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Laboratory of Environmental Biology
NYS ELAP Laboratory ID 10765
Division of Environmental Health Sciences
Albany, New York

NYS DOH LEB-613

Identification of Thermophilic Actinomycetes in Medical Marijuana Products
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1.0. Scope and Application

1.1. This method NYS DOH LEB-613, Identification of Thermophilic Actinomycetes in Medical Marijuana Products (ELAP ID 9978) the methods for detecting and identifying thermophilic actinomycetes in medical marijuana samples 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. It is used as a follow-up to NYS DOH LEB-604 section 9.6, and applies to sample enrichments showing growth in Trypticase Soy broth after incubation at 50-55°C.

2.0. Summary of the Method

2.1. Medical marijuana samples showing growth in Trypticase Soy Broth incubated at 50-55°C are transferred to Trypticase Soy Agar and incubated at 50-55°C for 3-5 days. Bacterial colonies suspected of being actinomycetes are microscopically examined for morphometrics typical of actinomycetes. Samples from which actinomycetes are isolated at 50-55°C are reported as positive for thermophilic actinomycetes.

3.0. Definitions

3.1. TSB stands for Trypticase Soy Broth
3.2. TSA stands for Trypticase Soy Agar

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 Envirocide®, 10% bleach, or 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. SDSs are located within the laboratory. These guidelines must be made available to all personnel involved in microbiological analyses.
4.2. 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
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. This procedure is initiated upon completion of the Presence/Absence procedure (see NYS DOH LEB-604).

5.4. Preservation
5.4.1. Presence-Absence test aliquots that are presumptive positive for thermophilic actinomycetes are stored refrigerated until it has been determined that they are not needed for additional microbiological evaluation.

5.5. Storage
5.5.1. If storage is required prior to analysis, isolates or archived plates are stored refrigerated until it has been determined that they are not needed for additional microbiological evaluation.

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
7.1.1. Incubator, set at 50.0-55.0°C
7.1.2. Automatic pipetters and sterile aerosol-resistant micropipette tips
7.1.3. Sharpie or equivalent
7.1.4. Sterile inoculating loops, 10µL
7.1.5. Biosafety cabinet with HEPA filter

7.2. Reagents and Chemicals
7.2.1. TSA, 15 x 100mm, plates.
7.2.2. Gram Stain Kit – BD BBL, Fisher Scientific, cat. no. B12539 or equivalent.
7.2.3. Freshly grown positive and negative control cultures for Gram staining, e.g., *Enterococcus* (Gram positive) and *E. coli* (Gram negative).

7.2.4. Disinfectants such as Envirocide® (Fisher Scientific cat. no. 19898220), 70% ethanol, and/or Clorox

7.3. Forms

7.3.1. Thermophilic Actinomycetes Identification Result Sheet (e.g., LEB-RS-613A).

8.0. Quality Control/Assurance

8.1. Method Detection Limits

8.1.1. Method Detection Limits are product-specific and 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 50.0-55.0°C incubator is recorded on the Incubator Temperature Record.

8.2.1.1.1. If the incubator temperature does not stay within 50.0-55.0°C, laboratory-specific corrective actions are followed. Analytical results may be invalidated if the incubator temperature exceeds 55.0°C, at the discretion of the laboratory.

8.2.2. 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 Record.

8.2.2.1. If the cold room or refrigerator does not stay within 1.0-8.0°C, laboratory-specific corrective actions are followed.

8.2.2.2. The optimum temperature range for a cold room or refrigerator is 1.0-4.0°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 8.0°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 twice-daily temperature measurements are not possible, such as on holidays and weekends.

8.2.4. Thermometers must be calibrated against a NIST-certified thermometer as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.
8.2.5. The volumetric accuracy of automatic pipettors and serological pipettes is determined as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

8.2.6. The intensity and efficacy of the UV light in the biosafety cabinet is determined as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

8.2.7. Biosafety cabinets are certified annually.

8.3. Quality Control

8.3.1. Comparative recovery and sterility between lots of TSA will be determined.

8.3.2. Agar plates can be used for up to 2 weeks after preparation date if stored refrigerated in plastic bags and in the dark.

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

8.3.3. 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.

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

8.3.4. Sterility of disposable inoculation loops and spreaders are determined.

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.

9.1.2. All work surfaces are disinfected prior to subculturing and colony identification.

9.1.3. Subculturing and colony identification are performed in a physically different location than sample preparation and initial sample analyses to prevent cross-contamination of incoming products.
9.2. Subculture

9.2.1. If the sample shows turbidity in TSB that is incubated at 50.0-55.0°C (see NYS DOH LEB-604, section 9.6) and was confirmed to have growth (see LEB-604 section 9.7.), use the TSA plate from NYS DOH LEB-604 section 9.7. to confirm the presence of thermophilic actinomycetes by gram stain (section 9.3.).

