ANDREW M. CUOMO
Governor

HOWARD A. ZUCKER, M.D., J.D.
Commissioner

SALLY DRESLIN, M.S., R.N.
Executive Deputy Commissioner

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

Table of Contents

1.0. Scope and Application 3

2.0. Summary of the Method 3

3.0. Definitions 3

4.0. Health and Safety Warnings 3

5.0. Shipping Conditions, Receiving, Preservation and Storage 4

6.0. Interferences 4

7.0. Apparatus and Materials 4

8.0. Quality Control/Assurance (Laboratories must conform to sections 9020-9050 of Standard Methods for the Examination of Water and Wastewater.) 5

9.0. Procedure 6

10.0. Data Acquisition, Reduction, Analysis, Calculations, Acceptance Criteria and Documentation 8

11.0. Method Performance 8

12.0. Waste Management/Pollution Prevention 8

13.0. References 9

14.0. Appendices 9

1.0. Scope and Application

1.1. This method NYS DOH LEB-610, Identification of *Pseudomonas* in Medical Marijuana Products (ELAP Method ID 9990), describes methods for detecting and identifying *Pseudomonas* species 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 9.1, 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 are subcultured onto Cetrimide Agar and incubated at 30-35°C for 18-24 hours. Bacterial colonies are transferred to Trypticase Soy agar plates and identified using a commercially available bacterial identification system, e.g., API® 20E identification strips. Samples from which *Pseudomonas* sp. are isolated are reported as positive.

3.0. Definitions

3.1. TSA stands for Trypticase Soy Agar

3.2. 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. 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. This procedure is initiated upon completion of the Presence/Absence procedure (see NYS DOH LEB-604, section 9.1).

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 identification.

7.0. Apparatus and Materials

7.1. Equipment

7.1.1. Incubator, 30-35°C

7.1.2. Automatic pipetters and sterile tips

7.1.3. Sharpie or equivalent

7.1.4. Sterile inoculating loops, 10µL

7.2. Reagent and Chemicals

7.2.1. CEA plates

7.2.2. TSA plates

7.2.3. Commercially available bacterial identification system (e.g., API® 20E test strips, bioMérieux cat. No. 20100)

7.2.4. Any reagents required for the bacterial identification system

7.3. Forms

7.3.1. *Pseudomonas* Identification Result Sheet (e.g. LEB-RS-610A, 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 incubator temperature does not stay within 30-35°C, laboratory specific corrective actions. Analytical results are invalidated if the incubator temperature exceeds 35.0°C.

8.2.2. Temperatures of the cold room and refrigerators are observed and recorded at least 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. Invalidate lot of media if tests are not in accordance with acceptance criteria as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

8.3.2. Acceptability of supplies is tested as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

8.3.3. 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.

8.3.4. 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.

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

8.3.5.1. Agar plates can be stored for longer than 2 weeks 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. Subculture

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. For each turbid TSB sample enrichment produced according to NYS DOH LEB-604, section 9.1, remove three CEA plates from the cold room and warm to room temperature while drying in the biological safety cabinet.

9.1.2.1. Two plates will be used for the sample enrichment and one for the corresponding matrix spike.

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

9.1.4. Use an inoculating loop to streak sample from the corresponding turbid TSB matrix spike enrichment onto a CEA plate for colony isolation.

9.1.5. Once samples have dried, invert plates and incubate at 30-35°C for 24-48 hours.

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

9.1.6. After incubation, record colony characteristics and growth for the sample plates as growth-positive (bacterial colonies are present) or negative (bacterial colonies are absent) (e.g. LEB-RS-610A).

9.1.6.1. If the sample is positive, proceed to 9.2.

9.1.6.2. If there is no growth on the CEA plates, the sample is negative for the presence of *Pseudomonas.*

9.1.7. After incubation, record colony characteristics and growth of the matrix spike plates as growth-positive (presence of bacterial colonies) or negative (absence of bacterial colonies) and colony characteristics (e.g. LEB-RS-610A).

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

9.1.7.1.1. Typical *Pseudomonas* ATCC 9027 colonies produce a blue-green/greenish pigment and fluoresce under 365-366 nm light.

9.1.7.2. If the matrix spike is positive on CEA, proceed to 9.2.

9.1.7.3. If the matrix spike result is negative on CEA, the test results are invalidated.

9.1.7.3.1. 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.

