Columbia Agar Plate

**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/IP (Medium 15)

**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>Maize 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**

# Pancreatic digest of casein ;  ## Meat peptic digest ;  ### Heart pancreatic digest

**Directions**

Either streak, inoculate or surface spread the test inoculum (50-100 CFU) aseptically on the plate.

**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/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

**Specimen Collection and Handling**

For pharmaceutical samples, follow appropriate techniques for sample collection, processing as per guidelines (8,1,3,6,4). After use, contaminated materials must be sterilized by autoclaving before discarding.

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Please refer disclaimer Overleaf.
**Warning and Precautions:**
Read the label before opening the pack. 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. Individual strain of a microorganism may have unique growth requirements with respect to nutrients and physical conditions. Based on which the growth pattern of each varies on a medium and some even may display significant delay in development.

2) Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user’s unique requirement.

3) It is recommended to store the plates at 24-30°C to avoid minimum condensation.

**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 90 mm disposable plates.

**Colour of medium**
Light amber coloured medium

**Quantity of medium**
25 ml of medium in 90 mm disposable plates.

**pH**
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><em>Clostridium sporogenes</em> 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><em>Clostridium sporogenes</em> 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><em>Bacteroides vulgatus</em> ATCC 8482</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><em>Clostridium perfringens</em> 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><em>Bacteroides fragilis</em> 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>

**Additional Microbiological testing**

Please refer disclaimer Overleaf.
Key : (*) Corresponding WDCM numbers

Storage and Shelf Life
On receipt store between 20-30°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
5. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition

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.