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NYS DOH LEB-603

Preparation of Samples for Medical Marijuana Testing
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1.0. Scope and Application

1.1. This method, NYS DOH LEB-603, Preparation of Samples for Medical Marijuana Testing (ELAP Method ID 9926) describes the preparation of samples of medical marijuana products for the detection of microbial contaminants, including bile tolerant gram negative bacteria, Escherichia coli, Clostridium botulinum, Pseudomonas species, Enterococcus species, Salmonella species, Penicillium species, Aspergillus species, Mucor species, and thermophilic actinomycetes as required in Title 10 (Health), Subpart 55-2.15 and Chapter XIII, Section 1004.14 of the official Compilation of Codes, Rules, and Regulations, of the State of New York. Numbers of fungi and aerobic bacteria are also determined.

1.2. Numbers of fungi and aerobic bacteria are also determined.

1.3. This protocol also describes the policy and procedure for storage, re-sampling, and destruction of unused product.

1.4. Protocols for the identification of these organisms in samples of medical marijuana products can be found in the NYS DOH LEB-600 series. See Appendix A for Medical Marijuana Microbial Testing Plan flowcharts.

2.0. Summary of the Method

2.1. Twenty doses of individual lots of medical marijuana product are pooled. Fifteen doses are diluted at least 1:10 in PBST, and the remaining doses are archived. Samples containing materials that are not soluble in water are diluted with isopropyl myristate prior to adding PBST. Matrix spikes of microbial contaminants under test and positive and negative controls are prepared and analyzed in parallel with sample aliquots.

3.0. Definitions

3.1. PBST stands for Phosphate Buffered Saline, pH 7.2, containing 0.1% Tween® 80.

3.2. Polysorbate 80 (Tween® 80) is a nonionic surfactant and emulsifier.

3.3. A dose is determined by the Registered Organization and is listed in the paperwork that accompanies the delivery of the product. The dose is typically a weight or volume with the exception of capsules or tablets in which the dose is either one or multiple capsules/tablets. The fill weight of a capsule is not the dose.

3.4. Pooled product refers to the total combined doses of one lot of product. Typically, 20 doses are pooled.

3.5. Batched product refers to the combining of individual doses from the pooled product to be tested. Typically, 15 doses of the pooled product are batched for analysis.

3.6. Diluted sample aliquot is one measured dose of sample diluted, typically, 1:10 in PBST.

3.7. BioBalls® are small water-soluble balls containing a known number of microorganisms that are used for quality control.

3.8. RO stands for an organization that is registered to manufacture and dispense medical marijuana in New York State.

4.0. Health and Safety Warnings

4.1. Microbiological analyses involve the culturing of potentially pathogenic organisms.

4.1.1. All microbiologically contaminated materials, including media, shall be autoclaved after use.

4.1.2. Contaminated glassware and plastic ware shall be decontaminated prior to washing.

4.1.3. Laboratory equipment and benches shall be disinfected using either Envirocide®, 10% bleach, or 70% ethanol before and after use.

4.1.4. Mouth pipetting is prohibited.

4.1.5. All accidents, particularly those which may result in infection, shall be reported.

5.0. Shipping Conditions, Receiving, Preservation and Storage

5.1. Sample Shipping Conditions

5.1.1. The medical marijuana products from the Registered Organizations (ROs) are shipped as per manufacturer’s specifications and must adhere to all regulatory requirements.

5.2. Sample Receipt

5.2.1. Medical marijuana products from the RO are received, verified and documented ensuring that method, regulatory and Accreditation Body requirements are met.

5.3. Method Holding Times

5.3.1. Once samples are diluted, they are immediately analyzed according to NYS DOH LEB-604 and NYS DOH LEB-605.

5.4. Preservation

5.4.1. Remaining diluted sample aliquots after set-up of the presence/absence, APC, and MPC analyses are stored refrigerated until it has been determined that they are not needed for additional microbiological evaluation.

5.4.2. Unused, reconstituted BioBalls® can be stored for one week at 1.0-8.0°C.

5.5. Storage

5.5.1. All medical marijuana products must be stored under the conditions recommended by the manufacturer until analyses are completed. The storage is documented.

5.5.2. If storage is required prior to analysis, samples are refrigerated within a box having double locks in a locked refrigerator.

5.5.3. Once analysis is complete, remaining doses are maintained at room temperature within a box having double locks, in a locked room, until destroyed.

6.0. Interferences

6.1. Some components of medical marijuana products, e.g., ethanol, may inhibit the growth of microorganisms.
7.0. Apparatus and Materials

7.1. Equipment and Supplies

7.1.1. Water bath set at 35.0-40.0°C
7.1.2. Erlenmeyer flasks, foam plugged, various sizes, sterile
7.1.3. Graduated cylinders, various sizes, sterile
7.1.4. 50mL conical tubes, sterile – Krackeler Scientific cat. no. 3-352098, or equivalent
7.1.5. 1.7mL microcentrifuge tubes, sterile – Krackeler Scientific cat. no. 383-MCT175C, or equivalent
7.1.6. 250mL centrifuge bottles, sterile – Fisher Scientific cat. no 0553853, or equivalent
7.1.7. Automatic pipetters and sterile aerosol-resistant micropipette tips
7.1.8. Pipette-aid
7.1.9. Pipettes, sterile disposable, 5mL, 10mL, 25mL
7.1.10. Balance
7.1.11. NIST traceable weights
7.1.12. Forceps, either metal or disposable individually wrapped and sterile (Krackeler Scientific, Albany, NY, cat. no. 8-F37944-0000-PK, or equivalent)
7.1.13. Spatulas, either metal or disposable individually wrapped and sterile (Krackeler Scientific, Albany, NY, cat. no. 83-3012, or equivalent).
7.1.14. Pasteur pipettes, sterile
7.1.15. Pliers
7.1.16. Bunsen burner, or equivalent
7.1.17. Biosafety cabinet with HEPA filter

