte, as specified by an expert committee, which must be detected and identified by the candidate method with a specified probability of identification (POI).

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

Inclusivity.—Study involving pure target strains or species that shall be detected and identified by the alternative method.

Maximum time-to-result.—Maximum time to complete an analysis starting from the test portion preparation to assay result.

Probability of identification (POI).—Proportion of positive analytical outcomes for an identification method for a given matrix at a given analyte level or concentration.

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.

Venezuelan equine encephalitis (VEE) virus (VEEV).—VEEV encompasses several viruses all of which are within the Alphavirus genus of the *Togaviridae* family. For the purpose of this SMPR, VEEV includes the human pathogenic virus variants VEEV-1AB, VEEV-1C, VEEV-1D, and VEEV-1E.

4 Method Performance Requirements

See Table 1.

5 System Suitability Tests and/or Analytical Quality Control

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

Table 1. Minimum performance requirements

Parameter	Minimum performance requirement
AMIL	5000 genome copies/mL
POI at AMIL within sample collection buffer	≥0.95
POI at AMIL in an aerosol environmental matrix	≥0.95 (*see* OMA Appendix O)
System false-negative rate using spiked aerosol environmental matrix	≤5% (*see* OMA Appendix O)
System false-positive rate using aerosol environmental matrix	≤5% (*see* OMA Appendix O)
Inclusivity panel purified DNA	All inclusivity strains in Table 3 must be correctly identified as VEEV at 2x the AMIL[a]
Exclusivity panel purified DNA	All exclusivity strains (Table 4) and all environmental organisims (section 1.2 of OMA Appendix O) must test negative at 10x the AMIL[a]

[a] 100% correct analyses are expected. All discrepancies are to be retested following the *AOAC 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].

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 members must be characterized and documented to truly be the species and strains they are purported to be.

7 Maximum Time-to-Results

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 2, 2015. 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 2. 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.	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 3. Inclusivity panel[a]

Virus	Serotype/variant	Representative strain(s)	Human illness?
VEEV	VEEV-IAB	Trinidad Donkey MF-8	Yes
	VEEV-IC	ICVE93, ICVE95	Yes
	VEEV-ID	1DPA61, 1DPE98, IDPE06	Yes
	VEEV-IE	IEMX63, IEPA62	Yes

[a] Method developers/evaluators must select at least one strain per serotype/variant in this table for their inclusivity panel evaluation.

Table 4. Exclusivity panel (near-neighbor)[a]

Virus	Representative strain(s)
Mosso das Pedras[b]	78V 3531
Everglades[b,c]	Fe-3-7c
Mucambo[b]	A
	C (strain 71D-1252)
	D
Tonate[b]	Tonate
Pixuna[b]	Pixuna
Cabassou[b]	Cabassou
Rio Negro[b]	AG 80-663
EEEV	PE6
WEEV	CBA87

[a] Method developers/evaluators must select at least one strain per virus in this table for their exclusivity panel evaluation.

[b] Virus is related to VEEV and is in the same antigenic complex.

[c] Due to close genetic relationships, assays that detect Everglades virus may be considered, however this detection must be noted.