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HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

ANAEROBE NITRATE REAGENT

Cat. no. Z134	Anaerobe Nitrate Reagent A	15ml
Cat. no. Z135	Anaerobe Nitrate Reagent B	15ml
Cat. no. Z73	Nitrate Reagent C	5.0gm

INTENDED USE

Hardy Diagnostics Anaerobe Nitrate Reagents A, B, and C are recommended for use in determining the nitrate reduction reaction of anaerobic bacteria.

SUMMARY

The nitrate reduction test is a qualitative procedure for determining the ability of bacteria to reduce nitrate. In the reaction, nitrate is reduced to nitrite, which may then be further reduced to nitrogen gas or ammonia. Determination of nitrate reduction to nitrite is a two step process. First, the reduction of nitrate to nitrite is determined by the addition of Anaerobe Nitrate Reagents A and B, then if necessary, the reduction of nitrate beyond nitrite is determined by the addition of Nitrate Reagent C (zinc dust). Hardy Diagnostics Anaerobe Nitrate Reagents are more sensitive than regular nitrate reagents when used with microaerophilic, facultatively anaerobic, and obligately anaerobic isolates.

REAGENT FORMULA

Anaerobe Nitrate Reagent A:	
Sulfanilic Acid	5.0gm
Acetic Acid, 5N	1000.0ml
Anaerobe Nitrate Reagent B:	
5-Amino-2-naphthalenesulfonic Acid (1,6-Cleve's Acid)	3.0gm
Acetic Acid	1000.0ml
Nitrate Reagent C:	
Zinc Dust	5.0gm

STORAGE AND SHELF LIFE

Storage: Upon receipt store Anaerobe Nitrate Reagent A and Anaerobe Nitrate Reagent B at 2-30ºC. away from direct light. Nitrate Reagent C can be stored at 15-30ºC. away from direct light. Products should not be used if there are any signs of deterioration, contamination, or if the expiration date has passed.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: This product is not intended for primary isolation of patient specimens. This product is used in conjunction with other biochemical tests to identify cultures of isolated organism.

Method of Use:

1. Inoculate and incubate Indole Nitrate Medium (Cat. no. K147) according to the technical insert.

2. After incubation, add 5 drops of Anaerobe Nitrate Reagent A and 3 drops of Anaerobe Nitrate Reagent B to the medium. Shake gently to mix the reagents.

3. Examine for the appearance of a deep red color within 1-2 minutes. Positive reactions may fade rapidly (as early as five minutes after addition of reagents A and B).

4. If the result is negative, i.e. no color development, confirm the negative finding by sprinklilng a small amount (approximately 6mg) of Nitrate Reagent C (zinc dust) to the tube containing the previously added Nitrate Reagents A and B.

5. Examine for the appearance of a red to pink color within 5-10 minutes.

If using nitrate reagents for commercial identification strips, such as API®, consult the manufacturer's literature.

INTERPRETATION OF RESULTS

A positive nitrite reduction is denoted by the appearance of a deep red color change after the addition of Anaerobe Nitrate Reagents A and B. Lack of color development denotes a **presumptive** negative nitrite reduction test. Development of a pink or red color following the addition of Nitrate Reagent C (zinc dust), confirms the negative nitrite reduction test obtained in the first phase of the test. Lack of color development after the addition of zinc dust indicates that the nitrate was reduced beyond the nitrite reaction to nitrogen gas and constitutes a positive nitrate reduction reaction. Therefore a red color development after the zinc addition constitutes a negative result for nitrate

reduction.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

The nitrate reduction test may be used as an aid in the identification of bacteria.

Due to the possible presence of nitrite in culture media only a low nitrite media such as Indole Nitrate Medium (Cat. no. K147) should be used for the nitrate reduction test.

Interpretation of nitrate reduction color reactions should be made immediately, as color reactions with a positive test may fade rapidly (as early as five minutes after addition of reagents A and B).

To avoid false-negative nitrite reduction reactions, reactions must be verified by the addition of Reagent C to the medium.