7.2. Reagents and Chemicals

7.2.1. Phosphate Buffered Saline pH 7.2, 10mL aliquots in tubes, 100mL and 500mL aliquots in bottles, containing 0.1% Tween 80 (PBST). Ensure that the formulation is in agreement with that specified by USP.
7.2.2. Isopropyl myristate, Sigma Chemical Company, cat. no. M0757 or equivalent, pre-warmed to 35-40°C.
7.2.3. Aspergillus brasiliensis ATCC 16404, BioBall® MultiShot 550, bioMérieux cat. no. 56001 or 56011.
7.2.4. Clostridium sporogenes ATCC 11437, BioBall® MultiShot 550, bioMérieux cat. no. 56004 or 56014.
7.2.5. Enterococcus faecalis ATCC 29212, either lab prepared spiking solution or BioBall® MultiShot 550, bioMérieux cat. no. 56005 or 56015.
7.2.6. Escherichia coli ATCC 8739, either lab prepared spiking solution or BioBall® MultiShot 550, bioMérieux cat. no. 56006 or 56016.
7.2.7. Klebsiella pneumoniae ATCC 13883, lab prepared spiking solution.
7.2.8. Pseudomonas aeruginosa ATCC 9027, either lab prepared spiking solution or BioBall® MultiShot 550, bioMérieux cat. no. 56007 or 56017.
7.2.9. Salmonella typhimurium ATCC 14028, either lab prepared spiking solution or BioBall® SingleShot, bioMérieux cat. no. 413775 or 56044.
7.2.10. Ethanol for flame-sterilization
7.2.11. Disinfectants such as Envirocide® (Fisher Scientific cat. no. 19898220), 70% ethanol, and/or Clorox.
7.3. Forms
7.3.1. Medical Marijuana Sample Pooling Log (e.g., LEB-RS-603A)
7.3.2. Medical Marijuana Controlled Substance Inventory Log (e.g., LEB-RS-603B)
7.3.3. Medical Marijuana Sample Batch Calculations spreadsheet
7.3.4. Summary of Sample Preparation and Analysis Set-Up (e.g., LEB-AP-603A)
7.3.5. Medical Marijuana Microbial Testing Plan (e.g., LEB-AP-603B)
7.3.6. Medical Marijuana Sample Types and Preparation Instructions (e.g., LEB-AP-603C)
7.3.7. Homogenization of Whole Flower for Composite Analysis Instructions (LEB-AP-603D)
7.3.8. Balance Calibration Records

8.0. Quality Control/Assurance
8.1. Method Detection Limits
8.1.1. Method detection limits are product-specific.
8.2. Calibration and Standardization
8.2.1. Temperatures of the cold room and refrigerator are observed and recorded twice daily separated by at least 4 hours on either the Cold Room or Refrigerator Temperature Records.
   8.2.1.1. If the cold room or refrigerator does not stay within 1.0-8.0°C, the supervisor is notified.
   8.2.1.2. The optimum temperature range for a cold room or refrigerator is 1.0-4.0°C
   8.2.1.3. If the cold room or refrigerator was in a defrost cycle at the time that the temperature was recorded, and the temperature does not reach 8.0°C, re-testing of media is not required.
   8.2.1.4. Media may be re-tested for quality, depending on the number of degrees and the amount of time that the cold room temperature was out of compliance, at the discretion of the laboratory.
8.2.2. Temperature of the freezer is observed and recorded twice daily separated by at least 4 hours on the Freezer Temperature Record.
   8.2.2.1. If the temperature on the freezer exceeds -15.0°C, laboratory-specific corrective actions are followed.
   8.2.2.2. Temperature may be lower, but not higher than the targeted temperatures.
8.2.3. Water bath temperatures shall be observed and recorded twice daily, separated by at least 4 hours.
   8.2.3.1. Temperature of the 35.0-40.0°C water bath is observed and recorded on the Water Bath Temperature Record.
   8.2.3.1.1. If water bath temperature does not stay within 35.0-40.0°C, laboratory-specific corrective actions are followed. Analytical results may be invalidated if the temperature exceeds 40.0°C, at the discretion of the laboratory.
8.2.4. Max/min temperatures are recorded when twice-daily temperature measurements are not possible, such as on holidays and weekends.

8.2.4.1. Max/min temperatures of freezers may show transient excursions of temperature above 0.0°C due to defrost cycles.

8.2.4.2. This is acceptable, provided that the quality of BioBalls® are unaffected.

8.2.5. Thermometers must be verified against a NIST-certified thermometer as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

8.2.6. Sterility of disposable loops, spreaders, and spatulas is determined as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

8.2.7. The volumetric accuracy of automatic pipettors and serological pipettes is as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

8.2.8. The intensity and efficacy of the UV light in the biosafety cabinet is measured quarterly.

8.2.9. Biosafety cabinets are certified annually as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

8.3. Quality Control

8.3.1. The top-loading balance is calibrated prior to pooling and results are recorded.

8.3.2. Lab prepared spikes are counted at a minimum of once a week in as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

8.3.3. Comparative recovery and sterility between lots of PBST are determined.

8.3.4. Liquid media is stored in tightly-capped bottles in the dark at 4°C for up to 3 months from the date of preparation.

