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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 in accordance with LEB-603.
- 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.
- 3.9. SM refers to *Standard Methods for the Analysis of Water and Wastewater*, 22nd edition.

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 a minimum concentration of 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. Samples diluted in PBST that are not required for 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 pipettors 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, 100 mL 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* (also known as *A. niger*) 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 (e.g., LEB-XLS-603A)
- 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. 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.
- 8.2.3. 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 calibrated 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.
 - 8.3.5.** The use test for deionized 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.
 - 9.1.1.2. 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.

9.3. 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.
 - 9.3.2.2. 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 a minimum concentration of 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 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 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 less than or 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 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). 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 50 μ L 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, $10\text{g}/20 \text{ capsules} = 0.5\text{g}/\text{capsule}$.
- 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 $0.5\text{g}/\text{dose} \times 15 \text{ doses} = 7.5\text{g}$.
- 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 $7.5\text{g}/\text{batch} \times 10 = 75\text{mL}$.
- 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 $75\text{mL} - 7.5\text{mL} = 67.5\text{mL}$.
- 9.5.8.** The Medical Marijuana Sample Batch Calculations spreadsheet (e.g., LEB-XLS-603A) can be used to perform these calculations prior to sample preparation (see section 9.7).
- 9.5.9.** Aseptically add 15 pooled doses 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 $90\mu\text{L}/20 \text{ doses} = 4.5\mu\text{L}/\text{dose}$.
- 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 $4.5\mu\text{L}/\text{dose} \times 15 \text{ doses} = 67.5\mu\text{L}$. 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 $135\mu\text{L} \times 10 = 1350\mu\text{L}$.
- 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 $1350\mu\text{L} - 135\mu\text{L} = 1215\mu\text{L}$.

- 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 (e.g., LEB-XLS-603A) can be used to perform these calculations prior to sample preparation (see section 9.7).
- 9.6.9.** Aseptically add 15 pooled doses 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 (e.g., LEB-XLS-603A), 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. 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.

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

9.9.7. 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.

- 9.9.8. 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).
- 9.9.9. 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 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

- 13.1.** United States Pharmacopeia. USP38-NF33, The United States Pharmacopeial Convention, General chapters <61>, <62>, <1111>.
- 13.2.** *Standard Methods for the Examination of Water and Wastewater*, 22nd edition, section 9050C.
- 13.3.** NYS DOH LEB-604, Microbial Presence/Absence Test for Medical Marijuana Samples
- 13.4.** NYS DOH LEB-605, Aerobic Bacteria and Mold Plate Counts for Medical Marijuana Testing
- 13.5.** 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

Summary of Sample Preparation and Analysis Set-up (LEB-AP-603A)					
Analysis Number	Product Needed	Target	Matrix Spike Organism	Medium	Temperature
1	Yes	Aerobic bacteria	n/a	TSA	30-35°C
2	Yes	Aerobic bacteria, MS	<i>E coli</i>	TSA	30-35°C
3	NO	Aerobic bacteria, positive control	<i>E coli</i>	TSA	30-35°C
4	NO	Aerobic bacteria, negative control	n/a	TSA	30-35°C
5	Yes	Fungi	n/a	SDA	20-25°C
6	Yes	Fungi, MS	<i>A. brasiliensis</i>	SDA	20-25°C
7	NO	Fungi, positive control	<i>A. brasiliensis</i>	SDA	20-25°C
8	NO	Fungi, negative control	n/a	SDA	20-25°C
9	Yes	Bile tolerant gram-negative organisms	n/a	EEBM	30-35°C
10	Yes	Bile tolerant gram-negative organisms, MS	<i>K. pneumoniae</i>	EEBM	30-35°C
11	Yes	<i>E coli, Salmonella spp., Pseudomonas spp.</i>	n/a	TSB	30-35°C
12	Yes	<i>E coli, Salmonella spp., Pseudomonas spp., MS</i>	<i>E coli, S. typhimurium., Ps. aeruginosa</i>	TSB	30-35°C
13	Yes	<i>Clostridium botulinum</i>	n/a	RCM	30-35°C, anaerobic
14	Yes	<i>Clostridium botulinum*</i>	n/a	RCM	30-35°C, anaerobic
15	Yes	<i>Clostridium botulinum, MS</i>	<i>Clostridium sporogenes</i>	RCM	30-35°C, anaerobic
16	Yes	<i>Enterococcus spp.</i>	n/a	TSB	30-35°C, 5% CO ₂
17	Yes	<i>Enterococcus spp., MS</i>	<i>E. faecalis</i>	TSB	30-35°C, 5% CO ₂
18	Yes	Thermophilic actinomycetes	n/a	TSB	50-55°C
19	Yes	Thermophilic actinomycetes, MS	<i>T. vulgaris</i>	TSB	50-55°C
20	Yes	Archived sample	n/a	n/a	Room temp
21	Yes	Archived sample	n/a	n/a	Room temp
22	Yes	Archived sample	n/a	n/a	Room temp
23	Yes	Archived sample	n/a	n/a	Room temp
24	Yes	Archived sample	n/a	n/a	Room temp

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.

