NITRATE SUBSTRATE BROTH

INTENDED USE
Remel Nitrate Substrate Broth is a liquid medium recommended for use in qualitative procedures to determine nitrate utilization by *Mycobacterium* species.

SUMMARY AND EXPLANATION
The nitrate test is one of a battery of biochemical tests used to identify members of the genus *Mycobacterium* based on the presence of nitrate reductase. The nitrate reduction procedure using chemical reagents is recommended by the Centers for Disease Control and Prevention and Clinical Laboratory Standards Institute to aid in the identification of *Mycobacterium* spp.[1,2]

PRINCIPLE
Certain mycobacteria produce the enzyme nitroreductase.[3] The reduction of nitrate to nitrite is indicated by the presence of a catabolic end product or the absence of nitrate in the medium. A positive test occurs when nitrite reacts with the three reagents: hydrochloric acid, sulfanilic acid, and naphthylethylenediamine dihydrochloride to form a diazonium compound, indicated by a red color. To confirm a negtive test (no color change), add zinc dust to the tube. Zinc dust reduces diazonium salt in the presence of acetic acid to produce a red colored compound, arhydrazine.[4]

REAGENTS (CLASSICAL FORMULA)*

Disodium Phosphate	4.85 g	Sodium Nitrate	0.85 g
Monopotassium Phosphate	1.17 g	Demineralized Water	1000.0 ml

pH 7.0 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE
1. Prepare a positive and negative control and an uninoculated tube with each test.
2. Place 3 or 4 drops (0.2 ml) of sterile demineralized water in each sterile, screw-cap test tube.
3. Emulsify two loopfuls or spadefuls of a mature, 2-4-week old culture from a solid medium in the water. A heavy suspension yields the best results.[2]
4. Add 2 ml of Nitrate Substrate Broth to each test tube.
5. Shake by hand to mix and incubate in ambient air at 35-37°C for 2 hours.
6. Add 1 drop of 50% concentrated hydrochloric acid to each tube and shake to mix.
7. Add 2 drops of Nitrate A for AFB (R21243) and 2 drops of Nitrate B for AFB (R21244) to each tube.
8. Examine immediately for a pale pink (+/-) to deep red (5+) color within 30-60 seconds. Positive color controls used for comparison in the nitrate procedure are outlined in the CDC manual.[3] Only 3+ to 5+ are considered positive.
9. Confirm negative results (nitrate not reduced) by adding a pinch of zinc dust to the tube. The development of a red color following the addition of zinc dust confirms the negative result; nitrate was not reduced. If no color change occurs after adding zinc dust, the result was positive; nitrate was reduced beyond nitrite to a colorless compound. In such cases, the test should be repeated to confirm the results.

INTERPRETATION OF THE TEST
Positive Test - 3+ to 5+ pink/red color development (refer to CDC Manual for color controls)[2]

Negative Test - No color change or pale pink (+/-) color development

QUALITY CONTROL
All lot numbers of Nitrate Substrate Broth have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
Mycobacterium tuberculosis ATCC® 25177	Ambient, 2 h @ 33-37°C	Positive
Mycobacterium marinum ATCC® 927	Ambient, 2 h @ 33-37°C	Negative

LIMITATIONS
1. A positive test for nitrate reduction (red color development) may flash instantly or quickly fade.[2]
2. The ability of acid-fast bacilli to reduce nitrate is influenced by age of the colonies, temperature, pH, and enzyme inhibitors. Rapid growers can be tested within 2 weeks; slow growers should be tested after 3 to 4 weeks of luxuriant growth.[3]
3. For best results, use a heavy suspension of organisms.

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BIBLIOGRAPHY

1. Clinical and Laboratory Standards Institute (CLSI). 2008. Laboratory Detection and Identification of Mycobacteria; Approved Guideline. M48-A. CLSI, Wayne, PA.
2. Kent, GA. Kent, P.T. and G.P. Kubica. 1985. Public Health Mycobacteriology, A Guide for the Level III Laboratory. U.S. Dept. of H.H.S. and CDC, Atlanta, GA.
3. MacFaddin, J.F. 2000. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Lippincott Williams & Wilkins, Philadelphia, PA.
4. Garcia, L.S. 2010. Clinical Microbiology Procedures Handbook. 3rd ed. ASM Press, Washington D.C.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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