AOAC SMPR® 2019.001

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

Intended Use: Consensus-Based Reference Method

1 Purpose

AOAC SMPRs describe the minimum recommended performance characteristics 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 and adopted by AOAC composed of representatives from industry, regulatory organizations, contract laboratories, test kit manufacturers, and academic institutions. AOAC SMPRs are used by AOAC expert review panels in their evaluation of validation study data for methods being considered for *Performance Tested Methods*SM or AOAC *Official Methods of Analysis*SM and can be used as acceptance criteria for verification at user laboratories. [Refer to Appendix F: *Guidelines for Standard Method Performance Requirements*, *Official Methods of Analysis of AOAC INTERNATIONAL* (2019) 21st Ed., AOAC INTERNATIONAL, Rockville, MD, USA.]

2 Applicability

Candidate methods used to detect *Aspergillus* (*Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus terreus*) in cannabis (plants/flowers) and/or cannabis products (concentrates, infused edibles, and infused nonedibles). Candidate methods may be validated for specific matrices, categories or broader claims. *See* Table 1 for matrix/category claim acceptance criteria.

3 Analytical Technique

Any analytical technique that meets the method performance requirements is acceptable.

4 Definitions

Aspergillus.—Filamentous, cosmopolitan, and ubiquitous fungus found in nature producing colonies typically of 1–9 cm in size (select species produce 0.5–1 cm colonies). Colonies are powdery in texture and color varies based on species. Reverse color is typically uncolored to pale yellow. Growth is typical at 20–30°C. *Aspergillus fumigatus* is thermotolerant and can grow at a temperature range of 20 to 50°C. For all species, hyphae are septate and hyaline. The conidiophores originate from the basal foot cell located on the supporting hyphae and terminate in a vesicle at the apex. Vesicle is the typical formation for the genus *Aspergillus*. The morphology and color of the conidiophore vary from one species to another. Covering the surface of the vesicle entirely (“radiate” head) or partially only at the upper surface (“columnar” head) are the flask-shaped phialides, which are either uniseriate and attached to the vesicle directly or are biseriate and attached to the vesicle via a supporting cell, metula. Over the phialides are the round conidia (2–5 µm in diameter) forming radial chains. Other microscopic structures include sclerotia, cleistothecia, aleuriconidia, and Hulle cells are of key importance in identification of some *Aspergillus* species. Cleistothecium is a round, closed structure enclosing the asci which carry the ascospores. The asci are spread to the surrounding when the cleistothecium bursts. Cleistothecium is produced during the sexual reproduction stage of some *Aspergillus* species. Aleuriconidium is a type of conidium produced by lysis of the cell that supports it. The base is usually truncate and carries remnants of the lysed supporting cell. These remnants form annular frills at its base. Hulle cell is a large sterile cell bearing a small lumen. Similar to cleistothecium, it is associated with the sexual stage of some *Aspergillus species*. *See* Tables 2 and 3 for more macroscopic and microscopic information on *Aspergillus* species.

Chen, S.C.A., Meyer, W., Sorrell, T.C., & Halliday, C.L. (2019) *Manual of Clinical Microbiology*, 12th Ed., Landry, M.L., McAdam, A.J., Patel, R., & Richter, S.S. (Eds) ASM Press, Washington, DC, USA, pp 2103–2131

Anaissie, E.J., McGinnis, M.R., & Pfaller, M.A. (2009) *Clinical Mycology*, 2nd Ed., Churchill Livingstone, New York, NY, USA, 687 pp

Walsh, T.J., Hayden, R.T., & Larone, D.H. (2018) *Larones Medically Important Fungi: A Guide to Identification*, 6th Ed, ASM Press, Washington, DC, USA, 500 pp

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 4 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]

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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 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* Table 5 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. The difference in POD values between presumptive and confirmed results is termed $dPOD_{CP}$.

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 used. A larger test portion can be used in validation studies when appropriate. *See* Table 6 for minimum test portion requirements.

