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NYS DOH LEB-605

Aerobic Plate Counts for Medical Marijuana Testing

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1.0. Scope and Application

1.1. This method NYS DOH LEB-605, Aerobic Plate Counts for Medical Marijuana Testing (ELAP Method ID 9985) is used to estimate the number of heterotrophic aerobic bacteria in samples of medical marijuana products 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.

1.2. Aerobic plate counts are determined for all samples submitted for analysis in the Medical Marijuana Testing Program.

1.3. 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. Sample aliquots prepared according to NYS DOH LEB-603(ELAP Method ID 9926) are spread-plated onto Trypticase Soy Agar and incubated for 72 hours at 30-35°C. Colonies are enumerated with the aid of a Quebec colony counter and the number of CFU/dose is reported.

3.0. Definitions

3.1. For the purposes of this test, aerobic bacteria are defined as aerobic or facultatively anaerobic bacteria capable of growth on Trypticase Soy Agar within 72 hours at 30-35°C.

3.2. TSA stands for Trypticase Soy Agar

3.3. APC stands for Aerobic Plate Count

3.4. CFU stands for colony forming unit. Because a colony theoretically arises from a single bacterial cell, the number of CFUs reflects the number of culturable bacteria in the sample.

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. Laboratory equipment and benches shall be disinfected before and after use with a minimum concentration of 70% ethanol.

4.1.3. Mouth pipetting is prohibited.

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

4.1.5. All accidents, particularly those which may result in infection, shall be reported according to laboratory specific policies and procedures.

4.1.6. Laboratory safety procedures shall be followed at all times. Regulations required by federal, state and local government agencies shall be implemented and followed.

4.1.7. The analyst must be familiar with any possible hazards from the reagents and standards used for sample preparation and analysis.

4.1.8. Always follow guidelines listed in safety data sheets (SDS) for proper storage, handling, and disposal of samples, solvents, reagents, and standards. SDS are located within the laboratory. These guidelines must be made available to all personnel involved in microbiological analyses.

4.1.9. Appropriate lab coat, safety glasses and gloves must be worn when performing standard or sample preparations, working on instrumentation, disposing of waste, and cleaning laboratory equipment.

5.0. Shipping Conditions, Receiving, Preservation and Storage

5.1. Sample shipping conditions - 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 - Medical marijuana products from the RO are received, verified and documented ensuring that method, regulatory and Accreditation Body requirements are met. All medical marijuana products must be stored under the conditions based on the manufacturer's recommendation. The storage is documented.

5.3. Method holding times

5.3.1. Not determined.

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.

5.5. Storage

5.5.1. Samples are analyzed upon receipt.

5.5.2. If storage is required, samples are maintained at room temperature in a secure location.

6.0. Interferences

6.1. The presence of spreading colonies or confluent growth can interfere with accurate colony enumeration.

7.0. Apparatus and Materials

7.1. Equipment

7.1.1. Incubator, set at 30-35°C

7.1.2. Automatic pipetters and sterile micropipette tips.

7.1.3. Disposable sterile inoculum spreader, or equivalent

7.1.4. Quebec colony counter

7.1.5. Hand tally

7.2. Reagent and Chemicals.

7.2.1. Trypticase Soy Agar plates, 15 x 100 mm or 15 x 150 mm.

7.3. Forms

7.3.1. Medical Marijuana Aerobic Plate Count Result Sheet (e.g. LEB-RS-605A, Appendix B)

8.0. Quality Control/Assurance (Laboratories must conform to sections 9020-9050 of Standard Methods for the Examination of Water and Wastewater.)

8.1. Method Detection Limits

8.1.1. Detection limits are determined in accordance with relevant standards, regulations and accreditation body requirements.

8.2. Calibration and Standardization

8.2.1. Incubator temperatures shall be observed and recorded twice daily, separated by at least 4 hours.

8.2.1.1. Temperature of the 30-35°C walk-in is recorded.

8.2.1.1.1. If the incubator temperature does not stay within 30-35°C, laboratory specific corrective actions are followed. Analytical results are invalidated if the incubator temperature exceeds 35.0°C.

