New York State Department of Health - Wadsworth Center
Laboratory of Environmental Biology
NYS ELAP Laboratory ID 10765

Division of Environmental Health Sciences
Albany, New York

NYS DOH LEB-604

Microbial Presence/Absence Test for Medical Marijuana Samples

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1.0 Scope and Application

1.1. This method, NYS DOH LEB-604, Microbial Presence/Absence Test of Medical Marijuana Samples (ELAP Method ID 9986) is used after completion of NYS DOH LEB-603 (ELAP Method ID 9926) , "Preparation of Samples for Medical Marijuana Testing", and describes the inoculation of several types of bacteriological media for determining the presence or absence of bile tolerant gram negative bacteria, *Escherichia coli*, *Clostridium* species, *Pseudomonas* species, *Streptococcus* species, *Salmonella* species, the molds *Penicillium*, *Aspergillus* and *Mucor*, and thermophilic actinomycetes 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. Evaluation of medical marijuana products for aerobic bacteria (NYS DOH LEB-605) and molds (NYS DOH LEB-609) is also required.

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. Portions of the prepared sample of medical marijuana product and associated matrix spikes are inoculated into four different liquid media and incubated at temperatures and times specific for the detection of bile tolerant gram negative bacteria, *Escherichia coli*, *Clostridium* species, *Pseudomonas* species, *Streptococcus* species, *Salmonella* species, the molds *Penicillium*, *Aspergillus* and *Mucor*, and thermophilic actinomycetes.

2.2. Any growth (turbidity) observed is considered a presumptive positive result and is further investigated using the NYS DOH LEB-600 series.

3.0. Definitions

3.1. TSB stands for Trypticase Soy Broth

3.2. EEBM stands for Enterobacteria Enrichment Broth Mossel

3.3. RCM stands for Reinforced Clostridial Medium

3.4. SDB stands for Sabouraud Dextrose Broth

3.5. PBST stands for phosphate buffered saline containing 0.1% Tween 80

3.6. RT-PCR stands for real time-polymerase chain reaction

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

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

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

4.4. 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.

4.5. 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 and Supplies

7.1.1. Incubator, 30-35°C

7.1.2. Incubator, 20-25°C

7.1.3. Incubator, 50-55 ^{o}C

7.1.4. Incubator, 30-35 ^{o}C, 5% CO_2

7.1.5. Water Bath, 80°C

7.1.6. Pipette-aid

7.1.7. Pipettes, sterile – 25 mL, 10 mL, and 5 mL

7.1.8. Micropipetters and sterile tips
7.1.9. NIST certified laboratory timer – Krackeler Scientific, Albany, NY, cat no. 291-5004 or equivalent.
7.1.10. Disposable powder-free latex gloves
7.1.11. Anaerobic incubation chambers
7.1.12. Glassware or plasticware, sterile, for measurement and incubation of samples

7.2. Reagents and Chemicals

7.2.1. Trypticase Soy broth, sterile 1X and 10X, in e.g., 100 mL aliquots/Wheaton bottle and 10 mL aliquots/tube.
7.2.2. Enterobacteria Enrichment Mossel broth, sterile 1X and 2X, in e.g., 100 mL and 50 mL aliquots, respectively, in Wheaton bottles.
7.2.3. Reinforced Clostridial Medium broth, sterile, 1X and 2X, in e.g., 100 mL and 50 mL aliquots, respectively, in Wheaton bottles.
7.2.4. GasPak™ EZ Gas Generating sachets – Fisher Scientific, Hanover Park, IL, cat. no. B260678 or equivalent.
7.2.5. Anaerobic Indicator Strips – Fisher Scientific, Hanover Park, IL, cat. no. B71051 or equivalent.
7.2.6. Sample aliquots, and positive and negative controls of medical marijuana samples, prepared according to NYS DOH LEB-603.
7.2.7. PBST, sterile, in e.g., 100 mL and 1 L aliquots.
7.2.8. Lab prepared spikes or BioBalls® (prepared, e.g., according to LEB-603) of *Aspergillus brasiliensis*, ATCC 16404; *Clostridium sporogenes*, ATCC 11437; *Enterococcus faecalis*, ATCC 29212; *Escherichia coli*, ATCC 8739; *Klebsiella pneumoniae* ATCC 13883; *Mucor hiemalis*, ATCC 28932; *Penicillium chrysogenum*, ATCC 11709; *Pseudomonas aeruginosa*, ATCC 9027; *Salmonella typhimurium*, ATCC 14028; *Thermoactinomyces vulgaris* ATCC 43649.