8.3.4.1. Analyses must be completed prior to the expiration date of the media and analyses must not be initiated on the day media expires.

8.3.4.2. Liquid media can be used after 3 months storage if ongoing QC demonstrates no loss in selectivity or growth promotion.

8.3.5. The use test for reagent water is performed annually, when cartridges are changed, or repairs are made to the deionized water systems.

8.4. Corrective/Preventive Actions

8.4.1. The laboratory will initiate non-conformances and/or corrective/preventive actions in accordance with laboratory-specific procedures and as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

9.0. Procedure

9.1. General

9.1.1. Aseptic technique is used for all procedures.

9.1.1.1. Aseptic technique can be found in a general microbiology textbook or on-line.
9.1.2. Laboratory-specific procedures are followed.

9.1.2. All work surfaces are disinfected prior to preparation of spiking solutions, sample pooling/batching, and sample preparation.

9.1.3. Preparation of spiking solutions are performed in a physically different location than sample preparation and initial sample analyses to prevent cross-contamination of incoming products.

9.2. Matrix Spikes

9.2.1. Every RO has different brands of products which can come in different forms. For example, a RO may have an “Extra Strength” brand that comes in capsules, tinctures, and vaporizers. Each one of the forms under the “Extra Strength” brand is considered a separate product type and is analyzed as such.

9.2.2. Every new product type analyzed for microbial contaminants will have corresponding matrix spikes for the first three times that specific product type is analyzed or until results are consistent. After at least three consistent matrix spike results, that specific product type will only need corresponding matrix spikes every 20th time it is received for analysis.

9.2.2.1. This matrix spike policy applies only to the presence/absence analyses. All APC and MPC analyses (LEB-605) will have matrix spikes regardless of how many times a specific sample type has been analyzed.

9.3. Preparation of Spiking Solutions

9.3.1. Prior to any sample preparation or analysis, the spiking solutions must be prepared either in the laboratory or by using BioBalls®.

9.3.2. Laboratory Prepared Spiking Solutions

9.3.2.1. Follow laboratory-specific procedures.

9.3.2.1.1. Laboratory prepared spikes should be prepared so theoretically a 100µL aliquot is equivalent to 50 CFUs.

9.3.2.1.2. The volume of the laboratory prepared spike may be adjusted to ensure the target concentration of the spiked organism is approximately 50 CFUs.

9.3.2.2. Lab prepared spikes are counted at a minimum of once a week.

9.3.3. BioBall® Rehydration

9.3.3.1. For each BioBall® label a 1.7mL sterile microcentrifuge tube with the organism name and date of rehydration.

9.3.3.2. Using a 1mL sterile serological pipette and Pipette-Aide, add 1.1mL of PBST to each tube.

9.3.3.3. Remove the BioBall® vial from the freezer, slowly uncap, and tip the BioBall into the tube containing PBST.

9.3.3.4. Once the BioBall® dissolves, invert the tube several times to mix gently.

9.3.3.5. Incubate the tubes for 4 hours at room temperature.

9.3.3.6. Vortex to mix prior to use.

9.3.3.7. The reconstituted BioBall® can now be used in 100µL aliquots which is theoretically equivalent to a 50 CFUs.
9.3.3.7.1. The BioBall® spike should not be more than 100 CFUs.

9.3.3.7.2. If historical results indicate that the 100μL aliquots are more than 100 CFUs or less than 50 CFUs, the volume of the aliquot should be adjusted to meet the target of 50 CFUs.

9.3.3.8. BioBall® preparations of K. pneumoniae and T. vulgaris are not available, so laboratory prepared spikes must be used.

9.4. Pooling Accepted Samples

9.4.1. Pooling of accepted samples is performed in a biological safety cabinet.

9.4.1.1. Prior to pooling, place all supplies needed for pooling in the biological safety cabinet, close the sash, and UV disinfect the cabinet for 15 minutes.

9.4.2. When the samples arrive, the containers are disinfected using either Envirocide®, 10% bleach, or 70% ethanol prior to placing them in the biosafety cabinet.

9.4.3. Aseptically add 20 doses from the same production date/lot number to a sterile, labeled, vessel that has been tared, and record the weight on the sterile vessel.

9.4.3.1. Occasionally a RO will provide a dispensing tool (i.e. syringe) with the product. Use the provided dispensing tool to pool the doses.

9.4.3.2. Pool an equal number of doses from each of the containers submitted for the lot of product being analyzed.

9.4.3.3. Occasionally a sample matrix is too viscous to accurately pool 20 doses. In this instance, more than 20 doses can be pooled to ensure there are enough doses for analysis.

9.4.4. On the Medical Marijuana Sample Pooling Log (e.g., LEB-RS-603A) record the sample accession number, sample type, dose amount, combined pool weight, analyst initials, and date. Confirm the dose amount with the incoming paperwork, and check the box verifying the dose amount on the Medical Marijuana Sample Pooling Log (e.g., LEB-RS-603A).

