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-608

Identification of *Escherichia coli* in Medical Marijuana Products
## 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 .................................................................................................................. 9  
12.0. Waste Management/Pollution Prevention .................................................................................. 9  
13.0. References ................................................................................................................................. 10  
14.0. Appendices ................................................................................................................................. 11
1.0. Scope and Application

1.1. This method NYS DOH LEB-608, Identification of *Escherichia coli* in Medical Marijuana Products, describes methods for detecting and identifying *Escherichia coli* (ELAP ID 9988) in 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. 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.

1.3. 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 transferred to MacConkey Broth and incubated at 42-44°C for 24-48 hours. Samples that show growth in MacConkey broth are subcultured onto MacConkey Agar and incubated at 30-35°C for 18-24 hours. Bacterial colonies are transferred to Trypticase Soy Agar and identified using a commercially available bacterial identification system, e.g. API® 20E identification strips. Samples are reported as positive if *E. coli* is identified.

3.0. Definitions

3.1. TSB stands for Trypticase Soy Broth
3.2. TSA stands for Trypticase Soy Agar
3.3. MCB stands for MacConkey Broth
3.4. MCA stands for MacConkey 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 a minimum concentration of 70% ethanol.

4.1.3. Mouth pipetting is prohibited.

4.1.4. All accidents, particularly those which may result in infection, shall be reported according to laboratory specific procedures.

4.1.5. The analyst must be familiar with any possible hazards from the reagents and standards used for sample preparation and analysis.

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.

4.1.9. 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 method (NYS DOH LEB 604, section 9.3).

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 locked laboratory within a box having double locks.

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. Water bath, 42-44°C

7.1.3. Automatic pipettors and sterile tips

7.1.4. Sharpie or equivalent

7.1.5. Sterile inoculating loops, 10µL

7.2. Reagent and Chemicals

7.2.1. MCB

7.2.2. MCA plates

7.2.3. TSA plates

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

7.2.5. Any reagents required for the bacterial identification system
7.3. Forms
  7.3.1. E. coli Identification Result Sheet (e.g. LEB-RS-608A, 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 the incubator temperature does not stay within 30-35°C, laboratory specific corrective actions are followed. 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. Water bath temperatures shall be observed and recorded twice daily, separated by at least 4 hours.
    8.2.3.1. Temperature of the 42-44°C water bath is recorded on the Water Bath Temperature Record.
    8.2.3.1.1. If the water bath temperature does not stay within 42-44°C, laboratory specific corrective actions are followed. Analytical results are invalidated if the water bath temperature exceeds 44.0°C.

  8.2.4. Max/min temperatures are recorded when daily temperature measurements are not possible, such as on holidays and weekends.

  8.2.5. Thermometers must be calibrated as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

  8.2.6. Sterility of disposable loops and spreaders is determined as prescribed by the Accreditation Body and in accordance with relevant regulations and standards.

  8.2.7. 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 used after 2 weeks storage 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. Enrichment in MacConkey Broth (MCB)

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 two 100 mL aliquots of MCB from the cold room and warm to room temperature.

9.1.2.1. One aliquot will be used for the TSB sample enrichment and one for the corresponding matrix spike.

9.1.3. Inoculate 1 mL from the turbid TSB sample enrichment into a 100 mL MCB aliquot.

9.1.4. Inoculate 1 mL from the corresponding turbid TSB matrix spike enrichment into a 100 mL MCB aliquot.

9.1.5. Mix and incubate at 42-44°C for 24-48 hours.

9.1.6. After incubation, record the results of MCB sample enrichments either as positive (turbid) or negative (no turbidity) (e.g. LEB-RS-608A).

9.1.6.1. Record any color change of the medium.

9.1.6.2. Growth of *E. coli* in MCB is typically paralleled by a change in medium color from red/purple to yellow.
9.1.7. If the sample result is turbid, proceed with 9.2.
9.1.8. If the sample result is negative (no turbidity), the sample is negative for the presence of *E. coli*.
9.1.9. After incubation, record the results of MCB matrix spike enrichments as turbid or negative (no turbidity) (e.g. LEB-RS-608A).
9.1.10. If the matrix spike enrichment is turbid in MCB, and the sample is turbid, proceed with 9.2.
9.1.11. If the matrix spike enrichment result is negative in MCB, the test results are invalidated.

9.2. Subculture

9.2.1. For each turbid MCB sample enrichment, remove three MCA plates from the cold room and warm to room temperature while drying in the biological safety cabinet.
  
  9.2.1.1. Two plates will be used for the sample enrichment and one for the corresponding matrix spike.

9.2.2. Use an inoculating loop to streak the sample from the turbid MCB enrichment onto two MCA plates for colony isolation.

9.2.3. Use an inoculating loop to streak the corresponding turbid MCB matrix spike onto one MCA plate for colony isolation.

9.2.4. Once the plates have dried, invert plates and incubate at 30-35°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 plates as growth-positive (bacterial colonies are present) or negative (bacterial colonies are absent) and colony characteristics (e.g. LEB-RS-608A).
  