9.1.7.3.2. 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.

9.1.7.3.3. 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.

9.2. Colony Identification

9.2.1. Streak well-isolated colonies having distinct morphologies from positive CEA plates onto TSA plates that have been warmed to room temperature and dried in a biological safety cabinet.

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

9.2.1.2. If growth is confluent on the CEA plate, re-streak for isolation of individual colonies and proceed with 9.2.1.

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

9.2.2.1. If growth is confluent, re-streak for isolation of individual colonies and proceed with 9.2.2.

9.2.3. Invert TSA plates and incubate at 30-35°C for 18-24 hours.

9.2.3.1. Do not stack more than four high.

9.2.4. After incubation, choose a well-isolated colony from each TSA plate and proceed with a cpommercially available bacterial identification system, e.g., the API® Identification Test Strip method to identify the organism using the API® 20E Test Strips.

9.2.5. Attach all API® 20E Identification Results sheets (or relevant paperwork from other identification systems) to the *Pseudomonas* Identification Results Sheet (e.g. LEB-RS-610A).

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 , start and end dates and times, source of colony (matrix spike or sample), bacterial identification system start and end dates and times, colony morphology, colony identification and results (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 as negative samples showing no growth on CEA.

10.5. Invalidate the test results for samples lacking growth in the matrix spike at any point in the analysis or from which *Pseudomonas* sp. was not identified.

10.5.1. See 9.1.7.3.1.

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

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

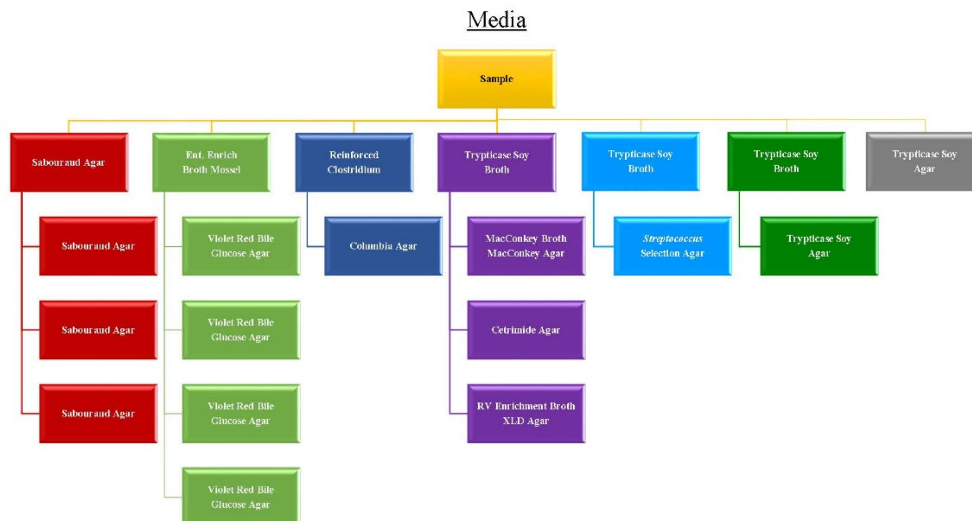
14.0. Appendices

Appendix A – Flowcharts

Medical Marijuana Microbial Testing Plan

4/7/16

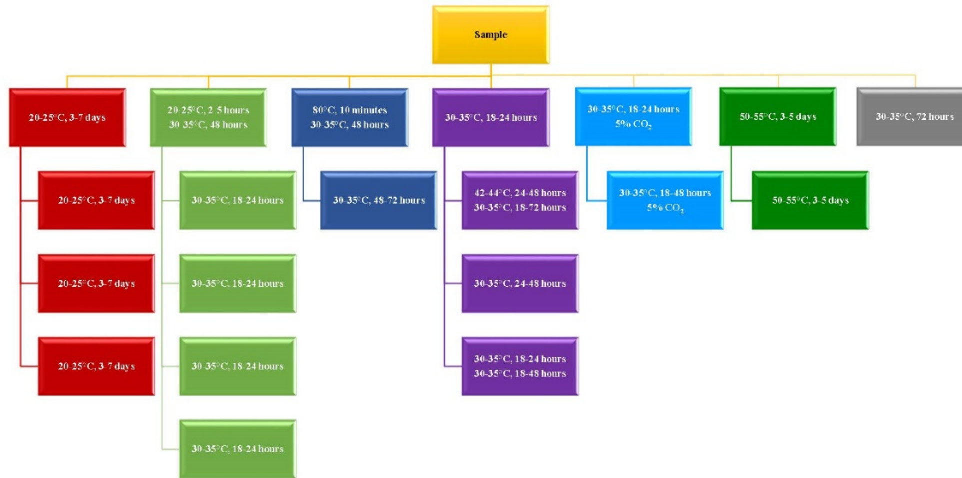
Medical Marijuana Microbial Testing Plan