9.4.5. If the viscosity of the pooled doses will require the addition of isopropyl myristate prior to dilution (see section 9.6), check the corresponding box on the Medical Marijuana Sample Pooling Log (e.g., LEB-RS-603A).

9.4.6. Record the number of doses that have been pooled on the Medical Marijuana Sample Pooling Log (e.g., LEB-RS-603A).

9.4.6.1. If the number of doses is more than 20, the number of doses pooled is calculated by dividing the total weight or volume by the weight or volume of one dose.

9.4.6.1.1. For example, if one dose of a product is 4.5mg, and the weight of the pooled product is 99mg, 99/4.5 = 22 doses.

9.4.7. Once 20 or more doses have been pooled, the combined pool is stored refrigerated in a double locked box until analysis (see section 9.9.).
9.5. Preparation of Products Soluble in Water

9.5.1. If the sample requires the addition of isopropyl myristate go to section 9.6.

9.5.2. Determine the number of individual doses that will be analyzed (these are now batched doses). At least 15 doses are diluted. Refer to section 9.2. and use LEB-AP-603A to determine when matrix spikes are needed and for a summary of sample preparation and analyses set up.

9.5.2.1. Viscous samples with small doses (less than 50µL or 50mg) may prevent accurate pipetting because a small portion of the sample may stick to the insides of pipette tips. In this case it is recommended that all pooled doses be diluted prior to sample analysis to ensure there is enough volume for all sample analyses. See the Medical Marijuana Sample Types and Preparation Instructions (e.g., LEB-AP-603C).

9.5.3. Determine the dilution factor for the sample using the Medical Marijuana Sample Types and Preparation Instructions (e.g., LEB-AP-603C) as a guide.

9.5.4. Calculate the weight or volume of a single dose by dividing the combined pool weight or volume (e.g., LEB-RS-603A) by the number of doses that were pooled.

9.5.4.1. For example, if the combined pool weight is 10g and 20 capsules were pooled, 10g/20 capsules = 0.5g/capsule.

9.5.5. Calculate the weight or volume of the doses being batched.

9.5.5.1. For example, if the sample is 0.5g/dose and 15 doses are being batched for analysis, then 0.5g/dose x 15 doses = 7.5g.

9.5.6. Calculate the final volume of the batched doses when they are diluted with PBST (weight/volume for pills or solids or volume/volume for liquid samples) by multiplying the batched weight or volume (calculated in 9.5.5) by the dilution (determined in 9.5.3.).

9.5.6.1. For example, if the weight of the batched doses is 7.5g and they have to be diluted 1:10, then 7.5g/batch x 10 = 75mL.

9.5.7. Calculate the volume of PBST required to produce the necessary dilution by subtracting the batched weight/volume of the doses (calculated in 9.5.5.) from the final volume of the diluted batch (calculated in 9.5.6.).

9.5.7.1. For example, if the volume of the diluted batch is 75mL, then 75mL – 7.5mL = 67.5mL.

9.5.8. The Medical Marijuana Sample Batch Calculations spreadsheet can be used to perform these calculations prior to sample preparation (see section 9.7).

9.5.9. Aseptically add the appropriate number of pooled doses (at least 15) to a labeled, sterile vessel and add the calculated volume of PBST (9.5.7.) to the batched doses and vortex to mix.

9.5.9.1. Pills or capsules may require heating to enhance dissolution.

9.5.9.2. If needed, use PBST preheated to 35.0-40.0°C and heat the diluted aliquots to 35.0-40.0°C in a water bath with intermittent vortexing until the material is dissolved.
9.6. Preparation Products Insoluble in Water

9.6.1. If, due to matrix viscosity, an emulsion cannot be created when the batched sample is diluted, add a volume of filter-sterilized isopropyl myristate (pre-warmed to 35.0-40.0°C) that is equal to the weight or volume of to the batched doses prior to dilution with PBST.

9.6.2. Determine the number of individual doses that will be analyzed (these are now batched doses). Refer to section 9.2. and LEB-AP-603A to determine when matrix spikes are needed and for a summary of sample preparation and analyses set up.

9.6.2.1. Viscous samples with small doses (less than 50µL or 50mg) may prevent accurate pipetting because a small portion of the sample may stick to the insides of pipette tips. In this case it is recommended that all 20 doses be diluted prior to sample analysis to ensure there is enough volume for all sample analyses. See the Medical Marijuana Sample Types and Preparation Instructions (e.g., LEB-AP-603C).

9.6.3. Determine the dilution factor for the sample using the Medical Marijuana Sample Types and Preparation Instructions (e.g., LEB-AP-603C) as a guide.

9.6.4. Calculate the weight or volume of a single dose by dividing the combined pool weight or volume (e.g., LEB-RS-603A) by the number of doses that were pooled.

9.6.4.1. For example, if the combined volume of 20 doses is 90µL, then 90µL/20 doses = 4.5µL/dose.

9.6.5. Calculate the volume of the combined doses being batched and the volume of isopropyl myristate being added. The addition of the isopropyl myristate is included in the final volume/weight of the combined doses.

9.6.5.1. For example, if the sample is 4.5µL/dose and 15 doses are being batched, then 4.5µL/dose x 15 doses = 67.5µL. Therefore, 67.5µL of isopropyl myristate are added so the final volume/weight of the combined doses is 135µL.

