FNA Medium (Fluorescein Denitrification Agar)

M565

Intended Use:

Recommended 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
Peptone	5.000
Tryptone	5.000
Magnesium sulphate	1.500
Dipotassium hydrogen 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 purified / 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 (5). Fluorescence-Denitrification (FN) Media is formulated to detect fluorescein pigment (4) 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 (1).

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

Type of specimen

Isolated Microorganism

Specimen Collection and Handling:

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. 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. Colonies do not fluoresce if medium is poured in Petri dishes.

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 tubes as slants

Cultural Response
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 (00025*)	50-100	good-luxuriant	positive	positive reaction,red colour developed within 1-2 minutes

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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 (2,3).

Reference

1. 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.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. I, Williams and Wilkins, Baltimore.
5. Pickett M. J. and Pedersen M. M., 1968, Appl. Microbiol., 16:1631.

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