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-609

Mold Plate Counts and Identification for Medical Marijuana Testing

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1.0. Scope and Application

1.1. This method NYS DOH LEB-609, Mold Plate Counts and Identification for Medical Marijuana Testing (ELAP Method ID 9989), is used to estimate the number of molds in samples of medical marijuana products and identify *Aspergillus, Mucor* and *Penicillium* spp 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. Mold plate counts are determined for all samples submitted for analysis in the Medical Marijuana Testing Program. Samples are also enriched for subsequent identification of molds using real-time PCR, as required.

1.3. See Appendix A for Medical Marijuana Microbial Testing Plan flowcharts.

2.0. Summary of the Method

2.1. Sample aliquots prepared according to NYS DOH LEB-603 are spread-plated onto Sabouraud Dextrose Agar and incubated for 3-7 days at 20-25°C. Colonies are enumerated and reported as CFUs/dose product. Also, one sample aliquot is inoculated into Sabouraud Dextrose broth for enrichment prior to identification using real-time PCR, as required.

3.0. Definitions

3.1. SDA stands for Sabouraud Dextrose Agar

3.2. SDB stands for Sabouraud Dextrose Broth

3.3. CFU stands for colony forming unit. Because a colony theoretically arises from a single bacterial cell/mold spore, the number of CFUs reflects the number of culturable mold spores in the sample.

3.4. RT-PCR stands for real-time polymerase chain reaction amplification

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 before and after use with a minimum concentration of 70% ethanol.

4.1.4. Mouth pipetting is prohibited.

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. 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

7.1.1. Incubator, set at 20-25°C

7.1.2. Automatic pipetters and sterile micropipette tips

7.1.3. Disposable sterile inoculum spreader, or equivalent

7.1.4. Extraction tubes containing sterile glass beads – e.g., Generite Cat. no. S0205

7.1.5. 1.7 mL microcentrifuge tubes, sterile

7.1.6. 15 mL conical tubes, sterile

7.1.7. 8-position mini bead beater – Biospec Products Inc., Cat. no. 693 or equivalent.

7.1.8. Rotamix rotating mixer – Appropriate Technical Resources, Laurel MD, cat no. RKVSD, or equivalent

7.1.9. Spectrophotometer and quartz cuvettes

7.2. Reagent and Chemicals.

7.2.1. Sabouraud Dextrose Agar plates, 15 x 150 mm

7.2.2. Sabouraud Dextrose broth, 10mL and 100mL aliquots

7.2.3. AE buffer, pH 9.0 – e.g., Qiagen Cat. no. 19077.

7.2.4. Salmon testes DNA – Sigma-Aldrich, Cat. no. D1626 or equivalent

7.2.5. PCR grade water, OmniPur water from VWR (EM-9610) or equivalent. Water must be DNase/RNase free.

7.3. Forms

7.3.1. Medical Marijuana Aerobic Plate and Mold Count Result Sheet (e.g. LEB-RS-605A)

7.3.2. Medical Marijuana Mold Air Density Monitoring Log (e.g. LEB-RS-605A)

7.3.3. Mold Identification By RT-PCR Result Sheet (e.g. LEB-RS-609B, Appendix B)

7.3.4. Mold Identification RT-PCR Targets Appendix (e.g. LEB-AP-609A, Appendix C)

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 20-25°C incubator is recorded.

8.2.1.1.1. If the 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.

8.2.2. Temperature of the cold room/refrigerator is 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 daily when 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

8.2.7. The spectrophotometer is calibrated at least annually as prescribed by the Accreditation Body and in accordance with relevant regulations and standards

8.3. Quality Control

8.3.1. Invalidate lot of medium 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. As an additional control, perform the air density test on days during which bacteriological analyses are performed.

8.3.2.1. Remove the lid from a SDA plate and expose the agar surface to ambient air for 15 minutes.

8.3.2.2. Record exposure time (e.g. LEB-RS-605A).

8.3.2.3. Replace the lid and incubate in parallel with heterotrophic plate count analyses for 3-7 days at 20-25°C.

8.3.2.4. Record results on the Mold Air Density Monitoring Log (e.g. LEB-RS-605A).

8.3.2.5. Should the number of surface colonies exceed 10, follow laboratory specific corrective actions.

8.3.2.5.1. If high numbers of surface colonies would interfere with accurate determination of sample APCs, the results will be qualified as a QC failure.

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.

