AOAC SMPR 20XX.XX, DRAFT VERSION 11-2024

Standard Method Performance Requirements **(SMPRs®) for Detection and/or Enumeration of *Listeria monocytogenes* in Cannabis Infused Edibles**

Intended Use

Surveillance and Monitoring 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 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 composed of volunteer subject matter experts representing academia, government, industry, and nonprofit sectors from around the world.*

Use*: AOAC uses SMPRs in its core science programs in which they are 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℠ and AOAC Performance Tested Methods℠ programs. Additionally, AOAC SMPRs may be used to provide acceptance criteria for the verification of methods and serve as a resource to guide method development and optimization.*

1. Applicability

Methods used to detect *Listeria monocytogenes* (see Table 1.) in cannabis infused edibles with the option of enumeration upon detection.

2. Analytical Technique

Any analytical technique that can meet the requirements of this SMPR.

3. Definitions:

Candidate Method.—The method submitted for validation [Appendix J: *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces*, *Official Methods of Analysis of AOAC INTERNATIONAL*, (2023) 22nd Ed., AOAC INTERNATIONAL, Rockville, MD, USA]

Candidate Method Presumptive Result.—Preliminary result for a test portion produced by following a candidate method's instructions for use.

Candidate Method Confirmed Result.—Final result obtained for a test portion after cultural confirmation of a candidate method.

Cannabis.—Genus of flowering plants within the Cannabinaceae family that commonly contain 9-tetrahydrocannabinol (THC), cannabidiol (CBD), and other cannabinoids and terpenes. Cannabis includes, but is not limited to, high-THC and high-CBD cultivars.

Cannabis Infused Edibles.—Food and drinks containing extracts of cannabis and/or cannabis materials (Category III).

Enumeration.—Determination of the number of colony-forming units (cfu) of *Listeria monocytogenes* per gram, per milliliter, per square centimeter, or per sampling device, when the analysis is carried out in accordance with this document.

Exclusivity.—Study involving pure nontarget strains, which are potentially cross-reactive, that shall be not detected or enumerated by the candidate method. *See* Table 2 for a list of recommended nontarget strains. [Appendix J: *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces*, *Official Methods of Analysis of AOAC INTERNATIONAL*, (2023) 22nd Ed., AOAC INTERNATIONAL, Rockville, MD, USA]

Fractional positive.—Validation criterion that is satisfied when an unknown sample yields both positive and negative responses within a set of replicate analyses. The proportion of positive responses should fall between 25 and 75% and should ideally approximate 50% of the total number of replicates in the set. A set of replicate analyses are those replicates analyzed by one method. Only one set of replicates per matrix is required to satisfy this criterion.

Inclusivity.—Study involving pure target strains that shall be detected or enumerated by the candidate method. *See* Tables 1 and 3 for a list of recommended target strains. [Appendix J: *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces*, *Official Methods of Analysis of AOAC INTERNATIONAL*, (2023) 22nd Ed., AOAC INTERNATIONAL, Rockville, MD, USA]

Laboratory probability of detection (LPOD).—The POD value obtained from combining all valid collaborator data sets for a method for a given matrix at a given analyte level or concentration. [Appendix H: *Probability of Detection (POD) as a Statistical Model for the Validation of Qualitative Methods*, *Official Methods of Analysis of AOAC INTERNATIONAL*, (2023) 22nd Ed., AOAC INTERNATIONAL, Rockville, MD, USA]

LCL.—Lower confidence limit.

Listeria monocytogenes.— An environmental pathogen that can contaminate foods and cause a mild, non-invasive illness, listerial gastroenteritis, or a severe, invasive illness, listeriosis.

Probability of detection (POD).—The portion of positive analytical outcomes for a qualitative method for a given matrix at a given analyte level or concentration. The difference in POD values between presumptive and confirmed results is termed $dPOD_{CP}$.

Test portion.— A specified quantity of the sample that is taken for analysis by the method. For cannabis infused edibles, a 25 g test portion is used. A larger test portion can be used in validation studies when appropriate. See Table 4 for minimum test portion requirements.