4/7/16

Medical Marijuana Microbial Testing Plan

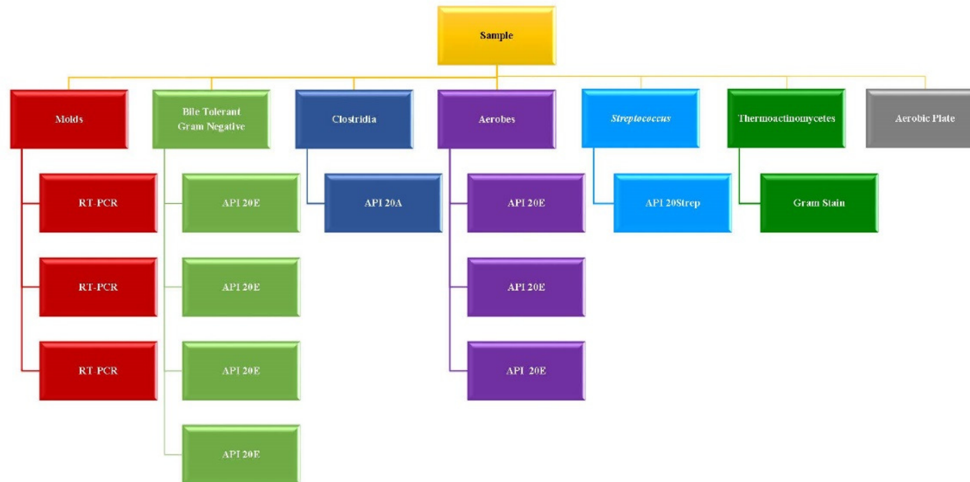
<u>Incubation Temperatures and Times</u>