8.3.4. The holding time for poured agar media in plates is 2 weeks if it is stored refrigerated in sealed plastic bags, in the dark.

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

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 and as prescribed by the laboratory, Accreditation Body and in accordance with relevant regulations and standards.

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. Mold counts

9.1.1. Remove enough SDA plates from the cold room to accommodate a PBST-diluted sample aliquot, the associated matrix spike containing *A. brasiliensis* ATCC 16404, and positive (*A. brasiliensis*) and negative controls prepared according to NYS DOH LEB-603 and allow to warm to room temperature while drying in the biohazard hood.

9.1.1.1. SDA in 15 x 150 mm plates are used.

9.1.1.2. If the sample dose is 2 mL or more (the diluted sample aliquot is 20mL or more after dilution with PBST), plate one fifth of the diluted sample aliquot volume.

9.1.1.2.1. For example, if the diluted sample aliquot is 150 mL, plate 30 mL total volume.

9.1.2. Label Petri plates with the sample accession number and dilution, if applicable.

9.1.2.1. If colony numbers are too numerous to count or overgrowth of certain mold species inhibits isolation of pure colonies, dilute an archived aliquot if available and repeat analysis.

9.1.2.2. Ten-fold serial dilutions may be prepared in PBST upon receipt if desired, e.g., when samples historically have contained numerous mold spores.

9.1.2.3. USP <61> recommends no more than 50 mold colonies per plate.

9.1.3. Using an automatic micropipetter and sterile tips, pipette equivalent aliquots of sample onto SDA plates.

9.1.3.1. Up to 1 mL can be plated onto a single plate.

9.1.4. Use a sterile spreader to spread the liquid over the entire agar surface.

9.1.5. Repeat 9.1.3-9.1.4 for the associated matrix spike, positive control and negative control.

9.1.6. After samples have dried, invert the plates and incubate in a plastic bag for 3-7 days at 20-25°C.

9.1.6.1. Stack plates no more than four high.

9.1.7. Examine and enumerate plates daily.

9.1.8. Include a description of colony morphology and the total number of similar colonies (e.g. LEB-RS-609B).

9.1.8.1. For plates having no colony forming units, record the count as less than one CFU/dose.

9.1.8.1.1. If one fifth of the volume was plated, record the count as less than five CFUs/dose.

9.1.8.2. If counting must be delayed, store plates at 4°C for no more than 48 hours.

9.1.8.3. If there are no colonies showing typical *Aspergillus brasiliensis* morphology on the matrix spike plates, the test results are invalidated.

9.1.8.3.1. Typical *A. brasiliensis* colonies grow rapidly and are initially white but form black centers upon aging.

9.1.8.4. If there are no colonies on the plated positive control showing typical *Aspergillus* morphology, the results are invalidated.

9.1.8.4.1. The total number of colonies in the positive control must be less than 100 CFUs/sample.

9.1.8.5. If the number of colonies on the matrix spike plates is less than half of the number of colonies on the positive control plates, the test results are invalidated.

9.1.8.6. If there are colonies on the negative control, laboratory specific corrective actions are followed.

9.1.8.6.1. Depending on the type and extent of contamination, the results may be invalidated.

9.1.9. Individually, total the number of colonies from each plated sample aliquot, matrix spike, positive and negative control.

9.1.9.1. Bacterial colonies are not enumerated and not included in the total number of mold colonies.

9.1.10. Record the total number of CFU/dose, the total number of CFU/matrix spike, the total number of CFU on the positive and negative controls and any notations (e.g. LEB-RS-605A).

9.1.11. Calculate and record the CFU/dose product.

9.1.12. For any sample containing greater than 5 mold colonies having similar morphology, record colony morphologies (e.g. LEB-RS-609B) and proceed to 9.2.

9.2. Identification of *Aspergillus* sp., *Penicillium* sp. and *Mucor* species using real-time PCR

9.2.1. Preparation of 1mg/mL Salmon DNA Stock Solutions

9.2.1.1. Place an empty, sterile 15 mL conical tube on the balance and tare the balance.

9.2.1.2. Using flame-sterilized scissors and forceps cut a small piece of lyophilized salmon testes DNA and place in the 15 mL conical tube.

9.2.1.3. Add enough PCR grade water to make a final concentration of 1mg/mL of DNA.

9.2.1.3.1. For example, if the piece of lyophilized DNA weighs 9.5 mg, add enough PCR grade water for a final volume of 9.5 mL.

