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Environmental Laboratory Approval Program (ELAP) and Laboratory of Environmental Biology (LEB)

Guidance Procedure on How to Perform an Initial Demonstration of Capability (iDOC) for Medical Marijuana Microbiological Contaminants

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Revision 1

1.0. Scope and Application

- 1.1. This protocol is used to set-up and analyze sixteen (16) samples for demonstration of capability analyses for testing of Medical Marijuana products for microbial contaminants.

2.0. Summary of the Method

- 2.1. The set-up analyst dilutes product 1:10 and spikes eight (8) of the diluted aliquots with a single target organism for presence/absence analyses.
- 2.2. The set-up analyst spikes two separate diluted aliquots with known numbers of *E. coli* and *A. brasiliensis* for enumeration analyses.
- 2.3. The set-up analyst adds six different extracted DNA to tubes for RT-PCR identification of molds.
- 2.4. Analyst follows NYS's approved Medical Marijuana methods (NYS DOH LEB-604 through NYS DOH LEB-618) to identify the unknown organisms in each tube prepared by the set-up analyst.

3.0. Definitions

- 3.1. DOC stands for Demonstration of Capability
- 3.2. LEB stands for NYS's Laboratory Environmental Biology
- 3.3. PBST stands for Phosphate Buffered Saline, pH 7.2, containing 0.1% Tween[®] 80.
- 3.4. Polysorbate 80 (Tween[®] 80) is a nonionic surfactant and emulsifier.
- 3.5. Diluted sample aliquot is one measured dose of sample diluted 1:10 in PBST in accordance with NYS DOH LEB-603.
- 3.6. EEEM stands for Enterobacteria Enrichment Broth Mossel
- 3.7. RCM stands for Reinforced Clostridial Medium
- 3.8. TSB stands for Trypticase Soy Broth
- 3.9. SDA stands for Sabouraud Dextrose Agar
- 3.10. TSA stands for Trypticase Soy Agar
- 3.11. APC stands for Aerobic Plate Count
- 3.12. MPC stands for Mold Plate Count

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. Contaminated glassware and plastic ware shall be decontaminated prior to washing.
 - 4.1.3. Laboratory equipment and benches shall be disinfected using either Envirocide[®], 10% bleach, or a minimum concentration of 70% ethanol before and after use.
 - 4.1.4. Mouth pipetting is prohibited.
 - 4.1.5. All accidents, particularly those which may result in infection, shall be reported to the Laboratory Director or Supervisor.

5.0. Shipping Conditions, Receiving, Preservation and Storage

5.1. Sample Shipping Conditions

5.1.1. None.

5.2. Sample Receipt

5.2.1. None.

5.3. Method Holding Times

5.3.1. None.

5.4. Preservation

5.4.1. Unused, reconstituted BioBalls® can be stored for one week at 1.0-8.0 °C.

5.4.1.1. If lab is not using BioBalls®, then include preservation for lab prepared suspensions.

5.4.2. Laboratory prepared spiking solutions are stored according to lab specific protocols.

5.5. Storage

5.5.1. BioBalls® are stored, frozen, at -20.0 °C and used on or before the expiration date marked on the package, if applicable. If not using BioBalls®, then include storage requirements for lab prepared suspensions.

5.5.2. Extracted DNA is stored, frozen, at -20.0 °C.

6.0. Interferences

6.1. None.

7.0. Apparatus and Materials

7.1. Equipment and Supplies

7.1.1. Pipette-aid

7.1.2. Pipettes, sterile – 25-mL, 10-mL, and 5-mL

7.1.3. Automatic pipettors and sterile aerosol-resistant micropipette tips

7.1.4. NIST certified laboratory timer – Krackeler Scientific, Albany, NY, cat no. 291-5004 or equivalent.

7.1.5. Disposable powder-free nitrile gloves

7.1.6. Disposable sterile inoculum spreader, or equivalent

7.1.7. Glass tubes with kaputs, 16x150mm size, sterile, or equivalent

7.1.8. 1.7mL microcentrifuge tubes, sterile

7.1.9. 15mL conical tubes, sterile – Krackeler Scientific cat. no. 3-352099, or equivalent