UCL. —Upper confidence limit.

4. Method Performance Requirements

See Table 3 for inclusivity and exclusivity performance requirements
See Table 4 for category test portion requirement
See Table 5 for acceptance criteria for detection method validation
See Table 6 for acceptance criteria for enumeration method validation
See Table 7 for matrix claims acceptance criteria
See Table 8 for descriptions of MPN analysis

5. Reference Materials

Reference cultures. — The use of live cultures and/or fungal spores (liquid stressed/non-stressed, lyophilized) is required for inclusivity and exclusivity testing and for inoculation of test matrices during the matrix studies. Extracted DNA is not suitable for use in validating methods against this SMPR but may be used to develop supplemental information.

Selective broth and agar. — See Table 9 for selective broth and agar recommendations
Inclusivity organisms. — See Table 1 for inclusivity organisms
Exclusivity organisms. — See Table 2 for exclusivity organisms

6. System suitability tests and/or analytical quality control

Positive and negative controls shall be embedded in assays as appropriate. Inhibition controls should be used for method verification for each new matrix. Manufacturer must provide written justification if controls are not appropriate to an assay.

7. Validation Guidance

Appendix F 'Guidelines for Standard Method Performance Requirements', in Dr. George W Latimer, Jr. (ed.), *Official Methods of Analysis of AOAC INTERNATIONAL*, 22 (New York, 2023; online edn, Oxford Academic, 4 Jan. 2023), https://doi.org/10.1093/9780197610145.005.006

Appendix J: 'AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces', in Dr. George W Latimer, Jr. (ed.), *Official Methods of Analysis of AOAC INTERNATIONAL*, 22 (New York, 2023; online edn, Oxford Academic, 4 Jan. 2023), https://doi.org/10.1093/9780197610145.005.010

At the time of the publication, no regulatory reference method exists for the confirmation *of Listeria monocytogenes* from cannabis infused edibles. Until a suitable reference method is established the following is recommended for method developers:

To screen samples for the presence or absence of the target analyte, two methods that employ different technologies (e.g., agar plate, PCR, ELISA, etc.) must be used. It is recommended that a combination of two or more confirmational methods (i.e., cultural, biochemical, molecular) be used in the validation.

To ensure the viability of the inoculating organism (both confirming presumptive results or determining false negative results) a secondary and/or extended enrichment (minimum total time of 48 h of enrichment) followed by plating of the sample to a minimum of two types of agar plates, one of which is recommended to be chromogenic agar (Table 10). Final confirmation can be achieved via matrix assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectroscopy, sequencing, or other suitable confirmatory procedures (e.g., biochemical analysis).

When performing the validation, bulk inoculation of test material is required. In certain instances (e.g., lollipop, gummies, etc.) individual item inoculation may be acceptable.

For the Single Laboratory Validation with artificial contamination, matrix naturally contaminated with non-target organisms (when available) shall be used. When available, matrix naturally contaminated with the target should be used. For at least one matrix evaluated during the single laboratory validation, competing non-target microflora must be at least 10x the level of the target microorganism. If the concentration of competing microflora does not exceed 10x the target organism for any matrix, artificial contamination of one matrix with non-target organism(s) is required.

A minimum three level most probable number (MPN) study should be performed to determine the concentration of the target organism used in the validation. Use the test portions from the matrix study as one level in the MPN study. See AOAC Appendix J guidelines and Table 8 for details on performing the MPN study.

Technical Guidance

Figure 1.

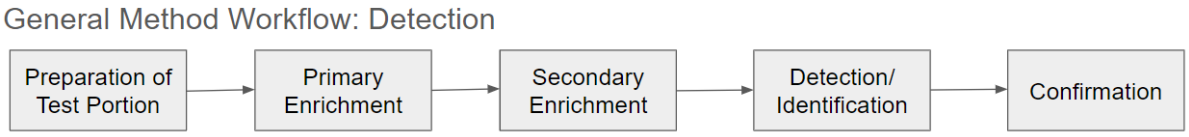
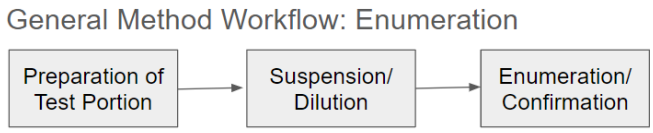


Figure 2.



