EMB AGAR (Levine)

INTENDED USE

EMB Agar, originally recommended by Levine, is used to isolate and identify *Escherichia coli* and *Enterobacter*, as well as Gram-negative intestinal bacteria in pharmaceutical products, dairy and other food products. It is also used as an isolation and identification medium in tests of water quality, after culture in liquid medium (presumptive tests).

HISTORY

In 1916, Holt-Harris and Teague used the combination of eosin and Methylene blue to differentiate microorganisms as a function of whether or not they could ferment lactose. Levine subsequently modified the formula by removing sucrose and increasing the lactose concentration, which led to the easy differentiation between *Escherichia coli* and *Enterobacter aerogenes*.

PRINCIPLES

- Eosin Y and Methylene blue have low selective capacities, since they only partially inhibit the development of Gram-positive bacteria such as enterococci.
- The dyes allow to the differentiation between lactose-positive and lactose-negative bacteria. Coliform strains form violet to brown colonies, while salmonellae are colorless, transparent or amber.

PREPARATION

- Suspend 37.5 g of dehydrated medium (BK056) in 1 liter of distilled or deionized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense in tubes or flasks.
- Sterilize in an autoclave at 121°C for 15 minutes.

NOTE:

Incomplete agar melting during preparation will invariably lead to significant inconsistency in the gel strength of the solidified agar, after sterilization and cooling.
INSTRUCTIONS FOR USE

- Cool and maintain the medium at 44-47°C.
- Mix well to oxidize the methylene blue and insure the homogeneous suspension of the precipitate.
- Pour into sterile Petri dishes.
- Let solidify on a cold surface.
- Dry in an incubator with the covers partially removed.
- Inoculate by streaking.
- Incubate at 37°C for 18 to 24 hours.

RESULTS

Colonies have the following appearance:

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Microorganisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dark violet colonies, convex, low confluence, 2-3 mm in diameter with a black center reaching more than 3/4 of the diameter and which exhibit a greenish metallic sheen in reflected light</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>Bluish flattened colonies, relatively confluent, 4-6 mm in diameter with a dark brown center, occasionally with a metallic sheen</td>
<td><em>Enterobacter aerogenes</em></td>
</tr>
<tr>
<td>Violet colonies with slight metallic sheen</td>
<td><em>Citrobacter</em></td>
</tr>
<tr>
<td>Brownish mucous colonies</td>
<td><em>Klebsiella</em></td>
</tr>
<tr>
<td>Transparent amber colonies</td>
<td><em>Salmonella</em> and <em>Shigella</em></td>
</tr>
</tbody>
</table>

TYPICAL COMPOSITION

(can be adjusted to obtain optimal performance)

For 1 liter of medium:

- Pancreatic digest of gelatin ............................................................. 10.0 g
- Lactose .................................................................................................... 10.0 g
- Dipotassium phosphate ......................................................................... 2.0 g
- Eosin Y .................................................................................................. 0.4 g
- Methylene blue .................................................................................... 65.0 mg
- Bacteriological agar ......................................................................... 15.0 g

pH of the ready-to-use medium at 25°C : 7.0 ± 0.2.
QUALITY CONTROL

- Dehydrated medium: violet powder, free-flowing and homogeneous.
- Prepared medium: claret agar, which may contain a slight precipitate after autoclaving.
- Typical culture response after 24 hours of incubation at 37°C (qualitative method of inoculation):

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Growth</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Escherichia coli</strong> ATCC®25922</td>
<td>good, score 2</td>
<td>violet colonies with greenish metallic sheen</td>
</tr>
<tr>
<td><strong>Enterobacter aerogenes</strong> ATCC 13048</td>
<td>good, score 2</td>
<td>violet-pink colonies</td>
</tr>
<tr>
<td><strong>Salmonella Typhimurium</strong> ATCC 14028</td>
<td>good, score 2</td>
<td>colorless colonies</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong> ATCC 27853</td>
<td>good, score 2</td>
<td>colorless colonies</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong> ATCC 25923</td>
<td>inhibited, score 0</td>
<td></td>
</tr>
</tbody>
</table>

STORAGE / SHELF LIFE

**Dehydrated medium**: 2-30°C.
- The expiration date is indicated on the label.

**Prepared medium** (benchmark value*):
- Media in vials: 6 months at 2-8°C.
- Media in plates: 1 month at 2-8°C.

PACKAGING

Dehydrated medium:
- 500 g bottle
  
  **Code**: BK056HA

BIBLIOGRAPHY


PHOTO SUPPORT

Product reference: BK056HA

Media used for: Isolation and identification of *Escherichia coli* and other Gram negatives.

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*Escherichia coli*

Eosin methylene blue agar (EMB - Levine)

Ref: BK056HA

Incubation: 24 hours / 37°C

Characteristics: Dark violet colonies with an intense green metallic sheen in reflected light.

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*Benchmark value refers to the expected shelf life when prepared under standard laboratory conditions following manufacturer’s instructions. It is provided as a guide only and no warranty, implied or otherwise is associated with this information.*

The information provided on the package take precedence over the formulations or instructions described in this document. The information and specifications contained in this technical data sheet date from 2010-01-12. They are susceptible to modification at any time, without warning.