A negative zinc reduction (no color change) test, in combination with a negative nitrite reaction, is presumptive indication that the nitrate was reduced beyond the nitrite stage. Although a common end product of nitrite reduction is nitrogen gas, other end products may be formed. Additional testing may be required to determine the final end products of the reaction.

Excess zinc dust has been reported to cause false-positive nitrite reduction reactions due to complete reduction of previously unreduced nitrate to ammonia.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, Indole Nitrate Medium (Cat. no. K147), incinerators, incubators, pasteur pipets, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Reaction
Cutibacterium acnes ATCC® 29399*	Positive nitrate reduction; deep red color seen after Reagents A and B are added
Paeniclostridium sordellii ATCC® 9714*	Negative nitrate reduction; no color change seen after Reagents A and B are added, and red color forms after addition of Reagent C

* Refer to the document "Inoculation Procedures for Media QC" for more information.

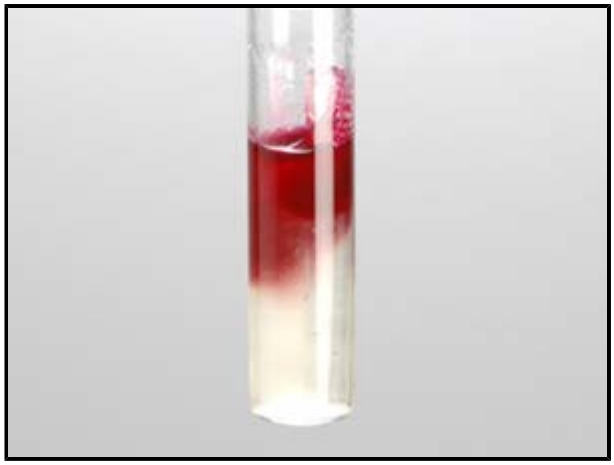
USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis

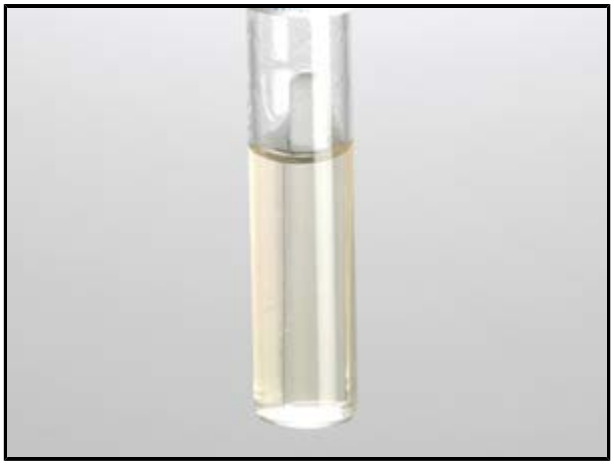
(CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

- Nitrate Reagent A should appear clear and colorless.
- Nitrate Reagent B should appear clear and pink.
- Nitrate Reagent C should appear as a free-flowing gray powder.



Showing positive nitrate reduction.
Cutibacterium acnes (ATCC® 29399) grown in Indole Nitrate Medium (Cat. no. K147) aerobically for 24 hours at 35ºC. Picture reflects appearance after addition of Reagent A (Cat. no. Z134) and Reagent B (Cat. no. Z135).



Showing negative nitrate reduction.
Paeniclostridium sordellii (ATCC® 9714) grown in Indole Nitrate Medium (Cat. no. K147) aerobically for 24 hours at 35ºC. Picture reflects appearance after addition of Reagent A (Cat. no. Z134) and Reagent B (Cat. no. Z135).

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

6. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*,, Lipincott Williams & Wilkins, Philadelphia, PA.

7. Summanen, P., et al. 2002. *Wadsworth Anaerobic Bacteriology Manual*, 6th ed. Veterans Administration Wadsworth Medical Center, and Departments of Medicine, and Microbiology and Immunology, UCLA School of Medicine, Los Angeles, CA.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]