AOAC SMPR® 2020.002

Standard Method Performance Requirements (SMPRs®) for Detection of *Salmonella* species in Cannabis and Cannabis Products

Intended Use: Consensus-Based Reference Method

1 Purpose

AOAC SMPRs describe the minimum recommended performance characteristics and suggested inclusivity/exclusivity organisms to be used during the evaluation of a method. The evaluation may be an on-site verification, a single-laboratory validation, or a multi-site collaborative study. SMPRs are written by AOAC working groups which are composed of representatives from industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs may be used for method development and optimization. Additionally, AOAC SMPRs are used by AOAC expert review panels in their evaluation of validation study data for methods being considered for *Performance Tested Methods*℠ or AOAC *Official Methods of Analysis*℠ and can be used as acceptance criteria for verification at user laboratories.

2 Applicability

Alternative methods used to detect *Salmonella* species and their serovars in cannabis and cannabis products.

3 Analytical Technique

Any analytical technique that can meet the requirements.

4 Definitions

Candidate method.—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* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA]

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

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

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 concentrates.—Extracts (primarily composed of cannabinoids and/or terpenes) manufactured through the extraction and concentration of compounds derived from the cannabis plant or flower. Final products can be many forms, including oils, wax, or hash (Category II).

Cannabis-infused edibles.—Food and drinks containing extracts of cannabis and/or cannabis materials (Category III).

Cannabis-infused nonedibles.—Products containing extracts of cannabis and/or cannabis materials intended to be applied to the human body or any part thereof. Final products can be many forms including creams, ointments, cosmetics and therapeutic pads (Category IV).

Cannabis plant and flower.—General terms for the structural and flowering unadulterated parts of the cannabis plant (Category I).

Cannabis products.—Products (edible and nonedible) extracted or infused with compounds derived from the cannabis plant including but not limited to CBD and THC.

Exclusivity.—Study involving pure nontarget strains, which are potentially cross-reactive, that shall be not detected or enumerated by the candidate method. *See* Table 1 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* (2019) 21st 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. Proportion of positive responses should fall within 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 2 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* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA]

Laboratory probability of detection (LPOD).—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* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA]

LCL.—Lower confidence limit.

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

Salmonella.—Straight rods, 0.7–1.5 × 2–5 µm. Gram negative. Usually motile by peritrichous flagella. Facultative anaerobic. Chemoorganotrophic, having both a respiratory and fermentative metabolism. D-glucose and other carbohydrates are catabolized with the production of acid and usually gas. Oxidase negative, catalase positive, indole and Voges-Proskauer negative, methyl red and Simmons citrate positive. Lysine and ornithine decarboxylase positive, there is a variable arginine dihydrolase reaction. H_2S is produced, urea is not hydrolyzed, and growth on KCN and utilization of malonate are variable. Reduce nitrates. Carbohydrates usually fermented include L-arabinose, maltose, D-mannitol, D-mannose, L-rhamnose, D-sorbitol, trehalose, and D-xylose. Occur in humans, warm- and cold-blooded animals, food, and the environment. Pathogenic for humans and many animal species. Causative agent of typhoid fever, enteric fevers, gastroenteritis, and septicemia. [*Bergey's Manual of Determinative Bacteriology,* 9th Ed., John G. Holt (Ed)]

Test portion.—Sample size used in most validation studies. For cannabis flower/plant and cannabis infused nonedible products, a 10 g test portion is used. For cannabis concentrates, a 5 g test portion is used. For cannabis-infused edibles, a 25 g test portion is

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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.

5 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.

6 Reference Material(s)

The use of live cultures and/or fungal spores (liquid stressed/nonstressed, 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.

