Nitrate Broth

Intended Use

Nitrate Broth is recommended as an aid in the identification of aerobic and facultative anaerobic gram-negative microorganisms by means of the nitrate reduction test.

Summary and Explanation

Microorganisms may be differentiated according to their metabolism of certain substrates. The ability to reduce nitrate to nitrite is characteristic of the family *Enterobacteriaceae*.[1] Nonfermenters and other miscellaneous gram-negative bacilli vary in their ability to reduce nitrates. Some members of this group are capable of denitrification, which is a reduction of nitrate to nitrogen gas. The production of gas from nitrate is an important differential test for glucose-nonfermenting gram-negative bacilli. The end product of reduction depends upon the bacterial species.[2]

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 denitrification. The medium is evaluated for nitrate reduction by the addition of two reagents, Nitrate A Reagent (0.8% sulfanilic 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.[2]

User Quality Control

Identity Specifications

Difco™ Nitrate Broth

Dehydrated Appearance:	Light to medium tan, free-flowing, homogeneous.
Solution:	0.9% solution, soluble in purified water. Solution is light to medium amber, clear.
Prepared Appearance:	Light to medium amber, clear.
Reaction of 0.9% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ Nitrate Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. Test for nitrate reduction using Nitrate A Reagent, Nitrate B Reagent and Nitrate C Reagent following label directions.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	NITRATE REDUCTION
Acinetobacter calcoaceticus	19606	10^2-10^3	Good	–
Enterobacter aerogenes	13048	10^2-10^3	Good	+
Escherichia coli	25922	10^2-10^3	Good	+
Pseudomonas aeruginosa	27853	10^2-10^3	Good	+

Uninoculated Tube | *Acinetobacter calcoaceticus* ATCC™ 19606 | *Escherichia coli* ATCC™ 25922

Principles of the Procedure

Reduction of nitrate is generally an anaerobic respiration in which an organism derives its oxygen from nitrate. Depending upon environmental conditions, the end products of this metabolic process are usually not further oxidized or assimilated into cellular metabolism, but are excreted into the surrounding medium. *Enterobacteriaceae* characteristically reduce nitrate to nitrite which reacts with sulfanilic acid and N, N-dimethyl-alpha-naphthylamine to produce a red color (Griess reaction). The formation of other end products (ammonia, nitrous oxide, hydroxylamine, etc.) is also possible; therefore, the addition of zinc dust is used to detect unreduced nitrate. The formation of nitrogen gas, an end product typical of certain organisms, is evidenced by displacement of the medium from the Durham tube by the gas produced.[2]

Formula

Difco™ Nitrate Broth

Approximate Formula* Per Liter		
Beef Extract	3.0	g
Peptone	4.0	g
Proteose Peptone No. 3	1.0	g
Potassium Nitrate	1.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Dissolve 9 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Prior to inoculation of Nitrate Broth, the organism to be tested must have been previously isolated on some other suitable solid medium. The use of a pure culture is essential to correct performance of the test.

Using a sterile inoculating loop remove several similar isolated colonies from the agar medium and inoculate into a tube of Nitrate Broth. Replace cap loosely and incubate at 35-37°C.

Examine the tubes after 18-24 and 42-48 hours for growth and presence of gas in the Durham tube. After 24-48 hours add reagents as described in "Expected Results."

Expected Results

If growth is apparent after 24-48 hours of incubation, examine for presence of gas in the Durham tube. If gas is present and the test organism is a nonfermenter, the test is positive for denitrification (nitrate was reduced to nitrogen gas). If the organism is a fermenter, gas may or may not be present. Add 10 drops of Nitrate A Reagent and 10 drops of Nitrate B Reagent to the tube. Development of a red color within 2 minutes denotes a positive test for nitrate. If there is no color development, add a small amount (approximately 20 mg on the tip of an applicator stick) of Nitrate C Reagent. If no color develops within 5-10 minutes, nitrate was reduced beyond nitrite and the test is positive. The development of a red color indicates the presence of unreduced nitrate and the test is negative.

Limitations of the Procedure

1. 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.[3-5]
2. Allow at least 2 minutes for the color to develop before considering the nitrate test negative.
3. 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.[2]
4. The addition of too much zinc dust may result in a false-negative reaction or just a fleeting color reaction.[6]

References

1. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., New York, N.Y.
2. MacFaddin. 2000. Biochemical tests for the identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
3. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
4. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
5. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
6. Porres and Porter. 1974. Am. J. Med. Technol. *40*:257.

Availability

Difco™ Nitrate Broth

AOAC BAM COMPF ISO SMD USDA

Cat. No. 226810 Dehydrated – 500 g

BBL™ Nitrate Broth with Durham Tube

Cat. No. 221830 Prepared Tubes (K Tubes) – Pkg. of 10

Difco™/BBL™ Nitrate A Reagent

Cat. No. 261197 Droppers, 0.5 mL – Ctn. of 50

Difco™/BBL™ Nitrate B Reagent

Cat. No. 261198 Droppers, 0.5 mL – Ctn. of 50

Difco™/BBL™ Nitrate C Reagent

Cat. No. 261207 Droppers, 1 g – Ctn. of 50