NITRATE DISKS

- For in vitro use only -

Catalogue No. DN45

Our Nitrate Disks are used to detect an anaerobic organism's ability to reduce nitrate. In 1977, the nitrate disk test was devised by Wideman, Citronbaum, and Sutter for the testing of anaerobic bacteria, and showed approximately 90% agreement with tests performed in conventional indole-nitrate mediums. Many bacteria possess the enzymes needed to reduce nitrate, which usually occurs under anaerobic conditions. The reduction of nitrate provides the organisms with oxygen, which serves as the final electron acceptor in the respiration process used to generate energy. Bacterial reduction of nitrate results in the production of various end products that commonly include nitrite, ammonia, nitrogen gas, nitric oxide, nitrous oxide, and hydroxylamine. The final end product observed depends on the bacterial species and the environmental conditions. The nitrate reduction characteristic of a particular species is more or less constant, making it a useful diagnostic tool.

Recommended Procedure

1. Using a sterile swab, streak a sample of the organism onto a non-selective blood agar plate in three directions to obtain a heavy, confluent growth.
2. Aseptically place a Nitrate Disk onto the agar surface.
3. Incubate anaerobically at 35°C for 48 hours.
4. Remove the disk and place in a clean petri dish.
5. Add one drop each of Nitrate “A” Reagent (Dalynn Catalogue No. RN75) and Nitrate “B” Reagent (Dalynn Catalogue No. RN 76).
6. Read results within 2 minutes.
7. If there is no color change, add a small amount (20-mg) of zinc dust. Wait five to ten minutes and read results.

Interpretation of Results

In the Nitrate Disk test, nitrate reduction is detected by either the presence of a catabolic end product, or the absence of nitrate in the medium. Reduction of nitrate to nitrite can be determined by the addition of Reagent A and Reagent B. Reagent A is a 0.8% sulfanilic acid solution, which reacts with nitrite to form a diazonium salt. The resultant diazonium salt reacts with Reagent B, a 0.6% N,N-dimethyl-α-naphthylamine solution, to form a red, water soluble azo dye. Therefore, if a red colour develops on addition of the reagents, then nitrate has been reduced to nitrite and this is a positive nitrate reduction test. If no colour change is observed then nitrite is not present and zinc dust must be added inorder for a final interpretation to be made.

Zinc dust is a reducing agent used to confirm the presence of unreduced nitrate. If unreduced nitrate is still present on the disk, zinc dust will reduce the nitrate to nitrite, which in turn reacts with the previously added reagents A and B to give a red color. Therefore, a red color change is indicative of a negative reaction indicating that nitrate was not reduced. However, if no color change is observed after the addition of zinc dust, then this indicates that nitrate has been reduced beyond nitrite to ammonia or nitrogen gas: a positive test for nitrate reduction.

- *In some instances nitrate may only be partially reduced or an organism may temporarily lose its ability to reduce nitrate*

- *Do not add an excessive amount of zinc dust as the large amount of hydrogen gas produced may reduce the nitrate beyond nitrate to ammonia resulting in erroneous observations*

- *Read reactions at the recommended times as fading of the color reactions do occur over prolonged waiting periods*

Quality Control

Organism	Expected Results	
Bacteroides ureolyticus ATCC 33387	+ve	Pink to red after addition of reagents
Peptostreptococcus asaccharolyticus ATCC 29743	-ve	No colour change after addition of reagents, red color on addition of zinc powder

Storage and Shelf Life

Our Nitrate Disks should be stored at 4°C to 8°C. At this temperature they have a shelf life of 52 weeks from the date of manufacture.

References

1. Wideman PA, Citronbaum DM, and Sutter VL. Simple disk technique for detection of nitrate reduction in anaerobic bacteria. J Clin Micro 1977; 5:315-9.
2. Lennette EH, Balows A, Hausler WJ et al. Manual of clinical microbiology. 4th ed. Washington, DC: ASM, 1985.
2. Baron EJ, Finegold SM. Bailey and Scott's diagnostic microbiology. 8th ed. St Louis: Mosby, 1990.

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