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

Microbiological Examination of Nonsterile Products: Tests for Specified Microorganisms (62), USP 40, United States Pharmacopeia, Rockville, MD, USA

Feng, P., Weagant, S.D., Grant, M.A., & Burkhardt, W. (2017) *Bacteriological Analytical Manual*, Ch. 4 Enumeration of *Escherichia coli* and the Coliform Bacteria, https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm064948.htm

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

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)

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 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]; or ISO 16140-2:2016

At the time of the publication, no national reference method exists for the confirmation of *Aspergillus* from cannabis products. Until a suitable reference method is established, the following is recommended for method developers:

(*1*) 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.

(*2*) To ensure the viability of the inoculating organism (both confirming presumptive results or determining false-negative results), an extended primary enrichment (up to at least 48 total hours) followed by plating of the sample to a minimum of two types of agar plates [examples: Dichloran rose bengal chloramphenicol (DRBC), Sabouraud dextrose (SAB-DEX), potato dextrose agar (PDA), Czapek's] is required. Final confirmation can be achieved via matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectroscopy, sequencing, or other suitable confirmatory procedures (microscopic examination, biochemical analysis, etc).

(*3*) When performing the validation, bulk inoculation of test material is required. In certain instances (for example, therapeutic patches), individual item inoculation may be required.

(*4*) 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 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 nontarget organism (s) is required.

(*5*) A minimum three-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* Appendix J guidelines for details on performing the MPN study.

8 Method Performance Requirements

See Table 7 for acceptance criteria for validation.

See Table 6 for category test portion requirement.

See Table 1 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 10 for inclusivity and exclusivity guidance.

Approved by attending stakeholders of the AOAC Cannabis Analytical Science Program (CASP) meeting on September 7, 2019. Final Version Date: October 3, 2019.

Posted: October 9, 2019

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Table 1. Acceptable matrix claims[a]

Matrix claim	Criteria	
	No. matrices	Minimum No. 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(es)	≥1	1

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

Table 2. Colony color in various *Aspergillus* species

Species	Surface	Reverse
A. clavatus	Blue-green	White, brownish with age
A. flavus	Yellow-green	Goldish to red brown
A. fumigatus	Blue-green to gray	White to tan
A. glaucus group	Green with yellow areas	Yellowish to brown
A. nidulans	Green, buff to yellow	Purplish red to olive
A. niger	Black	White to yellow
A. terreus	Cinnamon to brown	White to brown
A. versicolor	White at the beginning, turns to yellow, tan, pale green or pink	White to yellow or purplish red

Table 3. Microscopic features of various *Aspergillus* species

Species	Conidiophore	Phialides	Vesicle	S[a]	C[a]	HC[a]	A[a]
A. clavatus	Long, smooth	Uniseriate	Huge, clavate-shaped	–	–	–	–
A. flavus	Colorless, rough	Uni-/biseriate	Round, radiate head	+ (in some strains, brown)	–	–	–
A. fumigatus	Short (<300 μm), smooth, colorless or greenish	Uniseriate	Round, columnar head	–	–	–	–
A. glaucus group	Variable length, smooth, colorless	Uniseriate	Round, radiate to very loosely columnar head	–	+ (yellow -orange)	–	–
A. nidulans	Short (<250 μm), smooth, brown	Biseriate, short	Round, columnar head	–	+ (red)	+	–
A. niger	Long, smooth, colorless or brown	Biseriate	Round, radiate head	–	–	–	–
A. terreus	Short (<250 μm), smooth, colorless	Biseriate	Round, compactly columnar head	–	–	–	+ (solitary, round, produced directly on hyphae)
A. versicolor	Long, smooth, colorless	Biseriate	Round, loosely radiate head	–	–	+ (in some strains)	–

[a] S = Sclerotia; C = Cleistothecia; HC = Hulle cells; A = Aleuriconidia.

