Technical Information

Indole Nitrate MiVeg Medium (Tryptone Nitrate MiVeg Medium)

Product Code : VM1364

Application:- Indole Nitrate MiVeg Medium (Tryptone Nitrate MiVeg Medium) is used for identification of microoragnisms on the basis of nitrate reduction and indole tests.

Composition

Ingredients	Gms / Litre
MiVeg hydrolysate	20.0
Disodium phosphate	2.0
Dextrose	1.0
Potassium nitrate	1.0
Agar	1.0
Final pH (at 25°C)	7.2 ± 0.2

** Formula adjusted, standardized to suit performance parameters.

Principle & Interpretation

Indole Nitrate MiVeg Medium is prepared by using MiVeg hydrolysate in place of Casein enzymic hydrolysate thus making medium free from BSE/ TSE risks. This medium is the modification of Indole Nitrate medium for identification of microorganisms based on nitrate reduction and indole tests. Due to its nutritive content, the medium supports the growth of aerobes, microaerophiles, facultative as well as obligate anaerobes. The medium has low agar content which offers varying degree of anaerobiosis thereby allow organisms with various oxygen requirements to grow.

Certain microorganisms act upon tryptophan, an amino acid present in MiVeg hydrolysate thereby results in production of indole which is further detected with addition of Kovac's indole reagent (854500) or Ehrlich's reagent (829520). The formation of a pinkish red colour within 10 seconds in the reagent layer after gentle agitation indicates positive indole test (1, 2). Potassium nitrate in the medium acts as the substrate for nitrate reduction. Certain bacteria convert nitrate to nitrite, ammonia or nitrogen gas. The presence of nitrite is determined by addition of 0.5 ml of each of Sulphanilic Acid (894490) and alpha-Naphthylamine solution (868420). The development of red violet colour indicates nitrate reduction to nitrite. If no colour develops, it means that either nitrate is not reduced or further reduction to ammonia or nitrogen gas has taken place. This can be verified by adding a pinch of zinc dust to the tube. Zinc reduces nitrate to nitrite resulting in a red colour. The red olour indicates that nitrate results is still present and was not reduced previously, whereas absence of red colour indicates absence of nitrate. Therefore, the nitrate reduction test is evidenced by either the presence of a catabolic end product or the absence of nitate in the medium.

Although indole production is a useful test for baterial identification, this medium is not recommended for coliform and other enteric bacteria as they reduce nitrate to nitrite, which prevents the detection of indole (3). Duplicate tubes of Indole Nitrate MiVeg Medium may be inoculated and tested for the presence of nitrates and indole after incubation for various lengths of time.

Methodology

Suspend 25 grams of powder media in 1000 ml distilled water. Mix thoroughly and heat to boiling to dissolve the medium completely. Dispense in test tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Physical Appearance

Light yellow coloured, may have slightly greenish tinge, 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 semisolid gel forms in tubes as butts.

Reaction

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

pH Range

7.0 - 7.4

Cultural Response/Characteristics

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

Organisms (ATCC)	Inoculum (CFU)	Growth	Indole#	Nitrate Reduction *
Clostridium perfringens (12924)	10^2-2x10^2	luxuriant	–	+
Clostridium sordellii (9714)	10^2-2x10^2	luxuriant	+	–
Clostridium sporogenes (11437)	10^2-2x10^2	luxuriant	–	–
Escherichia coli (25922)	10^2-2x10^2	luxuriant	+	+
Staphylococcus aureus (25923)	10^2-2x10^2	luxuriant	–	+

Key: # = Red ring observed on addition of Kovac's Indole reagent.
* = Red colour observed on addition of 0.5% α-Naphthylamine solution and 0.8% Sulphanilic acid.

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 day.

Further Reading

1. MacFaddin J.F., 2000(ed), Biochemical Tests for Identification of MedicalBacteria, 3rd edition, Lippincott Williams and Wilkins, New York.
2. Murray PR, Baron, Pfaller, and Yolken (Eds.), 2003, In Manual of Clinical Micro- biology, 8th ed., ASM, Washington, D.C.
3. Smith, Rogers and Bettge, 1972, Appl. Microbiol., 23:423.

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