Columbia Agar

Intended use
Recommended for detection of *Clostridium sporogenes* from pharmaceutical products in accordance with the microbial limit testing by harmonized methodology of USP/EP/BP/JP/IP.

Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptone #</td>
<td>10.000</td>
</tr>
<tr>
<td>HM extract ##</td>
<td>5.000</td>
</tr>
<tr>
<td>HM hydrolysate ###</td>
<td>3.000</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>5.000</td>
</tr>
<tr>
<td>Maiz starch</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
</tbody>
</table>

If necessary adjust the pH so that after sterilization it is 7.3±0.2
* pH can also be measured after sterilization at 25°C
**Formula adjusted, standardized to suit performance parameters

Directions
Columbia Agar is a ready to use solid media in glass bottle. The medium is pre-sterilized, hence it does not need sterilization. Medium in the bottle can be melted either by using a pre-heated water bath or any other method. Slightly loosen the cap before melting. When complete melting of medium is observed dispense the medium in tubes as butts/slants or in plates as desired and allow to solidify. If on plate, either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically.

Principle And Interpretation
Columbia Agar is used as a general-purpose nutritious medium was devised by Ellner et al from Columbia University, which was further enriched by the addition of sheep blood (2). It can also be used for the isolation of organisms by addition of various supplements. Columbia Agar is prepared in accordance with the microbial limit testing harmonized methodology of USP/EP/BP/JP/IP (8,1,3,6,4). This medium is recommended to check the presence of *Clostridium* in non-sterile products like food, dietary, nutritional supplements related products. The genus *Clostridium* belongs to the family Clostridiaceae in the class Clostridia.

The product to be examined is initially enriched in Reinforced medium for clostridia. This medium contains 0.05% Agar and cysteine, which creates anaerobic conditions, thereby allowing anaerobic organisms to grow. The enriched sample is then subcultured on Columbia Agar Plate. Columbia Agar Plate is used as a base for media containing blood and for selective media formulations in which different combinations of antimicrobial agents are used as additives. This medium is highly nutritious as it contains tryptone, HM extract and HM hydrolysate and yeast extract provides carbonaceous and nitrogenous substances, long chain amino acids, vitamins of B complex group and other essential nutrients for the luxuriant growth of fastidious as well as non-fastidious organisms. Sodium chloride maintains osmotic balance of medium. Maize starch acts as an energy source and also neutralizes toxic metabolites if produced. It is used in detection of Clostridia from pharmaceutical products. Gentamicin (FD252) inhibits a number of contaminating gram-negative organisms and *Staphylococcus* species.

Clostridia grows under anaerobic conditions as gram positive rods giving a catalase negative test. Further confirmation is carried out by identification tests.

Type of specimen
Pharmaceutical samples; Clinical samples- wound samples

Please refer disclaimer Overleaf.
Specimen Collection and Handling
For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (8,1,3,6,4). For clinical samples follow appropriate techniques for handling specimens as per established guidelines (5,7). 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 specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations
1. Some Clostridium species may show poor growth due to nutritional variations.
2. Further biochemical tests must be carried out for complete 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
Sterile Columbia Blood Agar in glass bottle.

Colour of medium
Light amber coloured medium

Quantity of medium
100ml of medium in glass bottle.

Reaction
7.10-7.50

Sterility Test
Passes release criteria

Growth Promotion Test
Growth Promotion was carried out in accordance with the harmonized method of USP/EP/BP/JP, and growth was observed under anaerobic conditions after an incubation at 30-35°C for 24-48 hours. Recovery rate is considered as 100% for bacteria growth on Casein Soybean Digest Agar (Soybean Casein Digest Agar).

Growth promoting properties
Growth of microorganism comparable to that previously obtained with previously tested and approved lot of medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu under anaerobic conditions (at 30-35°C for <=48 hours).

Cultural Response

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Observed Lot value (CFU)</th>
<th>Recovery</th>
<th>Incubation temperature</th>
<th>Incubation period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Promoting</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium sporogenes ATCC 19404 (00008*)</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>30 -35 °C</td>
<td>&lt;=48 hrs</td>
</tr>
<tr>
<td>Clostridium sporogenes ATCC 11437</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>30 -35 °C</td>
<td>&lt;=48 hrs</td>
</tr>
<tr>
<td>Bacteroides vulgatus ATCC 8492</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>30 -35 °C</td>
<td>&lt;=48 hrs</td>
</tr>
<tr>
<td>Additional Microbiological testing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridium perfringens ATCC 13124 (00007*)</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>30 -35 °C</td>
<td>&lt;=48 hrs</td>
</tr>
<tr>
<td>Bacteroides fragilis ATCC 23745</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>25 -100</td>
<td>&gt;=50 %</td>
<td>30 -35 °C</td>
<td>&lt;=48 hrs</td>
</tr>
</tbody>
</table>

Please refer disclaimer Overleaf.
Storage and Shelf Life
On receipt store between 15-25°C 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 (5,7).

Reference
4. Indian Pharmacopoeia, 2018, Govt.of India, the Controller of Publication, New Delhi