7.3. Forms

7.3.1. Medical Marijuana Presumptive Presence/Absence Result Sheet (e.g. LEB-RS-604A, Appendix B).
7.3.2. Medical Marijuana Aerobic and Mold Plate Count Result Sheet (e.g. LEB-RS-605A)

8.0. Quality Control and Quality 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. Temperatures for the 20-25°C incubator are recorded.

8.2.1.1.1. If incubator temperature does not stay within 20-25°C, laboratory specific corrective actions are followed. Analytical results are invalidated if the incubator temperature exceeds 25.0°C.

8.2.1.2. Temperatures for the 30-35°C incubator are recorded.

8.2.1.2.1. 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.

8.2.1.3. Temperatures for the 50-55°C incubator are recorded.

8.2.1.3.1.1. If the incubator temperature does not stay within 50-55°C, laboratory specific corrective actions are followed. Analytical results are invalidated if the incubator temperature exceeds 55.0°C.

8.2.2. Temperatures of the cold room and refrigerators are observed and recorded at least once daily.

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

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

8.2.3.2. 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.

8.2.3.3. 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.

8.2.4. Water bath temperatures shall be observed and recorded twice daily, separated by at least 4 hours.

8.2.4.1. Temperature of the 80°C water bath is observed and recorded only on days that samples are analyzed.

8.2.4.1.1. If the water bath temperature does not stay within 78-80°C, laboratory specific corrective actions are followed. Analytical results are invalidated if the water bath temperature exceeds 81.0°C.

8.2.5. Max/Min temperatures are recorded when daily temperature measurement is not possible, such as on holidays and weekends.

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

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

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

8.3. Quality Control

8.3.1. Invalidate lot of media if tests are not in accordance with acceptance criteria prescribed by the laboratory, Accreditation Body and in accordance with relevant regulations and standards.

8.3.2. Acceptability of PBST is determined according to requirements prescribed by the laboratory, Accreditation Body and in accordance with relevant regulations and standards.

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

8.3.4. 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.

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

8.3.6. Agar plates can be used for 2 weeks if stored refrigerated in plastic bags and in the dark.

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

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. Aerobic Bacteria - *E. coli, Pseudomonas, Salmonella* (two sample aliquots)

9.1.1. Transfer two sample aliquots prepared in PBST according to NYS DOH LEB-603 into 2 labeled tubes, bottles, or flasks containing TSB (one for sample analysis and one for a matrix spike).

9.1.1.1. The volume of TSB chosen is dependent upon the volume of the sample aliquot being analyzed.

9.1.1.2. Do not dilute the TSB more than 10% with sample.

9.1.2. For a matrix spike, inoculate one of the sample aliquots diluted in TSB with a suspension of less than 100 CFUs each of *E. coli, Ps. aeruginosa* and *S. typhimurium*. Use either lab prepared spikes (prepared according to NYS DOH LEB-616) or 0.1mL BioBall® suspensions of *E. coli, Ps. aeruginosa* and *S. typhimurium* (prepared according to NYS DOH LEB-603).

9.1.3. Mix both portions by swirling or vortexing and incubate at 30-35°C for 18-24 hours without shaking.

9.1.4. After incubation record the results of both the sample and matrix spike analyses on the Sample Presumptive Presence/Absence Result Sheet (e.g. LEB-RS-604A).

9.1.4.1. Growth is indicated by the presence of turbidity and is recorded (e.g. LEB-RS-604A).

9.1.5. If there is turbidity, proceed to NYS DOH LEB-608, NYS DOH LEB-610 and NYS DOH LEB-611, for identification of *E. coli, Pseudomonas,* and *Salmonella* respectively.

9.1.6. If there is no turbidity, report results as negative (e.g., see the turbidity section of LEB-RS-604A).

9.1.7. If the positive and negative controls for aerobic plate counts and mold plate counts meet QC criteria (see NYS DOH-LEB 605 and NYS DOH LEB-609), results are considered valid in the absence of growth (turbidity) in the matrix spikes.

9.1.8. If the positive and negative controls for aerobic plate counts and mold plate counts do not meet QC criteria (see NYS DOH LEB-605 and NYS DOH LEB-609), results are considered invalid and the analyses must be repeated.