7 Validation Guidance

Appendix F: *Guidelines for Standard Method Performance Requirements* [*Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA, http://www.eoma.aoac.org/app_f.pdf]

Appendix J: *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces* [*Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA, http://www.eoma.aoac.org/app_j.pdf]; or ISO 16140-2:2016

Microbiological Examination of Nonsterile Products: Microbial Enumeration Tests (61), USP 40, United States Pharmacopeia, Rockville, MD, USA

Feng, P., Weagant, S.D., Grant, M.A., & Burkhardt, W. (2017) *Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms (62)*, USP 40, United States Pharmacopeia, Rockville, MD, USA

Bacteriological Analytical Manual, Chapter 4 Enumeration of Escherichia coli and the Coliform Bacteria, U.S. Food and Drug Administration, https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm064948.htm

Andrews, W.H., Wang, H., Jacobson, A., & Hammack, T. (2018) *Bacteriological Analytical Manual, Chapter 5 Salmonella,* U.S. Food and Drug Administration, https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070149.htm

At the time of publication, no national reference method exists for confirmation of *Salmonella* spp. from cannabis products. 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 (agar plate, PCR, ELISA) must be used.

To ensure the viability of the inoculating organism (both confirming presumptive results or determining false-negative results), a secondary 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, is required (Table 5). 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., therapeutic patches), individual item inoculation may be required.

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

A minimum 3-level most probable number (MPN) study should be performed to determine the concentration of the target organism used in the validation. If possible, the use of test portions included in the matrix study should be included as a level in the MPN study. *See* AOAC Appendix J guidelines for details on performing the MPN study [Appendix J: *AOAC INTERNATIONAL Methods Committee Guidelines for Validation of Microbiological Methods for Food and Environmental Surfaces, Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA, http://www.eoma.aoac.org/app_j.pdf]

8 Maximum Time-to-Determination

None

9 Method Performance Requirements

See Table 6 for acceptance criteria for validation.

See Table 4 for category test portion requirement.

See Table 7 for matrix claims acceptance criteria.

See Table 8 for descriptions of MPN analysis.

See Table 9 for condition of inoculating culture and stabilization of matrix for inoculation.

See Table 5 for selective broth and agar recommendations.

See Table 10 for inclusivity and exclusivity performance requirements.

See Tables 2 and 3 for inclusivity organisms. The tables list required and suggested subspecies and serovars that method developers can use to validate their methods. It is recommended that method developers reference Centers for Disease Control and Prevention's (CDC) revised *Atlas on Salmonella* (https://www.cdc.gov/salmonella/pdf/salmonella-atlas-508c.pdf) to incorporate as many serovars listed therein as possible. A minimum of 100 serovars are required for AOAC adoption. Additionally, requirements in Table 2 must be met.

See Table 1 for exclusivity organisms.

Approved by stakeholders of the AOAC Cannabis Analytical Science Program (CASP) on April 7, 2020.

Posted: April 28, 2020

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Table 1. Exclusivity panel

Organism	
Aeromonas hydrophila	*Hafnia* species
Additional *Aeromonas* species	*Klebsiella oxytoca*
Burkholderia species	*Klebsiella pneumonia*
Bacillus subtilis	*Listeria monocytogenes*
Campylobacter jejuni	*Morganella morganii*
Candida tropicalis	*Pantoea* species
Citrobacter braakii	*Proteus hauseri*
Citrobacter farmerii	*Proteus mirabilis*
Citrobacter freundii	*Proteus vulgaris*
Citrobacter murliniae	*Pseudomonas aeruginosa*
Citrobacter youngae	*Pseudomonas fluorescens*
Citrobacter species	*Pseudomonas* species
Edwardsiella tarda	*Ralstonia* species
Enterobacter aerogenes	*Rhanella* species
Enterobacter amnigenus	*Serratia marcesens*
Enterobacter cancerogenus	*Shigella dysenteriae*
Enterobacter cloacae	*Shigella flexneri*
Enterobacter gergoviae	*Shigella sonnei*
Enterobacter sakazakii	*Trichoderma harzianum*
Erwinia species	*Yersinia* species
Escherichia coli	*Vibrio vulnificus*
Escherichia coli O157:H7	
Escherichia fergusonii	
Escherichia hermanii	
Escherichia vulneris	