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Table 4. *Aspergillus* exclusivity panel[a]

Organism	Reference ID (where applicable)
Acinetobacter baumanii	
Alternia alternata	
Aspergillus aculeatus	
Aspergillus alabamensis	
Aspergillus brasiliensis Varga et al.	ATCC 9642[b]
Aspergillus caesiellus	
Aspergillus carbonarius	
Aspergillus carneus	
Aspergillus clavatus	
Aspergillus deflectus	
Aspergillus fijiensis Varga et al.	ATCC 20611[b]
Aspergillus fischeri	
Aspergillus glaucus	
Aspergillus janponicus	
Aspergillus nidulans	
Aspergillus oryzae (Ahlburg) Cohn	ATCC 10124[b]
Aspergillus parasiticus Speare	ATCC 15517[b]
Aspergillus pseudoterreus Peterson et al.	ATCC 10020[b]
Aspergillus steynii	
Aspergillus tamarii	
Aspergillus tubingensis (Schober) Mosseray	ATCC 1004[b]
Aspergillus tubingensis (Schober) Mosseray	ATCC 10550[b]
Aspergillus ustus	
Aspergillus versicolor	
Botrytis cinerea Persoon	
Candida albicans	
Cryptococcus laurentii	
Cryptococcus neoformans	
Fusarium proliferatum	
Fusarium oxysporum	
Fusarium solani	
Golovinomyces cichoracearum	
Mucor circinelloides	
Mucor hiemalis	
Penicillium chrysogenum	
Penicillium rubens	
Penicillium venetum	
Pseudomonas aeruginosa	
Rhizopus nigrans	
Rhizopus stolonifer	
Scopulariopsis acremonium	
Yarrowia lipolytica	

[a] List of suggested organisms method developers can use to validate their methods. A minimum of 30 nontarget organisms are required for AOAC adoption. Organisms utilized should be well characterized and information provided must include source, strain numbers and origin (if available)

[b] Genus/species listed is required. Recommended strains were previously misidentified as one of the four target strains and are strongly encouraged for inclusion. Alternative strains of the same genus/species may be substituted.

Table 5. *Aspergillus* inclusivity panel[a]

Aspergillus spp.	Minimum No. strains
Aspergillus niger	10
Aspergillus flavus	10
Aspergillus fumigatus	10
Aspergillus terreus	10

[a] Required species method developers must use to validate their methods. A minimum of 50 total strains, including 10 strains of each of the species are required for AOAC adoption. Strains utilized should be well-characterized and information provided must include source, strain, numbers and origin (if available).

Table 6. 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.

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Table 7. Validation acceptance criteria (plants/flowers, concentrates, infused edibles, infused nonedibles)

Parameter	Parameter requirements	Target test concentration[a]	Minimum acceptable results
Single-laboratory validation (SLV) with artificial contamination			
Fractional concn (low level)	Replicates per matrix: 20 Inoculation procedure: AOAC Appendix J	Low level to produce fractional positive results (example: 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 concn	Replicates: 5 Inoculation procedure: AOAC Appendix J	High level to produce consistently positive results (example: 2–10 CFU/test portion)	POD of 1.00[c]
Noninoculated (zero) concn	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	N/A	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	12	1–10 CFU/test portion	0.15 ≥ LPOD ≥ 0.85 $dPOD_{CP}$ 95% CI: LCL < 0 < UCL[b]
	12	10–50 CFU/test portion	LPOD ≥ 0.95
$LPOD_{(0)}$	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 8. Minimum most probable number (MPN) recommendation

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

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Table 10. Inclusivity/exclusivity performance requirements

Parameter	Parameter requirements	Final test concn, CFU/mL	Minimum acceptable results
Inclusivity	Single-laboratory validation (SLV) study: At least 10 strains per required *Aspergillus* spp. (Table 5) cultured by the candidate method enrichment procedure. A minimum of 50 strains is required.	10–100 x limit of detection of the candidate method	100% positive results[a]
Exclusivity	SLV study: At least 30 nontarget organisms (including those required in Table 4), 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, and 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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