9.1.8.1. Additional testing using USP methods designed to decrease inhibitory effects of product that could result in growth in matrix spike samples may be undertaken using archived samples.

9.1.9. Record the incubation start and end date and time, volume of sample inoculated, volume of TSB used, results and lot date of the TSB (e.g. LEB-RS-604A).

9.2. Bile Tolerant Gram Negative Bacteria (two sample aliquots)

9.2.1. Transfer two sample aliquots prepared in PBST according to NYS DOH LEB-603 into 2 labeled 10 mL tubes of TSB.

9.2.1.1. If the PBST-diluted sample aliquot volume is greater than 1 mL, instead add 1/10 volume of 10X TSB to both sample aliquots.

9.2.1.1.1. TSB (1X) should not be diluted by more than 10% with sample.

9.2.2. For a matrix spike, inoculate one sample aliquot with a suspension of less than 100 CFUs of *K. pneumoniae.*

9.2.3. Mix both portions by swirling or vortexing and incubate at 20-25°C for 2-5 hours without shaking to resuscitate bacteria.

9.2.4. After 2-5 hours incubation, inoculate the entire sample and matrix spike into separate 100mLs EEBM pre-warmed to room temperature.

9.2.5. If the volume of the pre-incubated sample aliquot and matrix spike exceeds 10 mLs, instead add the sample to an equal volume of 2X EEBM.

9.2.6. Mix both portions by swirling and incubate the EEBM at 30-35°C for 24-48 hours.

9.2.7. Samples are incubated until color change is evident, or a maximum of 48 hours.

9.2.8. After incubation record the results (e.g. LEB-RS-604A).

9.2.8.1. Growth is indicated by the presence of turbidity, and is recorded (e.g. LEB-RS-604A).

9.2.8.2. Record medium color.

9.2.8.2.1. Typically, medium color changes from green to yellow, and is recorded as "Y" in the color change section.

9.2.9. If there is turbidity, proceed to NYS DOH LEB-606 for identification of Bile Tolerant Gram Negative Bacteria. If there is no turbidity, report the results as negative (e.g. LEB-RS-604A).

9.2.10. If the positive and negative controls for aerobic plate counts and mold plate counts meet QC criteria (see NYS DOH LEB-605 and NYS DOH LEB-609), results are considered valid in the absence of growth (turbidity) in the matrix spikes.

9.2.11. If the positive and negative controls for aerobic plate counts and mold plate counts do not meet QC criteria (see NYS DOH LEB-605 and NYS DOH LEB-609), results are considered invalid and the analyses must be repeated.

9.2.11.1. Additional testing using United States Pharmacopeia (USP) methods designed to decrease inhibitory effects of product that could result in growth in matrix spike samples may be undertaken using archived samples.

9.3. *Clostridium* spp. (three sample aliquots)

9.3.1. Transfer three sample aliquots prepared in PBST according to NYS DOH LEB-603 into three 10 mL tubes (one for sample analysis, one for a heat-treat sample and one for a matrix spike).

9.3.1.1. Use sterile PBST to make the final volume 10 mL if required.

9.3.2. If the volume of the PBST-diluted aliquot exceeds 10 mLs, split the diluted sample aliquot designated for heat treatment into 10 mL sub-aliquots and leave the other two portions of sample in the original flasks.

9.3.2.1. The final volume of sample aliquots heated at 80 ^{o}C must not exceed 10 mL.

9.3.3. Heat the sample aliquot(s) designated for heat treatment at 80°C in a water bath for 10 minutes and cool on ice.

9.3.3.1. Leave the other 2 aliquots at room temperature.

9.3.4. For a matrix spike, inoculate one of the sample aliquots with 0.1 mL of a *C. sporogenes* BioBalls® suspension prepared according to NYS DOH LEB-603.

9.3.5. Transfer 10 mL sample aliquots and the matrix spike to separate bottles containing 100 mL of Reinforced Clostridial Medium (RCM) that have been pre-reduced overnight.

9.3.5.1. To pre-reduce media, incubate under anaerobic conditions by following the manufacturer's instructions for GasPak™ and Dry Anaerobic Indicator Strips.

9.3.5.1.1. Dry Anaerobic Indicator strips are blue under aerobic conditions and white under anaerobic conditions.

9.3.5.1.2. If the color strip indicates the presence of aerobic conditions after incubation overnight in the anaerobic

chamber, transfer the plates to a different anaerobic chamber and replace the GasPak™ and indicator strip.

