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MAST ID™

NITRATE DISCS

For the detection of nitrate reductase activity in anaerobes

Introduction

MAST ID™ Nitrate Discs provide a rapid qualitative method for the determination of nitrate reduction to nitrite by anaerobic bacteria and are suitable for use in the identification and differentiation of anaerobic bacteria.[1,2,3]

The test detects the ability of bacteria to utilise nitrate as a final electron acceptor in the respiratory chain.[2] During this process, nitrate is reduced to nitrite, which may then be further reduced to nitrogen gas or ammonia.[1,6] This reduction process is detected by the addition of sulfanilic acid and N,N-dimethyl alpha naphthylamine or 1,6-Cleve's Acid which combine with nitrite to form a red compound, ρ-sulfobenzene-azo-alpha-naphthylamine, which indicates a positive test. If no colour change occurs, zinc dust is added to determine if unreduced nitrate or other products such as ammonia, nitrogen, nitric oxide or hydroxylamine are present. If residual nitrate is present, zinc ions will reduce nitrate to nitrite, producing a red colour. Negative zinc and nitrite reactions presumptively indicate that the nitrate was reduced to end products beyond the nitrite stage.[1,4]

Description

MAST ID™ Nitrate Discs are filter paper discs impregnated with a solution containing 40% potassium nitrate and 0.1% sodium molybdate.

Directions

1. Subculture the test organism by spreading over a reduced blood agar plate so that a confluent lawn of growth is obtained.
2. Place a nitrate disc on the inoculated medium.
3. Incubate anaerobically at 35ºC for 24-48 hours.
4. Remove plates from incubation and add one drop each of N,N-dimethyl alpha naphthylamine (or 1,6-Cleve's Acid) and sulfanilic acid to the disc.
5. Examine the disc for the development of a red colour within 3-5 minutes.
6. If the results from step 5 are negative, confirm by the addition of a small amount of zinc dust to the disc.
7. Examine the disc for the development of a pink/red colour within 5-10 minutes.

In Use

The nitrate reduction test may be used as a diagnostic aid in the identification of bacteria. Additional biochemical and serological testing using pure cultures should be performed for full identification.

Rapidly growing organisms may cause the nitrate discs to turn a tan colour as a result of haemolysis and/or metabolism. Addition of test reagents may cause only a subtle colour change or no colour change at all. In the event that this reaction occurs, it is recommended that some other means of nitrate reduction testing is employed.

Organisms that produce only light or non-confluent growth may fail to produce a sufficient quantity of nitrate reductase and may therefore produce false negative results.

Only fresh, pure cultures should be tested. Cultures that produce little or no growth should be re-incubated before the addition of reagents.

Storage

Store at 2-8ºC in the containers provided. Allow to equilibrate to room temperature before opening.

Packing and Ordering Details

MAST ID™ Nitrate Discs are provided as a single vial containing 100 discs (Order Code D51) or as packs of 5 cartridges, each cartridge containing 50 discs (Order Code D51C).

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