For Technical Guidance for **Confirmation** refer to:

(1) ISO 11290-1:2017, *Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. Part 1: Detection Method* (2017) International Organization for Standardization, Geneva, Switzerland https://www.iso.org/standard/60313.html

(2) Anthony D. Hitchins, A., Jinneman, K., Chen, Y., (2022) *Bacteriological Analytical Manual: Chapter 10, Sections G-I, Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods,* U.S. Food and Drug Administration, https://www.fda.gov/food/laboratory-methods-food/bam-chapter-10-detection-listeria-monocytogenes-foods-and-environmental-samples-and-enumeration

(3) Health Products and Food Branch MFLP-01, *Isolation of Listeria monocytogenes from food samples* (2016) Franco Pagotto and Karine, Hébert Microbiology Research Division, Health Canada https://www.canada.ca/en/health-canada/services/food-nutrition/research-programs-analytical-methods/analytical-methods/compendium-methods/laboratory-procedures-microbiological-analysis-foods-compendium-analytical-methods.html

(4) Health Canada, Health Protection and Food Branch, Food Directorate, *Policy on Listeria monocytogenes in Ready-to-Eat Foods* (2023) https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/policies/listeria-monocytogenes-ready-eat-foods.html

(5) United States Department of Agriculture Food Safety and Inspection Service, Office of Public Health Science MLG 8.13, *Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Ready-to-Eat, Siluriformes (Fish) and Egg Products, Environmental Samples* (2021), https://www.fsis.usda.gov/sites/default/files/media_file/2021-09/MLG-8.13.pdf

(6) Public Health England, *Detection and Enumeration of Listeria monocytogenes and other Listeria species* (2018) National Infection Service Food, Water and Environmental Microbiology Standard Method https://assets.publishing.service.gov.uk/media/5c2e2c3540f0b66cf5a38e51/detection_and_enumeration_of_listeria_monocytogenes_and_other_listeria_species.pdf

For Technical Guidance for **Enumeration** refer to:

(1) ISO 11290-1:2017, *Microbiology of the food chain - Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. Part 2: Enumeration Method* (2017) International Organization for Standardization, Geneva, Switzerland https://www.iso.org/standard/60314.html

(2) Anthony D. Hitchins, A., Jinneman, K., Chen, Y., (2022) *Bacteriological Analytical Manual: Chapter 10 Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods,* U.S. Food and Drug Administration, https://www.fda.gov/food/laboratory-methods-food/bam-chapter-10-detection-listeria-monocytogenes-foods-and-environmental-samples-and-enumeration

(3) Health Products and Food Branch MFLP-74, *Enumeration of Listeria monocytogenes in foods* (2011) Franco Pagotto, Yvon-Louis Trottier, Jacqueline Upham and Irene Iugovaz, Research Division, Bureau of Microbial Hazards, Food Directorate, Health Canada https://www.canada.ca/en/health-canada/services/food-nutrition/research-programs-analytical-methods/analytical-methods/compendium-methods/laboratory-procedures-microbiological-analysis-foods-compendium-analytical-methods.html

(4) Health Canada, Health Protection and Food Branch, Food Directorate, *Policy on Listeria monocytogenes in Ready-to-Eat Foods* (2023) https://www.canada.ca/en/health-canada/services/food-nutrition/legislation-guidelines/policies/listeria-monocytogenes-ready-eat-foods.html

(5) Public Health England, *Detection and Enumeration of Listeria monocytogenes and other Listeria species* (2018) National Infection Service Food, Water and Environmental Microbiology Standard Method https://assets.publishing.service.gov.uk/media/5c2e2c3540f0b66cf5a38e51/detection_and_enumeration_of_listeria_monocytogenes_and_other_listeria_species.pdf

8. Maximum Time-To-Determination: None

Table 1. Inclusivity Panel

Listeria monocytogenes	Serotypes[a]: 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, 7

[a]At least one strain of each serotype of *Listeria monocytogenes* is **required** to be included.

