DIFFERENT TYPES OF AGAR

Mac Conkey’s Agar plate

**MacConkey** (also **McConkey**) agar is a culture medium designed to grow Gram-negative bacteria and stain them for lactose fermentation. It contains bile salts (to inhibit most Gram-positive bacteria, except *Enterococcus* and some species of *Staphylococcus*), crystal violet dye (which also inhibits certain Gram-positive bacteria), neutral red dye (which stains microbes fermenting lactose), lactose and peptone.

**QUALITY CONTROL**
Results after 24 hrs at 35º C

<table>
<thead>
<tr>
<th>Organisms</th>
<th>ATCC</th>
<th>Growth</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>25922</td>
<td>+</td>
<td>red</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>12453</td>
<td>±</td>
<td>colourless</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>14028</td>
<td>+</td>
<td>colourless</td>
</tr>
<tr>
<td><em>Streptococcus faecalis</em></td>
<td>29212</td>
<td>-</td>
<td>or partial</td>
</tr>
</tbody>
</table>

**Uses**

Acting as a visual pH indicator, the agar distinguishes those Gram-negative bacteria that can ferment the sugar lactose (Lac+) from those that cannot (Lac-).

This medium is also known as an "indicator medium" and a "low selective medium". Absence of electrolytes serves to inhibit swarming by Proteus species.

**Lac+**

By utilizing the lactose available in the medium, Lac+ bacteria such as *Escherichia coli*, *Enterobacter* and *Klebsiella* will produce acid, which lowers the pH of the agar below 6.8 and results in the appearance of red/pink colonies.
**Eosin methylene blue (EMB) agar –**

**Eosin methylene blue (EMB)** is a selective stain for Gram-negative bacteria. It is a blend of two stains, eosin and methylene blue in the ratio of 6:1. A common application of this stain is in the preparation of **EMB agar**, a differential microbiological medium, which inhibits the growth of Gram-positive bacteria and provides a color indicator distinguishing between organisms that ferment lactose (e.g., E. coli) and those that do not (e.g., Salmonella, Shigella).[1] Organisms that ferment lactose display "nucleated colonies" -- colonies with dark centers.[2]

- Lactose fermentation produces acids, which lower the pH. This encourages dye absorption by the colonies, which are now coloured purple-black.
- Lactose non-fermenters may increase the pH by deamination of proteins. This ensures that the dye is not absorbed.
- On EMB if *E. coli* is grown it will give a distinctive metallic green sheen (due to the metachromatic properties of the dyes, *E. coli* movement using flagella, and strong acid end-products of fermentation). It is one of the few bacteria that reacts this way to EMB.[3]

**Examples of EMB with different coliform bacteria.**
EMB agar plate inoculated with *Enterobacter aerogenes* (a gram-negative coliform bacterium) showing good growth of brown, dark-centered, mucoid colonies indicating lactose fermentation and acid production.
EMB agar plate inoculated with Klebsiella pneumoniae (a gram-negative coliform bacterium) showing good growth of brown, dark-centered, mucoid colonies (smaller than Enterobacter) indicating lactose fermentation and acid production.

EMB agar plate inoculated with (A) Escherichia coli, (B) Pseudomonas aeruginosa, (C) Klebsiella pneumoniae, and (D) Enterobacter aerogenes. All four gram negative bacteria grew exhibiting different morphology. Escherichia coli grew with typical lactose fermenter.
morphology with excessive acid production and precipitation of green metallic pigment (colonies with green metallic sheen). *Pseudomonas aeruginosa* grew exhibiting the nonfermenter morphology (pinkish colonies); both *Klebsiella pneumoniae* and *Enterobacter aerogenes* grew with lactose fermentation and acid production morphology (with purple dark centered mucoid colonies).

**Hektoen enteric agar**

**Hektoen enteric agar (HEK or HE or HEA)** is a selective and differential agar primarily used to recover Salmonella and *Shigella* from patient specimens. HE contains indicators of lactose fermentation and H₂S production; as well as inhibitors to prevent the growth of gram positive bacteria. The pattern of lactose fermentation and H₂S production aids in the identification of the organism subbed to the plate. HE agar is designed to isolate and recover fecal bacteria belonging to the Enterobacteriaceae family. HE is particularly useful in isolating *Salmonella* and *Shigella*.