9.2.1.4. Place the tube on a rotating mixer, and rotate for 2 hours, at room temperature, to allow the DNA to completely dissolve into solution.

9.2.1.5. Once dissolved, remove three, 10 µL aliquots and dilute each to 1000 µL with PCR grade water.

9.2.1.6. Using a spectrophotometer and quartz cuvette, check the absorbance (OD_{260}) of each aliquot.

9.2.1.7. Average the three absorbances, and calculate the amount of DNA in each aliquot by using the equation of an OD_{260} of 1 is equivalent to a concentration of 50 µg/mL.

9.2.1.7.1. For example:

OD_{260} of the 1:100 dilutions are 0.3050, 0.3056, and 0.3101

(0.3050+0.3056+0.3101)/3 x 100 x 50µg/mL = 1534.5µg/mL

9.2.1.8. Vortex to mix.

9.2.1.9. Adjust the stock solution to bring the final concentration to 1mg/mL DNA using DNase/RNase free water.

9.2.1.10. Aliquot the solution into 1.7 mL tubes and store at -20°C.

9.2.1.10.1. Make 10-20 aliquots of 20 µL and aliquot the rest as 1mL aliquots.

9.2.1.10.2. Label the tubes with the DNA concentration and date of preparation.

9.2.2. Preparation of Extraction Buffer

9.2.2.1. Extraction buffer is made fresh daily.

9.2.2.2. Thaw a 20 µL aliquot of a 1mg/mL concentration of salmon testes DNA.

9.2.2.2.1. Label the tube with the date it was thawed.

9.2.2.2.2. This aliquot is good for one month when stored at 2-8°C.

9.2.2.3. Vortex to mix and briefly centrifuge.

9.2.2.4. Using a pipette-aid and sterile 5 mL pipette, aseptically add 5 mL of AE buffer to a sterile 15 mL conical tube.

9.2.2.5. Using a micropipettor and sterile pipette tips, aseptically add 1 µL of the thawed salmon testes DNA to the conical tube containing 5mL AE buffer.

9.2.2.5.1. Increase the volumes of the solutions according to the number of samples being extracted.

9.2.2.5.1.1. For example, if there are 20 samples being extracted add 2 µL of salmon testes DNA to 10 mL of AE buffer.

9.2.2.6. Vortex to mix.

9.2.3. DNA Extraction for RT-PCR Identification

9.2.3.1. Aseptically remove the colony from the SDA plate using a sterile inoculation loop and add to an extraction tube containing 300mg glass beads and 0.5 mL of extraction buffer.

9.2.3.2. Cap the extraction tube and bead beat at maximum speed for one minute.

9.2.3.3. Centrifuge the extraction tube for 1 minute at 12,000 x g to pellet the debris.

9.2.3.4. Aseptically transfer as much supernatant as possible to a sterile 1.7 mL microcentrifuge tube.

9.2.3.5. Centrifuge the sample tube for 5 minutes at 12,000 x g.

9.2.3.6. Aseptically transfer as much supernatant as possible to a second sterile, labeled, 1.7 mL microcentrifuge tube taking care not to transfer any of the pellet or leftover glass beads.

9.2.3.7. Refrigerate the DNA lysates until identification by RT-PCR.

9.2.3.7.1. DNA lysates can be stored long term at -20°C.

9.2.3.8. Proceed to NYS DOH LEB-618 to set up and run the RT-PCR assay using primer and probe sets listed in Mold Identification RT-PCR Targets Appendix (e.g. LEB-AP-609A).

9.2.3.9. The method in this section is also used for extracting DNA from positive control organisms. Positive controls are listed in LEB-AP-609A.

9.2.3.9.1. Positive controls are prepared in the laboratory from spore suspensions

9.2.3.9.2. Either freshly prepared or frozen suspensions of spores may be used.

9.2.4. Results Recording

9.2.4.1. Record the number of colonies analyzed(e.g. LEB-RS-609B).

9.2.4.2. If individual colonies overlap, transfer a representative example of each fungal colony showing similar morphology onto SDA for re-isolation.

9.2.4.3. Follow NYS DOH LEB-618 to screen individual colony types for the presence of ribosomal DNA sequences indicative of organisms belonging to the *Penicillium, Aspergillus* or the *Mucor* genera.

9.2.4.4. Record the number of colonies of each type, source (matrix spike or sample), colony morphology and RT-PCR identification (e.g. LEB-RS-609B).