Table 2. Exclusivity Panel[a]

Listeria innocua[b]	*Enterobacter* spp.
Listeria ivanovii[b]	*Enterococcus* spp.
Listeria seeligeri[b]	*Hafnia* spp.
Listeria welshimeri[b]	*Klebsiella* spp.
Listeria grayi[b]	*Kocuria* spp.
Listeria fleischmanii	*Kurthia* spp.
Listeria marthii	*Jonesia* spp.
Listeria rocourtiae	*Lactobacillus* spp.
Listeria weihenstephanensis	*Lactococcus* spp.
Alicyclobacillus spp.	*Leuconostoc* spp.
Bacillus spp	*Marinilactibacillus* spp.
Brevibacterium spp.	*Micrococcus* spp.
Bronchothrix spp.	*Pediococcus* spp.
Candida spp.	*Pseudoclavibacter* spp.
Carnobacterium spp.	*Pseudomonas* spp.
Cellulosimicrobium spp.	*Rhodococcus* spp.
Citrobacter spp.	*Rhodotorula* spp.
Clostridium spp.	*Rothia* spp.
Cornebacterium spp.	*Saccharomyces* spp.
Cronobacter spp.	*Salmonella enterica*
Eryspielothrix spp.	*Staphylococcus* spp.
Escherichia spp.	*Streptococcus* spp.

[a]Non-*Listeria* species listed are **recommended**. More may be added if appropriate. Strain lists should be submitted for review and approval prior to conducting the validation study.
[b]These Listeria species are required for inclusion in the exclusivity study.

Table 3. Inclusivity/Exclusivity Performance Requirements

Parameter	Parameter requirements	Final test concentration (cfu/mL)	Minimum acceptable results
Inclusivity	Single-laboratory validation (SLV) study: A minimum of 50 strains is required to be cultured by the candidate method enrichment procedure (including those detailed in Table 9).	10–100 x limit of detection of the candidate method	100% positive results[a]
Exclusivity	SLV study: At least 30 non-target organisms, cultured under optimal conditions for growth[b]	Overnight growth undiluted	100% negative results[a]

[a]100% correct analyses are expected. Perform root cause analysis for unexpected results and report all findings. Some unexpected results may be acceptable if the unexpected results are investigated, and acceptable explanations can be determined and communicated to method users.
[b]In instances where an exclusivity culture produces a positive result by the candidate method, the culture may be reanalyzed after culture following the candidate method enrichment procedure. Both results (optimal growth conditions and candidate method enrichment) must be reported.

Table 4. Test Portion Requirements

Categories	Minimum test portion size[a]
Chocolate	25 g
Beverages	25 g
Baked goods	25 g
Tinctures	25 g
Gummies	25 g
Capsules/Pills	25 g
[a]Minimum test portion size required for validation. Alternatively, larger test portions may be validated.	

Table 5. Validation Acceptance Criteria – Detection methods

Validation material[a]	Replicate test portions	Study design requirements	Acceptance criterion
Single laboratory validation with artificial contamination			
Low contamination level	20	Fractional positive results, POD 0.25–0.75 Ex. 0.2–2 cfu/test portion	$dPOD_{CP}$ 95% CI: LCL < 0 < UCL[b]
High contamination level	5	POD 1.00[c] Ex. 2–10 cfu/test portion	
Non-inoculated material (control)	5	POD 0.00[c] Non-inoculated	
Single Laboratory Validation with natural contamination (minimum two materials required)			
Material 1	20	Fractional positive results, POD 0.25–0.75	$dPOD_{CP}$ 95% CI: LCL < 0 < UCL[b]
Material 2	20	No requirement	
Multi Laboratory Validation			
Low contamination level	12	LPOD 0.2–0.8 Ex. 0.2–2 cfu/test portion	$dLPOD_{CP}$ 95% CI: LCL < 0 < UCL[b]
High contamination level	12	LPOD ≥ 0.95 Ex. 2–10 cfu/Test Portion	
Non-inoculated material (control)	12	LPOD ≤ 0.05 Non-inoculated	
[a]Follow Table 6 for appropriate inoculum preparation and matrix stabilization for each matrix.			
[b]The range between the lower and upper confidence interval should encompass 0. If not, the results must be investigated, and an explanation provided.			