9.2.1.1. If growth is confluent on the TSA plate, re-streak for isolation of individual colonies and incubate at 50.0-55.0°C for 3-5 days.

9.3. Gram Stain

9.3.1. Select one or more well-isolated colonies having distinct morphologies from growth-positive TSA plates and record the sample source and colony morphology on the Thermophilic Actinomycetes Identification Results Sheet (e.g., LEB-RS-610A).

9.3.2. Place a drop of sterile water onto a clean microscope slide, and using a sterile inoculation loop, pick a small amount of growth from an isolated colony.

9.3.3. Mix the bacteria with the drop of sterile water on the microscope slide and spread it evenly over an area the size of a quarter.

9.3.4. Include recently grown positive (e.g., *Ent faecalis* ATCC 29212) and negative (e.g., *E. coli* ATCC 8739) control cultures on separate slides.

9.3.5. Air dry the slide and fix the sample by quickly passing the slide through the flame of a Bunsen burner.

9.3.5.1. Alternatively, place the slide on a slide warmer set to a moderate temperature until dry.

9.3.6. Flood the smear with the crystal violet solution for one minute.

9.3.7. Wash the slide gently with water and drain off excess.

9.3.8. Flood the slide with the iodine solution for one minute.

9.3.9. Wash the slide gently with water and drain off excess.

9.3.10. Decolorized the slide for 5-10 seconds, by tilting slide, until the solvent flows colorlessly from the slide.

9.3.11. Counterstain the slide by flooding the slide with safranin for 15 seconds.

9.3.12. Wash the slide gently with water and air dry.

9.3.13. Examine microscopically for gram reaction. Gram positive organisms will appear purple and gram negative organisms will appear pink.

9.3.14. If the control cultures do not give the appropriate staining reaction, repeat the test.

9.3.15. Record the colony morphology and results of the Gram stain on the Thermophilic Actinomycetes Results Sheet (e.g., LEB-RS-613A).

9.3.15.1. Actinomycetes are spore-forming and gram-positive, with branching filaments of less than 1 micron in diameter. They may also have a diphtheroid appearance.
9.3.16. If the organisms do not display morphology typical for thermophilic actinomycetes proceed to section 9.4.

9.4. Identification of Non-Target Organisms

9.4.1. The identification of non-regulated bacterial contaminants is required.

9.4.1.1. In cases where there is growth of a non-regulated analyte(s), consultation with the NYS Medical Marijuana Program is required.

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

10.1. Record the accession number, analyst initials, TSA lot date, start and end dates and times, source of colony (sample, matrix spike, or positive control), colony morphology, and gram stain results on the Thermophilic Actinomycetes Identification Results Sheet (e.g., LEB-RS-613A).

10.2. Report samples showing bacterial growth typical of actinomycetes on TSA at 50.0-55.0°C that result in identification of thermophilic actinomycetes as positive for thermophilic actinomycetes.

10.3. Report samples showing growth on TSA at 50.0-55.0°C that do not result in identification of thermophilic actinomycetes as negative for thermophilic actinomycetes.

10.4. Report samples showing no growth on TSA at 50.0-55.0°C as negative for thermophilic actinomycetes.

10.4.1. A note is added to the final report if unregulated contaminants are identified.

10.5. Invalidate the test results for samples lacking growth in the matrix spike at any point in the analysis or from which thermophilic actinomycetes was not identified only if the positive and negative controls for aerobic plate counts and mold plate counts do not meet QC criteria (see NYS DOH LEB-605).

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.

11.2. **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 aqueous 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**


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

13.3. 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**
Thermophilic Actinomycetes Identification Results Sheet (LEB-RS-613A)

Incubate TSA for 3-5 days
(50-55°C Incubator)

<table>
<thead>
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<th>Accession Number:</th>
<th>Analyst Initials:</th>
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Final Results (circle one):

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<tr>
<th>Negative for Thermophilic Actinomycetes</th>
<th>Positive for Thermophilic Actinomycetes (list organism)</th>
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<table>
<thead>
<tr>
<th>TSA Start Date/Time:</th>
<th>Sample Growth: Y N</th>
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<th>M.S. Growth: Y N</th>
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<tr>
<th>Gram Stain Lot/Exp Date:</th>
<th>Negative Control Result:</th>
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Source (Sample, M.S., or P.C.), Colony Morphology and Gram Stain Results

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TSA = Trypticase Soy Agar, M.S. = matrix spike, P.C. = positive control.
*T. vulgaris* ATCC 43649, used as a matrix spike, appears as dry, white powdery colonies.

Reviewed by __________________________ Date __________________________

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