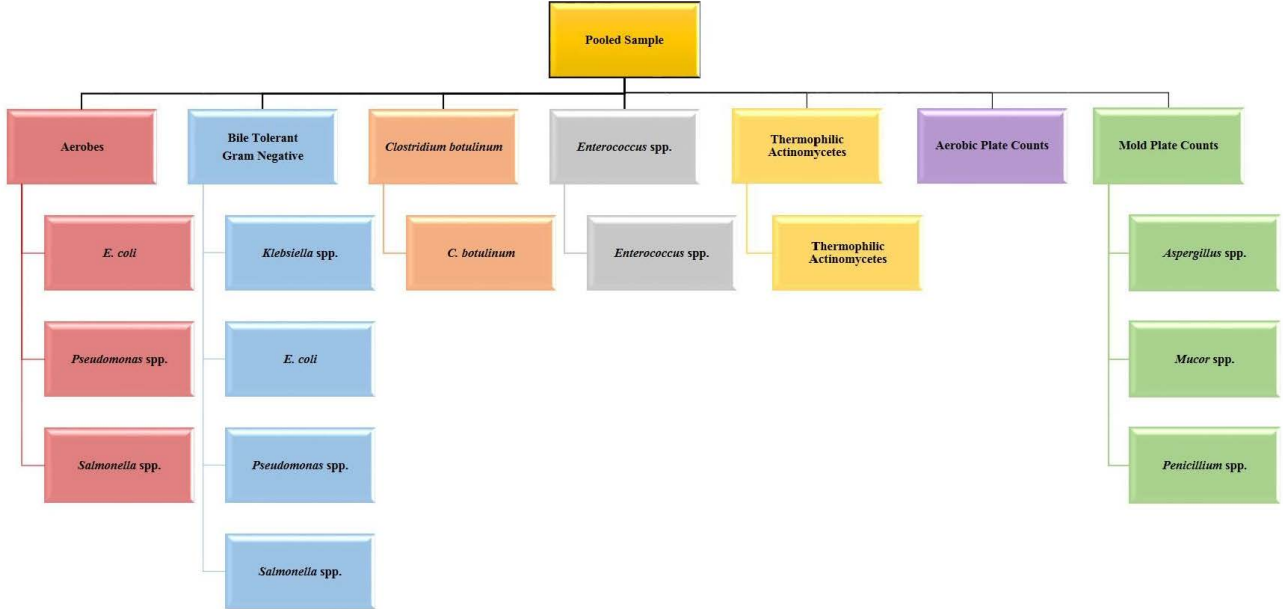
*Heat sample at 80°C for 10 minutes



Medical Marijuana Microbial Testing Plan (LEB-AP-603B)

Medical Marijuana Microbial Testing Plan

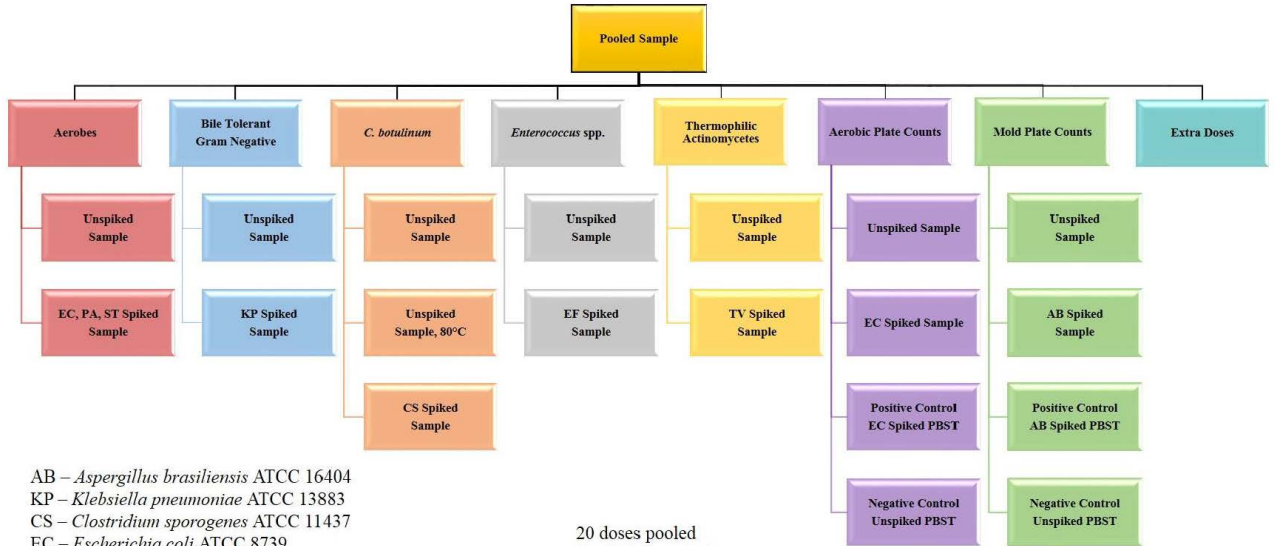
Target Organisms





Medical Marijuana Microbial Testing Plan

General Set-Up



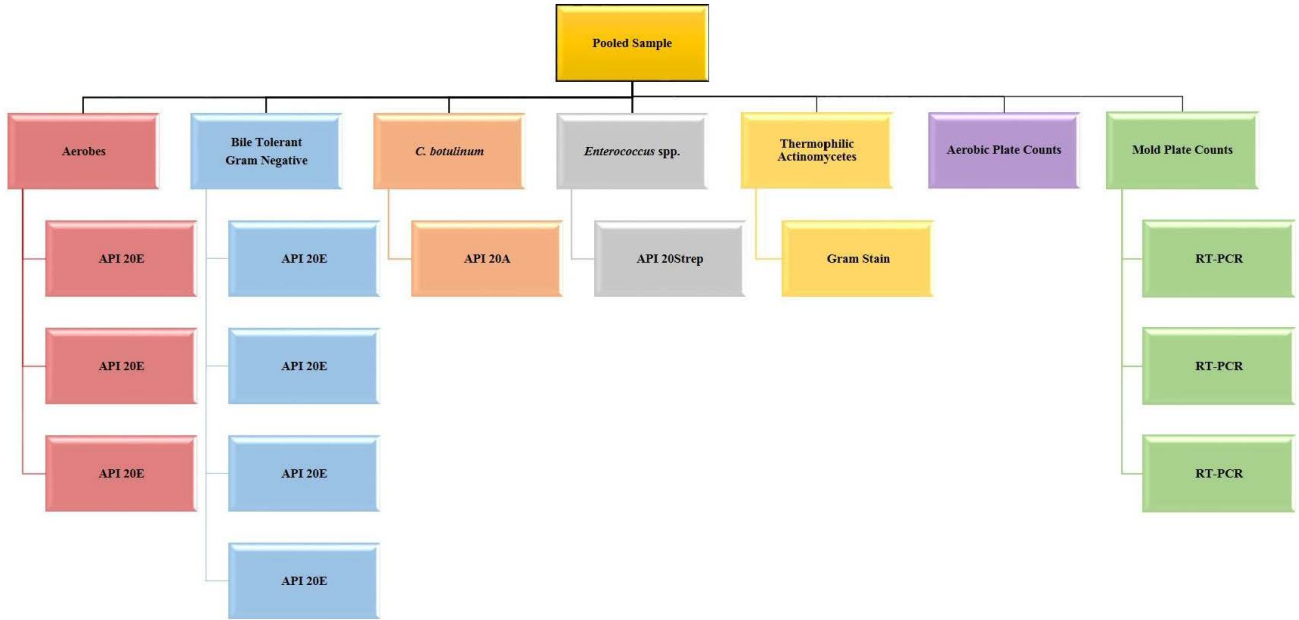
AB – *Aspergillus brasiliensis* ATCC 16404
 KP – *Klebsiella pneumoniae* ATCC 13883
 CS – *Clostridium sporogenes* ATCC 11437
 EC – *Escherichia coli* ATCC 8739
 PA – *Pseudomonas aeruginosa* ATCC 9027
 ST – *Salmonella typhimurium* ATCC 14028
 EF – *Enterococcus faecalis* ATCC 29212
 TV – *Thermoactinomyces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

20 doses pooled
 10-15 doses tested
 5-10 doses archived



Medical Marijuana Microbial Testing Plan

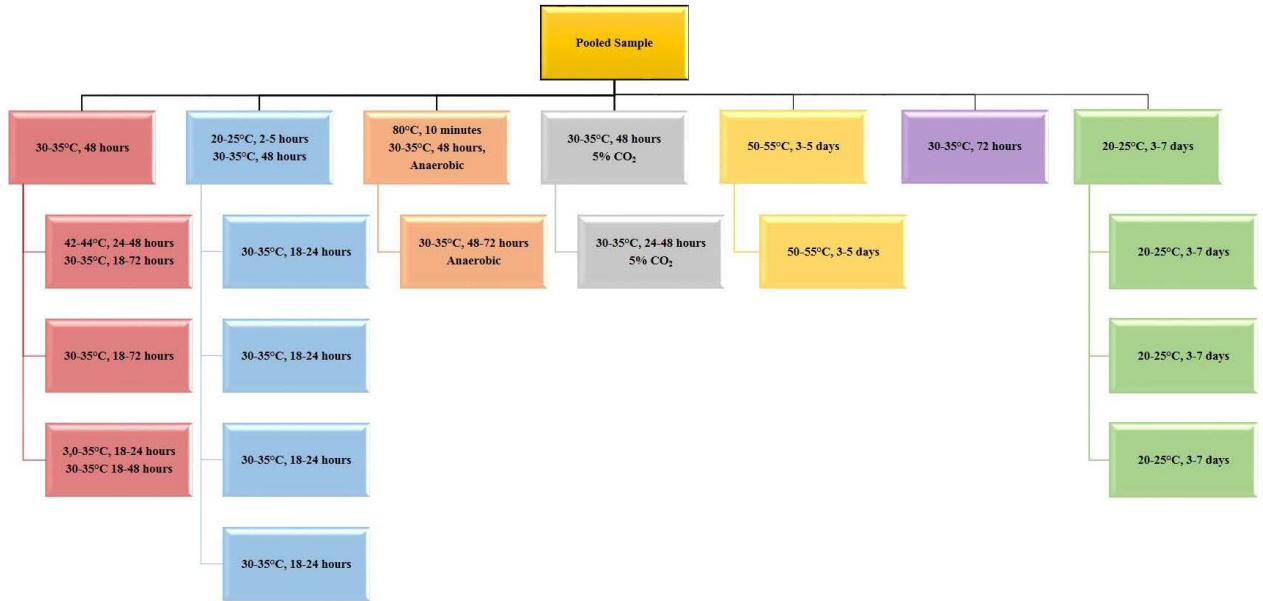
Organism Identification Assays





Medical Marijuana Microbial Testing Plan

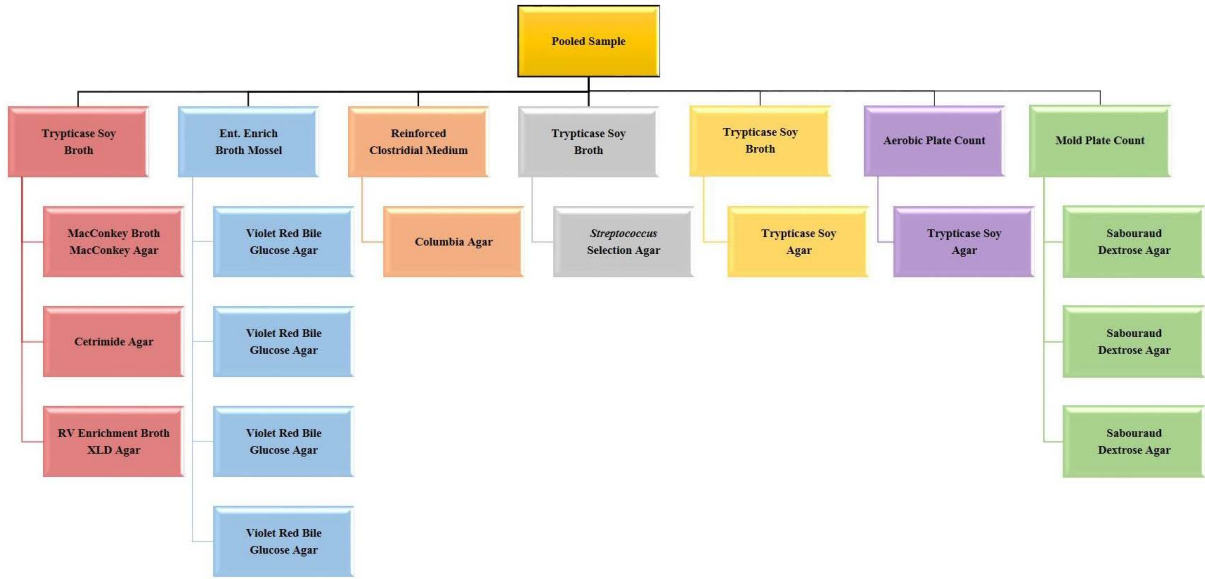
Incubation Conditions





Medical Marijuana Microbial Testing Plan

Media Needed





Medical Marijuana Sample Types and Preparation (LEB-AP-603C)

Product Type	Isopropyl Myristate Added	PBST Dilution	Heat and Vortex	Comments
Balm	X	1:10	X	Dilute all 20 doses
Capsules – Cellulose Fill		1:20	X	
Capsules – Oil Fill		1:10	X	
Syrup		1:10		
Tincture/Oral Solution/Oral Spray – Ethanol Base		1:10		
Tincture/Oral Solution/Oral Spray – Oil Base		1:10		
Vaporizer – Liquid viscosity		1:10	X	Dilute all 20 doses
Vaporizer – Waxy viscosity	X	1:10	X	Dilute all 20 doses
Lotion	X	1:10	X	Dilute all 20 doses
Lozenge		1:10	X	
Powder		1:10		
Plant Material		1:100		Dilute all 20 doses
Suppository		1:10	X	
Tablets – Cellulose Base		1:20	X	



Medical Marijuana Sample Pooling Log (LEB-RS-603A)

Accession #	Sample Type	Dose Amount	No. of Pooled Doses	Combined Pool Weight	Diluted Aliquot Volume	Analyst Initials/Date	Comments
		<input type="checkbox"/> Verified					<input type="checkbox"/> Add isopropyl myristate Number of Containers Submitted:
		<input type="checkbox"/> Verified					<input type="checkbox"/> Add isopropyl myristate Number of Containers Submitted:
		<input type="checkbox"/> Verified					<input type="checkbox"/> Add isopropyl myristate Number of Containers Submitted:
		<input type="checkbox"/> Verified					<input type="checkbox"/> Add isopropyl myristate Number of Containers Submitted:
		<input type="checkbox"/> Verified					<input type="checkbox"/> Add isopropyl myristate Number of Containers Submitted:
		<input type="checkbox"/> Verified					<input type="checkbox"/> Add isopropyl myristate Number of Containers Submitted:
		<input type="checkbox"/> Verified					<input type="checkbox"/> Add isopropyl myristate Number of Containers Submitted:
		<input type="checkbox"/> Verified					<input type="checkbox"/> Add isopropyl myristate Number of Containers Submitted:



Medical Marijuana Controlled Substance Inventory Log (LEB-RS-603B)

Accession #	Pooled Weight Initials/Date	Initial Archived Weight Initials/Date	Second Archived Weight	Second Use Archived Weight	Analyst/Witness Initials	Comments
						<input type="checkbox"/> All used for initial testing <input type="checkbox"/> Transfer for destruction <input type="checkbox"/> Other (see back)
						<input type="checkbox"/> All used for initial testing <input type="checkbox"/> Transfer for destruction <input type="checkbox"/> Other (see back)
						<input type="checkbox"/> All used for initial testing <input type="checkbox"/> Transfer for destruction <input type="checkbox"/> Other (see back)
						<input type="checkbox"/> All used for initial testing <input type="checkbox"/> Transfer for destruction <input type="checkbox"/> Other (see back)
						<input type="checkbox"/> All used for initial testing <input type="checkbox"/> Transfer for destruction <input type="checkbox"/> Other (see back)
						<input type="checkbox"/> All used for initial testing <input type="checkbox"/> Transfer for destruction <input type="checkbox"/> Other (see back)
						<input type="checkbox"/> All used for initial testing <input type="checkbox"/> Transfer for destruction <input type="checkbox"/> Other (see back)
						<input type="checkbox"/> All used for initial testing <input type="checkbox"/> Transfer for destruction <input type="checkbox"/> Other (see back)
						<input type="checkbox"/> All used for initial testing <input type="checkbox"/> Transfer for destruction <input type="checkbox"/> Other (see back)
						<input type="checkbox"/> All used for initial testing <input type="checkbox"/> Transfer for destruction <input type="checkbox"/> Other (see back)

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