9.3.5.1.3. If anaerobic conditions are still not achieved, follow laboratory specific corrective actions.

9.3.5.2. Do not dilute the RCM more than 10% with sample.

9.3.5.2.1. For larger sample volumes, dilute 50 mLs sample into 50 mLs 2X RCM.

9.3.6. Incubate the RCM samples at 30-35°C for 48 ± 3 hours under anaerobic conditions.

9.3.7. After incubation record the results (e.g. LEB-RS-604A).

9.3.7.1. Growth is indicated by the presence of turbidity and is recorded (e.g. DOH LEB-RS-604A).

9.3.8. If there is turbidity, proceed to NYS DOH LEB-607 for identification of *Clostridium*.

9.3.9. If there is no turbidity, report the results as negative (e.g. LEB-RS-604A).

9.3.10. If the positive and negative controls for aerobic plate counts and mold plate counts meet QC criteria (see NYS DOH LEB-605 and NYS DOH LEB-609), results are considered valid in the absence of growth (turbidity) in the matrix spikes.

9.3.11. If the positive and negative controls for aerobic plate counts and mold plate counts do not meet QC criteria (see NYS DOH LEB-605 and NYS DOH LEB-609), results are considered invalid and the analyses must be repeated.

9.3.11.1. Additional testing using methods to decrease inhibitory effects of product that could result in growth in matrix spike samples may be undertaken using archived samples.

9.4. ***Streptococcus*** **species (two sample aliquots)**

9.4.1. Transfer two sample aliquots prepared in PBST according to NYS DOH LEB-603 into 2 labeled tubes, bottles, or flasks of TSB (one for sample analysis and one for a matrix spike).

9.4.1.1. The volume of TSB chosen is dependent upon the volume of the sample aliquot.

9.4.1.2. Do not dilute the TSB more than 10% with sample.

9.4.2. For a matrix spike, inoculate one of the sample aliquots with a suspension of less than 100 CFUs of *E. faecalis*. Use either lab-prepared spikes prepared according to NYS DOH LEB-616 or a 0.1 mL BioBall® suspension of *E. faecalis* prepared according to NYS DOH LEB-603.

9.4.3. Mix both by vortexing or swirling and incubate at 30-35°C for 48 ± 3 hours under 5% CO_2.

9.4.4. After incubation record the results (e.g. LEB-RS-604A).
Growth is indicated by the presence of turbidity and is recorded (e.g. LEB-RS-604A).

9.4.5. If there is turbidity, proceed to NYS DOH LEB-612 for identification of *Streptococcus*.

9.4.6. If there is no turbidity, record the results as negative (e.g. LEB-RS-604A).

9.4.7. If the positive and negative controls for aerobic plate counts and mold plate counts meet QC criteria (see NYS DOH LEB-605 and NYS DOH LEB-609), results are considered valid in the absence of growth (turbidity) in the matrix spikes.

9.4.8. If the positive and negative controls for aerobic plate counts and mold plate counts do not meet QC criteria (see NYS DOH LEB-605 and NYS DOH LEB-609), results are considered invalid and the analyses must be repeated.

9.4.8.1. Additional testing using USP methods designed to decrease inhibitory effects of product that could result in growth in matrix spike samples may be undertaken using archived samples.

9.5. Thermophilic Actinomycetes (two sample aliquots)

9.5.1. Add two diluted sample aliquots (prepared according to NYS DOH LEB-603) to two labeled flasks of TSB.

9.5.1.1. The volume of TSB chosen is dependent upon the volume of the sample aliquot.

9.5.1.2. It is important to use a flask regardless of sample volume to allow for greater exposure to air.

9.5.1.3 Do not dilute the TSB more than 10% with sample.

9.5.2. For a matrix spike, inoculate one of the flasks with a suspension of less than 100 CFUs *T. vulgaris.*

9.5.3. Mix by swirling and incubate at 50-55°C for 3-5 days without shaking.

9.5.3.1. The sample is incubated until the matrix spike shows turbidity for a maximum of 5 days.

9.5.4. After incubation record the results on the Sample Presumptive Presence/Absence Result Sheet (e.g., see LEB-RS-604A, Appendix B).

