IFU

Instructions for Use

NITRATE REAGENT

Cat. no. Z71	Nitrate Reagent A	15ml
Cat. no. Z72	Nitrate Reagent B	15ml
Cat. no. Z73	Nitrate Reagent C	5.0gm

INTENDED USE

Hardy Diagnostics Nitrate Reagents A, B, and C are recommended for use in determining the nitrate reduction reaction of bacteria, other than *Mycobacterium* species.

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 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).

REAGENT FORMULA

Nitrate Reagent A:	
Sulfanilic Acid	8.0gm
Acetic Acid, 5N	1000.0ml
Nitrate Reagent B:	
N,N-dimethylnaphthylamine	6ml
Acetic Acid	1000.0ml
Nitrate Reagent C:	
Zinc Dust	5.0gm

STORAGE AND SHELF LIFE

Storage: Upon receipt store Nitrate Reagent A and 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: Heavily inoculate Nitrate Broth (Cat. no. K42) or Nitrate Agar with the test organism. When using Nitrate Agar, inoculate both the slant and butt of medium. Incubate 24-48 hours at 35ºC. To determine nitrite production, add 5 drops of Nitrate Reagent A and 5 drops Nitrate Reagent B to the medium. Shake gently to mix the reagents. Examine for the appearance of a deep red color within 1-2 minutes. If the result is negative, i.e. no color development, confirm the negative finding by adding Nitrate Reagent C (approximately 6.0mg) to the tube containing the previously added Nitrate Reagents A and B. Examine for the appearance of a deep red 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 Nitrate Reagents A and B. Lack of color development denotes a **presumptive** negative nitrite reduction test. Development of a 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.

LIMITATIONS

The nitrate reduction test may be used as an aid in the identification of bacteria. Additional biochemical testing using pure culture is recommended for complete identification.

Nitrate Broth and Nitrate Reagents A and B are not recommended for use in determining nitrate utilization by *Mycobacterium* spp. (see Nitrate Substrate Broth , Cat. no. R97).

Due to the possible presence of nitrite in the culture media, a low nitrite media such as Nitrate Agar or Nitrate Broth

should be used for the nitrate reduction test.

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 very 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.

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

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, 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**
Escherichia coli ATCC® 25922	Positive nitrate reduction; deep red color seen after Reagents A and B are added
Acinetobacter baumannii ATCC® 19606	Negative nitrate reduction; no color change seen after Reagents A and B are added, and red color forms after addition of Reagent C

USER QUALITY CONTROL

It is recommended that each new lot or shipment of reagent be tested with known positive and negative controls.[1]

PHYSICAL APPEARANCE

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

Escherichia coli (ATCC® 25922) grown in Nitrate Broth with Durham Tube (Cat. no. K42). Incubated aerobically for 24 hours at 35ºC. Five drops of Nitrate Reagent A (Cat. no. Z71) and five drops of Nitrate Reagent B (Cat. no. Z72) were added subsequent to incubation. The red color development was indicative of a positive reaction: the reduction of nitrate to nitrite.

Acinetobacter baumannii (ATCC® 19606) grown in Nitrate Broth with Durham Tube (Cat. no. K42). Incubated aerobically for 24 hours at 35ºC. Five drops of Nitrate Reagent A (Cat. no. Z71) and five drops of Nitrate Reagent B (Cat. no. Z72) were added subsequent to incubation. No red color development indicated that nitrate was not reduced to nitrite (presumptive negative). To ensure nitrate was not reduced to an end product other than nitrite, all negatives should be confirmed by adding Nitrate Reagent C (Cat. no. Z73). A red color development is indicative of a "true negative" for nitrate reduction.

Showing confirmation reaction for *Acinetobacter baumannii* (ATCC® 19606). Since there was not red color development after the addition of Nitrate Reagents A and B, a small amount of Nitrate Reagent C, zinc dust (Cat. no. Z73) was added using the end of a sterile wooden stick. The red color development was indicative as a "true negative" for nitrate reduction.

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. Versalovic, J., 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.

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

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