  9.2.5.1. If the sample is positive on MCA, proceed to 9.3.
  
  9.2.5.2. If there is no growth on MCA plates, the sample is negative for the presence of *E. coli*.

9.2.6. 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) (e.g. LEB-RS-608A).
  
  9.2.6.1. If the sample result is negative on MCA and the plated matrix spike shows colonies having morphology typical of *E. coli* ATCC 8739, it is not necessary to identify colonies according to 9.3.

  9.2.6.1.1. Typical *E. coli* ATCC 8739 colonies are red/pink or purple and are surrounded by a zone of bile precipitation (halo), depending on the medium manufacturer.

  9.2.6.2. If the matrix spike is positive on MCA, proceed with 9.3.

  9.2.6.3. If the matrix spike result is negative, the test results are invalidated.
9.2.6.3.1. 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.

9.2.6.3.2. 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.

9.2.6.3.2.1. 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.

9.3. Identification

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

9.3.1.1. Bacterial colonies can be selected from either of the MCA sample plates.

9.3.1.2. If growth is confluent on the MCA plate, re-streak for isolation of individual colonies and proceed with 9.3.1.

9.3.2. At a minimum, streak well-isolated colonies from matrix spikes showing characteristics typical of E. coli ATCC 8739 onto TSA plates that have been warmed to room temperature and dried in a biological safety cabinet.

9.3.2.1. If growth is confluent, re-streak for isolation of individual colonies and proceed with 9.3.2.

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

9.3.3.1. Do not stack more than four high.

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

9.3.5. Attach all API® 20E Identification Results sheets (or relevant paperwork from other identification systems) to the E. coli Identification Results Sheet (e.g. LEB-RS-608A).

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

10.1. Record the accession number, analyst initials, MCB lot date, start and end dates and times, MCA lot date, start and end dates and times, TSA lot date, start and end dates and times, bacterial identification system start and end dates and times,
colony morphology, source of colony (matrix spike or sample), colony identification and results of testing (e.g. LEB-RS-608A).

10.2. Report samples showing bacterial growth on MCA that result in identification of \textit{E. coli} as positive for \textit{E. coli}.

10.3. Report samples showing growth on MCA that do not result in identification of \textit{E. coli} as negative for \textit{E. coli}.

10.4. Report as negative samples showing no growth in MCB or MCA.

10.5. Invalidate the test results for samples lacking growth in the matrix spike at any point in the analysis or from which \textit{E. coli} was not identified.

10.5.1. See 9.2.6.3.1

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


13.2. Standard Methods for the Examination of Water and Wastewater, sections 9020-9050

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

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

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

13.6. NYS DOH LEB-609, Mold Plate Counts and Identification for Medical Marijuana Testing
14.0 Appendices

Appendix A – Flow Charts

Medical Marijuana Microbial Testing Plan

Organisms

Sample

Media

Sample

4/7/16
Medical Marijuana Microbial Testing Plan

Incubation Temperatures and Times

Sample

Medical Marijuana Microbial Testing Plan

Colony Identification Assays

Sample

4/7/16
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 – Closstridium perfringens ATCC 11317
KC – Escherichia coli ATCC 8739
PA – Pseudomonas aeruginosa ATCC 9027
ST – Salmonella typhimurium ATCC 14028
EF – Enterococcus faecalis ATCC 29212
TV – Thermotoga novellae ATCC 43649

20 doses pooled
16 doses tested
4 doses stored

4/7/16
Medical Marijuana Microbial Testing Plan

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

5.
- Aerobes
- E. coli
- MRSA
- Pseudomonas
- Staphylococci

6.
- Aerobic
- SBE
- TSA
- MPN

7.
- Aerobic filters
- TSA
- TSA
- TSA
- TSA filters

4/7/16
**Appendix B – Forms**

**E. coli Identification Results Sheet**

Incubate MCB for 24-48 hours and MCA for 18-72 hours

(30-35°C Incubator, E552 and 42-44°C Water Bath, 20028)

<table>
<thead>
<tr>
<th>Accession Number:</th>
<th>Analyst Initials:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MCB Start Date/Time:</th>
<th>Sample Result:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MCB End Date/Time:</th>
<th>M.S. Result:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MCB Lot Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MCA Start Date/Time:</th>
<th>Sample Result:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MCA End Date/Time:</th>
<th>M.S. Result:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MCA Lot Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TSA Start Date/Time:</th>
<th>API® 20E Start Date/Time:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TSA End Date/Time:</th>
<th>API® 20E End Date/Time:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TSA Lot Date:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
</tbody>
</table>

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.

MCA = MacConkey Agar, MCB = MacConkey Broth, TSA = Trypticase Soy Agar, TSB = Trypticase Soy Broth

*E. coli* ATCC 8739, used as a matrix spike, appears as red/violet/purple colonies with a purple halo on MCA

Reviewed by: Date: