HiCrome™ Bacillus Agar

Intended use

Recommended for isolation and differentiation between various species of *Bacillus* from a mixed culture in foods, clinical and non-clinical samples by chromogenic method.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10.000</td>
</tr>
<tr>
<td>HM extract #</td>
<td>1.000</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>10.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>10.000</td>
</tr>
<tr>
<td>Chromogenic mixture</td>
<td>3.200</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.025</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.1±0.2</td>
</tr>
</tbody>
</table>

**Formula adjusted, standardized to suit performance parameters**

Directions

Suspend 49.22 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 50°C and aseptically add rehydrated contents of 1 vial of Bacillus Selective Supplement (FD324) if desired. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Majority of *Bacillus* species apparently have little or no pathogenic potential and are rarely associated with disease in humans or lower animals. The principal exception to this are *Bacillus anthracis*, the agent of anthrax, and *Bacillus cereus*, but a number of other species, particularly those of the *B.subtilis* group, have been implicated in food poisoning and other human and animal infections (7). *Bacillus cereus* causes food poisoning due to consumption of contaminated rice (2,5,10), other starchy foods such as potato, pasta and cheese have also been implicated, eye infections and a wide range of other clinical conditions like abscess formation, meningitis, septicemia and wound infection.

HiCrome™ Bacillus Agar is based on the formulation of MYP Agar formulated by Mossel et al (6) used for enumeration of *Bacillus cereus* and *Bacillus thuringiensis* when present in large number in certain foodstuffs.

The medium contains peptone and HM extract, which provide nitrogenous, carbonaceous compounds, long chain amino acids, vitamins and other essential growth nutrients. Mannitol serves as the fermentable carbohydrate, fermentation of which can be detected by phenol red. Mannitol fermenting organisms like *B. megaterium* yield yellow coloured colonies. The chromogenic mixture present in the medium is cleaved by the enzyme beta-glucosidase found in *B.cereus* resulting in the formation of blue colonies. *B.thuringiensis* also grows as blue/green colonies on this medium as *B.cereus* and *B.thuringiensis* are biochemically identical, however *B.cereus* shows flat colonies with distinct blue centers, while *B.thuringiensis* shows irregular margins. If selective isolation of *B.cereus* or *B.thuringiensis* is required aseptically add Bacillus Selective supplement (FD324).

**Type of specimen**

Clinical samples - Blood, pus, eye exudates; Food and dairy 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 (1,8,9).

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

Please refer disclaimer Overleaf.
Warning and Precautions:
In Vitro diagnostic Use only. 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.

Limitations:
1. Due to variable nutritional requirements, some strains may show poor growth on this medium.
2. Slight colour variation may be observed depending upon the utilization of the substrate by the organism.

 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 1.5% Agar gel

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

Reaction
Reaction of 4.92% 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 30°C for 24-48 hours.

Cultural Response

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth w/o addition of FD324</th>
<th>Recovery w/o addition of FD324</th>
<th>Growth w/ addition of FD324</th>
<th>Recovery w/ addition of FD324</th>
<th>Colour of Colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>* Bacillus subtilis subsp. spizizenii ATCC 6633 (00003*)</td>
<td>50-100</td>
<td>fair</td>
<td>20-30%</td>
<td>inhibited</td>
<td>0%</td>
<td>yellowish green to green colonies</td>
</tr>
<tr>
<td>* Bacillus cereus ATCC 10876 50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>light blue,large,flat colonies with blue centre</td>
<td></td>
</tr>
<tr>
<td>* Bacillus thuringiensis ATCC 50-100 10792</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>light blue,large,flat colonies with irregular margins</td>
<td></td>
</tr>
<tr>
<td>* Bacillus megaterium ATCC 14581</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>inhibited</td>
<td>0%</td>
<td>yellow, mucoid colonies</td>
</tr>
<tr>
<td>* Bacillus coagulans ATCC 7050 (00002*)</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>inhibited</td>
<td>0%</td>
<td>pink,small, raised colonies</td>
</tr>
<tr>
<td>* Bacillus pumilis ATCC 14884</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>&gt;=50%</td>
<td>poor</td>
<td>10-20%</td>
<td>light green to green colonies</td>
</tr>
<tr>
<td>* Staphylococcus aureus subsp. aureus ATCC 25923 (00034*)</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>light green to green colonies</td>
<td></td>
</tr>
<tr>
<td>* Enterococcus faecalis ATCC 50-100 29212 (00087*)</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>luxuriant</td>
<td>&gt;=50%</td>
<td>light green to green colonies</td>
<td></td>
</tr>
</tbody>
</table>

* - Corresponding WDCM numbers

Please refer disclaimer Overleaf.
Storage and Shelf Life

Store dehydrated medium 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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