9.5.4.1. Growth is indicated by the presence of turbidity and is recorded (e.g. LEB-RS-604A).

9.5.5. If there is growth, proceed to NYS DOH LEB-613 for identification of thermophilic actinomycetes.

9.5.6. If there is no turbidity, record results as negative (e.g. LEB-RS-604A).

9.5.7. If the positive and negative controls for aerobic plate counts and mold plate counts meet QC criteria (see NYS DOH LEB-605 and NYS DOH LEB-609), results are considered valid in the absence of growth (turbidity) in the matrix spikes.

9.5.8. If the positive and negative controls for aerobic plate counts and mold plate counts do not meet QC criteria (see NYS DOH LEB-605 and NYS DOH LEB-609), results are considered invalid and the analyses must be repeated.

9.5.8.1. Additional testing using USP methods designed to decrease inhibitory effects of product that could result in growth in matrix spike samples may be undertaken using archived samples.

9.6. Enrichment for potential identification of fungi using qPCR (one sample aliquot).

9.6.1. Inoculate a sample aliquot prepared according to NYS DOH LEB-603 into a labeled tube, bottle or flask of SDB.

9.6.1.1. The volume of SDB chosen is dependent upon the volume of the sample aliquot.

9.6.1.2. Do not dilute the SDB more than 10% with sample.

9.6.2. Mix by vortexing or swirling and incubate at 20-25°C for 3-7 days.

9.6.3. After incubation record the results (e.g. LEB-RS-605A).

9.6.4. Growth is indicated by the presence of turbidity and is recorded (e.g. LEB-RS-604A).

9.6.5. If there is no turbidity, record results (e.g. LEB-RS-605A).

9.6.6. This sample aliquot is analyzed at the discretion of the laboratory and only if required, e.g., due to desired confirmation of the results of mold pate count analyses, or unacceptable results due to control failures or laboratory accidents.

9.6.7. If analysis is desired, proceed to NYS DOH LEB-609 and NYS DOH LEB-618 for DNA extraction and identification of fungi using RT-PCR.

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

10.1. Record the accession number, the incubation start and end dates and times for each analysis, media lot dates, results, and analyst initials (e.g. LEB-RS-604A).

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 state regulations and standards for additional information on performing a DOC for microbiological 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 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, sections 9020-9050

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

13.4 NYS DOH LEB-605, Aerobic Plate Counts for Medical Marijuana Testing

13.5 NYS DOH LEB-606, Identification of Bile Tolerant Gram Negative Bacteria in Medical Marijuana Products

13.6 NYS DOH LEB-607, Identification of *Clostridium* in Medical Marijuana Products

13.7 NYS DOH LEB-608, Identification of *Escherichia coli* in Medical Marijuana Products

13.8 NYS DOH LEB-609, Mold Plates Counts and Identification for Medical Marijuana Testing

13.9 NYS DOH LEB-610, Identification of *Pseudomonas* in Medical Marijuana Products

13.10 NYS DOH LEB-611, Identification of *Salmonella* in Medical Marijuana Products

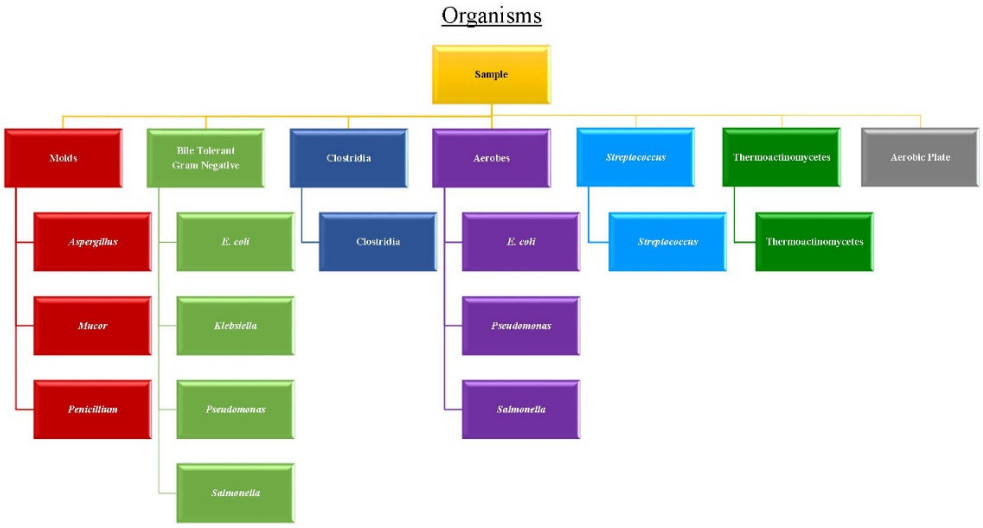
13.11 NYS DOH LEB-612, Identification of *Streptococcus* in Medical Marijuana Products