8.2.2. Temperature of the cold room/refrigerator are observed and recorded at least once daily.

8.2.2.1. If the cold room or refrigerator does not stay within 1-8°C, laboratory specific corrective actions are followed.

8.2.2.2. The optimum temperature range for a refrigerator is 1-4°C

8.2.2.3. 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°C, re-testing of media is not required.

8.2.2.4. 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.

8.2.3. Max/min temperatures are recorded when daily temperature measurements are not possible, such as on holidays and weekends.

8.2.4. Thermometers must be calibrated as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

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

8.2.6. Micropipetters are calibrated as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

8.3. Quality Control

8.3.1. Comparative recovery and sterility between lots of Trypticase Soy Agar will be determined as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

8.3.2. As an additional control, perform the air density test on days during which medical marijuana samples are analyzed.

8.3.2.1. Remove the lid from a TSA plate and expose the agar surface to ambient air for 15 minutes.

8.3.2.1.1. Use a plate of the same size as that used for sample analysis that day.

8.3.2.2. Record exposure time (e.g. LEB-RS-605A).

8.3.2.3. Replace the lid and incubate in parallel with aerobic plate count analyses for 72 ± 3 hours at 30-35°C.

8.3.2.4. Count the number of colonies and record the number of CFUs (e.g. LEB-RS-605A).

8.3.2.5. Should the number of surface colonies exceed 10, follow laboratory specific corrective actions.

8.3.2.5.1. If high numbers of surface colonies would interfere with accurate determination of sample APCs, the results will be qualified and reported as a QC failure.

8.3.2.5.2. The analysis for APC will be repeated if product is available.

8.3.3. The holding time for poured agar media in plates is 2 weeks if it is stored refrigerated in sealed plastic bags in the dark.

8.3.3.1. Agar plates can be used after 2 weeks storage if ongoing QC demonstrates no loss in selectivity or growth promotion.

8.3.4. Acceptability of supplies is tested according to requirements prescribed by the laboratory, Accreditation Body and in accordance with relevant regulations and standards

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 and as prescribed by the laboratory, Accreditation Body and in accordance with relevant regulations and standards.

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. Remove enough TSA plates from the cold room to accommodate a diluted sample aliquot, the associated matrix spike containing *E. coli* ATCC 8739, and positive (*E. coli*) and negative controls prepared according to NYS DOH LEB-603 and warm to room temperature while drying in the biohazard hood.

9.1.1. Depending on the sample volume to be plated, remove either 15 x 100 (small volume) or 15 x 150 mm (larger volume) plates.

9.1.2. If the sample dose is 2 mL or more (i.e., the diluted sample aliquot is 20 mLs or more after dilution with PBST), plate one tenth of the diluted sample aliquot volume.

9.1.2.1. For example, if the dose is 15 mL and the diluted sample aliquot volume is 150 mL, plate 15 mL total volume.

9.2. Label TSA plates with the sample accession number and dilution, if applicable.

9.3. Using an automatic micropipetter and sterile tips, pipette equivalent aliquots of sample onto each TSA plate.

9.3.1. Up to 0.5 mL can be plated onto a single 15 x 100 mm plate.

9.3.2. Up to 1 mL can be plated onto a single 15 x 150 mm plate.

9.4. Use a sterile inoculum spreader to spread the sample aliquot over the entire agar surface.

9.5. Repeat sections 9.3-9.4 for the associated matrix spike, positive control and negative control samples prepared in NYS DOH LEB-603.

9.6. After samples have dried, invert the plates and incubate in a plastic bag for 72 ± 3 hours at 30-35°C.

9.6.1. Stack plates no more than four high.

9.7. Count colonies on all plates inoculated with sample with the aid of a Quebec colony counter and hand tally, if required.

