FNA Medium (Fluorescein Denitrification Agar) M565

FNA Agar is used for differentiation of *Pseudomonas* from other bacilli by their ability to reduce nitrates or nitrites to nitrogen gas (denitrification) and detection of fluorescein pigment.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Casein enzymic hydrolysate	5.000
Magnesium sulphate	1.500
Dipotassium phosphate	1.500
Potassium nitrate	2.000
Sodium nitrite	0.500
Agar	15.000

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 30.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubed medium in a slanted position.

Principle And Interpretation

FNA Agar is based on the formula described by Pickett and Pedersen (1). Fluorescence-Denitrification (FN) Media is formulated to detect fluorescein pigment (2) and complete reduction of nitrate to nitrogen gas. These two characteristics are important in the identification of the pseudomonads and other non-fermentative bacilli. *Pseudomonas* species may represent a minority of the total microflora at the beginning of shelf life. However under certain conditions, their capacity for rapid growth decides their dominance. A problem associated with the use of media developed for isolation of *Pseudomonas* species from foods is the considerable interference from non-pseudomonads (3).

The medium contains potassium nitrate and sodium nitrite as the source of nitrate and nitrite respectively for the denitrification by *Pseudomonas* . Peptic digest of animal tissue and casein enzymic hydrolysate supply the necessary nutrients. Dipotassium phosphate maintains buffering conditions. Magnesium sulphate is the cationic salt and is an activator, which intensifies luminescence.

Using a sterile inoculating needle, streak the slant medium. Incubate the tubes with caps loosened, at 35°C for 18- 24 hours. If the isolate fails to grow, re-incubate at 25-30°C for upto 1 week. Examine daily for growth and pigment production. If pigmentation fails to develop, re-incubate the cultures at 22°C for 1 or more days. Examine under UV light for fluorescein, a greenish yellow fluorescent pigment by the colonies and surrounding the medium. Denitrification results in formation of gas bubbles in the butt.

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 tubes as slants

Cultural Response
M565: Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours .

Organism	Inoculum (CFU)	Growth	Fluorescence (under uv)	Nitrate Reduction

Acinetobacter calcoaceticus ATCC 43498	50-100	good-luxuriant	negative	negative reaction, no colour development
Pseudomonas aeruginosa ATCC 27853	50-100	good-luxuriant	positive	positive reaction,red colour developed within 1-2 minutes

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

Reference

1. Pickett M. J. and Pedersen M. M., 1968, Appl. Microbiol., 16:1631.
2. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
3. Corry J. E. L., Curtis G. D. W. and Baird R. M., Culture Media for Food Microbiology, Vol. 34, Progress in Industrial Microbiology, 1995, Elsevier, Amsterdam.

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