13.12 NYS DOH LEB-613, Identification of Thermophilic Actinomycetes in Medical Marijuana Products

14.0 Appendices

Appendix A – Flowcharts

Medical Marijuana Microbial Testing Plan



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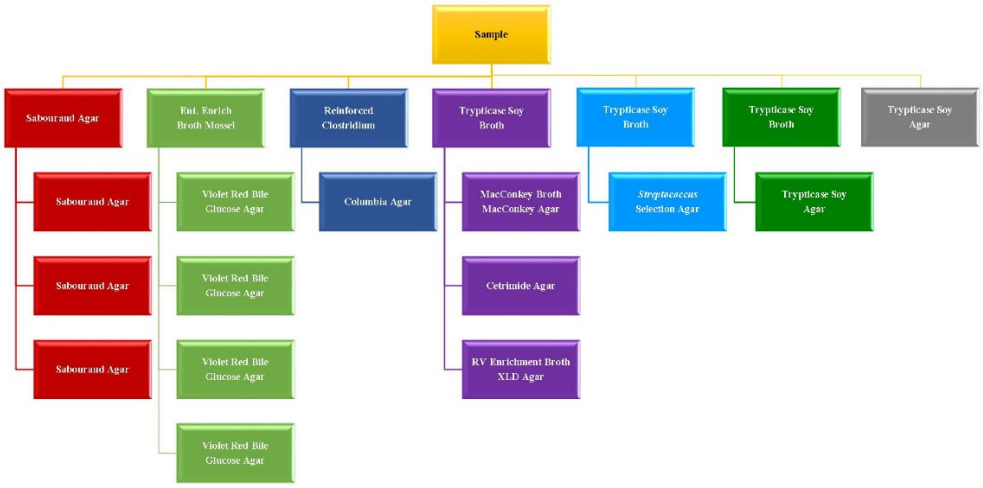
Medical Marijuana Microbial Testing Plan

Media



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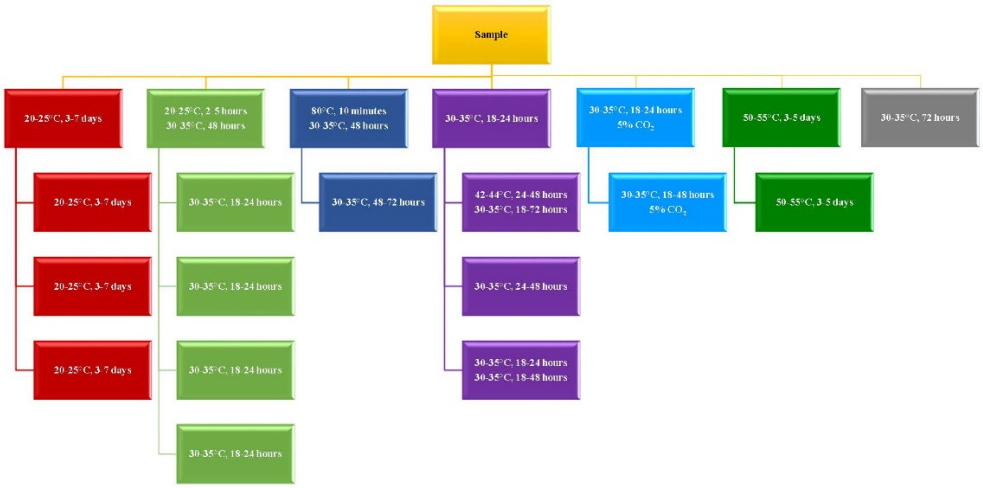
Medical Marijuana Microbial Testing Plan