9.2.4.5. If *Penicillium/Aspergillus* are identified using the RT-PCR screen for the presence of rDNA sequences belonging to the *Penicillium/Aspergillus* group (Pan Asp or PenAsp1mgb), then the DNA is screened for *Asp. terreus, Asp. flavus, Asp. fumigatus*, and *Asp. niger* according to NYS DOH LEB-618 and using the species-specific primer/probe sets listed in LEB-AP-609A.

10.0. Data Acquisition, Reduction and Documentation

10.1. Record the accession number, analyst, media lot date, and associated dilutions (if applicable), and start/end dates and times of incubation (e.g. LEB-RS-605A).

10.2. Record number of CFU/plate and the total number of CFUs/dose in the sample, matrix spike, positive and negative controls (e.g. LEB-RS-605A).

10.3. Report the total number of CFUs mold/dose.

10.3.1. Record the results of air density plate testing on SDA and the open/close times.

10.4. If subculture (colony isolation) was necessary, record SDA start/ end dates and times of incubation, and SDA lot date (e.g. LEB-RS-609B).

10.5. Record the analyst initials, RT-PCR run date, RT-PCR Run Name, number of colonies of each morphological type picked, source, colony morphology and RT-PCR identification (e.g LEB-RS-609B).

10.6. Report numbers of CFU of *Aspergillus/Penicillium* species, *Mucor* species, *Asp. niger, Asp. terreus, Asp. fumigatus,* and *Asp. flavus*.

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. NYS DOH LEB-603, Sample preparation

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

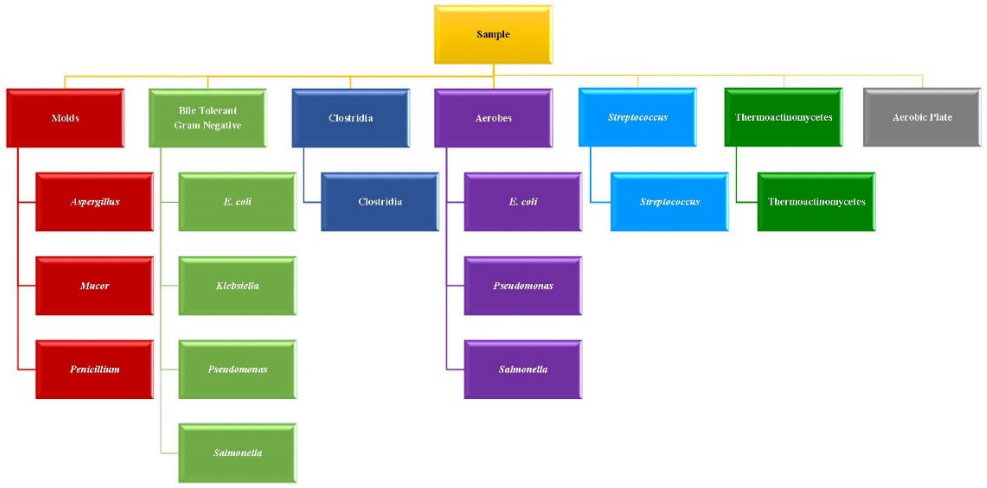
13.5. NYS DOH LEB-618, RT-PCR Identification of Molds

14.0. Appendices

Appendix A – Flow Charts

Medical Marijuana Microbial Testing Plan

Organisms



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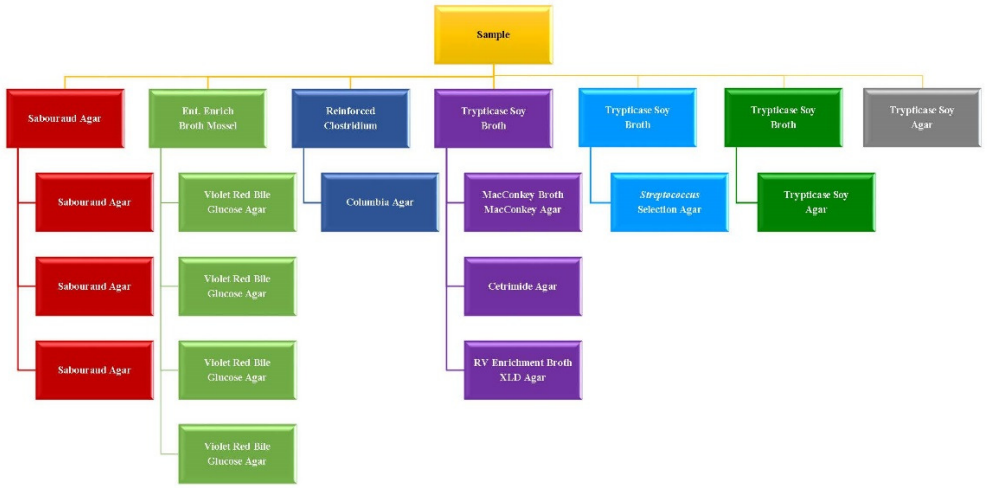
Medical Marijuana Microbial Testing Plan

