Indole Nitrate Medium (Tryptone Nitrate Medium)

M364

Indole Nitrate Medium (Tryptone Nitrate Medium) is used for identification of microorganisms on the basis of nitrate reduction and indole production.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	20.000
Disodium phosphate	2.000
Dextrose	1.000
Potassium nitrate	1.000
Agar	1.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 25 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Indole Nitrate Medium (Tryptone Nitrate Medium), due to the nutritive content, supports growth of aerobes, microaerophiles, and facultative as well as obligate anaerobes. It serves a dual purpose of detecting indole production and nitrate reduction in a wide range of microorganisms.

Casein enzymic hydrolysate contains tryptophan, which is acted upon by certain microorganisms, resulting in the production of indole. Potassium nitrate acts as the substrate for determining nitrate reduction by microorganisms. Duplicate tubes of Indole Nitrate Medium may be inoculated and tested for the presence of nitrates or indole after incubation for various lengths of time. Nitrate test is performed by addition of 0.5 ml each of Sulphanilic Acid (R015) and alpha - Naphthylamine (R009). The development of pink colour indicates nitrate reduction. The colour develops due to presence of nitrite generated from reduction of nitrate. When nitrate is further reduced to ammonia, no colour develops. Add a pinch of zinc dust to the tube. The formation of pink colour after addition of zinc dust indicates that nitrate is not reduced. Indole production can be tested by the addition of Kovacs Reagent (R008) or Ehrlich reagent (R005) (1,2). The formation of a deep red colour in the reagent layer after gentle agitation indicates positive indole test. Indole Nitrate Medium is not recommended for indole test in coliform and other enteric bacteria, as they reduce nitrate to nitrite, which prevents the detection of indole (3). Indole Nitrite Medium should not be used for detecting indole production by members of the *Enterobacteriaceae* . The tubed medium should be boiled for 2 minutes and cooled, without agitation, before use.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Semisolid, comparable with 0.1% Agar gel.

Colour and Clarity of prepared medium
Light amber coloured, clear to slightly opalescent gel forms in tubes as butts

Reaction
Reaction of 2.5% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH
7.00-7.40

Cultural Response
M364: Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	Indole production	Nitrate reduction
Cultural Response				
Bacteroides corrodens ATCC 23834	50-100	luxuriant	negative reaction	negative reaction
Bacteroides ovatus ATCC 8483	50-100	luxuriant	negative reaction	variable reaction
Clostridium perfringens ATCC 12924	50-100	luxuriant	negative reaction	positive reaction,red colour developed within 1-2 minutes
Clostridium sordellii ATCC 9714	50-100	luxuriant	positive reaction,red ring at the interface of the medium	negative reaction
Clostridium sporogenes ATCC 11437	50-100	luxuriant	negative reaction	negative reaction
Escherichia coli ATCC 25922	50-100	luxuriant	not applicable	positive reaction,red colour developed within 1-2 minutes
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	not applicable	positive reaction,red colour developed within 1-2 minutes
Staphylococcus aureus ATCC 25923	50-100	luxuriant	negative reaction	positive reaction,red colour developed within 1-2 minutes

Storage and Shelf Life

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

Reference

1.MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
2.Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
3.Smith R. F., Rogers R. R., and Bettge C. L., 1972, Appl. Microbiol., 23:423.

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