9.7.1. Record the number of colonies per plate inoculated with sample (e.g. LEB-RS-605A).

9.8. Count colonies on all plates inoculated with the positive control sample. The positive control plates should have up to 100 colonies showing typical *E. coli* morphology.

9.8.1. *E. coli* ATCC 8739 colonies are non-pigmented, shiny, round and have an entire margin when grown on TSA.

9.8.2. Record the total number of colonies from the positive control plates (sum of colonies on all plates) as CFUs (e.g. LEB-RS-605A).

9.8.2.1. If there are no colonies on the positive control showing typical *E. coli* ATCC 8739 morphology, the test results are invalidated.

9.8.2.2. If the total number of *E. coli* colonies is greater than 100 CFUs/sample aliquot, laboratory specific corrective actions are followed.

9.8.2.2.1. Depending on the number of colonies, the test results may be invalidated.

9.9. Count colonies on all plates inoculated with the matrix spike sample. Colonies of *E. coli* and any organisms indigenous to the sample aliquot will be observed.

9.9.1. Record the number of colonies per plate in the matrix spike as CFUs (e.g. LEB-RS-605A).

9.10. Count and record the number of colonies per plate in the negative control as CFUs (e.g. LEB-RS-605A).

9.10.1. If there are colonies on the negative control plate, laboratory specific corrective actions are followed.

9.10.1.1. Depending on the extent of contamination, the results may be invalidated.

9.10.2. If counting must be delayed, store plates at 4°C for no more than 48 hours.

9.10.3. For plates having no colony forming units, report the count as less than one CFU/dose plated.

9.10.3.1. If one tenth the volume was plated, report the count as less than ten CFU/dose plated

9.10.4. As a general practice, plates with more than 250 CFU should not be counted.

9.10.4.1. If there is no plate showing 250 or fewer colonies, results must be reported as estimated.

9.10.4.2. If colonies on plates are too numerous to count, dilute an archived sample from the same lot and repeat the analysis.

9.11. Colony identification is not required for any of the inoculated plates.

10.0. Data Acquisition, Reduction and Documentation

10.1. Record the accession number, analyst, sample type (sample, matrix spike, positive or negative control, size of plate used, number of CFUs per plate, total number of CFUs per dose, media lot date, and start/end dates and times of incubation (e.g. LEB-RS-605A).

10.2. Report results of aerobic plate counts as CFU/dose.

10.3. Results for samples from which *E. coli* was not recovered in the positive control are invalidated.

10.4. Record results of air density plate analyses (e.g. LEB-RS-605A).

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. Consult state regulations and standards for additional information on performing a DOC for microbiological contaminants.

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

11.2.1 Detection limits are determined in accordance with relevant standards, regulations and accreditation body requirements.

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.

13.0. References

13.1. Standard Methods for the Examination of Water and Wastewater, sections 9020-9050.

13.2. United States Pharmacopeia. USP38-NF33, The United States Pharmacopeial Convention, General chapters <61>, <62>, <1111>.

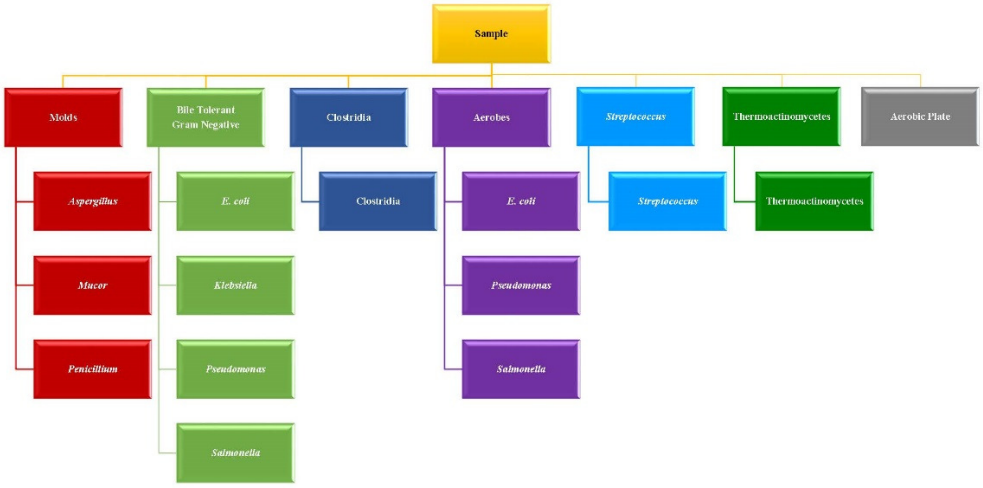
13.3. NYS DOH LEB-603, Preparation of Samples for Medical Marijuana Testing

14.0. Appendices

Appendix A – Flowcharts

Medical Marijuana Microbial Testing Plan

Organisms



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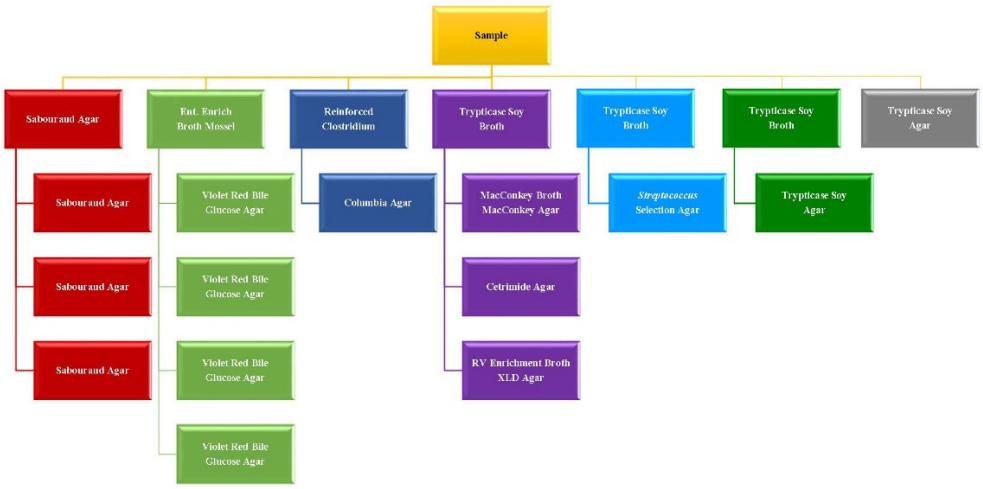
Medical Marijuana Microbial Testing Plan

Media



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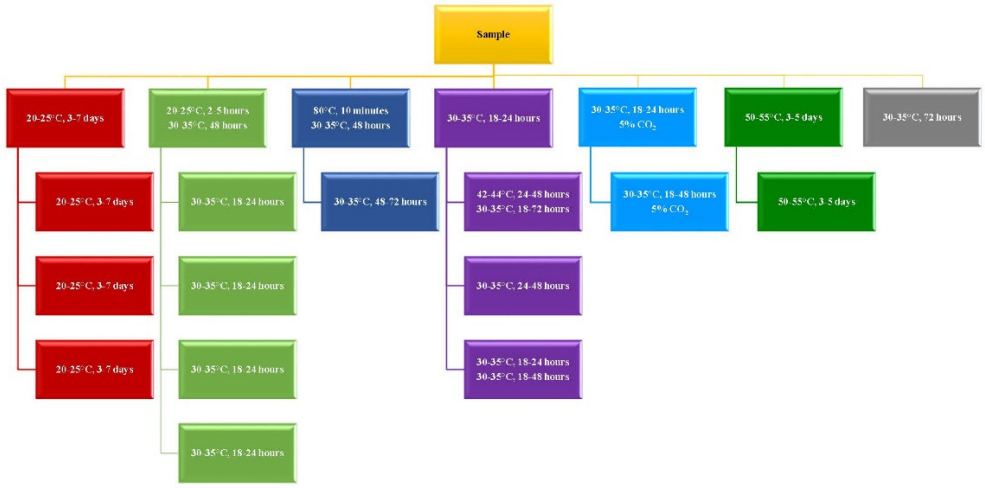
Medical Marijuana Microbial Testing Plan

Incubation Temperatures and Times



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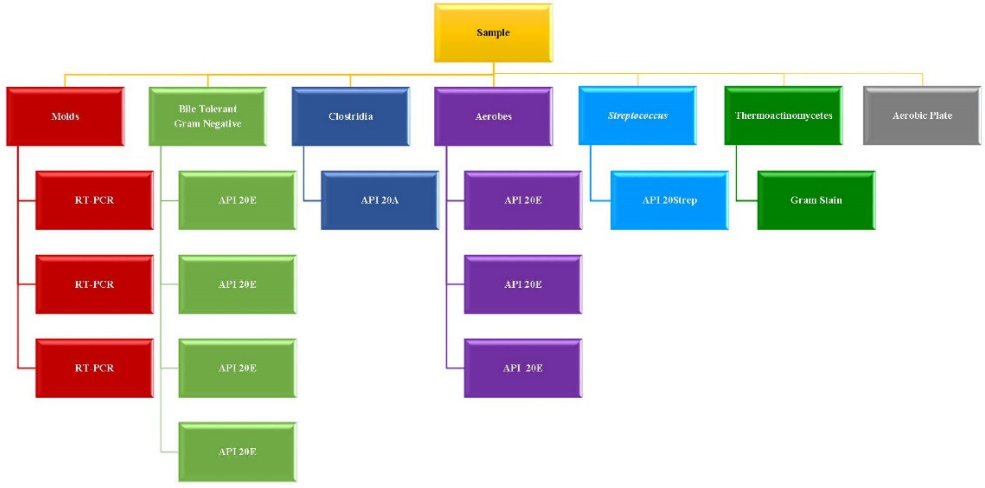
Medical Marijuana Microbial Testing Plan

Colony Identification Assays



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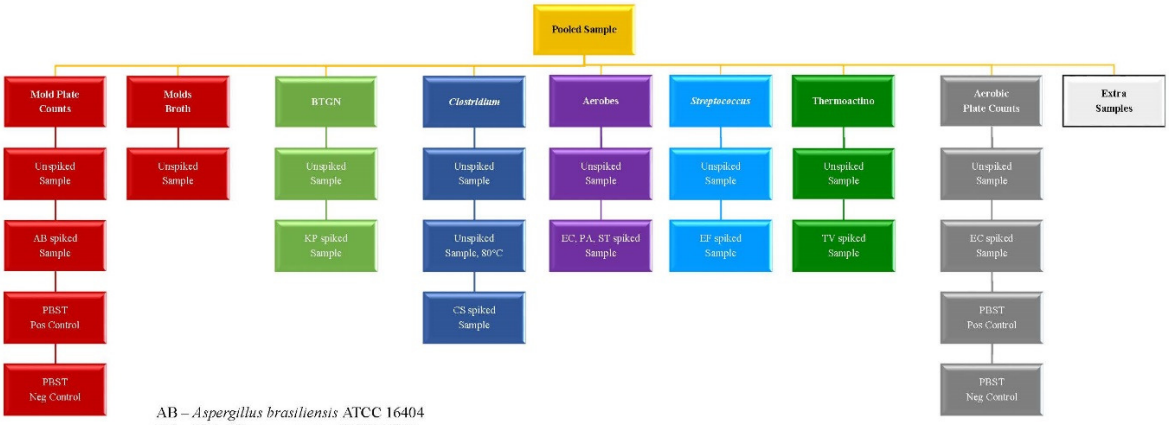
Medical Marijuana Microbial Testing Plan

General Set-Up for Presence/Absence and Plate Count Assays



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
16 doses tested
4 doses stored

4/7/16

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