9.6.6. Calculate the final volume of the batched doses when they are diluted with PBST (diluted batch) by multiplying the batched volume (calculated in 9.6.5.) by the dilution (determined in 9.6.3.).

9.6.6.1. For example, if the volume of the batched doses is 135µL and they have to be diluted 1:10, then 135µL x 10 = 1350µL.

9.6.7. Calculate the volume of PBST required to produce the necessary dilution (weight/volume for pills or solids or volume/volume for liquid samples by subtracting the batched volume of the doses (calculated in 9.6.5.) from the final volume of the diluted batch (calculated in 9.6.6.).

9.6.7.1. For example, if the volume of the diluted batch is 1350µL and the volume of the batched doses is 135µL, then 1350µL - 135µL = 1215µL.

9.6.7.2. Occasionally a product will require more than a 1:10 dilution to allow for accurate dispensing for sample analyses. Use the Medical Marijuana Sample Types and Preparation Instructions (e.g., LEB-AP-603C) as a guide for sample dilutions.
9.6.8. The Medical Marijuana Sample Batch Calculations spreadsheet can be used to perform these calculations prior to sample preparation (see section 9.7).

9.6.9. Aseptically add the appropriate number of pooled doses (at least 15) to a labeled, sterile vessel, and aseptically add an equal volume of pre-warmed isopropyl myristate (calculated in 9.6.5.) to the batched doses.

9.6.10. Add the calculated volume of PBST to the batched samples with isopropyl myristate and vortex to mix.

9.6.10.1. The use of PBST preheated to 35.0-40.0°C can assist with creating an emulsion.

9.7. Sample Calculations Spreadsheet

9.7.1. Using the Medical Marijuana Sample Batch Calculations spreadsheet, enter the following information:

9.7.1.1. Sample accession ID number.
9.7.1.2. Name of the registered organization.
9.7.1.3. The sample brand (see section 9.2. for the description of a brand).
9.7.1.4. Sample type.
9.7.1.5. Combined weight or volume of the pool recorded on the Medical Marijuana Sample Pool Log (e.g., LEB-RS-603A).
9.7.1.6. Number of doses in the pool recorded on the Medical Marijuana Sample Pool Log (e.g., LEB-RS-603A).

9.7.1.6.1. The number of doses being batched for analysis as determined in section 9.2.

9.7.1.7. Volume of isopropyl myristate to add to the batched doses, if applicable. See section 9.6. for more information.
9.7.1.8. The dilution factor for the batched doses.

9.7.2. The Medical Marijuana Sample Calculations spreadsheet (e.g., LEB-XLS-603A) will generate the following information:

9.7.2.1. The weight or volume of a single dose.
9.7.2.2. The total weight or volume of the batched doses.
9.7.2.3. The final volume of the batched doses after isopropyl myristate is added (if applicable).
9.7.2.4. The total volume of the batched doses after diluting with PBST.
9.7.2.5. The volume of PBST to add to the batched doses.
9.7.2.6. The volume of a single diluted aliquot needed for subsequent analyses.

9.7.3. Print out a copy the Medical Marijuana Calculations spreadsheet (e.g., LEB-XLS-603A) and have a second analyst check for transcription errors.

9.7.4. Record the analyst initials, the reviewing analyst initials, and date on the printed copy.

9.8. Analysis of product

9.8.1. Once the samples have been batched and diluted, and the BioBalls® have rehydrated for 4 hours, immediately proceed with NYS DOH LEB-604 and NYS DOH LEB-605.
9.8.1.1. Sample analyses required are summarized in the Summary of Sample Preparation and Analysis Set-Up (e.g., LEB-AP-603A).

9.8.2. Un-used aliquots of rehydrated BioBalls® can be reused when stored at 1.0-8.0°C for up to one week.

9.9. Product archival and re-analysis

9.9.1. After pooling the 20 doses (see section 9.4), the pool is stored refrigerated in a double-lock box until analyzed.

9.9.2. When storing the pool, the accession ID, weight of the pool, date of storage, and the initials of the analyst and witness are recorded on the Medical Marijuana Controlled Substance Inventory Log (e.g., LEB-RS-603B).

9.9.3. After removing the appropriate number of doses for sample analyses, the remaining doses are stored refrigerated in a double-locked safe until analyses are completed.

9.9.4. When archiving the remaining doses, the weight of the archived doses, date of archival, and the initials of the analyst and witness are recorded on the Medical Marijuana Controlled Substance Inventory Log (e.g., LEB-RS-603B).

9.9.4.1. If all the doses are used for sample analyses (ie, viscous samples insoluble in water that require the addition of isopropyl myristate) then “n/a” is recorded in the archived weight section on the Medical Marijuana Controlled Substance Inventory Log (e.g., LEB-RS-603B) as they will not be stored. The box “All used for initial testing” (e.g., LEB-RS-603B) is checked to indicate this.

9.9.5. The recording of the initial archived weights and archiving the remaining doses are witnessed by a second analyst and both analyst initials are recorded in the “Analyst/Witness Initials” section on the Medical Marijuana Controlled Substance Inventory Log (e.g., LEB-RS-603B).

9.9.6. Samples are only re-analyzed if it has been determined that the sample was contaminated during analyses. This investigation is documented in a corrective action. If the analyses need to be repeated the Medical Marijuana program is notified for permission to request a new set of samples.

9.9.7. Archived doses are only analyzed to gather more information regarding contamination and are not part of the reported results.

