Technical Information

Nitrate Broth

Product Code: DM 1439S

Application: Nitrate Broth is recommended for detection of nitrate reduction by bacteria.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Meat extract	3.000
Potassium nitrate	1.000
Sodium chloride	30.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Nitrate Broth is prepared in according to the formula published in 'Pure Culture Study of Bacteria' of the Society of American Bacteriologist [(1)] and present modified formula is recommended by BIS [(2).] The ability to reduce nitrate is valuable for differentiating and identifying various types of bacteria especially those belonging to *Enterobacteriaceae* family [(3).] Recently ISO committee [(4)] has recommended Nitrate Broth for the enumeration of *Bacillus cereus* - colony count technique at 30°C [(4).] Non-fermenters and other miscellaneous gram-negative bacilli difer in their ability to reduce nitrates. Some members of this group are capable of denitrification which is a reduction of nitrate to nitrogen gas. For the glucose fermenting gram-negative bacilli, the production of nitrogen gas from nitrate is an important differential test [(4).] Reduction of nitrate is generally an anaerobic respiration in which an organism derives its oxygen from nitrate. Members of *Enterobacteriaceae* characteristically reduce nitrate to nitrite which reacts with sulfanilic acid and N,N-dimethyl-1-naphthylamine to produce the red colour. This reaction is known as Griess reaction. If an organism grows rapidly and reduces nitrate actively, the test should be performed after an early incubation period since the nitrite may be further reduced to nitrogen.

Preparation of Nitrate Test Reagents:

1. Sulfanilic Acid: Dissolve 8 grams of sulfanilic acid in 1 litre 5 N acetic acid.
2. Alpha-Naphthylamine reagent: Dissolve 5 grams of alpha-naphthylamine in 1 litre 5 N acetic acid.

For the test: Put few drops of each reagent into the tube containing culture to be tested. A distinct red or pink colour indicates nitrate reduction. The results should be recorded within 5 - 10 seconds as the colour fades on standing. A control (uninoculated) tube should also be tested. If there is no pink colour formation, add pinch of zinc dust to confirm the absence of nitrate in the medium (5). Nitrate reduction is not a confirmatory test. Complete identification should include the morphology, gram reaction, biochemical and serological tests. Addition of excess zinc may result in false-negative reaction.

Methodology

Suspend 39 grams of powder media in 1000 ml distilled water. Shake & heat if necessary to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Physical Appearance
Cream to yellow coloured homogeneous free flowing powder

Colour and Clarity of prepared medium
Light amber coloured clear to slightly opalescent solution forms in tubes.

Reaction
Reaction of 3.9% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH range
6.80-7.20

Cultural Response/Characteristics
DM 1439S: Cultural characteristics observed after an incubation at 35-37^{0}C for 18-24 hours.

Organism	Inoculum (CFU)	Growth	Nitrate Reduction
Bacillus cereus ATCC 10876	50-100	Luxuriant	Positive reaction red colour developed within 1-2 minutes
Enterobacter aerogenes ATCC 13048	50-100	Luxuriant	Positive reaction red colour developed within 1-2 minutes
Escherichia coli ATCC 25922	50-100	Luxuriant	Positive reaction red colour developed within 1-2 minutes
Salmonella Typhimurium ATCC 14028	50-100	Luxuriant	Positive reaction red colour developed within 1-2 minutes

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 days.

Further Reading

1. Society of American Bacteriologist, Pure Culture Study of Bacteria, 1994, 12 : Leaflet 11:8.
2. Bureau of Indian Standards IS : 5887 (Part IV) 1976.
3. Ewing, 1986, Edwards and Ewings Identification of Enterobacteriaceae, 4th ed., Elsevier Science Pub. Co., Inc., N.Y.
4. International Organization for Standardization (ISO), 1993, Draft ISO/DIS 7932.
5. MacFaddin, 1980, Biochemical Tests for the Identification of Medical Bacteria, 2nd ed.,Williams and Wilkins, Baltimore.

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- User must ensure suitability of the product(s) in their application prior to use.
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