Table 2. Required *Salmonella* subspecies for inclusivity

	Salmonella	Min. No. of strains included[a]
1	*Salmonella bongori*	2
2	*Salmonella enterica* subsp. *arizonae*	3
3	*Salmonella enterica* subsp. *diarizonae*	3
4	*Salmonella enterica* subsp. *houtenae*	3
5	*Salmonella enterica* subsp. *indica*	3
6	*Salmonella enterica* subsp. *salamae*	3
7	*Salmonella enterica* subsp. *enterica*	1 Strain per serovar

[a] Required number of strains per subspecies, per method claims.

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Table 3. Suggested *Salmonella* serovars for inclusivity

		Antigenic properties serotype				
	Salmonella (serovar included)	O	H	Year outbreak	CDC Top 20[a]	FDA ranking Top 40
1	*Salmonella bongori*, Serotype Brookfield	66	z_{41}:-			
2	*Salmonella bongori*	66				
3	*Salmonella enterica* subsp. *Salamae*	47				
4	*Salmonella enterica* subsp. *Salamae*	50				
5	*Salmonella enterica* subsp. *salamae*	53				
6	*Salmonella enterica* subsp. *salamae*	55				
7	*Salmonella enterica* subsp. *salamae serovar Artis*	56				
8	*Salmonella enterica* subsp. *salamae*	57				
9	*Salmonella enterica* subsp. *salamae* serovar *Basel*	58				
10	*Salmonella enterica* subsp. *salamae*	59				
11	*Salmonella enterica* subsp. *salamae*	60				
12	*Salmonella enterica* subsp. *Arizonae*[b]	40				41
13	*Salmonella enterica* subsp. *Arizonae*	51				
14	*Salmonella enterica* subsp. *Arizonae*	62				
15	*Salmonella enterica* subsp. *Arizonae*	63				
16	*Salmonella enterica* subsp. *Arizonae*	65				
17	*Salmonella enterica* subsp. *diarizonae*	35				
18	*Salmonella enterica* subsp. *diarizonae*	47				
19	*Salmonella enterica* subsp. *Diarizonae*[b]	48				29
20	*Salmonella enterica* subsp. *diarizonae* serovar *Eilbek*	61				
21	*Salmonella enterica* subsp. *houtenae* serovar *Halmstad*	3,{10}{15}{15,34}	g,s,t:-			
22	*Salmonella enterica* subsp. *houtenae* serovar *Harmelen*	51				
23	*Salmonella enterica* subsp. *houtenae* serovar *Ochsenzoll*	16				
24	*Salmonella enterica* subsp. *Indica*	1,6,14,25				
25	*Salmonella enterica* subsp. *Indica*	45				
26	*Salmonella enterica* subsp. *enterica* serovar *Paratyphi A*	1,2,12				
27	*Salmonella enterica* subsp. *enterica* serovar *Agona*[b]	1,4,[5],12	f,g,s:[1,2]	11	15	5
28	*Salmonella enterica* subsp. *enterica* serovar *Heidelberg*	1 ,4,[5],12	r:1,2	14, 13, 11	7	
29	*Salmonella enterica* subsp. *enterica* serovar *Paratyphi B*[b]	1,4,[5],12	b:1,2		16	34
30	*Salmonella enterica* subsp. *enterica* serovar *Derby*	1, 4,[5], 12	f,g:[1,2]			
31	*Salmonella enterica* subsp. *enterica Typhimurium*[b]	1,4 ,[5],12	i:1,2	13, 12, 11, 10	2	6
32	*Salmonella enterica* subsp. *enterica* serovar *Saintpaul*[b]	1,4,[5],12	e,h:1,2	13	12	15
33	*Salmonella enterica* subsp. *enterica* serovar *Sandiego*[b]	1,4,[5],12	e,h:e,n,z_{15}	13		24
34	*Salmonella enterica* subsp. *enterica I 4,[5],12:i:-*	1,4,[5],12	i:-	10	5	
35	*Salmonella enterica* subsp. *enterica Chester*	1,4,[5],12	e,h:e,n,x	10		
36	*Salmonella enterica* subsp. *enterica Stanley*[c]	1,4,[5],12,[27]	d:1,2	14		31
37	*Salmonella enterica* subsp. *enterica* serovar *Indiana*	1,4,12	z;1,7			
38	*Salmonella enterica* subsp. *enterica* serovar *Preston*	1,4,12	z:l,w			
39	*Salmonella enterica* subsp. *enterica* serovar *Bredeney*	1,4,12,27	l,v:1,7	12		