Incubation Temperatures and Times



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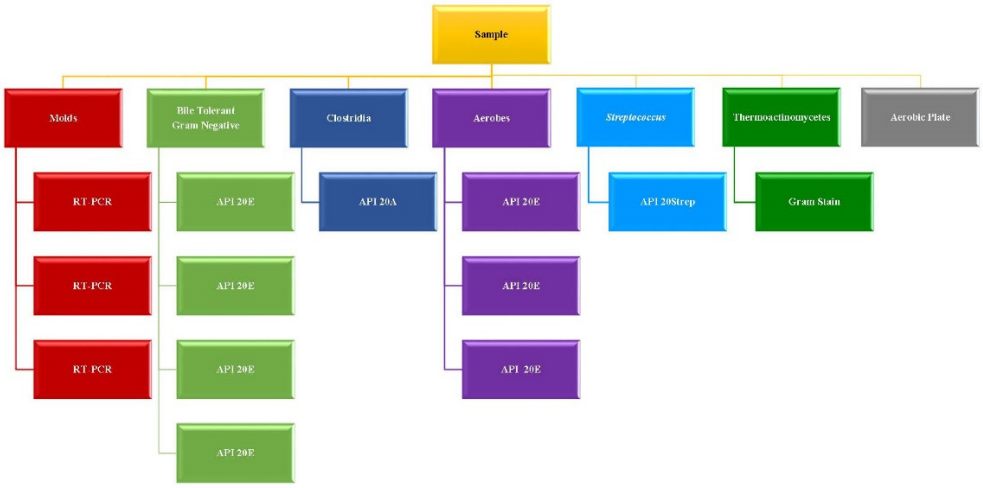
Medical Marijuana Microbial Testing Plan

Colony Identification Assays



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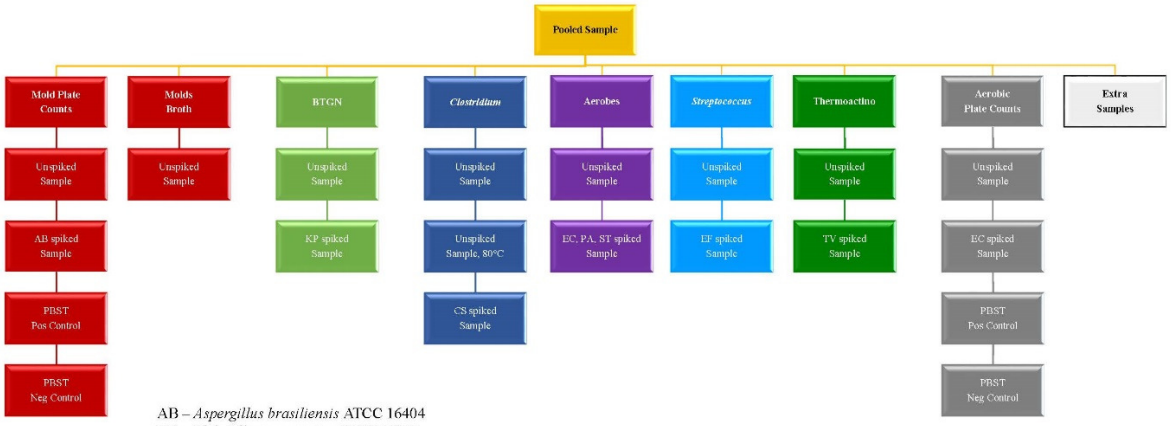
Medical Marijuana Microbial Testing Plan

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



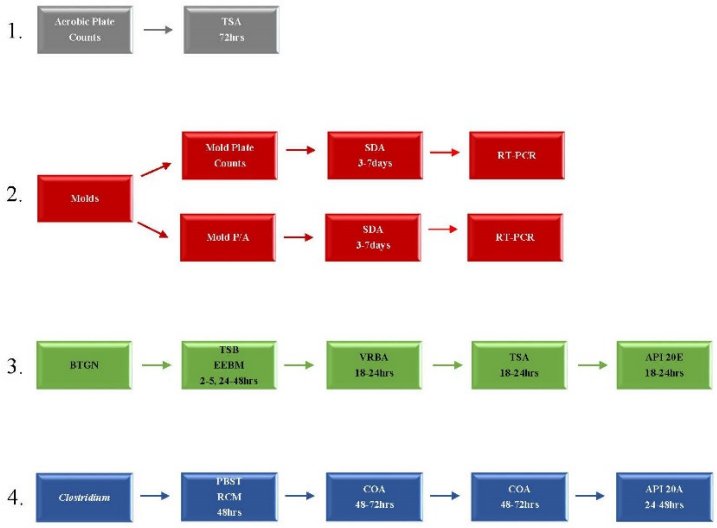
AB – *Aspergillus brasiliensis* ATCC 16404
KP – *Klebsiella pneumoniae* ATCC 13883
CS – *Clostridium sporogenes* ATCC 11437
EC – *Escherichia coli* ATCC 8739
PA – *Pseudomonas aeruginosa* ATCC 9027
ST – *Salmonella typhimurium* ATCC 14028
EF – *Enterococcus faecalis* ATCC 29212
TV – *Thermoactinomyces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

