Public Comments (Nov. 17 - Dec. 18, 2023) in response to the draft SMPR® for Detection and/or Enumeration of Listeria Monocytogenes in Cannabis Infused Edibles.

Comments	Reconciliation/Response
1. a. Line 123: 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. Q: Do these need to certified by AOAC already?	**1.** **a. Historically no.**
b. Line 129: ...results or determining false negative results) IN THE MATRIX a secondary and/or extended enrichment... Q: add capitalized text	**b. Emphasize according to AOAC's editorial guidelines**
c. Line 131: 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 6). Q: reference where formulas for media are contained	**c. add (FDA/BAM, etc)**
d. Line 139: For the Single Laboratory Validation with artificial contamination, matrix naturally contaminated with non-target organisms (when available) shall be used. Q: do background counts need to be determined?	**d. typically yes, listed in Appendix J (Constance to double check the reference is listed)**
e. Tables 1 & 2: Multi Laboratory validation study Q: List how many valid sets of data need to be submitted for ILS	**e. Appendix J has this recommendation – section after table 2. Reference to number of labs that participate.** ***table 2, make typo correction, low med and high in 1st column, do not repeat low.** **Per Appendix J, 12 labs per matrix are recommended. At least 10 valid data sets from 10 different laboratories must be included. A minimum number of data points is defined in Tables 1 and 2.**

f. Line 237: For beverages and tinctures, should this be 25 mL as opposed to 25 g for minimum test portion size? g. Line 3: Is there no validations allowed for Listeria spp. methods?	**f. Leave at 25 g (for consistency of dilution amounts).** **g. Decided to be specific for L-mono**
2. **a.** Table 2 on line 234 seems to have a copy and paste error in the validation material, as it goes from low to medium to low. Additionally, please double-check the target contamination levels for the same table, as other CASP SMPRs have specified lower 'low' contamination levels. **b.** Lines 119-121: This paragraph has sparked several discussions throughout the CASP and microbiology community as a whole. On one end of the spectrum, all other CASP SMPRs utilize the same method flow-through for the confirmation of the target microorganism. On the other hand, does the infusion of cannabinoids make a significant change in the matrix to justify a new method of analysis, or can food methods of confirmation be used while following ISO or BAM protocols? At the end of the day, I do not believe we have any published data stating that matrix types are different or similar with the addition of cannabis. So, by following precedent, we call them two different matrices. I would urge method developers and validation experts to utilize methods of confirmation from food alongside the traditional CASP micro confirmations to see if there would be any change in results.	**2.** **a. A low to med to low (should be high). Additionally, a statement was added to the footer in Table 2 to state, "Target contamination levels may be dependent on method developer requirements"** **b. Could be helpful to compare some of the food methods to see if they're comparable to cannabis/casp methods – more from a data standpoint. Does not need to be addressed in this SMPR just a point to think more on.** **ISO requirements up to the individual labs based on their goals/needs.**
3. a. Table 2. Validation Acceptance Criteria – Enumeration methods States "Low contamination level" for 1,000–	**3.** **a. same as editorial**

10,000 cfu/g (mL)- Is this correct?	
b. Table 3. Test Portion Requirement B1) Is 25g of Tinctures appropriate? B2) Is 25g of Capsules appropriate? B2.1) Is this before or after encapsulation? B3) Some products are tested with the concentrate, then added to the capsules (not tested in final form). How is concentrate to be tested? B3.1) Would a 1:10 ratio be appropriate or is there a risk of inhibition?	**b. (B2) Align with previous recommendations/ guidelines for capsules** **(B2.1) After encapsulation – final product** **(B3) Application of the SMPR is for the final product** **(B3.1) would be evaluated in the validation study**
c. Line 123&124 only mentions agar plate, PCR, and ELISA. C1) Is the term "PCR" an inclusive term? There are many types of PCR (e.g., qPCR, conventional PCR, dPCR, etc.) and because some regulators interpret these terms "literally," it may cause debate or restrictive if someone believes you can only use PCR vs. qPCR, dPCR, etc. C1.1) Would using the phrase "PCR methodology" or (something to that effect) demonstrate an allowance of the application of techniques such as qPCR, dPCR, and sequencing? C1.2) Is the LAMP method allowed? (This is not a "PCR" technique) C.2) Lines 123&124 do not explicitly say what order the agar plate and PCR/ELISA methods have to be used to perform screening. This ambiguity may allow for misinterpretation. For example, would screening with qPCR, then confirming with agar plating and subsequent biochemical analysis meet this guidance?	**c. (C1/.1/.2) Add in (e.g, etc.)**
d. Would it be beneficial to include/address the need for a policy that indicates how "positive" results may be interpreted when screened with molecular methods? As in there is a difference between a positive result	**d. SMPR is not meant to set policy – up to regulatory jurisdiction. How positive results could be interpreted could be included in future guidance doc's**