**Hektoen Enteric Agar**

Hektoen Enteric Agar (HE) is a selective and differential medium designed to isolate and differentiate members of the species *Salmonella* and *Shigella* from other Enterobacteriaceae. Bile salts and the dyes bromthymol blue and acid fuchsin inhibit the growth of most Gram positive organisms. Lactose, sucrose, and salicin provide fermentable carbohydrates to encourage the growth and differentiation of enterics. Sodium thiosulfate provides a source of sulfur. Ferric ammonium citrate provides a source of iron to allow production of hydrogen sulfide from sodium thiosulfate, which provides a source of sulfur. Ferric ammonium citrate also allows the visualization of hydrogen sulfide production by reacting with hydrogen sulfide gas to form a black precipitate.

Enterics that ferment one or more of the carbohydrates will produce yellow to salmon-colored colonies. Non-fermenters will produce blue-green colonies. Organisms that reduce sulfur to hydrogen sulfide will produce black colonies or blue-green colonies with a black center.
Klebsiella pneumoniae ferments lactose and produces salmon-colored growth. Micrococcus luteus does not grow. The white specks are crystals formed by a component of the media that precipitated out of solution when the plates were cooled. They look somewhat like a contaminant, but they do not interfere with the performance of the media.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Colonial Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coliforms (rapid lactose fermenters)</td>
<td>Moderately inhibited. When present bright-colored, orange to salmon-pink.</td>
</tr>
<tr>
<td>Citrobacter</td>
<td>Usually inhibited. When present, small and bluish-green in color.</td>
</tr>
<tr>
<td>Shigella</td>
<td>Moist, raised colonies, greener than Salmonella. Periphery of colony often lighter than central portion.</td>
</tr>
<tr>
<td>Proteus</td>
<td>Most strains are inhibited. P. mirabilis may produce dark centered greenish colonies somewhat similar to Salmonella, but smaller. Proteus strains not producing H2S resemble Shigella but are smaller. In general, colonies of Proteus have a more glistening appearance than do Salmonella or Shigella.</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>Most strains are inhibited. When present, colonies are small, flat irregular and green to brown in color.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organism</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shigella sonnei</td>
<td>Growth-green colonies</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>Growth-green colonies</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>Growth-Blue to blue-green, black centers</td>
</tr>
<tr>
<td><strong>Salmonella choleraesuis</strong></td>
<td>Growth-Blue to blue-green</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td>Growth-orange to salmon-pink</td>
</tr>
<tr>
<td><strong>Proteus vulgaris</strong></td>
<td>If growth-green, black centers</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td>Growth-Partially to completely inhibited</td>
</tr>
<tr>
<td><strong>Uninoculated Control</strong></td>
<td>No growth</td>
</tr>
</tbody>
</table>

This photograph depicts the colonial growth pattern displayed by *Salmonella typhimurium* bacteria cultured on a *Hektoen enteric (HE) agar medium*; *S. typhimurium* colonies grown on HE agar are blue-green in color, for this organism is a lactose non-fermenter, but it does produce hydrogen sulfide, (H2S), therefore there can be black-colored deposits present.

**Shigella Infections.** This photograph depicts the colonial morphology displayed by *Shigella boydii* bacteria cultivated on a *Hektoen enteric (HE) agar surface*; colonies of *S. boydii* bacteria grown on HE agar display a raised, green, and moist appearance. HE agar is the preferred medium designed for the isolation, and recovery of fecal bacteria belonging to the family *Enterbacteriaceae*. Four species from the genus *Shigella*, *S boydii, S dysenteriae, S flexneri*, and *S sonnei*, are the etiologic agents responsible for the infection, shigellosis. Most who are infected with *Shigella* spp. develop diarrhea, which is often bloody, fever, and stomach cramps starting a day or two after they are exposed to the bacterium. Shigellosis usually resolves in 5 to 7 days.
**Trypticase soy agar**

*Trypticase soy agar* is a bacterial growth medium.

The medium contains enzymatic digests of casein and soybean meal which provides amino acids and other nitrogenous substances making it a nutritious medium for a variety of organisms. Dextrose is the energy source. Sodium chloride maintains the osmotic equilibrium, while dipotassium phosphate acts as buffer to maintain pH. Agar extracted from any number of organisms is used as a gelling agent. The medium may be supplemented with blood to facilitate the growth of more fastidious bacteria or antimicrobial agents to permit the selection of various microbial groups from mixed flora. As with any media, minor changes may be made to suit specific circumstances.

A culture of *Serratia marcescens* grown on tryptic soy agar medium

My Unknown Samples.
HEA uncovered
EMB uncovered
Open MAC plate
Open TSA