9.9.8. If archived samples are analyzed after initial testing is completed, analysts first record the weight of the archived samples on the Medical Marijuana Controlled Substance Inventory Log (e.g., LEB-RS-603B) in the “Second Archived Weight/Date” section.

9.9.8.1. This is to indicate if there was any sample evaporation while the samples were archived.

9.9.9. Analysts then remove the amount of product needed for analysis and record the weight of the remaining doses on the Medical Marijuana Controlled Substance Inventory Log (e.g., LEB-RS-603B) in the “Second Use Archived Weight/Date” section.
9.9.10. The recording of the archived and second use weights are witnessed by a second analyst and both analyst initials and the date are recorded on the Medical Marijuana Controlled Substance Inventory Log (e.g., LEB-RS-603B).

9.9.11. In the comments section of the Medical Marijuana Controlled Substance Inventory Log (e.g., LEB-RS-603B) indicate that the archived doses are being used for re-analysis.

9.10. Product Destruction

9.10.1. When analysis of a lot of product is completed and released, and re-analysis is not necessary, the archived doses are destroyed in accordance with relevant regulations and standards.

9.10.2. The laboratory may destroy unused samples under the following conditions:

9.10.2.1. Archived samples whose total volume exceeds 50mLs

9.10.2.1.1. Samples are destroyed by diluting 1:1 with 100% bleach.

9.10.2.1.2. The sample:bleach mixture is left at room temperature for at least one hour and then poured down the drain with running water.

9.10.3. If archived samples are being destroyed, analysts first record the weight of the archived samples on the Medical Marijuana Controlled Substance Inventory Log (e.g., LEB-RS-603B).

9.10.3.1. This is to indicate if there was any sample evaporation while the samples were archived.

9.10.4. The recording of the archived weight is witnessed by a second analyst and both analyst initials are recorded on the Medical Marijuana Controlled Substance Inventory Log (e.g., LEB-RS-603B).

9.10.5. Analyst then checks the box for “Transfer for destruction” and writes “n/a” in the “Second Use Archived Weight/Date” column on the Medical Marijuana Controlled Substance Inventory Log (e.g., LEB-RS-603B).

10.0. Data Acquisition, Reduction, Analysis, Calculations, Acceptance Criteria and Documentation

10.1. Record the sample accession number, sample type, dose amount, dose amount verification, number of pooled doses, combined weight, diluted aliquot volume, analyst initials and date on the Medical Marijuana Sample Preparation Log (LEB-RS-603A).

10.2. Record the date, target weight, measured weight, calculated error, pass/fail, and initials on the Balance Verification Form.

10.3. Record the accession number, initial weight, initial archive weight, second archive weight, second use archived weight, dates and analyst and witness initials on the Medical Marijuana Controlled Substance Inventory Log (e.g., LEB-RS-603B).
11.0. Method Performance

11.1. Demonstration of Capability

11.1.1. Prior to acceptance and use of this method for data reporting, a satisfactory initial demonstration of capability (DOC) is required. Thereafter, an ongoing DOC is to be performed annually.

11.1.2. An initial DOC shall be made prior to using any method, and at any time there is a change in instrument type, personnel or method or any time that a method has not been performed by the laboratory or analyst in a twelve (12) month period.

11.1.3. All DOCs shall be documented, and all data applicable to the demonstration shall be retained and readily available at the laboratory.

11.1.4. Consult relevant standards, regulations and Accreditation Body requirements for additional information on performing DOCs for microbial contaminants.

11.2. Laboratory Detection Limits

See section 8.1.

12.0. Waste Management/Pollution Prevention

12.1. It is the responsibility of the laboratory to comply with all federal, state and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions.

12.2. Bacterial/fungal cultures and contaminated or potentially contaminated disposable materials are disposed of in biohazardous waste cans and autoclaved prior to discarding.

12.3. Dispose of non-hazardous water waste in the laboratory sink followed by flushing with tap water.

12.4. Dispose of glassware in appropriately labeled boxes. Be sure that, whenever possible, the glass has been thoroughly rinsed and is contaminant-free before disposal.

12.5. Consult federal, state and local regulations for additional information or for information on the disposal of products not described in this method.

12.6. Unused products are disposed of according to section 9.9.

13.0. References


13.4. TNI 2016 Standards – EL-V1M5-2016-Rev2.0: Microbiological Testing

13.5. NYS DOH LEB-604, Microbial Presence/Absence Test for Medical Marijuana Samples

13.6. NYS DOH LEB-605, Aerobic Bacteria and Mold Plate Counts for Medical Marijuana Testing

13.7. Title 10 (Health), Subpart 55-2.15 and Chapter XIII, Section 1004.14 of the official Compilation of Codes, Rules, and Regulations, of the State of New York
14.0. Appendices