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Table 3. (*continued*)

	Salmonella (serovar included)	Antigenic properties serotype		Year outbreak	CDC Top 20[a]	FDA ranking Top 40
		O	H			
40	*Salmonella enterica* subsp. *enterica Vellore*	1,4,12,27	z_{10}:z_{35}			
41	*Salmonella enterica* subsp. *enterica* serovar *Schwarzengrund*	1 ,4 ,12, 27	d:1,7			
42	*Salmonella enterica* subsp. *enterica* serovar *Abortusequi*	4,12	-:e,n,x			
43	*Salmonella enterica* subsp. *enterica* serovar *Abortusovis*	4,12	c:1,6			
44	*Salmonella enterica* subsp. *enterica* serovar *Choleraesuis*	6, 7	c:1,5			
45	*Salmonella enterica* subsp. *enterica Hartford*	6, 7	y:e,n,x	10		
46	*Salmonella enterica* subsp. *enterica Braenderup*[b]	6 , 7,14	This serovar is now recognized as Westhampton var 15+	12	10	40
47	*Salmonella enterica* subsp. *enterica* serovar *Bareilly*[b]	6,7,14	y:e,n,x	12	17	14
48	*Salmonella enterica* subsp. *enterica* serovar *Infantis*[b]	6,7,14	r:1,5	12	9	28
49	*Salmonella enterica* subsp. *enterica* serovar *Lille*	6,7,14	z_{38}:-	13,12		
50	*Salmonella enterica* subsp. *enterica* serovar *Mbandaka*[b]	6, 7, 14	z_{10}:e,n,z_{15}	13		2
51	*Salmonella enterica* subsp. *enterica* serovar *Oranienburg*[b]	6, 7, 14	m,t:[z_{57}]		11	10
52	*Salmonella enterica* subsp. *enterica* serovar *Thompson*[b]	6, 7, 14	K:1,5		14	19
53	*Salmonella enterica* subsp. *enterica* serovar *Montevideo*[b]	6 , 7,14,[54]	g,m,[p],s: [1,2,7]	13,12, 10	6	4
54	*Salmonella enterica* subsp. *enterica* serovar *Hadar*	6,8	z_{10}:e,n,x	12, 11		
55	*Salmonella enterica* subsp. *enterica* serovar *Muenchen*[b]	6, 8	d:1,2		8	12
56	*Salmonella enterica* subsp. *enterica* serovar *Newport*[b]	6, 8, 20	e,h:1,2	13,12, 10	3	1
57	*Salmonella enterica* subsp. *enterica* serovar *Haardt*	8	k:1,5			
58	*Salmonella enterica* subsp. *enterica* serovar *Kentucky*[b]	8, 20	l,z_6			18
59	*Salmonella enterica* subsp. *enterica Panama*[b]	1,9,12	l,v:1,5	11		39
60	*Salmonella enterica* subsp. *enterica* serovar *Berta*	1,9,12	[f],g,[t]:-		19	
61	*Salmonella enterica* subsp. *enterica* serovar *Enteritidis*[b]	1,9,12	g,m:-	12, 11, 10	1	17
62	*Salmonella enterica* subsp. *enterica* serovar *Gallinarum*	1,9,12	-:-			
63	*Salmonella enterica* subsp. *enterica* serovar *Javiana*[b]	1,9,12	l,z_{28}:1,5		4	13
64	*Salmonella enterica* subsp. *enterica* serovar *Neasden*	9,12	g,s,t:e,n,x			
65	*Salmonella enterica* subsp. *enterica* serovar *Typhi*	9,12[Vi]	d:-	10	18	
66	*Salmonella enterica* subsp. *enterica Baildon*	9,46	a:e,n,x	10		
67	*Salmonella enterica* subsp. *enterica* serovar *Anatum*[b]	3,{10}{15}{15,34}	e,h:1,6		20	8
68	*Salmonella enterica* subsp. *enterica* serovar *Anatum var. 15+*	3,{10}{15}	g,m,s:-			
69	*Salmonella enterica* subsp. *enterica* serovar *Give*[b]	3,{10}{15}{15,34}	l,v:1,7			11
70	*Salmonella enterica* subsp. *enterica Nchanga*	3 ,{10}{15}	l,v:1,2	12		
71	*Salmonella enterica* subsp. *enterica* serovar *Krefeld*	1,3,19	y;l,w			
72	*Salmonella enterica* subsp. *enterica* serovar *Senftenberg*[b]	1 ,3,19	g,[s],t:-			3
73	*Salmonella enterica* subsp. *enterica* serovar *Abaetetuba*[b]	11	k:1,5			38
74	*Salmonella enterica* subsp. *enterica* serovar *Poona*[b]	1,13,22	z:1,6	13		21
75	*Salmonella enterica* subsp. *enterica Cubana*[b]	1 ,13,23	z_{29}:-			27
76	*Salmonella enterica* subsp. *enterica Mississippi*	1 ,13,23	b:1,5		11	
77	*Salmonella enterica* subsp. *enterica* serovar *Bristol*	13,22	z:1,7			
78	*Salmonella enterica* subsp. *enterica* serovar *Putten*	13,23	d:l,w			

