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-610

Identification of *Pseudomonas* in Medical Marijuana Products

Contents

1.0. Scope and Application

1.1. This method NYS DOH LEB-610, Identification of *Pseudomonas* in Medical Marijuana Products, describes methods for detecting and identifying *Pseudomonas* species (ELAP Method ID 9990) 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.. It is used as a follow-up to NYS DOH LEB-604, section 9.2, and applies to sample enrichments showing growth in Trypticase Soy Broth at 30-35°C.

1.2. 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. Medical marijuana samples showing growth in Trypticase Soy Broth at 30-35°C are subcultured onto Cetrimide Agar and incubated at 30-35°C for 18-72 hours. Bacterial colonies are transferred to Trypticase Soy agar plates and identified using API® 20E identification strips. Samples producing bacterial colonies on Cetrimide Agar that are identified as *Pseudomonas* spp. are reported as positive.

3.0. Definitions

3.1. TSB stands for Trypticase Soy Broth

3.2. TSA stands for Trypticase Soy Agar

3.3. CEA stands for Cetrimide agar

4.0. Health and Safety Warnings

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

4.1.1. All microbiologically contaminated media in the laboratory shall be autoclaved prior to disposal.

4.1.2. Laboratory equipment and benches shall be disinfected before and after use with at least 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.

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 aerobic bacteria 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 30.0-35.0°C
7.1.2. Automatic pipetters and sterile aerosol-resistant micropipette tips
7.1.3. Sharpie or equivalent
7.1.4. Disposable sterile inoculating loops, 10µL
7.1.5. Disposable sterile inoculum spreader, or equivalent
7.1.6. Biosafety cabinet with HEPA filter

7.2. Reagents and Chemicals

7.2.1. CEA plates, 15 x 100mm. Ensure that the formulation is in agreement with that specified by USP.
7.2.2. TSA plates, 15 x 100mm
7.2.3. Disinfectants such as Envirocide® (Fisher Scientific cat. no. 19898220), 70% ethanol, and/or Clorox.

7.3. Forms

7.3.1. *Pseudomonas* Identification Result Sheet (e.g., LEB-RS-610A).

8.0. Quality Control/Assurance

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 30.0-35.0°C walk-in is recorded.

8.2.1.1.1. If the incubator temperature does not stay within 30.0-35.0°C, laboratory-specific corrective actions are followed. Analytical results may be invalidated if the incubator temperature exceeds 35.0°C, at the discretion of the laboratory.

8.2.2. Temperatures of the cold room and refrigerators 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 or refrigerator 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 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 CEA, TSA, and TSAB will be determined as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

8.3.2. Agar plates can be used for up to 2 weeks after the 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 as prescribed by the 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.

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. For each turbid TSB sample enrichment produced according to NYS DOH LEB-604, section 9.2, and confirmed to have growth (see NYS DOH LEB-604 section 9.7.), remove three CEA plates from the cold room and warm to room temperature while drying in the biological safety cabinet.

9.2.2. Use an inoculating loop to streak the sample from the turbid sample TSB onto two CEA plates for colony isolation.

9.2.3. Use a separate inoculating loop to streak the matrix spike from the corresponding turbid TSB onto one CEA plate for colony isolation.

9.2.3.1. If there isn't a corresponding matrix spike, streak a loopful of the positive control *Ps. aeruginosa* ATCC 9027 from the monthly transfer plates (prepared according to LEB-602).

9.2.4. Once the plates have dried, invert and incubate at 30.0-35.0°C for 18-72 hours.

9.2.4.1. Do not stack the plates more than four high.

9.2.5. After incubation, record colony characteristics and growth for the sample CEA plates as "Y" for growth-positive (bacterial colonies are present) or "N" for negative (bacterial colonies are absent) in the "CEA Sample Result" section of the *Pseudomonas* Identification Results Sheet (e.g., LEB-RS-610A).

9.2.5.1. If the sample is growth-positive on CEA, proceed to 9.3.

9.2.5.2. If there isn't any growth on the CEA plates, the sample is negative for the presence of *Pseudomonas* spp.

9.2.5.3. Proceed to 9.4 to identify any organisms isolated on nonselective agar plates in NYS DOH LEB-604 section 9.7.

9.2.6. After incubation, record the results of either the matrix spike or positive control CEA plate as "Y" for growth-positive (presence of bacterial colonies) or "N" for negative (absence of bacterial colonies) in the "CEA M.S./P.C. Result" section of the *Pseudomonas* Identification Results Sheet (e.g., LEB-RS-610A).

9.2.6.1. If the sample result is negative on CEA, it is not necessary to identify colonies on either the matrix spike or positive control plate that show morphology typical of *Ps. aeruginosa* ATCC 9027.

