



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Nitrate Broth is a medium which aids in the identification of microorganisms by means of the nitrate reduction test.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. Using 0.01 mL calibrated loops, inoculate tubes with 10⁻¹ dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures.
 - b. Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere. An uninoculated tube should be incubated in parallel as a control and tested for the presence of nitrite.
2. Examine tubes after 18–24 and 42–48 h for growth and gas production.
3. Nitrate Reduction Test (on cultures incubated 42–48 h).
 - a. Add 0.5 mL of sulfanilic acid solution.
 - b. Add 0.5 mL of N,N-dimethyl-1-naphthylamine solution.
 - c. Observe for the production of a pink to red color within 1–2 min (positive nitrate reduction test). Since some organisms further reduce nitrite to nitrogen gas, add a small amount of zinc dust to tubes exhibiting no color. A pink color in this part of the test indicates no nitrate reduction, whereas, a colorless reaction indicates that nitrates have been completely reduced to nitrogen gas.
4. Expected Results

Organisms	ATCC™	Nitrite Reaction	Gas
* <i>Pseudomonas stutzeri</i>	17588	+	+
* <i>Acinetobacter baumannii</i>	19606	-	-

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes aerobically at 20–25°C and 30–35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV 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.

V 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 with Durham Tube is a basal medium containing potassium nitrate. The microorganism under evaluation is inoculated into the medium and after incubation, nitrate reduction may be determined. The inverted Durham tube serves to trap nitrogen gas produced through denitrification. The medium is evaluated for nitrate reduction by the addition of two reagents which detect the presence of a catabolic end product and by the addition of zinc dust which detects the absence of remaining nitrate in the medium.²

VI 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-1-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 gas produced.²

VII REAGENTS

Nitrate Broth

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Gelatin	20.0 g
Potassium Nitrate	2.0 g

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–25°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

This product is not intended for use directly with specimens or mixed cultures. The organism to be tested must first be in pure culture.

IX PROCEDURE

Material Provided: Nitrate Broth with Durham Tube

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Prior to inoculation of Nitrate Broth with Durham Tube 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 in an aerobic atmosphere.

Examine the tubes after 18–24 and 42–48 h for growth and presence of gas in the Durham tube. After 42–48 h add reagents as described in “Results.”

User Quality Control: See “Quality Control Procedures.”

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

If growth is apparent after 24–48 h 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 0.5 mL of the nitrate reagents (see II. 3. above) to the tube. Development of a red color within 1–2 min denotes a positive test for nitrite. If there is no color development, add a pinch (approximately 20 mg on tip of an applicator stick) of zinc dust. If no color develops within 5–10 min, 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.

XI LIMITATIONS OF THE PROCEDURE

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.²⁻⁷

The addition of too much zinc dust may result in a false-negative reaction or just a fleeting color reaction.²

XII AVAILABILITY

Cat. No.	Description
221830	BBL™ Nitrate Broth with Durham Tube, Pkg. of 10 size K tubes

XIII REFERENCES

1. Ewing, W.H. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., New York.
2. MacFaddin, J.F. 2000. Biochemical tests for the identification of medical bacteria, 3rd ed. Lippincott Williams and Wilkins, Baltimore.
3. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
4. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.
5. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller and R.H. Tenover (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
6. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven, Philadelphia.
7. Isenberg, H.D. (ed.). 2004. Clinical microbiology procedures handbook, vol. 1, 2 and 3, 2nd ed. American Society for Microbiology, Washington, D.C.

Becton, Dickinson and Company
7 Loveton Circle
Sparks, Maryland 21152 USA
800-638-8663

ATCC is a trademark of the American Type Culture Collection.

BD, BD Logo, BBL and Trypticase are trademarks of Becton, Dickinson and Company. ©2006 BD.