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Table 3. (*continued*)

	Salmonella (serovar included)	Antigenic properties serotype		Year outbreak	CDC Top 20[a]	FDA ranking Top 40
		O	H			
79	*Salmonella enterica* subsp. *enterica* serovar *Kaitaan*	1,6,14,25	m,t:-			
80	*Salmonella enterica* subsp. *enterica* serovar *Schalkwijk*	6 ,14,[24]	i:e,n,z_{15}			
81	*Salmonella enterica* subsp. *enterica* serovar *Sundsvall*	[1], 6,14,[25]	z:e.n.x			
82	*Salmonella enterica* subsp. *enterica* serovar *Nottingham*	16	d:e,n,z_{15}			
83	*Salmonella enterica* subsp. *enterica* serovar *Matadi*	17	k:e,n,x			
84	*Salmonella enterica* subsp. *enterica* serovar *Cerro*	6,14,18	z_4,z_{23}:[1,5]			
85	*Salmonella enterica* subsp. *enterica* serovar *Minnesota*	21	b:e,n,x			
86	*Salmonella enterica* subsp. *enterica Pomona*[b]	28	y:1,7	13		37
87	*Salmonella enterica* subsp. *enterica Urbana*[b]	30	b:e,n,x			33
88	*Salmonella enterica* subsp. *enterica* serovar *Adelaide*	35	f,g:-			
89	*Salmonella enterica* subsp. *enterica* serovar *Inverness*	38	k:1,6			
90	*Salmonella enterica* subsp. *enterica* serovar *Champaign*	39	k:1,5			
91	*Salmonella enterica* subsp. *enterica* serovar *Johannesburg*	1,40	b:e,n,x	11		
92	*Salmonella enterica* subsp. *enterica* serovar *Waycross*	41	z_4,z_{23}:[e,n,z_{15}]			
93	*Salmonella enterica* subsp. *enterica* serovar *Kahla*	1, 42	z_{35}:1,6			
94	*Salmonella enterica* subsp. *enterica* serovar *Houten*	43	z_4,z_{23}:-			
95	*Salmonella enterica* subsp. *enterica* serovar *Niarembe*	44	a:l,w			
96	*Salmonella enterica* subsp. *enterica* serovar *Deversoir*	45	c:e,n,x			
97	*Salmonella enterica* subsp. *enterica* serovar *Dahlem*	48	k:e,n,z_{15}			
98	*Salmonella enterica* subsp. *enterica* serovar *Wassenaar*	50				
99	*Salmonella enterica* subsp. *enterica Utrecht*	52	d:1,5			
100	*Salmonella enterica* subsp. *enterica* serovar *Uccle*	54	g,s,t:-			
101	*Salmonella enterica* subsp. *enterica Tranora*	55	k:z_{39}			