9.2.6.1.1. Typical *Ps. aeruginosa* ATCC 9027 colonies produce a blue-green/greenish pigment that fluoresces under 365-366 nm light.

9.2.6.2. If the sample and matrix spike results are negative on CEA, turbidity in the TSB may have been caused by matrix characteristics. The test results are valid if the positive and negative controls for aerobic plate counts and mold plate counts meet QC criteria (see NYS DOH LEB-605).

9.2.6.3. If the matrix spike result is negative on CEA, and the positive and negative controls for aerobic plate counts and mold plate counts do not meet QC criteria (see NYS DOH LEB-605), results are considered invalid and the analyses must be repeated.

9.2.6.3.1. 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, at the discretion of the laboratory.

9.2.6.4. If a positive control is analyzed instead of a matrix spike and the results are negative, the results are considered invalid and analyses must be repeated.

9.3. Colony Identification

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

9.3.1.1. If growth is confluent, re-streak for isolation of individual colonies and proceed with 9.3.1.

9.3.2. For each colony being identified, remove one TSA plate from the cold room and warm to room temperature while drying.

9.3.3. Streak well isolated colonies having the positive sample CEA plates onto TSA plates for colony isolation.

9.3.3.1. Bacterial colonies can be selected from either of the two CEA sample plates.

9.3.4. At a minimum, streak well-isolated colonies from either the matrix spike or positive control showing characteristics typical of *Ps. aeruginosa* ATCC 9027 onto TSA plates that have been warmed to room temperature and dried in a biological safety cabinet.

9.3.4.1. Record the sample source and colony morphology on the *Pseudomonas* Identification Results Sheet (e.g., LEB-RS-610A).

9.3.4.2. If growth is confluent on the CEA plate, re-streak for isolation of individual colonies and proceed with 9.3.4.

9.3.5. Once the plates have dried, invert TSA plates and incubate at 30.0-35.0°C for 18-24 hours.

9.3.5.1. Do not stack more than four high.

9.3.6. After incubation, use the growth on the TSA plates to perform a gram stain using instructions given in NYS DOH LEB-613.

9.3.7. Record the results of the gram stain on the *Pseudomonas* Identification Results Sheet (e.g., LEB-RS-610A).

9.3.8. If the organisms are gram negative rods, use the growth on the TSA plates to proceed with the API® Identification Test Strip method to identify the organisms using the API® 20E Test Strips.

9.3.8.1. Attach all API® 20E Identification Results sheets to the *Pseudomonas* Identification Results Sheet (e.g., LEB-RS-610A).

9.3.9. If the organisms are not gram negative rods proceed to 9.4.

9.3.10. If the API® 20E Identification Test kits fail to identify the isolate proceed to 9.4.

9.4. Identification of Non-Target Organisms

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

9.4.2. 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, CEA lot date, start and end dates and times, TSA lot date, start and end dates and times, colony morphology, source of colony (matrix spike, positive control, or sample), gram stain results, colony identification and results on the *Pseudomonas* Identification Results Sheet (e.g., LEB-RS-610A).

10.2. Report samples showing bacterial growth on CEA that result in identification of *Pseudomonas* sp. as positive for *Pseudomonas*.

10.3. Report samples showing growth on CEA that do not result in identification of *Pseudomonas* sp. as negative for *Pseudomonas.*

10.4. Report samples showing no growth on CEA as negative for *Pseudomonas*.

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 *Pseudomonas* 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.

11.1.4. 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 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

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

13.2. API® 20E Test Strips Instructions for Use, bioMérieux

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

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

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

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

14.0. Appendices - Forms

Pseudomonas **Identification Results Sheet (LEB-RS-610A)**

Incubate CEA for 18-72 hours and TSA for 18-24 hours
(30-35°C)

Accession Number: _____ Analyst Initials: _____

Final Results (circle one): Negative for *Pseudomonas* Positive for *Pseudomonas* _____ (list organism)

CEA Start Date/Time: _____ Sample Growth: Y N
CEA End Date/Time: _____ M.S./P.C. (circle one) Growth: Y N
CEA Lot Date: _____

TSA Start Date/Time: _____ Gram Stain Results: _____
TSA End Date/Time: _____ API® 20E Start Date/Time: _____
TSA Lot Date: _____ API® 20E End Date/Time: _____

	Source (Sample, M.S., or P.C.), Colony Morphology and API® 20E Colony Identification
1	
2	
3	
4	
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6	
7	
8	
9	
10	

All API® 20E Result Sheets are attached. CEA = Cetrimide Agar, TSA = Trypticase Soy Agar, M.S. = matrix spike, P.C. = positive control plate used in lieu of a matrix spike. *Ps. aeruginosa* ATCC 9027, used as a matrix spike, appears as blue/green colonies that fluoresce under 365-366 nm light.

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Reviewed by Date