



Technical Data

Aspergillus Differentiation Medium Base

M1127

Intended Use:

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

Composition**

| Ingredients | Gms / Litre |
|-------------------------|-------------|
| Peptone | 10.000 |
| Yeast extract | 20.000 |
| Ferric ammonium citrate | 0.500 |
| Dichloran | 0.002 |
| Agar | 15.000 |
| Final pH (at 25°C) | 6.3±0.2 |

**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.

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.

| Organism | Inoculum (CFU) | Growth | Colour of colony |
|---|----------------|----------------|--|
| <i>Aspergillus brasiliensis</i> ATCC 9642 | 50-100 | good-luxuriant | pale yellow colour on the reverse side of colonies with black heads on the top of the colonies |
| <i>Aspergillus flavus</i> ATCC 22547 | 50-100 | good-luxuriant | yellowish orange colour on the reverse side of colonies |
| <i>Aspergillus parasiticus</i> ATCC 28285 | 50-100 | good-luxuriant | yellowish orange colour on the reverse side of colonies |

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

1. Assante G. et al., 1981, J. Ag. Food Chem., 29:7
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
4. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Editio
5. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
6. Koneman E. W., (Ed.), Mycology, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed, 1992, J. B. Lippincott Company.
7. McGinnis, 1980, Laboratory Handbook of Medical Mycology, Academic Press, New York, N.Y.
8. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Tenover F. C., Tenover J. C., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
9. Pitt J., Hocking D., and Glenn D. R., 1983
10. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

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