Media



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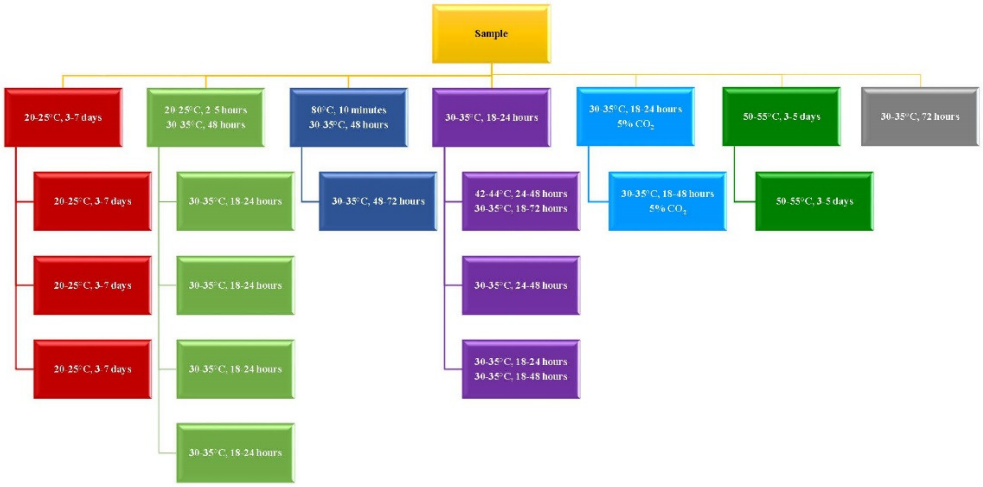
Medical Marijuana Microbial Testing Plan

Incubation Temperatures and Times



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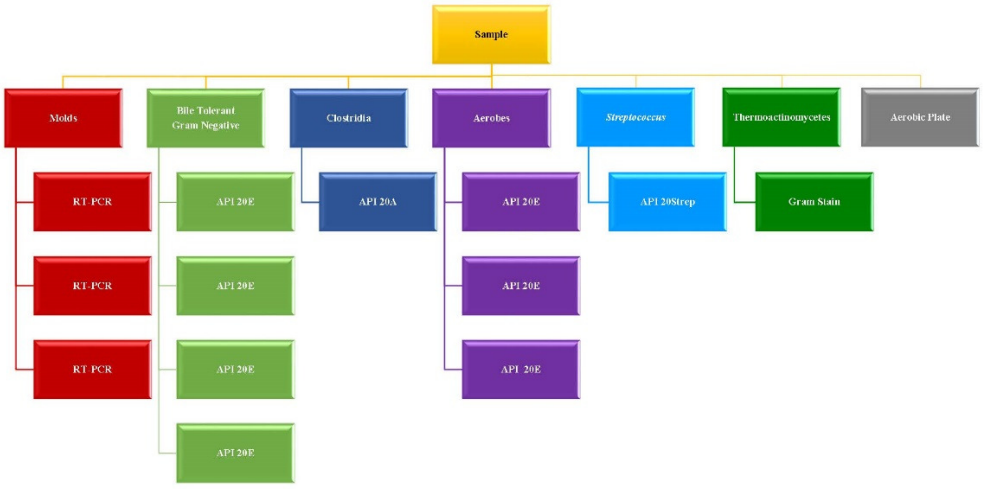
Medical Marijuana Microbial Testing Plan

