Indole Nitrite Medium (Trypticasein Nitrate Medium)

Cat. 1504

For the differentiation of microorganism on the basis of indole production and the reduction of nitrate to nitrite

Practical information

Aplications	Categories
Differentiation	Enterobacteria

Industry: General cultivation

Principles and uses

Indole Nitrite Medium (Trypticasein Nitrate Medium) is a semisolid medium used to determine nitrate reduction and indole production by a wide variety of organisms. The reduction of nitrate is an important biochemical tool in the identification of many microorganisms. Nitrate reduction is an important characteristic of most members of the Enterobacteriaceae family.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Potassium nitrate acts as the substrate for determining nitrate reduction. Dextrose is the fermentable carbohydrate providing carbon and energy.

This medium is used to identify Gram-negative bacilli using 2 tests: one for Indole production and another for Nitrate reduction. Indole Nitrite Medium can be used for nitrite tests with members of the Enterobacteriaceae family but is not recommended for the indole test with these organisms since they reduce nitrate to nitrite, thus preventing indole detection.

Formula in g/L

Dextrose	1	Bacteriological agar	1
Casein peptone	20	Disodium phosphate	2
Potassium nitrate	1		

Preparation

Suspend 25 grams of the medium in one liter of distilled water. To perform motility and gas detection tests add 2 grams of agar. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into regular test tubes, half-fill them and sterilize in autoclave at 121 ºC for 15 minutes. If the prepared medium is semisolid allow solidification in tubes in a vertical position. Use the medium during the first 2 days after preparation. If kept longer, heat until boiling in a water bath to regenerate the medium.

Instructions for use

The indole test should be performed after 24-48 hours of incubation (or after a good bacterial growth) at a temperature of 35±2 ºC and by the addition of a few drops of Kovacs reagent (Cat. 5205). A positive test is indicated by the formation of a pink to red color in the reagent layer after several minutes.

To investigate the reduction of nitrates, use 3 separate tubes: a positive control (Escherichia coli), a negative control (Acetobacter calcoaceticus) and a third comparison tube:
- Inoculate by puncturing each tube abundantly.
- Incubate at 35 ºC for 8, 12 and 24 hours.
- Add a few drops of Griess Reagent.
- The formation of a red color in 1-2 minutes indicates the reduction of nitrates to nitrites (positive test).
- If no color appears, add a pinch of zinc powder (free of nitrates and nitrites) to the tubes.
- Observe if the color appears red or if the medium remains colorless.

a) If there is no nitrate reduction, the zinc will be reduced to nitrite and will form a red color when reacted with the Griess reagent. The tested organism is negative (absence of nitrates).
b) If there is no appearance of color, this indicates that the organism reduced the nitrate present in the culture medium to nitrite, possibly leading the reaction to nitrogen gas. The tested organism is positive (presence of nitrates).

Quality control

Solubility	Appareance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25ºC)
w/o rests	Fine powder	Beige	Clear amber	7,2±0,2

Microbiological test

Incubation conditions: (35±2 ºC / 24 h).

Microrganisms	Specification	Characteristic reaction
Salmonella typhimurium ATCC 14028	Good growth	Nitrite (+), Indol (-)
Escherichia coli ATCC 25922	Good growth	Nitrite (+), Indol (+)

Storage

Temp. Min.:2 ºC
Temp. Max.:25 ºC

Bibliography

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