INTRODUCTION
Levine EMB Agar is a slightly selective and differential medium for the isolation, cultivation and differentiation of gram-negative enteric microorganisms isolated from both clinical and nonclinical specimens. It is widely used for the examination of materials of sanitary importance for the presence of coliforms.

PERFORMANCE TEST PROCEDURE
1. Inoculate representative samples with dilutions of the cultures listed below.
   a. Using an 18- to 24-h broth culture of \textit{Enterococcus faecalis} diluted to yield $10^4$ to $10^5$ CFU/plate, spread-inoculate using a sterile glass spreader. For the remaining organisms, use an 18- to 24-h broth culture diluted to yield $10^3$ to $10^4$ CFU/plate. Streak inoculate \textit{E. coli}; spread inoculate the remaining organisms.
   b. Incubate plates at 35 ± 2 °C in an aerobic atmosphere.
   c. Include \textit{Trypticase}™ Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for amount of growth, colony size, pigmentation and selectivity.

Expected Results

<table>
<thead>
<tr>
<th>CLSI Organisms</th>
<th>ATCC®</th>
<th>Recovery</th>
<th>Colony Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>*\textit{Escherichia coli}</td>
<td>25922</td>
<td>Growth</td>
<td>Blue-black with green metallic sheen</td>
</tr>
<tr>
<td>*\textit{Salmonella choleraesuis} subsp. \textit{choleraesuis} serotype Typhimurium</td>
<td>14028</td>
<td>Growth</td>
<td>Colorless to amber</td>
</tr>
<tr>
<td>*\textit{Enterococcus faecalis}</td>
<td>29212</td>
<td>Inhibition (partial)</td>
<td></td>
</tr>
<tr>
<td>Additional Organism</td>
<td>12022</td>
<td>Moderate to heavy growth (colonies large)</td>
<td>Colorless to amber</td>
</tr>
</tbody>
</table>

ADDITIONAL QUALITY CONTROL
1. Examine plates as described under “Product Deterioration.”
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.1 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 35 ± 2 °C for 72 h and examine for microbial contamination.

INTENDED USE
Levine EMB Agar is a selective and differential plating medium for the isolation of gram-negative enteric bacteria.

SUMMARY AND EXPLANATION
Shortly following the publication by Holt-Harris and Teague of a paper describing a new culture medium for the differentiation of enteric microorganisms through the use of eosin and methylene blue dyes,\footnote{Levine described a modification of their formulation which he claimed gave better differentiation between what are now referred to as \textit{Escherichia} and \textit{Enterobacter} species. The two formulations differ in that Levine EMB Agar does not contain sucrose. Both of these formulations were developed to improve upon the differentiating properties of Endo Agar, which was developed previously.} Levine EMB Agar utilizes dyes as selective agents. It is listed for use in the microbiological examination of dairy products and foods by the American Public Health Association.\footnote{Levine EMB Agar utilizes dyes as selective agents. It is listed for use in the microbiological examination of dairy products and foods by the American Public Health Association.}

PRINCIPLES OF THE PROCEDURE
The eosin Y and methylene blue dyes in Levine EMB Agar render the medium slightly selective in that they inhibit gram-positive bacteria to a limited degree. These dyes also play a role in differentiating between lactose fermenters and lactose-nonfermenters due to the presence or absence of dye uptake in the bacterial colonies. Coliforms, as lactose fermenting organisms, are visualized as blue-black colonies whereas colonies of \textit{Salmonella} and \textit{Shigella}, as lactose nonfermenters, appear colorless, transparent or amber in color.

REAGENTS
Levine EMB Agar
Approximate Formula* Per Liter Purified Water
\begin{align*}
\text{Pancreatic Digest of Gelatin} & \quad \text{10.0 g} \\
\text{Lactose} & \quad \text{10.0 g} \\
\text{Dipotassium Phosphate} & \quad \text{2.0 g} \\
\text{Eosin Y} & \quad \text{0.4 g} \\
\text{Methylene Blue} & \quad \text{0.065 g} \\
\text{Agar} & \quad \text{15.0 g}
\end{align*}

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For \textit{in vitro} Diagnostic Use.
If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.
Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. “Standard Precautions”6-9 and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

**Storage Instructions:** On receipt, store plates in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

**Product Deterioration:** Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration.

**VIII SPECIMEN COLLECTION AND HANDLING**
Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.10,11 Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

**IX PROCEDURE**

**Material Provided:** Levine EMB Agar

**Materials Required But Not Provided:** Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

**Test Procedure:** Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate plates, protected from light, at 35 ± 2 °C in an aerobic atmosphere for 18–24 h.

**User Quality Control:** See “Quality Control Procedures.”

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory’s standard quality control procedures.

**X RESULTS**

After incubation most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a “dilution” technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Better isolation is obtained due to the inhibitory action of the medium.

Typical colonial morphology on Levine EMB Agar is as follows:

- **E. coli**......................................................Large, blue-black, green metallic sheen
- **Enterobacter/Klebsiella** .......................Large, mucoid, blue-black
- **Proteus**...............................................Large, colorless
- **Salmonella**.............................................Large, colorless
- **Shigella**................................................Large, colorless
- **Pseudomonas** ........................................Irregular, colorless

Gram-positive bacteria .........................No growth to slight growth

**XI LIMITATIONS OF THE PROCEDURE**

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.10-15

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

**XII AVAILABILITY**

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>221170</td>
<td>BD BBL™ Levine EMB Agar, Pkg. of 20 plates</td>
</tr>
<tr>
<td>221268</td>
<td>BD BBL™ Levine EMB Agar, Ctn. of 100 plates</td>
</tr>
</tbody>
</table>
XIII REFERENCES


Technical Information: In the United States, contact BD Technical Service and Support at 800-638-8663 or www.bd.com/ds.