Aspergillus Differentiation Medium Base

Intended Use:
Recommended for detection of aflatoxin producing *Aspergillus* species from food samples.

**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>Yeast extract</td>
<td>20.000</td>
</tr>
<tr>
<td>Ferric ammonium citrate</td>
<td>0.500</td>
</tr>
<tr>
<td>Dichloran</td>
<td>0.002</td>
</tr>
<tr>
<td>Agar</td>
<td>15.000</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>6.3±0.2</td>
</tr>
</tbody>
</table>

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

**Directions**

Suspend 22.75 grams in 500 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 sterile rehydrated contents of 1 vial of Chloramphenicol Selective Supplement (FD033). Mix well and pour into sterile Petri plates.

**Principle And Interpretation**

Aspergilli are hyaline moulds that commonly cause opportunistic infections in humans. Allergic bronchopulmonary disease is a manifestation of hypersensitivity to fungal spores or products, a common manifestation of *Aspergillus* species (particularly *A. flavus*) (6). Aspergillus Differentiation Medium Base formulated by Pitt et al (9) is a modification of the medium formulated by Bothast and Fennel (2). *Aspergillus flavus* develops intense yellow orange colour at the base of the colonies, which is a differential characteristic of this species. This pigmentation helps in differentiating *A. flavus* from other *Aspergillus* species (2,3,7). Assante et al (1) showed that the orange yellow coloration was due to the reaction of ferric ions (from ferric ammonium citrate) with aspergillic acid or neoaspergillic acid forming a colored complex.

A mixture of chloramphenicol and dichloran restricts the spreading of moulds. It also inhibits bacterial growth and helps in the identification of fungi. Peptone and yeast extract serve as sources of nitrogen, amino acids and B complex vitamins. Ferric ammonium citrate aids in the production of yellow orange pigment characteristic of *A.flavus*. *A. parasiticus*, associated with aspergillosis also produces a yellow orange pigment similar to the one produced by *A. flavus* (8).

**Type of specimen**

Food samples

**Specimen Collection and Handling:**

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (10).

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. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
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.

**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.

Please refer disclaimer Overleaf.
Quality Control
Appearance
Cream to yellow 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 4.55% w/v aqueous solution at 25°C. pH : 6.3±0.2
pH
6.10-6.50
Cultural Response
Cultural characteristics observed with added 1 vial of Chloramphenicol Selective Supplement (FD033) after an incubation at 25-30°C for 48-72 hours.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Colour of colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus brasiliensis</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>pale yellow colour on the reverse side of colonies with black heads on the top of the colonies</td>
</tr>
<tr>
<td>ATCC 9642</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus flavus ATCC</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>yellowish orange colour on the reverse side of colonies</td>
</tr>
<tr>
<td>22547</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus parasiticus</td>
<td>50-100</td>
<td>good-luxuriant</td>
<td>yellowish orange colour on the reverse side of colonies</td>
</tr>
<tr>
<td>ATCC 28285</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 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. 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
2. Bothast and Fennel, 1974, Mycologia. 66:36
3. Haley and Callaway, 1978, Laboratory methods in medical mycology, 4th Ed., Center for Disease Control, Atlanta, G
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.