

**NITRATE BROTH****TM 239**

For detection of nitrate reduction by bacteria and enumeration of *Bacillus cereus*

Composition

Ingredients	Gms/Ltr.
Beef extract	3.00
Peptone	4.00
Proteose peptone	1.00
Potassium nitrate	1.00

* Dehydrated powder, hygroscopic in nature, store, in a dry place in tightly- sealed containers below 25°C and protect from direct Sunlight.

Instructions for Use

Dissolve 9gms in 1000ml of purified water or distilled water. Gently heat to boiling with gentle swirling and dissolve the medium completely. Sterilize by autoclaving at 15 psi (121°C) for 15 minutes. Cool to 45 – 50°C and dispense into sterile culture tubes.

Appearance: Light to medium amber colour, clear

pH (at 25°C): 7.0 ± 0.2

Principle

NITRATE BROTH is used for detection of nitrate reducing microorganisms or acts as an aid in the identification of aerobic and facultative anaerobic gram-negative microorganisms by means of the nitrate reduction test. This is the ability to reduce nitrate to nitrite is characteristic of the family *Enterobacteriaceae*. Non-fermenters and other miscellaneous gram-negative bacilli vary in their ability to reduce nitrates. Some members of this group are capable of de-nitrification, which is a reduction of nitrate to nitrogen gas. The production of gas from nitrate is an important differential test for glucose-non-fermenting gram-negative bacilli. The end product of reduction depends upon the bacterial species. Nitrate Broth is a basal medium containing potassium nitrate. The microorganism under evaluation is inoculated into the medium and after incubation, nitrate reduction may be determined. An inverted Durham fermentation tube in the prepared tubed medium serves to trap nitrogen gas produced through de-nitrification. The medium is evaluated for nitrate reduction by the addition of two reagents, Nitrate A Reagent (0.8% sulphanilic acid in 5N acetic acid) and Nitrate B Reagent (0.6% N, N-dimethyl- α -naphthylamine in 5N acetic acid), which detect the presence of a catabolic end product, and by the addition of Nitrate C Reagent, zinc dust, which detects the absence of remaining nitrate in the medium. Nitrate reduction is an aid to identification and is not a confirmatory test. Complete identification should include determination of Gram reaction, morphology, biochemical and serological tests. Appropriate texts should be consulted for additional information. Allow at least 2 minutes for the color to develop before considering the nitrate test negative. The nitrate test is very sensitive. An uninoculated nitrate control should be tested with reagents to determine whether the medium is nitrate-free and that the glassware and reagents have not been contaminated with nitrous oxide. The addition of too much zinc dust may result in a false negative reaction or just a fleeting color reaction.

Interpretation

Cultural characteristics observed after inoculating (10^3 CFU/ml), on incubation at $35 \pm ^\circ\text{C}$ for 5 days. Examine the tubes after 18-24 and 42-48 hours for growth and presence of gas in the Durham tube.

Microorganisms	ATCC	Inoculum (CFU/ml)	Growth
<i>Enterobacter aerogenes</i>	13048	10^3	Luxuriant
<i>Escherichia coli</i>	25922	10^3	Luxuriant
<i>Pseudomonas aeruginosa</i>	27853	10^3	Luxuriant

References

1. Ewing. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., New York, N.Y. (1986).
2. MacFaddin. Biochemical tests for the identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md. (2000).
3. Forbes, Sahm and Weissfeld. Bailey & Scott's diagnostic microbiology, 10th ed. Mosby, Inc., St. Louis, Mo. (1998).
4. J.S. Knapp, V.L. Clark, Anaerobic growth of *Neisseria gonorrhoeae* coupled to nitrite reduction, Infect. Immun. 46,176-181. (1984).
5. V.B.D. Skerman, A guide to the identification of the genera of bacteria, The Williams & Wilkins Co., Baltimore, MD, p.218 - 220. (1967).