4/7/16

Medical Marijuana Microbial Testing Plan

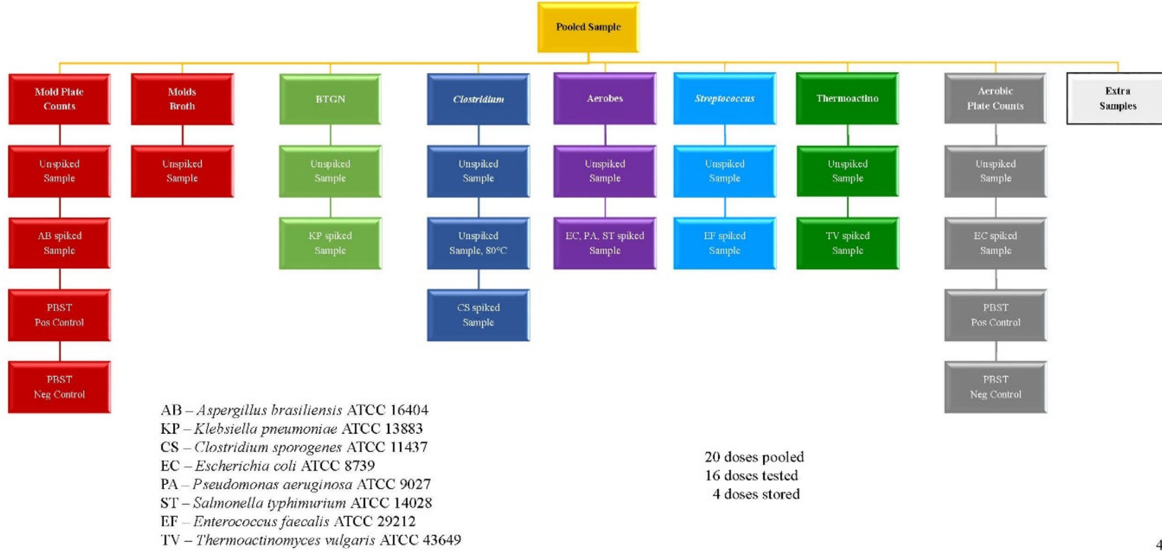
<u>Colony Identification Assays</u>



4/7/16

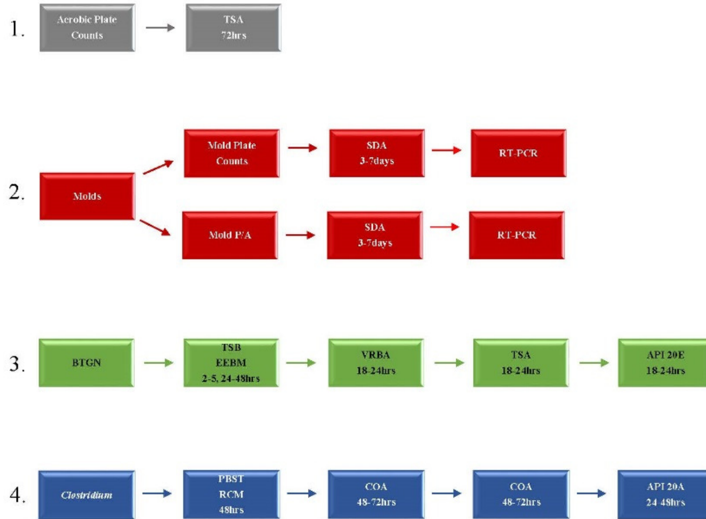
Medical Marijuana Microbial Testing Plan

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



Medical Marijuana Microbial Testing Plan

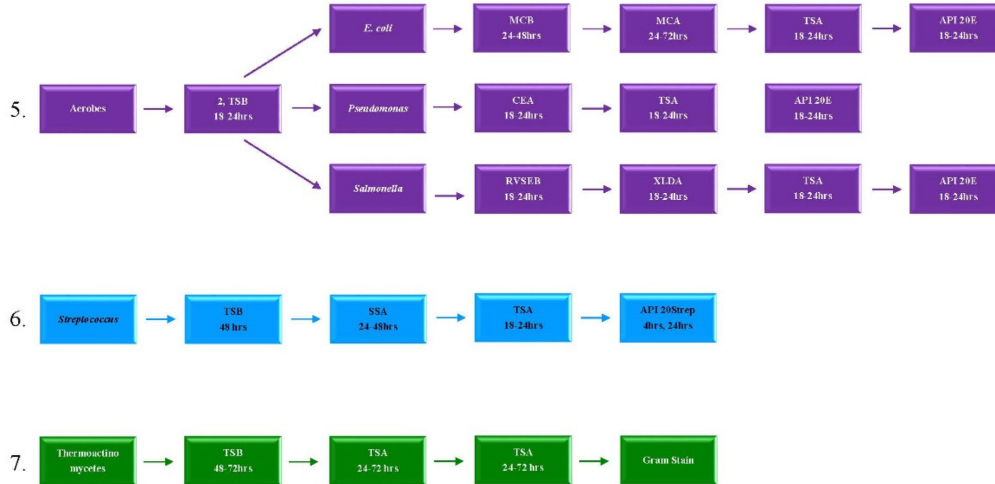
General Set-Up for Presence/Absence, Colony Identifications, and Plate Count Assays



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

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

Incubate CEA for 18-24 hours and TSA for 18-24 hours
(30-35°C Incubator, E552)

Accession Number: ______________________ Analyst Initials: ______________________

CEA Start Date/Time: ______________________ Sample Result: ______________________
CEA End Date/Time: ______________________ M.S. Result: ______________________
CEA Lot Date: ______________________

TSA Start Date/Time: ______________________ API® 20E Start Date/Time: ______________________
TSA End Date/Time: ______________________ API® 20E End Date/Time: ______________________
TSA Lot Date: ______________________

	Source (M.S. or Sample), Colony Morphology and API® 20E Colony Identification
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	

All API® 20E Result Sheets are attached.
CEA = Cetrimide Agar, TSA = Trypticase Soy Agar
Ps. aeruginosa ATCC 9027, used as a matrix spike, appears as blue/green colonies that fluoresce under 365-366 nm light.
Reviewed by: Date: