AOAC SMPR 2026.001

Standard Method Performance Requirements (SMPRs®) for Identification of Microorganisms in Food and Environmental Samples by Metagenomic Sequencing

Intended Use: Metagenomic Sequencing for Biosurveillance and Testing by Trained Technicians

Purpose

What: AOAC *Standard Method Performance Requirements* (SMPRs®) are voluntary consensus standards developed in accordance with the AOAC policy, "AOAC Due Process for Development of AOAC Non-Method Consensus Standards and Documents." SMPRs describe a scientific community's recommended minimum method performance characteristics and analytical requirements for a specific method-related intended use.

Who: Drafted by AOAC working groups, SMPRs are adopted by AOAC by a consensus of stakeholders affiliated with its integrated science programs and projects which are comprised of volunteer subject matter experts representing academia, government, industry, and nonprofit sectors from around the world.

Use: AOAC SMPRs are used in the AOAC core science programs as a resource for AOAC method experts, including expert review panels, in the evaluation of validation study data for methods submitted to the AOAC *Official Methods of Analysis*℠ (OMA) and AOAC *Performance Tested Methods*℠ programs. AOAC SMPRs may also be used to provide acceptance criteria for the verification of methods and serve as a resource to guide method development and optimization.

1 Applicability

Identification of microorganisms in samples, or their enrichments, from the food and agricultural farm-to-fork continuum.

2 Analytical Technique

Metagenomic sequencing (MGS) of nucleic acids. Methods can include target enrichment or nontarget depletion by various means.

3 Definitions

Identification.—Establishment of the taxonomical classification of an organism.

Identification accuracy (IA).—Proportion of organisms tested that are correctly identified in mixed culture testing without matrix present.

Limit of detection (LOD).—True net concentration or amount of the analyte in the material to be analyzed that will lead, with probability (1-β), to the conclusion that the concentration or amount of the analyte in the analyzed material is larger than that in the blank material.

Maximum time-to-result.—Maximum time to complete an analysis starting from the laboratory sample to assay result.

Probability of identification (POI).—Proportion of correct identification outcomes for a given matrix at a specified analyte level or concentration (1).

Single nucleotide polymorphism (SNP).—Variation of a single base pair in a DNA sequence.

Validation.—Establishment of the performance characteristics of a method and provision of objective evidence that the performance requirements for a specified intended use are fulfilled.

4 Method Performance Requirements

See Table 1.

5 Method Quality Controls

Suitable methods will include blanks and appropriate quality control measures as described in Table 2. Method developers must provide written justification if controls are not embedded in the method or assay. Controls should be representative of the matrix type and serve as controls for the entire process.

Negative controls should span sampling, extraction, library preparation, and sequencing to contextualize low-abundance signals. Spiked matrix controls utilizing a bioengineered organism

Table 1. Method performance requirements—matrix testing

Study[a]	Inoculation level[b]	Replicates	Performance parameter[c]	Acceptance criteria
Matrix—SLV	Blank	20	POI	POI ≤ 5%
	2–5 CFU/test portion	20		POI ≥ 95%
	20–50 CFU/test portion	20		POI ≥ 95%
	200–500 CFU/test portion	20		POI ≥ 95%
Matrix—MLV (≥10 data sets)	Blank	12	POI	POI ≤ 5%
	2–5 CFU/test portion	12		POI ≥ 95%
	20–50 CFU/test portion	12		POI ≥ 95%
	200–500 CFU/test portion	12		POI ≥ 95%
Mixed culture[d]	10^5 CFU/mL per strain or 10–100x LOD	1 per group	IA	IA ≥ 95%

[a] SLV = Single-laboratory validation; MLV = multi-laboratory validation; no more than three data sets per laboratory in the MLV.

[b] Methods may require target enrichment.

[c] POI = Probability of identification; IA = identification accuracy.

[d] 250–1000 strains total depending on the size of the database; test in groups, for example, 25 strains in a mixed culture for wet lab testing. Up to 90% of strains may be evaluated by in silico analysis.

Table 2. Method quality controls

Control	Description	Implementation
Positive	Designed to demonstrate appropriate test response. Positive control material, preferably organism with unique engineered tag not normally seen in the matrix environment, should be included at a low but reliably detectable concentration. The purpose of using a low concentration of positive control is to demonstrate that the methods are performing at a previously determined level of sensitivity.	Must be analyzed for every sample or set of samples tested
Negative	Designed to rule out causes of false positives, such as contamination	Must be analyzed for every sample or set of samples tested

containing a unique sequence tag are recommended as positive controls for each matrix being analyzed in a batch.

6 Reference Materials

The use of live cultures (liquid stressed/nonstressed, lyophilized) is required for inoculation of test matrices during matrix studies. All organisms used for evaluation must be characterized and documented to truly be the species and strains they are purported to be. Refer to OMA Appendix R (2).

7 Validation Guidance

Method developers should make an effort to optimize method parameters to meet the performance requirements in Table 1 when a cocktail of representative foodborne organisms is inoculated in food matrices. Method claim statements or applicability statements should be clear as to the scope of food, beverage, and environmental matrices, and/or enrichments, that have been validated as well as the scope and version of the sequence database.

Matrix Study

Matrices used must be representative of a typical sample of the matrix. They should reflect the expected variability in the physical, chemical, and microbiological composition of samples encountered during routine testing. Examples of matrix categories, types, and items can be found elsewhere (3–5).

Organisms of interest, such as food pathogens or spoilage organisms, shall be artificially contaminated at a low level approximating 2–5 CFU/test portion (or swab or sponge), medium level of 20–50/test portion (or swab or sponge), and high level of 200–500 CFU/test portion (or swab or sponge). Use different organisms of interest for each matrix validated. Methods may include enrichment to increase the levels of organisms of interest and/or suppress growth of background organisms, if needed.

Cocktails of organisms of interest for inoculation of matrices will depend on the scope of the method and the level of discrimination claimed (genus/species/serotype/SNP). For each claimed matrix, a cocktail of at least 10 organisms of interest should be inoculated into a food batch or onto a swab or appropriate surface that will be swab tested and aged to mimic environmental stressors. Organisms chosen should represent different genera, species, serotype and/or SNP. Variables to consider in preparing cocktails include, but are not limited to, growth rates, genome size, genome type (dsDNA, ssRNA, circular, linear, etc.), and extraction efficiency. For example, a cocktail for a Gram-negative method scope may include *Salmonella* Kentucky (8,20,:i:z_6), *Salmonella* Bargny (8,20:i:1,5), *Salmonella enterica* subsp. *arizonae*, *Escherichia coli* O26:H11 (stx1, eaeß1), *Escherichia coli* O26:H11 (stx2, eaeß1), *Escherichia coli* O157:H7 (stx1+2, eaeγ1), *Escherichia coli* K12, *Escherichia hermanii*, *Citrobacter freundii*, and *Shigella boydii*.

Background contamination should be at least 100-fold higher than the contamination level of organisms of interest. A "background cocktail" of 10 organisms typically found in the food matrix or food/farm environment can be co-inoculated if needed. Matrices can also be aged or temperature abused to mimic natural environmental stresses that can impact the viability of background microorganisms.

Report POI for each organism of interest as the number of correct identifications divided by the number of replicate test portions analyzed. Table 1 provides the acceptance criteria for single-laboratory validation (SLV) and multi-laboratory validation (MLV). The MLV may include up to three analysts working independently with independent test portion sets from one laboratory and requires a minimum of 10 data sets.

Mixed Culture Study

Mixed culture testing evaluates the breadth of organisms claimed to be identified by the method. This testing is performed without matrix at 10^5 CFU/mL/organism or a level 10–100 times the specific culture LOD for the method. Depending on the size of the database associated with the method, 250–1000 strains should be tested. The requirement for a species-level distinction is to test one strain per species such that 25% of genera and 10% of species contained in the database are tested, but with a minimum of 250 strains and maximum of 1000 strains. Up to 90% of the strains required may be evaluated through in silico analysis. When reference strains become available, wet lab testing should be performed to corroborate in silico analysis results. Wet lab testing should be performed with known cocktails of organisms, for example, 25 strains per cocktail. Test each cocktail once. The mixed culture study is typically performed in a single laboratory, but the testing may be divided among several laboratories as needed, depending on availability of organism strains.

