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*) (1). Aspergillus Differentiation Medium Base formulated by Pitt et al (2) is a modification of the medium formulated by Bothast and Fennel (3). *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 (3-5). Assante et al (6) 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 dicholoran 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* (7).

Type of specimen

Food samples

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (1,7,8).

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 clinical 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

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colourr 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
**Aspergillus brasiliensis ATCC 9642*	50-100	good-luxuriant	pale yellow colour on the reverse side of colonies with black heads on the top of the colonies
Aspergillus flavus ATCC 22547	50-100	good-luxuriant	yellowish orange colour on the reverse side of colonies
Aspergillus parasiticus ATCC 28285	50-100	good-luxuriant	yellowish orange colour on the reverse side of colonies

*Key: Formerly known as *Aspergillus niger*

Reference

1. Koneman E. W., (Ed.), Mycology, Colour Atlas and Textbook of Diagnostic Microbiology, 4th Ed, 1992, J. B. Lippincott Company.
2. Pitt J., Hocking D., and Glenn D. R., 1983
3. Bothast and Fennel, 1974, Mycologia. 66:365.
4. Haley and Callaway, 1978, Laboratory methods in medical mycology, 4th Ed., Center for Disease Control, Atlanta, Ga.
5. McGinnis, 1980, Laboratory Handbook of Medical Mycology, Academic Press, New York, N.Y.
6. Assante G. et al., 1981, J. Ag. Food Chem., 29:785
7. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.

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