AOAC SMPR® 2016.011

Standard Method Performance Requirements (SMPRs®) for Detection of Botulinum Neurotoxins A1 and A2 in Field-Deployable, Department of Defense (DoD) Aerosol Collection Devices

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

1 Applicability

Detection of botulinum neurotoxins A1 and A2 in collection buffers from aerosol collection devices. Field-deployable assays are preferred.

2 Analytical Technique

Any analytical method that can detect the protein and meets the requirements of this SMPR.

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).

Maximum time-to-assay result.—Maximum time to complete an analysis starting with recovery of toxins from the collection matrix and ending with the 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.

Selectivity study.—A study designed to demonstrate a candidate method's ability to detect the various forms of botulinum neurotoxin A, and at the same time, demonstrate that a candidate method does not detect nontarget compounds and related nontarget toxins.

4 System Suitability Tests and/or Analytical Quality Control

Controls listed in Table 1 shall be made available in assays as appropriate. Manufacturer or method developer must provide written justification if controls are not available 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*, 2019, 21st Ed., Appendix I).

Equal numbers of botulinum neurotoxin A1 and A2 and botulinum neurotoxin A1 and A2 complex samples must be represented in the selectivity study. Use pristine buffer solution. Samples with target and nontarget compounds must be: (*1*) blind coded; (*2*) randomly mixed together; (*3*) evaluated at the same time; and (*4*) masked, so that the sample identity remains unknown to the analysts. Batches are permissible provided that these four conditions are met.

Information on other subtypes is desirable but not required.

6 Method Performance Requirements

See Table 2.

7 Maximum Time-to-Assay 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 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. 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 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 (or sample set) run

Table 2. Method performance requirements	
Parameter	Minimum performance requirement
AMDL	1.25 ng/mL recovered botulinum neurotoxin A1 and A2 complexes in collection buffers
Selectivity study	POD ≥0.95 at AMDL for botulinum neurotoxin A1 and A2 complex
	Tetanus toxin must test negative at 10× the AMDL[a]
System false-negative rate using spiked aerosol environmental matrix at the AMDL	≤5% (OMA Appendix O, Part 2)
System false-positive rate using aerosol environmental matrix at the AMDL	≤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.