Colony Identification Assays



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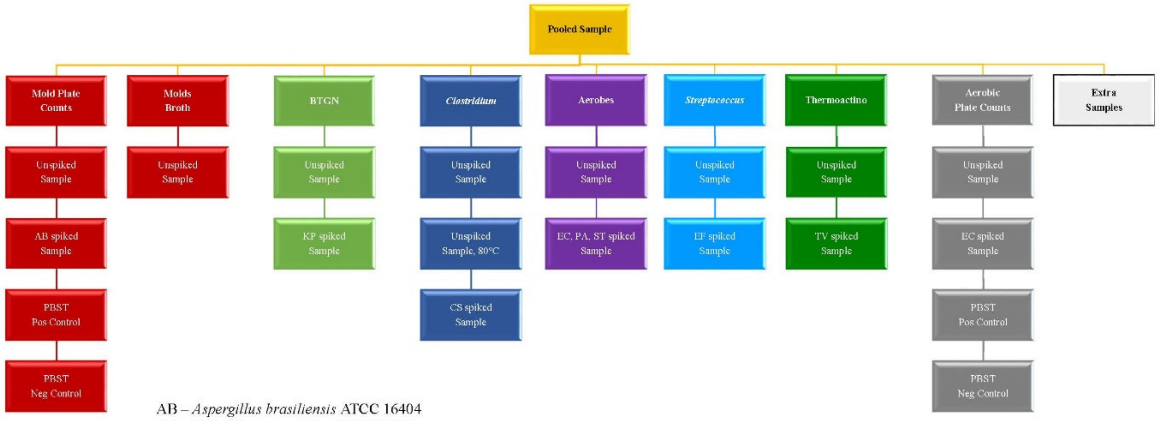
Medical Marijuana Microbial Testing Plan

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

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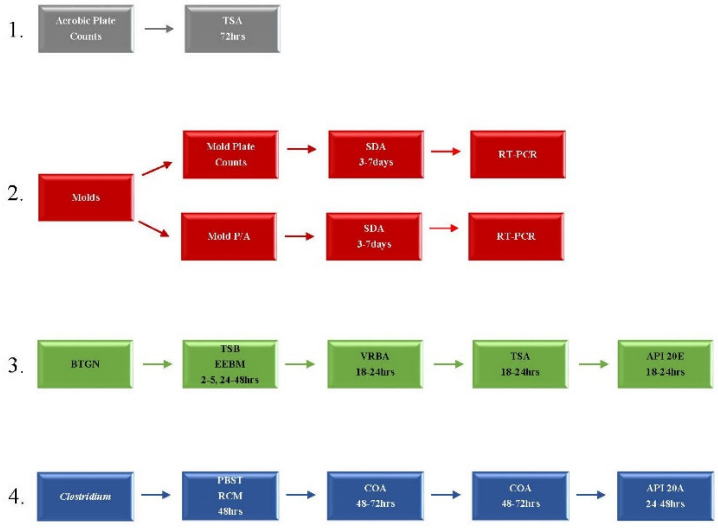
Medical Marijuana Microbial Testing Plan

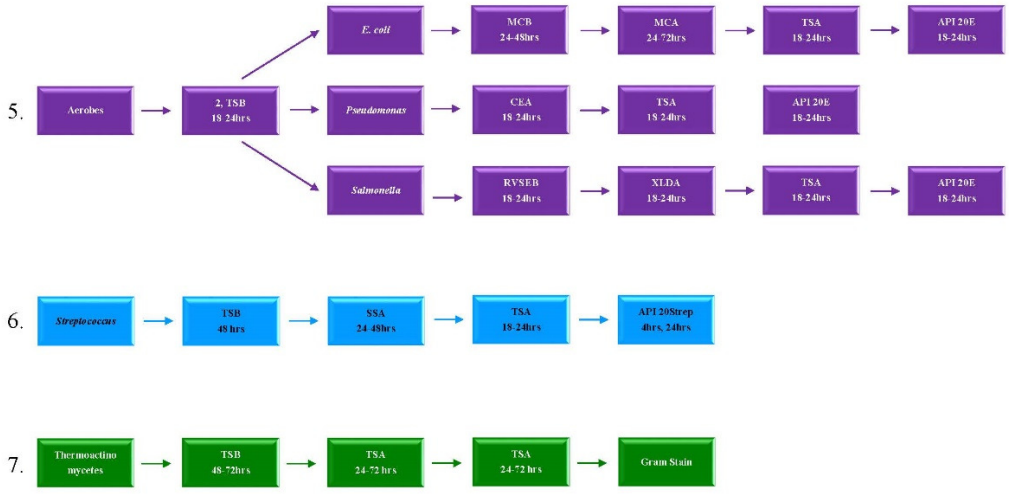
General Set-Up for Presence/Absence, Colony Identifications, and Plate Count Assays