7.2. Reagents and Chemicals

7.2.1. PBST pH 7.2, 100-mL and 1-L aliquots in tubes/bottles

7.2.2. TSB, 1X, 10-mL aliquots

7.2.3. EEBM, sterile, 1X, 100 mL aliquots

7.2.4. RCM, sterile, 1X, 100 mL aliquots

7.2.5. SDA plates, 15 x 150 mm

7.2.6. TSA plates, 15 x 100 mm

7.2.7. Laboratory prepared suspensions or BioBalls® (prepared according to NYS DOH LEB-603) for *Aspergillus brasiliensis* ATCC 16404;

Clostridium sporogenes, ATCC 11437; *Enterococcus faecalis*, ATCC 29212; *Escherichia coli*, ATCC 8739; *Klebsiella pneumoniae* ATCC 13883; *Pseudomonas aeruginosa*, ATCC 9027; *Salmonella typhimurium*, ATCC 14028; *Thermoactinomyces vulgaris* ATCC 43649.

7.2.8. DNA extracts (prepared according to NYS DOH LEB-609 and NYS DOH LEB-618) for *A. flavus* ATCC 16883; *A. fumigatus* ATCC 34506; *A. niger* ATCC 16888; *A. terreus* ATCC 1012; *Mucor hiemalis* ATCC 28932; *Penicillium chrysogenum* ATCC 11709.

7.3. Forms

7.3.1. "Demonstration of Capability Set-Up" Record as developed by the lab

7.3.2. "Demonstration of Capability" Results Sheet as developed by the lab

7.3.3. Each laboratory will use their own sample results sheets to record individual sample analyses.

8.0. Quality Control/Assurance

8.1. Method Detection Limits

8.1.1. Not determined.

8.2. Calibration and Standardization

8.2.1. Sterility of spreaders is determined as described in lab's procedures.

8.2.2. The volumetric accuracy of automatic pipettors and serological pipettes is determined according to lab's procedures.

8.3. Quality Control

8.3.1. Comparative recovery and sterility between lots of TSA and SDA are determined in accordance lab's procedures.

8.3.2. As an additional control, perform an air density test while adding the samples to their respective media according to NYS DOH LEB-605 section 8.3.

8.3.3. Acceptability of PBST is determined according to lab's procedures.

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 after preparation date if stored refrigerated in plastic bags and in the dark.

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

8.3.6. The use test for deionized water is performed annually, when cartridges are changed, or repairs are made to the deionized water systems following the lab's procedures.

8.4. Corrective/Preventive Actions

8.4.1. The analyst, Laboratory Director, or Laboratory Quality Assurance Officer will initiate corrective/preventive actions in accordance with lab's procedures.

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.
 - 9.1.1.2. Contact the laboratory Director, designee or supervisor with questions.
- 9.1.2. All work surfaces are disinfected prior to sample set-up.
- 9.1.3. Sample set-up for analyses is performed in a physically different location than preparation of spiking solutions to prevent cross-contamination of incoming products.
- 9.1.4. Pre-reduce media, as necessary, according to NYS DOH LEB-604 section 9.1.
- 9.1.5. Each of the 16 individual DOC samples prepared in 9.2. may be accessioned as part of the lab's LIMS, but it is not required, or given accession IDs manually.