with a molecular method when the genomic material is present vs. being viable. This phenomenon has a dramatic impact on if products are recommended/required to be green wasted. The policy doesn't have to be outlined, but would it behoove the validation process to have the need for a policy addressed.	
e. Lines 146&147 mention the use of MPN to determine the concentration of the target organism. Are other methods allowed that can also determine the concentration?	**e. could use an additional method to compare but need the MPN for statistical analysis**
f. In terms of inclusivity/exclusivity panels, would it be helpful to consider the feasibility of using that many organisms? F1) How are laboratories supposed to acquire this many organisms without the burden of cost? Many of these CRMs cost between 200-500+ and acquiring them from other labs/institutions does not ensure traceability of the CRM.	**f. WG did a lot of background research on this (inclusivity), exclusivity there is a list of required (9) and others listed are recommended but not required** **# of organisms it has been industry standard in Appendix J and other SMPRs.** **Inclusivity/exclusivity is meant to be performed outside of the matrix study.** **Should verify some of the representative strains, do not need to repeat the inclusivity/exclusivity test**
4. a. Line 119: The proposed SMPR for the detection of L monocytogenes in cannabis-infused edibles does not seem intricate enough given the nature and pathogenicity of the analyte being tested. We have seen (in recent Aspergillus testing of cannabis) that having only a culture confirmation validation scheme is not sufficient to evaluate methods (increased false negatives). It is recommended that although there are no specific federal guidelines for a reference method for cannabis, the general L mono guidelines (for individual subsample analysis) as outlined in the FDA BAM Chapter 10:Detection of Listeria monocytogenes in Foods and Environmental Samples, and	**4.** **a. N/A. Will continued to be discussed in future WG's**

Enumeration of Listeria monocytogenes in Foods (April 2022) be used as a reference method template for validation studies of L mono in cannabis-infused edibles. b. Line 128: We also recommend that an option to use suitable (certified) molecular-based methods (Eg. PCR) be allowed for confirmation of the secondary/extended enrichment and final confirmation	**b. Meant to ensure viability of organism, it does allow for molecular-based methods.** - **consider for validation guidance**
5. Table 10 Requirement of all non-Listeria monocytogenes strains for exclusivity testing as listed in table 10 does not align with current microbial guidance from AOAC, includes strains that have not been shown to be found prevalent in the environment, nor been shown to correlate to the presence of Listeria monotycogenes. In addition, some of these strains are only found in a single repositor and it is cost prohibitive (both a license is required in addition to purchasing the strains) to technology providers. Recommendation is to require the 5 common non-L. mono strains to be included as required (innocua, ivanovii, grayii, seelgeri, welshmeri) and make the remaining strains of Listeria listed as recommended.	**5. if only available from 1 source could cause issue with supply/demand or businesses closing… look at other AOAC guidance to confirm before making the change (Table 10, could add in footnote required/recommended as available). A footnote was added to the five common Listeria species (innocua, ivanovii, grayii, seelgeri, welshmeri) stating that they are required for inclusion in the exclusivity study.**
6. Line 119: A cultural reference procedure should be included for the matrix study as a method comparison, as is required in OMA Appendix J. Table 1: Footnote "c" should be added, "If acceptance criteria are not observed, results must be investigated and an explanation provided."	**6. Compare with what's written in App. J. to ensure it's aligned. Cross reference with guidance doc.** **Table1: align with previous SMPRs (that statement is true)** **Earlier in Table 6 should be Table 7**
7. I agree with the need to create a consensus reference method. Edibles are processed as food and could pose even greater food safety risk as many edible producers are not as experienced in food production.	**7. N/A**
8.	**8.**

a. Since cannabis edibles are food, I would request the use of the full FDA BAM, FSIS MLG, and/or ISO methods for Listeria monocytogenes as a reference method as we normally do for method validations used for any other foods-right now there is no use of a reference method in this or the other microbiological SMPRs for cannabis edibles. Without this, the limit of detection or full detection capability of the methods cannot be determined. Additionally, if and when cannabis is federally regulated, the methods evaluated under these SMPRs may not fulfill the criteria needed. The use of a 25 g sample size was a step in the right direction, but the method validation will still fall short-after a few years experience with the methods being validated for these products, the time has come to work towards a more robust validation. b. Line 264-Table 10-change "Non-monocytogenes Listeria species listed are required." to "Listeria species listed are required."	**a. Table for future discussions – would be good to have more data on this** **b. Change editorially. (may only be requiring the 5)**
9. a. Lines 138-140 are confusing. Please clarify. b. When using MALDI-TOF, ensure that the MALDI-TOF method has been validated for the confirmation of Listeria monocytogenes from the agars used in the validation study. c. Lines 146-149. MPNs are only needed for binary methods, not for validation of enumeration methods. Please clarify. d. I suggest including a full reference or consensus method for comparison for both qualitative and enumeration methods. Since these are edibles, a standard food micro method should be used starting with the reference method primary enrichment (qualitative) or specified diluent (quantitative).	**9.** **a. It is most beneficial to use matrix sample in the validation sample that is closest to a routine sample – not always available. Reference App.J** **b. Needs to be verified with App.J.** **c. Table this for larger discussion**
10. a. Line 53 - If running an MPN, the results should be reported as MPN/g, mL, cm^2, etc.	**10.** **a. Check with other SMPR's for consistency of definition**

Enumeration definition should include MPN and CFU for LM. b. Line 125 - The use of chromogenic agar should be included as an option for 2 or more confirmatory methods. For example, enrichments could be streaked to a chromogenic agar and typical colonies can be picked for an additional confirmation like biochemical, molecular, etc. Chromogenic agar should not be grouped with traditional agar plates as these have more selectivity and specificity than tradition culture agars. Plus, the colony colors can serve as an identifier of typical LM colonies unlike agars like OXA, MOX, PALCAM. c. Line 251 - Table 7 lists Modified Fraser broth. This should be changed to Half Fraser (Demi-Fraser) Broth	**b. See line 128. Currently consistent with other SMPRs.** **c. Update was made to Demi-Fraser broth with wording to match FDA. Additionally, Fraser broth was added to align with FDA recommendations and nomenclature.**
11. If a candidate method receives approval, and the manufacturer has done all of the inclusivitiy/exclusivity studies, does a single laboratory seeking to implement this method need to repeat those studies? Table 8, referencing a single-laboratory validation study, suggests that an individual user laboratory wanting to implement this method would need to repeat the experiment with 80 target and non-target strains. This would be beyond the capability of many labs. Can they rely on the manufacturer's data to meet this criteria:?	**11. SLV is in reference to the lab that is performing the validation (method developer). Often the requirement is a verification.**
12. Flowchart showing the process for detection and enumeration of Listeria monocytogenes and other Listeria species must be included for more clarity My Recommendations a. Water activity can be used as a marker for overall microbial levels:	**13.** **a. Add a workflow diagram to provide clarity** **b. Recommendations for general testing regulations for multiple matrices of cannabis, but does not fall under the scope of this specific SMPR**

Cannabis with water activity levels above Aw 0.65 should be returned to producers. b. Fresh Cannabis requires additional testing, which should include Pseudomonas aeruginosa, Clostridium botulinum, and toxigenic E. coli. c. Edible Cannabis products should be regulated by local health departments. They carry the same microbiological risks as any food product, and heated Cannabis extracts do not increase this risk… d. Cannabis should be tested for four species of Aspergillus: Aspergillus flavus, Aspergillus fumigatus, Aspergillus Niger, and Aspergillus terreus. e. Cannabis should be tested for total generic E.Coli. Samples with levels above 100 CFU/gram should be rejected. f. Cannabis should be tested for Salmonella: Samples with detectable Salmonella should be rejected. g. There is no need to test cured Cannabis for Pseudomonas aeruginosa, Listeria, toxigenic E. Coli (e.g., H7:0157), or other bacterial pathogens besides Salmonella. h. There is no need to test Cannabis for “total yeast and mold”. i. There is no need to test Cannabis for aflatoxins. j. Statistical sampling procedures must be used for microbial testing. A total of at least 5 grams randomly distributed throughout each pound of flower material must be collected. These subsamples for the entire batch should then be combined, thoroughly homogenized, and the appropriate volume of this mixture utilized for each assay. Batch sizes should be 5-6 lbs. k. Cannabis extracts made with hydrocarbon solvents, CO_2, or alcohol	

should be tested for Aspergillus if they are intended for direct inhalation. They do not need microbial screening prior to use in edible products. Extracts made with water or without solvents should be screened for the same microbes as cured Cannabis flowers: four Aspergillus species, generic E. coli, and generic Salmonella.	