4/7/16

Medical Marijuana Microbial Testing Plan

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



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Appendix B – Forms

Mold Identification By RT-PCR Results Sheet (LEB-RS-609B)

(20-25°C Incubator)

Accession Number: _____ Analyst Initials: _____

Colony Isolation (if necessary)

SDA Start Date/Time: _____ SDA Lot Date: _____
SDA End Date/Time: _____

RT-PCR Run Date: _____ RT-PCR Run Name: _____

	No. of colonies	Source (M.S. or Sample), Colony Morphology, and RT-PCR Identification
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		

SDA = Sabouraud Dextrose Agar, SDB = Sabouraud Dextrose Broth
All RT-PCR assay bench sheets (LEB-RS-618A) are attached.
Total no. colonies: ***Aspergillus/Penicillium _____; Mucor _____; Asp. niger _____; Asp. terreus _____; Asp. flavus _____;***

Asp. fumigatus_____

Reviewed by Date:

Appendix C – Reference

Mold Identification RT-PCR Targets and Positive Controls Appendix

Target Name: Pan-Asp (*Aspergillus/Penicillium* general)
Forward Primer: 5'-gTggAgTgATTTgTCTgCTTAATTg-3'
Reverse Primer: 5'-TCTAAgggCATCACAgACCTgTT-3'
Probe: 5'-6FAM-CggCCCTTAAATAgCCCggTCCg-QSY-3'
Positive Control: *A. brasiliensis* ATCC 16404 and/or *P. chrysogenum* ATCC 11709

Target Name: PenAsp1mgb (*Aspergillus/Penicillium* general)
Forward Primer: 5'-CggAAggATCATTACTgAgTg-3'
Reverse Primer: 5'-gCCCgCCgAAgCAAC-3'
Probe: 5'-6FAM-CCAACCTCCCACCCgTg-MGBNFQ-3'
Positive Control: *A. brasiliensis* ATCC 16404 and/or *P. chrysogenum* ATCC 11709

Target Name: Muc1 (*Mucor/Rhizopus*)
Forward Primer: 5'-CACCgCCCgTCgCTAC-3'
Reverse Primer: 5'-CCTAgTTTgCCATAgTTCTCAgCAg-3'
Probe: 5'-6FAM-CCgATTgAATggTTATAgTgAgCATATgggATC-TAMRA-3'
Positive Control: *M. hiemalis* ATCC 28932

Target Name: Aflav (*A. flavus*)
Forward Primer: 5'-CgAgTgTAgggTTCCTAgCgA-3'
Reverse Primer: 5'-CCggCGgCCATgAAT-3'
Probe: 5'-FAM-TCCCACCCgTgTTTACTgTACCTTAgTTgCT-TAMRA-3'
Positive Control: *A. flavus* ATCC 16883

Target Name: Afum (*A. fumigatus*)
Forward Primer: 5'-gCCCgCCgTTTCgAC-3'
Reverse Primer: 5'-CCgTTgTTgAAAgTTTTAACTgATTAC-3'
Probe: 5'-FAM-CCCgCCgAAgACCCCAACATg-TAMRA-3'
Positive Control: *A. fumigatus* ATCC 34506

Target Name: Anigr (*A. niger*)
Forward Primer: 5'-gCCggAgACCCCAACAC-3'
Reverse Primer: 5'-TgTTgAAAgTTTTAACTgATTgCATT-3'
Probe: 5'-FAM-AATCAACTCAgACTgCACgCTTTCAgACAg-TAMRA-3'
Positive Control: *A. niger* ATCC 16888

Target Name: Aterr (*A. terreus*)
Forward Primer: 5'-ATCATTACCgAgTgCgTgTCTTTA-3'
Reverse Primer: 5'-CCCgCCgAAgCAACAAg-3'
Probe: 5'-FAM-CCCAACCTCCCACCCgTgACTATTg-TAMRA-3'
Positive Control: *A. terreus* ATCC 1012

Target Name: Sketa (Internal control)
Forward Primer: 5'-ggTTTgTTgAggATgAgCTTCTg-3'
Reverse Primer: 5'-CgAAgCgAAAgggAAAggA-3'
Probe: 5'-FAM-CTACTTCCgTTCCCCCgTgCgC-TAMRA-3'
Positive Control: Extraction buffer