9.2. DOC Sample Set-Up

- 9.2.1. This section is completed by laboratory personnel that **are not** performing the DOC analyses.
- 9.2.2. Record the individual accession numbers, or unique identifiers, in the "Accession ID" column of the Demonstration of Capability Set-Up record.
- 9.2.3. Pool 8.5-mLs or 8.5-g of product. This can consist of several different lots of the same product.
- 9.2.4. Record the product type (i.e. ethanol tincture, oil tincture, etc.) and the accession number(s) of the product(s) on the Demonstration of Capability Set-Up record.
- 9.2.5. Label 8, 15-mL conical tubes with IDs created in 9.1. for the Presence/Absence analyses.
 - 9.2.5.1. To seven of the 15-mL tubes add the following:
 - 9.2.5.1.1. 1-mL or 1-g of product
 - 9.2.5.1.2. Enough spike volume for ten (10) analyses. A single tube will contain a spike of one of the following spiked organisms:
 - 9.2.5.1.2.1. *C. sporogenes*
 - 9.2.5.1.2.2. *E. coli*
 - 9.2.5.1.2.3. *E. faecalis*
 - 9.2.5.1.2.4. *Ps. aeruginosa*
 - 9.2.5.1.2.5. *K. pneumoniae*
 - 9.2.5.1.2.6. *S. typhimurium*
 - 9.2.5.1.2.7. *T. vulgaris*
 - 9.2.5.1.3. Bring the final volume to 10-mLs with PBST.

- 9.2.5.1.3.1. For example, if the spike is from a BioBall®, add 1-mL of product and 1-mL of reconstituted BioBall® to 8-mLs of PBST.
- 9.2.5.1.4. Record the ID and the organisms spiked into the individual tubes in the “P/A Spiked Organism” column of the Demonstration of Capability Set-Up record.
- 9.2.5.2. To the eighth 15-mL tube (blank) add the following:
 - 9.2.5.2.1. 1-mL or 1-g of product
 - 9.2.5.2.2. 9-mLs PBST
 - 9.2.5.2.3. Record the accession number and the blank in the “P/A Spiked Organism” column of the Demonstration of Capability Set-Up record.
- 9.2.6. Label 2, 1.7-mL tubes with IDs for the APC and MPC Analyses
 - 9.2.6.1. Each of the two tubes contain
 - 9.2.6.1.1. 100-µL or 100-mg of product
 - 9.2.6.1.2. Volume of spike (either *E. coli* or *A. brasiliensis*).
 - 9.2.6.1.3. Bring the final volume to 1-mL with PBST.
 - 9.2.6.2. After adding the spikes to the APC and MPC tubes, plate an equivalent spike volume between two plates (TSA and SDA, respectively) and incubate according to NYS DOH LEB-605.
 - 9.2.6.3. Record the IDs and organisms spiked into the individual tubes in the “Organism Enumeration” column of the Demonstration of Capability Set-Up record.
 - 9.2.6.4. After incubation, record the total number of colonies on both plates on the Demonstration of Capability Set-Up record.
- 9.2.7. Label 6, 1.7-mL tubes with IDs created in 9.1.5 by LIMS or manually.
 - 9.2.7.1. Each tube contains 50-µL of the following extracted DNA:
 - 9.2.7.1.1. *A. flavus*
 - 9.2.7.1.2. *A. fumigatus*
 - 9.2.7.1.3. *A. terreus*
 - 9.2.7.1.4. *A. niger*
 - 9.2.7.1.5. *P. chrysogenum*
 - 9.2.7.1.6. *M. hiemalis*
 - 9.2.7.2. Record the accession numbers, DNA, and date of extraction for the individual tubes in the “DNA Identification” column of the Demonstration of Capability Set-Up record.
- 9.2.8. Record the set-up analyst initials, date, spike prep dates or lot and expiration dates on the Demonstration of Capability Set-Up record.

