Technical Data

Indole Nitrate HiVeg™ Medium (Tryptone Nitrate HiVeg™ Medium) MV364

Intended Use:

Recommended for identification of microorganisms by means of nitrate reduction and indole production.

Composition**

Ingredients	g / L
HiVeg™ hydrolysate	20.000
Disodium hydrogen phosphate	2.000
Dextrose (Glucose)	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 25.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense in test tubes or flasks as desired. 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. ISP HiVeg™ Medium No. 6 (Peptone Yeast Extract Iron HiVeg™ Agar) is prepared by using vegetable peptones in place of animal based peptones which make the media free of BSE/TSE risks. HiVeg™ 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.

Type of specimen

Food and dairy samples; Water samples

Specimen Collection and Handling

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 α - 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 Kovac's 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.

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. Isolated microorganism must be used which are 18-24 hours old.

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

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

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

Organism	Inoculum (CFU)	Growth	Indole production	Nitrate reduction
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 (00013*)	50-100	luxuriant	not applicable	positive reaction,red colour developed within 1-2 minutes
Klebsiella pneumoniae ATCC 13883 (00097*)	50-100	luxuriant	not applicable	positive reaction,red colour developed within 1-2 minutes
Staphylococcus aureus subsp. *aureus* ATCC 25923 (00034*)	50-100	luxuriant	negative reaction	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 15-25°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. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
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3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. 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.
5. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
6. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
7. Smith R. F., Rogers R. R., and Bettge C. L., 1972, Appl. Microbiol., 23:423.
8. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.

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Please recheck reference

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