NITRATE AGAR TM 796

For detection of nitrate reduction by bacteria

Composition

Ingredients	Gms/Ltr.
Agar	12.00
Pancreatic digest of Gelatin	5.00
Beef extract	3.00
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 21gms 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^{0}C and dispense into sterile petri plates.

Appearance: Light yellow colour, clear to slightly opalescent gel
pH (at 25^{0}C): 7.0 ± 0.2

Principle

NITRATE AGAR 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 Agar is a basal medium containing potassium nitrate. This medium contains Pancreatic digest of Gelatin and Beef extract for the sources of nitrogen and essential vitamins. Agar is solidifying agent. The microorganism under evaluation is inoculated into the medium and after incubation, nitrate reduction may be determined. 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-alpha-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 (10^3CFU / ml), on incubation at 35 ± °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
Enterobacter aerogenes	13048	10^3	Luxuriant
Escherichia coli	25922	10^3	Luxuriant
Pseudomonas aeruginosa	27853	10^3	Luxuriant

References

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5. J.S. Knapp, V.L. Clark, Anaerobic growth of Neisseria gonorrhoeae coupled to nitrite reduction, Infect. Immun. 46,176-181. (1984).
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