102	*Salmonella enterica* subsp. *enterica* serovar *Crossness*	67	r:1,2			
103	*Salmonella enterica* subsp. *enterica* serovar *Weltevreden*[b]	3 , {10}{15}	r:z_6			7
104	*Salmonella enterica* subsp. *enterica* serovar *Tennessee*[b]	6,7,14	z_{29}:[1,2,7]			9
105	*Salmonella enterica* subsp. *enterica* serovar *Rubislaw*[b]	11	r:e,n,x			16
106	*Salmonella enterica* subsp. *enterica* serovar *Virchow*[b]	6,7,14	r:1,2			20
107	*Salmonella enterica* subsp. *enterica* serovar *Hvittingfoss*[b]	16	b:e,n,x			22
108	*Salmonella enterica* subsp. *enterica* serovar *Gaminara*[b]	16	d:1,7			23
109	*Salmonella enterica* subsp. *enterica* serovar *Aberdeen*[b]	11	i:1,2			25
110	*Salmonella enterica* subsp. *enterica* serovar *Mgulani*	38	i:1,2			26
111	*Salmonella enterica* subsp. *enterica* serovar *Havana*[b]	1,13,23	f,g,[s]:-			30
112	*Salmonella enterica* subsp. *enterica* serovar *Wandsworth*[b]	39	b:1,2			32
113	*Salmonella enterica* subsp. *enterica* serovar *Caracas*[b]	[1],6,14,[25]	g,m,s:-			35
114	*Salmonella enterica* subsp. *enterica* serovar *Rissen*[b]	6,7,14	f,g:-			36
115	*Salmonella enterica* subsp. *enterica* serovar *Michigan*[b]	17	l,v:1,2			42
116	*Salmonella enterica* subsp. *enterica* serovar *Meleagridis*[b]	3,{10}{15}{15,34}	e,h;l,w			43

[a] Number indicates numerical position on Centers for Disease Control and Prevention (CDC) list.

[b] Serotypes isolated from vegetable products, including spices. Courtesy of U.S. Food and Drug Administration-Center for Food Safety and Applied Nutrition (FDA-CFSAN).

[c] Strains identified from outbreaks, shorten dates as (2011 = “11”; 2012 = “12).

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Table 4. Category test portion requirements

Category	Minimum test portion size, g[a]
Plants and flowers	10
Concentrates	5
Infused edibles	25
Infused nonedibles	10

[a] Minimum test portion size required for validation. Alternatively, larger test portions may be validated.

Table 5. Recommended secondary selective broths and agar

Media name	Media type
Rappaport-Vassiliadis (RV) (alternately Rappaport-Vassiliadis R10)	Broth
Tetrathionate (TT)	Broth
Selenite cysteine (SC)	Broth
Xylose lysine desoxycholate (XLD)	Agar
Hektoen enteric (HE)	Agar
Bismuth sulfite (BS)	Agar
Chromogenic *Salmonella*	Agar
MacConkey	Agar

Table 6. Validation acceptance criteria (plants/flowers, concentrates, infused edibles, infused nonedibles)

Parameter	Requirement	Target test concn[a]	Minimum acceptable results
Single-laboratory validation (SLV) with artificial contamination			
Fractional concentration (low level)	Replicates per matrix: 20 Inoculation procedure: AOAC Appendix J	Low level to produce fractional positive results Ex. 0.2–2 CFU/test portion	Fractional positive results, 25–75% (5–15 positive test replicates) $dPOD_{CP}$ 95% CI: LCL < 0 < UCL[b]
High concentration	Replicates: 5 Inoculation procedure: AOAC Appendix J	High level to produce consistently positive results Ex. 2–10 CFU/test portion	POD of 1.00[c]
Noninoculated (zero) concentration	Replicates: 5	0 CFU/test portion	POD of 0.00[c]
SLV with natural contamination			
Acceptable minimum detection level (low level)	2 Separate lots of 20 replicates	NA	Fractional positive results, 25–75% (5–15 positive test replicates) for minimum 1 lot $dPOD_{CP}$ 95% CI: LCL < 0 < UCL[b]
Multilaboratory validation			
LPOD	Replicates: 12	1–10 CFU/test portion	0.15 ≥ LPOD ≥0.85 $dPOD_{CP}$ 95% CI: LCL < 0 < UCL[b]
	Replicates: 12	10–50 CFU/test portion	LPOD ≥0.95
$LPOD_{(0)}$	Replicates: 12	0 CFU/test portion	LPOD ≤0.05

[a] Determined through MPN procedures (*see* Table 8).

[b] Range between lower and upper confidence interval should encompass 0. If not, results must be investigated and an explanation provided.

[c] If acceptance criteria is not observed, results must be investigated and an explanation provided.

Table 7. Acceptable matrix claims

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

AOAC Technical Bulletin: TB02MAY2016: *Acceptable Validation Claims for Proprietary/Commercial Microbiology Methods for Foods and Environmental Surfaces*.

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Table 8. Minimum most probable number (MPN) recommendation

Category	Inoculation level	Test portions, g		
		Large	Medium	Small
Plants and flowers concentrates	Low	20 × 10[a]	3 × 5	3 × 1
	High	5 × 10[a]	3 × 5	3 × 1
Concentrates	Low	20 × 5	3 × 2.5	3 × 1
	High	5 × 5[a]	3 × 2.5	3 × 1
Infused edibles	Low	20 × 25[a]	3 × 10	3 × 5
	High	5 × 25[a]	3 × 10	3 × 5
Infused nonedibles	Low	20 × 10[a]	3 × 5	3 × 1
	High	5 × 10[a]	3 × 5	3 × 1

[a] Test portions from matrix study.

Table 9. Condition of inoculating culture and stabilization of matrix

Matrix	Inoculating cells	Stabilization conditions
Perishable product	Liquid nonstressed culture	4°C, 48–72 h
Heat-processed perishable product	Liquid heat stressed	4°C, 48–72 h
Frozen product	Liquid nonstressed 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 nonstressed culture (if shelf stable product is processed, cells must be heat stressed)	Ambient temperature (20–25°C), 2 weeks

Table 10. Inclusivity/exclusivity performance requirements

Parameter	Requirement	Final test concn, CFU/mL	Minimum acceptable results
Inclusivity	Single-laboratory validation (SLV) study: Minimum of 100 strains is required to be cultured by candidate method enrichment procedure (including those detailed in Table 2)	10–100 × LOD of candidate method	100% positive results[a]
Exclusivity	SLV study: At least 30 nontarget organisms, cultured under optimal conditions for growth[b]	Overnight growth undiluted	100% negative results[a]

[a] 100% correct analyses are expected. All unexpected results are to be retested following internationally recognized guidelines (ISO 16140, AOAC OMA Appendix J, or *The Compendium of Analytical Methods of Health Canada*). 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.

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