Anaerobic Egg Agar Base

**Intended use**
Anaerobic Egg Agar Base supplemented with egg yolk emulsion is recommended for detection of *Clostridium perfringens* in foods.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteose peptone</td>
<td>20.000</td>
</tr>
<tr>
<td>Tryptone</td>
<td>5.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Agar</td>
<td>20.000</td>
</tr>
<tr>
<td><strong>Final pH (at 25°C)</strong></td>
<td>7.0±0.2</td>
</tr>
</tbody>
</table>

**Directions**
Suspend 55 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 and aseptically add 80 ml sterile Egg Yolk Emulsion (FD045). Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

*Clostridium perfringens*, ranked behind *Salmonella* species and *Staphylococcus aureus*, has been the third most common etiological agent of food-borne disease (2). *Clostridium* species are spore forming, gram-positive rods occurring naturally in soil (3). *C. perfringens* food poisoning results from eating contaminated food. The major virulence factor of *C. perfringens* is the CPE enterotoxin, which is secreted upon invasion of the host gut, and contributes to food poisoning and other gastrointestinal illnesses (3). *C. perfringens* cells may lose viability if the suspected food samples are refrigerated, thereby making it difficult to incriminate the organisms in food poisoning outbreaks (9). Anaerobic Egg Agar is one of the media recommended by APHA (8) for detecting *C. perfringens* in foods.

Tryptone and proteose peptone supply long chain amino acids and other complex nitrogenous nutrients. Yeast extract provides essential B-complex vitamins. Egg yolk emulsion is added to the medium by which the lipase and lecithinase activity can be observed. Lecithinase of *C. perfringens* degrades lecithin of egg yolk, forming an insoluble opaque precipitate (4). Lipase breaks down free fats present in the egg yolk causing iridescent sheen to form on the colony surface. For the lipase reaction, plates may be kept up to a week for incubation (4). Proteolysis is indicated by clear zones in the medium surrounding the growth (7).

**Type of Specimen**
Food and dairy samples

**Specimen Collection and Handling**
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,8).

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.

**Limitations:**
1. Due to nutritional variations, some strains may show poor growth.
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
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium
Basal medium - Light yellow coloured, clear to very slightly opalescent gel. After addition of Egg Yolk Emulsion - Light yellow coloured, opaque gel forms in Petri plates

Reaction
Reaction of 5.5% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH
6.80-7.20

Cultural Response
Cultural characteristics observed with added Egg Yolk Emulsion (FD045) when incubated anaerobically, 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>Lecithinase</th>
<th>Lipase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium perfringens</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>positive reaction, opaque zone around the colony</td>
<td>negative reaction</td>
</tr>
<tr>
<td>ATCC 12924</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium sporogenes</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>negative reaction</td>
<td>positive reaction, iridescent sheen on the colony</td>
</tr>
<tr>
<td>ATCC 11437</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 (5,6).

Reference


Revision : 03/2019

Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.