<table>
<thead>
<tr>
<th>Analysis Number</th>
<th>Product Needed</th>
<th>Target</th>
<th>Matrix Spike Organism</th>
<th>Medium</th>
<th>Temperature</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Yes</td>
<td>Aerobic bacteria</td>
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<td>TSA</td>
<td>30-35°C</td>
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<td>2</td>
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<tr>
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<td>E coli</td>
<td>TSA</td>
<td>30-35°C</td>
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<tr>
<td>4</td>
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<td>Aerobic bacteria, negative control</td>
<td>n/a</td>
<td>TSA</td>
<td>30-35°C</td>
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<td>5</td>
<td>Yes</td>
<td>Fungi</td>
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<td>SDA</td>
<td>20-25°C</td>
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<td>6</td>
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<td>Fungi, MS</td>
<td>A. brasilensis</td>
<td>SDA</td>
<td>20-25°C</td>
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<tr>
<td>7</td>
<td>No</td>
<td>Fungi, positive control</td>
<td>A. brasilensis</td>
<td>SDA</td>
<td>20-25°C</td>
</tr>
<tr>
<td>8</td>
<td>No</td>
<td>Fungi, negative control</td>
<td>n/a</td>
<td>SDA</td>
<td>20-25°C</td>
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<td>9</td>
<td>Yes</td>
<td>Bile tolerant gram-negative organisms</td>
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<td>EEBM</td>
<td>30-35°C</td>
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<td>11</td>
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<td>E coli, Salmonella spp., Pseudomonas spp., MS</td>
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<td>12</td>
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<td>E coli, Salmonella spp., Pseudomonas spp., MS</td>
<td>E coli, S. typhimurium., Ps. aeruginosa</td>
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<td>30-35°C</td>
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<td>13</td>
<td>Yes</td>
<td>Clostridium botulinum</td>
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<td>RCM</td>
<td>30-35°C, anaerobic</td>
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<td>14</td>
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<td>Clostridium botulinum*, MS</td>
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<td>30-35°C, anaerobic</td>
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<td>Clostridium sporogenes</td>
<td>RCM</td>
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<td>Enterococcus spp.</td>
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<td>TSB</td>
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<td>17</td>
<td>Yes</td>
<td>Enterococcus spp., MS</td>
<td>E. faecalis</td>
<td>TSB</td>
<td>30-35°C, 5% CO₂</td>
</tr>
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<td>18</td>
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<td>Thermophilic actinomycetes</td>
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<td>TSB</td>
<td>50-55°C</td>
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<td>19</td>
<td>Yes</td>
<td>Thermophilic actinomycetes, MS</td>
<td>T. vulgaris</td>
<td>TSB</td>
<td>50-55°C</td>
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<tr>
<td>20</td>
<td>Yes</td>
<td>Archived sample</td>
<td>n/a</td>
<td>n/a</td>
<td>Room temp</td>
</tr>
<tr>
<td>21</td>
<td>Yes</td>
<td>Archived sample</td>
<td>n/a</td>
<td>n/a</td>
<td>Room temp</td>
</tr>
<tr>
<td>22</td>
<td>Yes</td>
<td>Archived sample</td>
<td>n/a</td>
<td>n/a</td>
<td>Room temp</td>
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<tr>
<td>23</td>
<td>Yes</td>
<td>Archived sample</td>
<td>n/a</td>
<td>n/a</td>
<td>Room temp</td>
</tr>
<tr>
<td>24</td>
<td>Yes</td>
<td>Archived sample</td>
<td>n/a</td>
<td>n/a</td>
<td>Room temp</td>
</tr>
</tbody>
</table>

MS = matrix spike, TSB = tryptic soy broth, TSA = tryptic soy agar, SDA = Sabouraud Dextrose agar, EEBM = Enterobacterial Enrichment Broth Mossel, RCM = Reinforced Clostridial Medium.

Matrix spikes are prepared according to LEB-603 section 9.3, and should contain less than 100 organisms.

*Heat sample at 80°C for 10 minutes
Medical Marijuana Microbial Testing Plan (LEB-AP-603B)
Media Needed

Pooled Sample

- Tryptose Soy Broth
  - MacConkey Broth
    - MacConkey Agar
  - Cetrimide Agar
  - RV Enrichment Broth
    - XLD Agar
  - Violet Red Bile Glucose Agar

- Enteric Enrich Broth Medium
  - Columbia Agar
  - Proteose Peptone Selection Agar

- Reinforced Clostridial Medium

- Tryptose Soy Broth
  - Tryptose Soy Agar

- Aerobic Plate Count
  - Sabouraud Dextrose Agar

- Mold Plate Count
  - Sabouraud Dextrose Agar
Organism Identification Assays

Pooled Sample

- Aerobes
  - API 20E, MALDI-TOF MS, sequencing, etc.
  - API 20E, MALDI-TOF MS, sequencing, etc.
  - API 20E, MALDI-TOF MS, sequencing, etc.

- Bile-Tolerant Gram Negative
  - API 20A, MALDI-TOF MS, sequencing, etc.

- C. tetani
  - API 20A, MALDI-TOF MS, sequencing, etc.

- Enteroccoci spp.
  - API 2000 ms, MALDI-TOF MS, sequencing, etc.

- Thermophilic Actinomyces
  - Gram stain, MALDI-TOF MS, sequencing, etc.

- Aerobic Plate Counts
  - RT-PCR

- Mold Plate Counts
  - RT-PCR
  - RT-PCR
General Set-Up

AB – Aspergillus brasiliensis ATCC 16404
KP – Klebsiella pneumoniae ATCC 13883
CS – Clostridium sporogenes ATCC 11437
EC – Escherichia coli ATCC 8739
PA – Pseudomonas aeruginosa ATCC 9027
ST – Salmonella typhimurium ATCC 14028
EF – Enterococcus faecalis ATCC 29212
TV – Thermactinomyces vulgaris ATCC 43649

20 doses pooled
At least 15 doses tested
5 doses archived
# Medical Marijuana Sample Types and Preparation (LEB-AP-603C)

<table>
<thead>
<tr>
<th>Product Type</th>
<th>Isopropyl Myristate Added</th>
<th>PBST Dilution</th>
<th>Heat and Vortex</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balm</td>
<td>X</td>
<td>1:10</td>
<td>X</td>
<td>Dilute all 20 doses</td>
</tr>
<tr>
<td>Capsules – Cellulose fill</td>
<td></td>
<td>1:20</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Capsules – Oil fill</td>
<td></td>
<td>1:10</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Chews or Gummies</td>
<td></td>
<td>1:10</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Inhaler</td>
<td></td>
<td>1:100</td>
<td></td>
<td>Dilute all 20 doses</td>
</tr>
<tr>
<td>Lotion</td>
<td>X</td>
<td>1:10</td>
<td>X</td>
<td>Dilute all 20 doses</td>
</tr>
<tr>
<td>Lozenge</td>
<td></td>
<td>1:10</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Powder</td>
<td></td>
<td>1:10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant Material (ground flower)</td>
<td></td>
<td>1:100</td>
<td></td>
<td>Dilute all 20 doses</td>
</tr>
<tr>
<td>Plant Material (whole flower)</td>
<td></td>
<td>1:100</td>
<td></td>
<td>See LEB-AP-603D</td>
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<tr>
<td>Suppository</td>
<td></td>
<td>1:10</td>
<td>X</td>
<td></td>
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<tr>
<td>Syrup</td>
<td></td>
<td>1:10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tablets – Cellulose base</td>
<td></td>
<td>1:20</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Tablets – Chewable</td>
<td></td>
<td>1:10</td>
<td>X</td>
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<tr>
<td>Tablets – Effervescent</td>
<td></td>
<td>1:10</td>
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<td>Use a container twice the size of the volume of the diluted doses</td>
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<tr>
<td>Tablets – Water soluble</td>
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<tr>
<td>Tincture/Oral Solution/Oral Spray – Ethanol Base</td>
<td></td>
<td>1:10</td>
<td></td>
<td></td>
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<tr>
<td>Tincture/Oral Solution/Oral Spray – Oil Base</td>
<td></td>
<td>1:10</td>
<td></td>
<td></td>
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<tr>
<td>Vaporizer – Liquid viscosity</td>
<td></td>
<td>1:10</td>
<td>X</td>
<td>Dilute all 20 doses</td>
</tr>
<tr>
<td>Vaporizer – Waxy viscosity</td>
<td>X</td>
<td>1:10</td>
<td>X</td>
<td>Dilute all 20 doses</td>
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</table>
Homogenization of Whole Flower for Composite Analysis (LEB-AP-603D)

1. General
   a. Create a homogenized composite sample from the submitted testing samples for each lot (Figure 1 and Figure 2).
   b. The blender (jar, lid, blade, and collar) must be autoclavable. It is recommended that the blender be made of glass or stainless steel.

2. Grinding
   a. In a biosafety cabinet, aseptically combine the samples from each final product container into the sterile blender jar.
      i. Depending on the amount of product used to create a composite sample, multiple blender jars may be used.
   b. Use the “pulse” feature to homogenize at brief intervals (approximately 1-3 seconds) and allowing for a few seconds in between pulses to minimize the heating effect of the composite sample.
      i. The number of pulses will vary with blender types and the amount of product being homogenized.

3. Quartering and Compositing
   a. Once the desired particle size has been achieved (approximately 3-5 mm as in Figures 1 and 2), place the entire composite onto a sterile surface and form a square-shaped heap.
   b. Divide the composite diagonally into four equal parts.
   c. Aseptically combine two opposite quarters and create a second square shaped heap.
   d. Repeat the quartering steps until the composite has been reduced to an acceptable sample size.
   e. Aseptically remove at least 20 doses for microbiological analyses. The remainder of the sample can be used for other testing (e.g., potency testing, testing for other contaminants).

4. Cleaning Equipment
   a. Disassemble the blender and wash the jar, lid, blade, and collar using the laboratory’s glassware cleaning procedure.
   b. Once dried, rinse all pieces with reagent grade ethanol three times to remove any residues.
   c. Rinse all pieces five times with DI water, re-assemble, and autoclave.

Figure 1 - Blending 5g of Whole Flower. Panel A is the product before blending. Panel B is the product after 10, 1 second pulses.

Figure 2 - Blending 20g of Whole Flower. Panel A is the product before blending. Panel B is the product after 20, 1 second pulses.
5. References
# Medical Marijuana Sample Pooling Log (LEB-RS-603A)

<table>
<thead>
<tr>
<th>Accession #</th>
<th>Sample Type</th>
<th>Dose Amount</th>
<th>No. of Pooled Doses</th>
<th>Combined Pool Weight</th>
<th>Diluted Aliquot Volume</th>
<th>Analyst Initials/Date</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
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</table>

- Add isopropyl myristate Number of Containers Submitted:
<table>
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<th>Accession #</th>
<th>Pooled Weight Initials/Date</th>
<th>Initial Archived Weight Initials/Date</th>
<th>Second Archived Weight</th>
<th>Second Use Archived Weight</th>
<th>Analyst/Witness Initials</th>
<th>Comments</th>
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<tbody>
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</tr>
</tbody>
</table>

*Archived samples are re-weighed upon removal from the lock-box to document any losses due to evaporation during storage.*