**Endo HiVeg\textsuperscript{TM} Agar**

**Intended Use**

Recommended for confirmation of the presumptive test for members of the coliform group from clinical and non-clinical samples.

**Composition\textsuperscript{**}

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiVeg\textsuperscript{TM} peptone</td>
<td>10.000</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.000</td>
</tr>
<tr>
<td>Dipotassium hydrogen phosphate</td>
<td>3.500</td>
</tr>
<tr>
<td>Sodium sulphite</td>
<td>2.500</td>
</tr>
<tr>
<td>Basic fuchsin</td>
<td>0.500</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.5±0.2</td>
</tr>
</tbody>
</table>

**Directions**

Suspend 41.5 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. If the solidified culture medium is somewhat too red, then to remove the colour add a few drops (max. 1 ml/litre) of a freshly prepared 10% Sodium sulphite solution and boil. Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

Endo Agar was developed by Endo to differentiate gram-negative bacteria on the basis of lactose fermentation, while inhibiting gram-positive bacteria (2). Inhibition of the later was achieved without the use of bile salts as was traditionally used. Endo was successful in inhibiting gram-positive bacteria on his medium by the incorporation of sodium sulphite and basic fuchsin. The resulting Endo Agar, also known as Fuchsin Sulphite and Infusion Agar, was used to isolate the typhoid bacilli. Many modifications of this media have been done over the years. Endo Agar is recommended by APHA as an important medium in the microbiological examination of water and wastewater, dairy products and foods (1,5,6). Endo Agar is used to confirm the detection and enumeration of coliform bacteria following presumptive test of drinking water. It is also used for the detection and isolation of coliforms and faecal coliforms from milk, dairy products and food. Endo HiVeg\textsuperscript{TM} Agar is same as Endo Agar except that the animal based peptones are completely replaced with vegetable peptones to avoid BSE/TSE risks associated with animal peptones.

The medium contains HiVeg\textsuperscript{TM} peptone which provide nitrogen, carbon, vitamins and minerals required for bacterial growth. Sodium sulphite and basic fuchsin make this medium selective by suppressing gram-positive organisms. Coliforms produce pink colonies on fermentation of lactose while lactose non-fermenters produce colourless colonies on the medium. With *Escherichia coli*, this reaction is very pronounced as the fuchsin crystallizes, exhibiting a permanent greenish metallic luster (fuchsin luster) to the colonies. Medium should be stored away from light to avoid photo-oxidation.

**Type of specimen**

Clinical samples - urine; Food and dairy samples; Water samples

**Specimen Collection and Handling**

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (3,4).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (5,6).

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(1)

After use, contaminated materials must be sterilized by autoclaving before discarding.

**Warning and Precautions**

In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

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Please refer disclaimer Overleaf.
Limitations:
1. Besides Enterobacteriaceae, other gram negative bacteria and yeasts may also grow.
2. Avoid exposure of the medium to light, as it may lead to photo oxidation and decrease productivity of the medium.
3. Overheating of the medium must be avoided, as it may destroy the productivity of the medium.
4. Further biochemical tests must be carried out for further confirmation.

Performance and Evaluation
Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control
Appearance
Light pink to purple homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Orangish pink coloured, clear to slightly opalescent gel with fine precipitate forms in Petri plates.

Reaction
Reaction of 4.15% w/v aqueous solution at 25°C. pH : 7.5±0.2

pH
7.30-7.70

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Recovery</th>
<th>Colour of Colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>* Klebsiella aerogenes ATCC 29212 (00087*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>pink</td>
</tr>
<tr>
<td>Enterococcus faecalis ATCC 50-100 29212 (00087*)</td>
<td>none-poor</td>
<td>&lt;=10%</td>
<td>pink, small</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli ATCC 25922 (00013*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>pink to rose red with metallic sheen</td>
</tr>
<tr>
<td>Klebsiella pneumoniae ATCC 13883 (00097*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>pink, mucoid</td>
</tr>
<tr>
<td>Proteus vulgaris ATCC 13315</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless to pale pink</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa ATCC 27853 (00025*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless, irregular</td>
</tr>
<tr>
<td>Salmonella Typhi ATCC 6539</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless to pale pink</td>
</tr>
<tr>
<td>Staphylococcus aureus subsp.aureus ATCC 25923 (00034*)</td>
<td>&gt;=10⁴</td>
<td>inhibited</td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>Enterobacter cloacae ATCC 50-100 13047 (00083*)</td>
<td>good</td>
<td>40-50%</td>
<td>pink</td>
<td></td>
</tr>
<tr>
<td>Salmonella Typhimurium ATCC 14028 (00031*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless</td>
</tr>
<tr>
<td>Salmonella Enteritidis ATCC 50-100 13076 (00030*)</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless</td>
<td></td>
</tr>
<tr>
<td>Shigella flexneri ATCC 12022 (00126*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>colourless</td>
</tr>
</tbody>
</table>

#- Formerly known as Enterobacter aerogenes

Key: *Corresponding WDCM numbers.

Please refer disclaimer Overleaf.
Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Use before expiry date on the label.
Product performance is best if used within stated expiry period.

Disposal
User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

Disclaimer:
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