20 doses pooled
16 doses tested
4 doses stored

4/7/16

Medical Marijuana Microbial Testing Plan

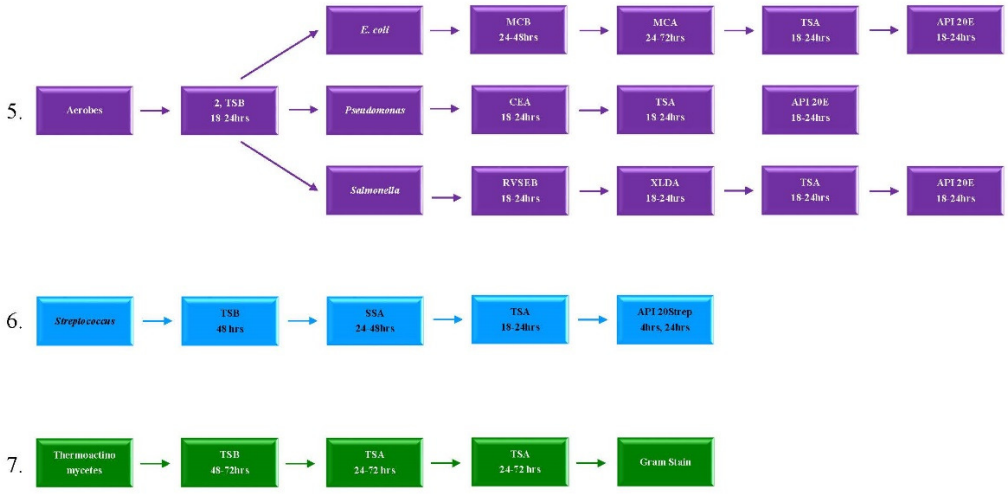
General Set-Up for Presence/Absence, Colony Identifications, and Plate Count Assays



4/7/16

Medical Marijuana Microbial Testing Plan

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



4/7/16

Appendix B – Forms

Medical Marijuana Presence/Absence Results Sheet (LEB-RS-604A)

Accession #: _____ Analyst Initials: _____ Start Date/Time: _____

Sample Type/Volume of Inoculum: _____ Sample Prep Date: _____

Aerobic Bacteria (*E. coli, Pseudomonas, Salmonella*), 30-35°C, E552, 18-24 hours

	Turbidity†		End Date/Time:
Sample	Y	N	
Matrix Spike	Y	N	TSB Lot Date*:

Bile Tolerant Gram Negative Bacteria, 30-35°C, E552, 24-48hrs

	Turbidity†		Color Change		End Date/Time:
Sample	Y	N	Y	N	
Matrix Spike	Y	N	Y	N	EEBM Lot Date*:

***Clostridium*, 80°C Water Bath, S/N 208556, 30-35°C Incubator, E552, 48±3hrs**

	Turbidity†		End Date/Time:
Sample	Y	N	Gas Pak Lot:
80°C Sample	Y	N	Indicator Lot:
Matrix Spike	Y	N	RCM Lot Date*:

***Streptococcus*, 30-35°C with 5% CO_2 Incubator, S/N 37111-5139, 24-48hrs**

	Turbidity†		End Date/Time:
Sample	Y	N	
Matrix Spike	Y	N	TSB Lot Date*:

Thermoactinomycetes, 50-55°C Incubator, S/N 0302193, 48-72hrs

	Turbidity†		End Date/Time:
Sample	Y	N	
Matrix Spike	Y	N	TSB Lot Date*:

* Expiration dates of liquid media is 3 months
EEBM = Enterobacteria Enrichment Broth Mossel, RCM = Reinforced Clostridia Medium, TSB = Trypticase Soy Broth, M.S. = Matrix Spike
† Samples with no turbidity are reported as "Negative"

Comments:

Lab Prepared Spike Date or BioBall Lot/Exp. Dates:

C. sporogenes: *E. coli:*
Ent. faecalis: *K. pneumoniae:*
Ps. aeruginosa: *S. typhimurium:*
T. vulgaris:

_____
Reviewed by Date