**Esculin Iron Agar**

**Intended Use:**
Recommended for verifying enterococcal colonies on membrane filters through which water samples have been filtered and which have been incubated on M-Enterococcus Agar, Modified (M1048).

**Composition**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esculin</td>
<td>1.000</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.500</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
</tbody>
</table>

**Final pH (at 25°C)** 7.1±0.2

**Directions**

Suspend 16.5 grams in 1000 ml purified / distilled water. Heat to boiling with frequent stirring. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and pour into sterile Petri plates to a depth of 4-5 mm.

**Principle And Interpretation**

Enterococci are indicators of the sanitary quality of recreational waters, since they occur in faeces of humans and warm-blooded animals (6). Detection and quantitation of Enterococci is necessary because gastroenteritis is associated with swimming in recreational water, which is dependant of enterococcal densities (2). Esculin Iron Agar is used in conjunction with M-Enterococcus Agar, Modified, (M1048) for verification of enterococcal colonies in fresh and marine recreational water, as recommended by APHA (3). Esculin in the medium is hydrolyzed by Enterococci to form esculetin and dextrose. Esculetin reacts with the iron salt (ferric ammonium citrate) and produces a dark brown to black complex, which appears around the colonies.

In the membrane filtration technique, two media, namely M-Enterococcus Agar, Modified (M1048) and Esculin Iron Agar (M1044) are used in conjunction, where the former serves as a selective medium while the later confirms the identification of colonies on the basis of its ability to hydrolyze esculin. The membrane filter used to filter the test water sample is aseptically placed on M-Enterococcus Agar, Modified (M1048) and incubated at 40-42°C for 48 hours. After incubation the membrane is aseptically transferred to Esculin Iron Agar (M1044) plate and incubated at 40-42°C for 20 minutes. After incubation count and record the number of pink to red colonies with black or reddish brown precipitate on the underside of the membrane. If required, magnifying glass or fluorescent lamp may be used for counting the visible colonies. Following formula is used for the final calculation (3).

\[
\text{Enterococci / 100 ml} = \frac{\text{No of enterococcal colonies}}{\text{Volume of sample filtered}} \times 100
\]

**Type of specimen**

Water samples

**Specimen Collection and Handling**

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:**

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 specimens. Safety guidelines may be referred in individual safety data sheets.

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*Please refer disclaimer Overleaf.*
Limitations:
1. Further biochemical and serological tests must be carried out for further identification.

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 yellow to light brown homogeneous free flowing powder

Gelling
Firm comparable with 1.5% Agar gel

Colour and Clarity of prepared medium
Medium amber coloured, clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 1.65% w/v aqueous solution at 25°C. pH: 7.1±0.2

pH
6.90-7.30

Cultural Response
Cultural characteristics observed after an incubation at 40-42°C for 18-24 hours on M-Enterococcus Agar, Modified (M1048) and after 20 minutes at 40-42°C on Esculin Iron Agar (M1044).

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
<th>Colour of Colony</th>
<th>Esculin Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em> ATCC 25922 (00013*)</td>
<td>none-poor</td>
<td></td>
<td>negative reaction</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> ATCC 29212 (00087*)</td>
<td>good-lustrous</td>
<td>pink to red</td>
<td>positive reaction, brown to black precipitate around colonies</td>
</tr>
</tbody>
</table>

Key: *Corresponding WDCM numbers.

Storage and Shelf Life
Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (4,5).
Reference