Refer to OMA Appendix T (6).

8 Maximum Time-to-Results

None.

References

(1) "Appendix K (Part II): AOAC Guidelines for Validation of Botanical Identification Methods" (2023) *Official Methods of Analysis of AOAC INTERNATIONAL*, 22nd Ed., AOAC INTERNATIONAL, Rockville, MD, USA, https://academic.oup.com/aoac-publications/book/45491

(2) "Appendix R: Guidelines for Verifying and Documenting the Relationships Between Microbial Cultures" (2023) *Official Methods of Analysis of AOAC INTERNATIONAL*, 22nd Ed., AOAC INTERNATIONAL, Rockville, MD,

USA, https://academic.oup.com/aoac-publications/book/45491
(3) ISO (2016) Microbiology of the food chain—Method validation, Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method (ISO 16140-2:2016), https://www.iso.org/standard/54870.html, accessed February 2026
(4) U.S. Food and Drug Administration (2019) *Guidelines for the Validation of Analytical Methods for the Detection of Microbial Pathogens in Foods and Feeds*, Edition 3.0, https://www.fda.gov/media/83812/download, accessed February 2026
(5) Feldsine, P., Abeyta, C. & Andrews, W.H. (2002) *J. AOAC Int*. **85**, 1187–1200, https://doi.org/10.1093/jaoac/85.5.1187
(6) "Appendix T: Standard Requirements for Nucleotide Sequences used in Biothreat Agent Detection, Identification, and Quantification: Verified Next Generation Sequences (VNGS)" (2023) *Official Methods of Analysis of AOAC INTERNATIONAL*, 22nd Ed., AOAC INTERNATIONAL, Rockville, MD, USA, https://academic.oup.com/aoac-publications/book/45491

Acknowledgments

AOAC Stakeholder Program on Agent Detection Assays (SPADA) Working Group for Metagenomic Sequencing volunteer their time and expertise to the development of the SMPR:

Joseph Russell (Co-chair)
Shanmuga Sozhamannan (Co-chair)
Kholoud Abu-Rumman
Jeffery J. Ambrose Jr.
Angela Anandappa
Maria Arevalo
Nick Batora
Robert Cory Bernhards
Sharon Brunelle
Kayla Cannon
Catherine Carrillo
Federica Cattapan
Stephen Cendrowski
Mike Connolly
James Crill
Lisa Foddrill
Brett Forshey
Bala Ganesan
Atul Kumar Gupta
Saravanan J
Santhosh Kumar J U
Jonathan Jacobs
Karen Jarvis
Malcolm Johns
Kamil Khanipov
Ishi Keenum
Julia Kelliher
Kelsey Kropp
Eleanor Kuntz
Joe Lacirignola
Kerin Law
Eun Mi Lee
John Lee
Jennifer Mallek
Luis Maroto Martin
Stephen McLaughlin
Dev Mittar
Bryan Murphy
Sharvari Narendra
Joao Carlos Gomes Neto
Anna Niewiadomska
Thomas O'Brien
Thais Oliveira
Cameron Parsons
Jonathan Phillips
Colin Price
Christine Rygiel
Haley Sanderson
Casey Schlenker
Stephanie Servetas
Akanksha Awadhesh Pratap Singh
Jason Smith
Jennifer Stone
Sidney Sudberg
Michael Sussman
Celina To
Christine Tomlinson
Paul Turack
Kathleen Verratti
Trina Vian
Andrew Wall
Amy Xiao
Rahat Zaheer

Technical facilitation and logistical coordination and support: Sharon Brunelle (AOAC technical consultant), Veronica Kays (AOAC senior program manager), and AOAC staff.

Developed by AOAC Stakeholder Program on Agent Detection Assays (SPADA) Working Group for Metagenomic Sequencing. Approved by AOAC SPADA on March 26, 2026. Final version: March 16, 2026. Effective date: March 26, 2026