9.3. DOC Analyses

9.3.1. Presence/Absence Target Organisms

- 9.3.1.1.** For each of the eight (8) 15-mL Presence/Absence tubes (prepared in 9.2.5.) label:
 - 9.3.1.1.1.** 4, 10-mL TSB tubes (32 total tubes of 10-mL TSB)
 - 9.3.1.1.2.** 2, 9-mL PBST tubes (16 total tubes of 9-mL PBST)
 - 9.3.1.1.3.** 1, 100-mL bottle of EEBM (8 total bottles)
 - 9.3.1.1.4.** 2, 100-mL bottles of RCM (16 total bottles)
- 9.3.1.2.** Open air density plates and start timer. This is used for Quality Control.
- 9.3.1.3.** Vortex the first 15-mL tube to and add 1-mL from the tube to:
 - 9.3.1.3.1.** 10-mL TSB for detection of aerobic bacteria.
 - 9.3.1.3.2.** 10-mL TSB for detection of BTGN bacteria.
 - 9.3.1.3.3.** 9-mL PBST for detection of clostridia.
 - 9.3.1.3.4.** 9-mL PBST for detection of heat-treated clostridia.
 - 9.3.1.3.5.** 10-mL TSB for detection of enterococci.
 - 9.3.1.3.6.** 10-mL TSB for detection of thermophilic actinomycetes.
 - 9.3.1.3.7.** Vortex in between adding the 1mL aliquots to each tube because the product may separate from the PBST.
- 9.3.1.4.** Repeat the steps in section 9.3.1.3 for the remaining seven (7) 15-mL tubes prepared in 9.2.5.
- 9.3.1.5.** Incubate and analyze the samples according to NYS DOH LEB-604.
 - 9.3.1.5.1.** Organisms isolated from the presence/absence analyses are identified using the appropriate NYS DOH LEB Methods.

9.3.2. Organism Enumerations

- 9.3.2.1.** For the 1.7-mL tube labeled “APC” (prepared in 9.2.6.) label two (2) TSA plates for the DOC tube and one plate for the PBST control.
 - 9.3.2.1.1.** Vortex the 1.7-mL DOC tube to mix.
 - 9.3.2.1.2.** Plate the entire 1-mL volume between two (2) TSA plates.
 - 9.3.2.1.3.** Plate 0.1-mL of the PBST onto one (1) TSA plate.
 - 9.3.2.1.4.** Analyze the plates according to NYS DOH LEB-605.
- 9.3.2.2.** For the 1.7-mL tube spiked labeled “MPC” (prepared in 9.2.6.) label two SDA plates for the DOC tube and one plate for the PBST control.

- 9.3.2.2.1. Vortex the 1.7-mL DOC tube to mix.
- 9.3.2.2.2. Plate the entire 1-mL volume between two (2) SDA plates.
- 9.3.2.2.3. Plate 0.1-mL of the PBST onto one (1) SDA plate.
- 9.3.2.2.4. Analyze the plates according to NYS DOH LEB-605.

9.3.3. Mold Identification

- 9.3.3.1. Select one (1) colony from MPC plates and extract the DNA according to NYS DOH LEB-609.
- 9.3.3.2. For the 1.7-mL tubes containing DNA (prepared in 9.2.7.) and the DNA extracted from the MPC plate, analyze the DNA according to NYS DOH LEB-618.

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

- 10.1. Record the set-up date and analyst, sample ID, spiked organism/DNA, spike prep dates or lot/exp dates, APC and MPC results, product type and ID on a Demonstration of Capability Set-Up record.
- 10.2. Record the analyst, completion date, accession number, identified organism, air density results, agar negative control results, and media/supply lots and expiration dates on a Demonstration of Capability Results sheet.

11.0. Method Performance

- 11.1. Demonstration of Capability
 - 11.1.1. See Laboratory Quality Manual.
- 11.2. Laboratory Detection Limits
 - 11.2.1. Not determined.

12.0. Waste Management/Pollution Prevention

- 12.1. Bacterial/fungal cultures and contaminated or potentially contaminated disposable materials are disposed of in biohazardous waste cans and handled according to the lab's waste disposal procedures.

13.0. References

References to be used are the laboratory's quality manual and its procedures related to:

- 13.1. Corrective and Preventative Action
- 13.2. Water Suitability Test
- 13.3. Equipment Calibration and Maintenance
- 13.4. Volumetric Sterility and Volume Quality Control
- 13.5. Supply Control and Inventory
- 13.6. Quality Assurance of Agar Plates
- 13.7. Quality Assurance of Enrichment Broths
- 13.8. Quality Assurance of Dilution Buffers

13.9. Laboratory Prepared Spikes
13.10. Waste Disposal