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

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

Composition ** :

Ingredients	Grams/Litre
HiVeg 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.

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 :

This medium is prepared by replacing Casein enzymic hydrolysate with HiVeg hydrolysate which is free of BSE/TSE risks. This medium can be used against animal based Indole Nitrate medium (M364) 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 and obligate anaerobes. The medium has low agar content which offers varying degree of anaerobiosis and enables organisms with various oxygen requirements to grow.
Certain bacteria decomposes amino acid tryptophan from the protein source (HiVeg hydrolysate) to indole which accumulates in the medium. Indole production can be detected by the addition of Kovac's Indole reagent (R008) or Ehrlich's aldehyde reagent (R005). 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 by microorganisms. 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 (R015) and alpha - Naphthylamine solution (R009). 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 colour indicates that nitrate is still present and was not reduced previously. An absence of red colour after the addition of zinc dust indicates that no nitrate is present, and thus the nitrate was reduced further than nitrite. Therefore the nitrate reduction test is evidenced by either the presence of a catabolic end product or the absence of nitrate in the medium.
Though production of indole has been a useful test in identification of bacteria, this medium is not recommended for coliform and other enteric bacteria as they reduce nitrate to nitrite, which prevents the detection of indole (3).

Product Profile :		
Vegetable based (Code MV)◉		**Animal based** (Code M)
MV364 HiVeg hydrolysate		**M364** Casein enzymic hydrolysate
Recommended for	:	Identification of microorganisms on the basis of nitrate reduction and indole tests.
Reconstitution	:	25.0 g/l
Quantity on preparation (500g)	:	20.0 L
(100g)	:	4.0 L
pH (25°C)	:	7.2 ± 0.2
Supplement	:	None
Sterilization	:	121°C / 15 minutes.
Storage : Dry Medium - Below 30°C, Prepared Medium 2 - 8°C.		

Duplicate tubes of Indole Nitrate HiVeg Medium may be inoculated and tested for the presence of nitrates and indole after incubation for various lengths of time.

Quality Control :

Appearance of powder
Light yellow coloured, may have slightly greenish tinge, homogeneous, free flowing powder.
Gelling
Semisolid, comparable with 0.1% Agar gel.
Colour and Clarity
Light amber coloured, clear to slightly opalescent gel forms in tubes as butts.
Reaction
Reaction of 2.5% w/v aqueous solution is pH 7.2 ± 0.2 at 25°C.
Cultural Response
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.

References :

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

◉ Prepared from GMO free Vegetable proteins replacing Animal based peptones. Freedom from BSE/TSE worries.