remel

NITRATE REAGENT A for AFB

INTENDED USE
Remel Nitrate Reagent A for AFB is recommended for use in qualitative procedures to determine nitrate utilization by *Mycobacterium* spp.

SUMMARY AND EXPLANATION
The nitrate reduction test is useful in differential identification of some mycobacteria that possess similar characteristics in morphology, pigmentation, and growth rate. Virtanen found that mycobacteria differ in their ability to reduce nitrate by means of nitrate reductase based on the age of the culture, temperature, enzyme inhibitors, and hydrogen ion concentration.[1] The nitrate reduction procedure using chemical reagents is recommended by Kent and Kubica in *A Guide for the Level III Laboratory*.[2]

PRINCIPLE
The end products of nitrate reduction are many and depend upon the bacterial species. The most common end product is molecular nitrogen by way of nitrite reduction which is indicated by the presence of a catabolic end product or the absence of nitrate in the medium. The development of a red color after the addition of three reagents: hydrochloric acid, sulfanilic acid (Nitrate A), and N-naphthylethylene-diamine (Nitrate B) indicates that nitrate has been reduced to nitrite. The red color reaction is due to the formation of a diazonium compound. When confirming a negative test with zinc dust, the reduction of the diazonium salt by zinc in the presence of acetic acid produces a colored compound, arylhydrazine.

REAGENTS (CLASSICAL FORMULA)*

Sulfanilamide (CAS 63-74-1)	2.0	g
Demineralized Water (CAS 7732-18-5)	1000.0	ml

*Adjusted as required to meet performance standards.

PRECAUTIONS
This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Take precautions against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully.

STORAGE
This product is ready for use and no further preparation is necessary. Store product in its original container at 2-8°C until used. Allow product to equilibrate to room temperature before use. Protect from light.

PRODUCT DETERIORATION
This product should not be used if (1) the yellow color has changed, (2) a precipitate has formed, (3) the expiration date has passed, or (4) there are other signs of deterioration.

SPECIMEN COLLECTION, STORAGE, TRANSPORT
Specimens should be collected and handled following recommended guidelines.[3]

MATERIALS REQUIRED BUT NOT SUPPLIED
(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alter-ative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Sterile screw cap test tubes, (7) Pipettes, (8) Hydrochloric acid (conc.), (9) Nitrate Reagent B for AFB (R21244), (10) Zinc dust, (11) Nitrate Substrate Broth (R061548), (12) Mycobacteriological safety equipment, (13) Sterile demineralized water.

PROCEDURE
1. Place 3-4 drops (0.2 ml) of sterile demineralized water into each sterile screw cap test tube.
2. Emulsify two loopfuls of 4-week old colonies from a solid medium in the water. A heavy suspension yields the best results.[2]
3. Add 2 ml of Nitrate Substrate Broth to each tube.
4. Shake to mix and incubate in a water bath or incubator at 35-37°C for 2 hours.
5. Add 1 drop of 50% concentrated hydrochloric acid to each tube and shake to mix (1:2 dilution of concentrated hydrochloric acid).
6. Add 2 drops each of Nitrate Reagent A for AFB and Nitrate Reagent B for AFB, to each tube.
7. Examine for a pale pink (+/-) to deep red (5+) color within 30-60 seconds. Positive color controls used for comparison are outlined in the CDC manual.[2] A positive test for nitrate reduction (red color) may flash instantly or quickly fade.
8. 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 initially. If no color change occurs upon 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 observation.

INTERPRETATION

Positive Test -	3+ to 5+ pink/red color after addition of Nitrate Reagents A and B; no color change after addition of zinc dust
Negative Test -	No color change after addition of Nitrate Reagents A and B; red color after addition of zinc dust

QUALITY CONTROL

All lot numbers of Nitrate Reagent A for AFB 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, 2h @ 35-37°C	Positive
Mycobacterium marinum ATCC® 927	Ambient, 2h @ 35-37°C	Negative

LIMITATIONS

1. Rapid growers can be tested within 2 weeks; slow growers should be tested after 3 to 4 weeks of luxuriant growth.

BIBLIOGRAPHY

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2. 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.
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5. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.
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PACKAGING

REF R21243, Nitrate Reagent A for AFB ..... 25 ml/Btl

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

ATCC® is a registered trademark of American Type Culture Collection.
CAS (Chemical Abstracts Service Registry No.)

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