Table 6. Validation Acceptance Criteria – Enumeration methods

Validation material[a]	Target contamination level[b]	Replicate test portions	Acceptance criterion[c]
Single laboratory validation study			
Low contamination level	10–100 cfu/g (mL)	5	90% CI on DOM within -0.5, 0.5
Medium contamination level	100–1,000 cfu/g (mL)	5	
High contamination level	1,000–10,000 cfu/g (mL)	5	
Non-inoculated (control) level[d]	Non-inoculated	5	
Multi laboratory validation study			
Low contamination level	10–100 cfu/g (mL)	5	90% CI on DOM within -0.5, 0.5
Medium contamination level	100–1,000 cfu/g (mL)	5	
High contamination level	1,000–10,000 cfu/g (mL)	5	
Non-inoculated (control) level[d]	Non-inoculated	5	
[a]Follow Table 6 for appropriate inoculum preparation and matrix stabilization for each matrix.			
[b]Target contamination levels may be dependent on method developer requirements. Contamination levels should be at least 1 $\log_{10}$ apart.			
[c]Report mean, standard deviation of repeatability, and difference of means (DOM) with 90 and 95% confidence intervals. If acceptance criterion is not observed, results must be investigated, and an explanation provided.			
[d]Non-inoculated level is not required for naturally contaminated materials.			

Table 7. Acceptable Matrix Claims

Matrix claim	Criteria	Minimum No. of categories
Broad range of cannabis infused edibles	15 (minimum 3 matrices/category	4
Variety of cannabis infused edibles	≥10 (minimum 2 matrices/category)	4
Select cannabis infused edibles	≥5	2
Specific category	≥5	1
Specific matrix(s)	≥1	1

Table 8. Minimum Most Probable (MPN) Number Recommendation

Category	Inoculation level	Large test portions	Medium test portions	Small test portions
Infused Edibles	Low	3 x 50 g	20 x 25 g[a]	3 x 10 g
	High	5 x 25 g[a]	3 x 10 g	3 x 5 g

[a]Test portions from matrix study

Table 9: Recommended agars and broths

Media name	Media type
Agar *Listeria* according to Ottaviani & Agosti (ALOA)	Agar
BBL™ Chromagar™ *Listeria*	Agar
Brilliance™ Listeria Agar	Agar

Lithium chloride-phenylathanol-moxalactam medium (LPM)	Agar
Modified Oxford Agar (MOX)	Agar
PALCAM Agar (PAL)	Agar
RAPID*L'mono*™	Agar
Modified University of Vermont broth (UVM1)	Broth
Buffered *Listeria* Enrichment Broth (BLEB)	Broth
Fraser broth	Broth
Demi-Fraser broth	Broth

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289 **Table 10. Condition of inoculating culture and matrix stabilization**

Matrix	Inoculating cells	Stabilization conditions
Perishable product	Liquid non-stressed culture	4°C, 48–72 h
Heat processed perishable product	Liquid heat stressed	4°C, 48–72 h
Frozen product	Liquid non-stressed culture (If frozen food is processed, cells must be heat stressed)	-20°C, 2 weeks
Shelf stable dry product	Dried culture	Ambient temperature (20–25°C), 2 weeks
Shelf stable liquid product (heat processed)	Liquid non-stressed culture (If shelf stable product is processed, cells must be heat stressed)	Ambient temperature (20–25°C), 2 weeks

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