remel

Nitrate Disk (Anaerobic)

INTENDED USE
Remel Nitrate Disk is a reagent-impregnated disk recommended for use in qualitative procedures for the determination of nitrate reduction by anaerobic bacteria.

SUMMARY AND EXPLANATION
In 1977, Wideman et al. utilized a nitrate disk for the detection of nitrate reductase production.[1] They reported it to be comparable (89% agreement) to the more conventional and time-consuming indole nitrate medium assay. Filter paper disks impregnated with potassium nitrate are recommended for presumptive testing of anaerobes.[2,3]

PRINCIPLE
Organisms that possess nitrate reductase can reduce nitrate to nitrite. Nitrate serves as the source of nitrogen for many bacteria. Molybdate is reported to stimulate synthesis of nitrate reductase.[4] The first step in nitrate utilization is reduction to nitrite by the removal of one oxygen molecule. Nitrite combines with sulfanilic acid and amino-2-naphthaline to form a red colored end product. Certain organisms are able to further reduce nitrite to nitrogen, yielding a colorless result. An additional test for the presence of unreacted nitrate must be performed to validate the colorless result. Zinc dust catalyzes the reaction of nitrate to nitrite. Therefore, when zinc dust is added, a red color indicates the presence of unreacted nitrate.

REAGENTS
Reactive Ingredients: Potassium Nitrate
Sodium Molybdate

PRECAUTIONS
This product is For *In Vitro* Diagnostic Use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and media after use. Directions should be read and followed carefully. Established laboratory procedures for processing anaerobes should be followed. Virginia Polytechnic Institute (VPI) recommends that nichrome loops not be used in processing anaerobes.[5]

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 come to room temperature before use. Do not incubate prior to use.

PRODUCT DETERIORATION
This product should not be used if (1) the color has changed from white, (2) the expiration date has passed, (3) the desiccant has changed from blue to pink, or (4) there are other signs of deterioration. Protect disks from moisture by removing from the vial only those disks necessary for testing. Promptly replace the cap and return the vial to 2-8°C.

SPECIMEN COLLECTION, STORAGE, TRANSPORT
Specimens should be collected and handled following recommended guidelines.[6]

MATERIALS REQUIRED BUT NOT SUPPLIED
(1) Loop sterilization device, (2) Inoculating loop, swabs, collection containers, (3) Incubators, alternative environmental systems, (4) Supplemental media, (5) Quality control organisms, (6) Forceps, (7) Anaerobic Nitrate Reagent A (REF 21201), (8) Anaerobic Nitrate Reagent B (REF 21211), (9) Zinc dust, (10) Clean petri dish.

PROCEDURE
1. Use established laboratory procedures for processing anaerobic organisms.
2. Allow disk(s) to equilibrate to room temperature.
3. Use a nonselective anaerobic blood agar plate [e.g., Anaerobic Reducible Blood Agar (REF 01060) or Brucella Blood Agar w/ Hemin & Vitamin K (REF 01254)].
4. Test suspected organisms from a primary anaerobic culture or use a pure subculture from an anaerobic isolate.
5. Select one well-isolated colony and streak the first quadrant of the plate back and forth several times to ensure an even lawn of growth. Streak the other quadrants for isolation.
6. Place the Nitrate Disk on the heavily inoculated part of the plate using forceps. Subsequent disks for presumptive identification of anaerobes may be placed on the plate. (Consult appropriate laboratory procedures or texts.)
7. Aerotolerance testing of the same colony used for the disk test should be performed at this time if not already established.
8. Incubate the plate anaerobically at 35-37°C for 24-48 hours (until good growth of the organism is evident).
9. Remove disk from surface of plate and place in a clean petri dish before adding reagents.
10. Add one drop each of Anaerobic Nitrate Reagents A and B to the disk. If no color develops within 3-5 minutes, add a small amount of zinc dust and wait for 5 minutes. Observe for color change.

INTERPRETATION
Positive Test - Red color development after addition of Anaerobic Nitrate A and B; no color change after the addition of zinc dust
Negative Test - No color change after the addition of Anaerobic Nitrate A and B; red color development after addition of zinc dust

EXPECTED VALUES[2]

Organism	Nitrate Reduction
Actinomyces israelii	+⁻
Actinomyces meyeri	–
Actinomyces naeslundii	+
Actinomyces odontolyticus	+
Actinomyces viscosus	+⁻
Bacteroides fragilis group	–
Bacteroides ureolyticus-like group	+
Eubacterium lentum	+
Fusobacterium species	–
Propionibacterium acnes	+⁻
Propionibacterium avidum	–
Propionibacterium granulosum	–
Veillonella	+
Other gram-negative cocci	V

+ = positive, – = negative, V = variable, superscripts = reactions of occasional strains. (The Nitrate Test is useful in the rapid identification of *V. parvula, E. lentum,* and *B. ureolyticus*-like group.)

QUALITY CONTROL

All lot numbers of Nitrate Disk 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
Veillonella parvula ATCC® 10790	Anaerobic, 48 h @ 35-37°C	Positive
Bacteroides fragilis ATCC® 25285	Anaerobic, 48 h @ 35-37°C	Negative

PERFORMANCE CHARACTERISTICS

An evaluation of 62 strains of anaerobic bacteria showed 100% agreement with expected values.[7]

LIMITATIONS

1. A rapidly growing organism may turn the disk a tan color during incubation as a result of hemolysis and/or metabolism. When the test reagents are then added, occasionally, only a very subtle color change will be discernable, or no color change will occur at all. When this occurs, a tube test or other means of nitrate reduction is suggested.
2. The quantity of nitrate reductase formed is directly related to the rate of growth of the test organism.[8] Only fresh, pure cultures should be used. If after 48 hours incubation, little or no growth is observed, the plates should be reincubated before the test reagents are added. False negative reactions may occur with lighter, nonconfluent growth.
3. Nitrate reductase is diffusible. Inaccurate results may occur when testing mixed cultures or multiple isolates per plate.

BIBLIOGRAPHY

1. Wideman, P.A., D.M. Citron, and V.L. Sutter. 1977. J. Clin. Microbiol. 5:315-319.
2. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey and Scott's Diagnostic Microbiology. 11th ed. Mosby, St. Louis, MO.
3. Summanen, P., E.J. Baron, D.M. Citron, C.A. Strong, H.M. Wexler, and S.M. Finegold. 1993. Wadsworth Anaerobic Bacteriology Manual. 5th ed. Star Publishing Co., Belmont, CA.
4. Enoch, H.G. and R.L. Lester. 1972. J. Bacteriol. 110:1032-1040.
5. Holdeman, L.V., E. P. Cato, and W.E.C. Moore. 1977. Anaerobe Laboratory Manual. 4th ed. Virginia Polytechnic Institute and State Univ., Blacksburg, VA.
6. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken. 2003. Manual of Clinical Microbiology. 8th ed. ASM, Washington, D.C.
7. Data on file. 1991. Remel Inc., Lenexa, KS.
8. Payne, W.J. 1973. Bacteriol. Rev. 37:409-452.

PACKAGING

REF 21093, Nitrate Disk (Anaerobic) .....25 Disks/Vial

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)

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12076 Santa Fe Drive, Lenexa, KS 66215, USA
General Information: (800) 255-6730 Technical Services: (800) 447-3641 Order Entry: (800) 447-3635
Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128
Website: www.remel.com Email: remel@remel.com