Nitrate Discs

REF CK9850

Nitrate Reagent A

REF CK9841

Nitrate Reagent B

REF CK9842

Intended Use
The nitrate test is used to demonstrate the ability of the test organism to reduce nitrate to nitrite and to reduce nitrite to nitrogen.

Background
Nitrates often serve as a source of nitrogen for bacteria. The ability of organisms to reduce nitrates to nitrites and to further reduce nitrite to nitrogen and oxygen is used in various identification schemes.
Most bacteria encountered in the clinical laboratory reduce nitrate and this test is therefore a useful marker for species that do not. These include a number of Gram negative non-fermenters such as *Acinetobacter* spp., *Alcaligenes faecalis* and *Sphingobacterium* spp. The nitrate test is also highly useful for division of mycobacteria where only a minority of species, including *Mycobacterium tuberculosis*, are able to reduce nitrate.

The test involves preparing a heavy suspension of the test organism in the presence of a nitrate source, following incubation and the addition of reagents A and B the development of a pink or reddish colour is a positive reaction for nitrate reduction. If the result is negative, the addition of the zinc reagent will identify if the test organism has reduced the nitrate to nitrite then reduced the nitrite to nitrogen and oxygen.

Precautions
This product is for in-vitro diagnostic use and should be used by properly trained laboratory professionals. Universal precautions should be taken in the handling, processing and discarding of all materials used to perform the test. Do not use reagents after the expiration date shown on the product label has expired.

Methods
Use a fresh 18-24 hour culture as older cultures could be less metabolically active and results from these may be unreliable.
Tube:
Add 3 to 5 drops of sterile distilled water to a reaction tube.
Using a loop, wire or stick take a heavy inoculum (McFarland No. 4) of the test organism and thoroughly mix in the reaction test tube.
Incubate uncovered at 37°C for 2 hours or 18-24 hours, following incubation add 1 drop of Nitrate Reagent A and 1 drop of Nitrate Reagent B. Mix and read the result within 2 minutes. Observe the colour of the

suspension, a pink or reddish colour is a positive result.
Plate:
Place 1 disc onto an area of heavy growth of the test organism
Incubate at 37°C for 2 hours (no longer), following incubation add 1 drop of Nitrate Reagent A and 1 drop of Nitrate Reagent B to the disc. Mix gently with a loop and read the result within 2 minutes. Observe the colour of the disc, a pink or reddish colour is a positive result.

Results
On addition of Nitrate Reagent A & Nitrate Reagent B:
Positive Reaction – pink or reddish colour
Negative Reaction – light pink or colourless.
On addition of zinc reagent to isolates giving a negative reaction:
Positive reaction – no colour change (indicating nitrate was reduced to nitrite and the nitrite further reduced to nitrogen)
Negative reaction – pink or reddish colour (the zinc reagent reduces nitrate to nitrite)

Limitations
This test should be performed on micro-organisms grown on media appropriate to the specimen.

Quality Control
A quality control should be undertaken daily or immediately prior to use.
Positive control-
Escherichia coli ATCC 25922
Negative control-
Staphylococcus saprophyticus ATCC 15305

Shelf Life & Storage
The expiry date, storage temperature and storage conditions are indicated on the outer package labels.

Materials provided
Supplied separately – each pack of discs contains 50 tests. Reagent A and reagent B are supplied individually, each pack containing 5x3ml dropper bottles

Materials required but not provided
Sterile loops or needles
Reaction tube
Sterile distilled water
Zinc reagent (0.5g zinc powder in 5ml of isopropyl alcohol)

References
Barrow, G.I. & Feltham, R.K.A. Cowan and Steel's Manual for the Identification of Medical Bacteria. Third edition.
Ford, M. Medical Microbiology. Oxford University Press.

REF	Catalogue number
LOT	Batch number
	Use by date
IVD	In-Vitro Diagnostic device
	Contains sufficient for <n> tests
	Temperature storage limitations
	Consult instructions for use
	Manufacturer

Issue	Date	Comments
3	07/09/2021	IFU format revision.

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