Nitrite Detection Kit

Intended Use
For differentiating and identifying various types of bacteria by their ability to reduce nitrate.

Summary
Bacterial species may be differentiated on the basis of their ability to reduce nitrate to nitrite or nitrogenous gases. Many bacteria have respiratory enzyme systems that can use nitrate as a terminal electron acceptor forming nitrite. Bacterial reduction of nitrate results in the production of various end products like nitrite, nitrogen gas, ammonia, nitrous oxide and hydroxylamine. The nitrate reduction test is valuable for differentiating and identifying various types of bacteria, especially *Enterobacteriaceae* making it a useful diagnostic tool.

Principle
Reduction of nitrate to nitrite is generally an anaerobic respiration in which an organism derives its oxygen from nitrate. Depending upon environmental conditions, the end products of this metabolic process are usually not further oxidized or assimilated into the cellular metabolism, but are excreted into the surrounding medium. The presence of nitrite in the medium is indicated by the change in colour of the strip to pale pink, deep red or violet colour. No change in colour of the strip only indicates that nitrite is not present in the medium.

There may be two reasons for this observation:
1. The nitrate may not have been reduced to nitrite and hence the strain under test is nitrate-reductase negative.
2. The nitrate may have been reduced to nitrite and this has been further completely reduced to nitric oxide, nitrous oxide or other end products that will not react with the Nitrite Detection Strips to give a colour change and hence the strain is nitrate reductase positive.

Any test medium that gives a negative result for the Nitrite detection strip must be further tested to determine which of the two interpretations is correct. This is carried out by adding a small amount of Zinc dust to all negative tests. The Zinc Dust will catalyze the reduction of nitrate to nitrite chemically. Thus, if the organisms have not reduced the nitrate, i.e. the strains are nitrate-negative; it will be reduced by zinc dust. This can be seen in the colour change from colourless to pink/red/violet in the nitrite detection strip. If no colour change is observed in the nitrite detection strip after the addition of zinc dust, it can be concluded that the organisms have not only reduced nitrate to nitrite, but have further reduced nitrite to nitrogenous gases. These organisms are therefore nitrate positive.

Reagents/contents
The Microxpress® Nitrite Detection Kit is a reagent set for laboratory use only.
The Nitrite Detection Kit comprises of:
1. Nitrite detection strips - 25 Nos.
2. Zinc dust reagent - 2 g.

Storage and stability
1. Store the Nitrite Detection Kit at 2°C-8°C away from light.
2. Stability of the Nitrite Detection Kit is as per the expiry date mentioned on the label.

Procedure
Preparation of Inoculum
1. Isolate the organism to be identified on Nutrient Agar or Brain Heart Infusion Agar.
2. Pick up a single isolated colony and inoculate it in 4-5 mL Brain Heart Infusion Broth.
3. Incubate at 37°C for 6-8 hours until inoculum turbidity is between 0.1- 0.2 at 620 nm. Alternatively, a homogenous suspension made in 2-3 mL sterile saline adjusted to a turbidity of 0.1- 0.2 at 620 nm can also be used as inoculum.

Test procedure

1. Retrieve the required number of strip pouch from the carton.
2. Bring the strip pouch to room temperature (25°C-30°C) prior to testing.
3. Inoculate an aliquot (1 mL) of a suitable medium like Nitrate Broth with the above-prepared inoculum (approx. 100 mL) and incubate for 6-8 hours at 35°C-37°C.
4. Observe for growth.
5. Dip the nitrite detection strip in this inoculated broth.
6. Alternatively, put one drop of the incubated broth on the reaction pad.
7. Observe for colour change. If no colour change is observed, add a pinch of Zinc Dust.

Appearance: R1 Nitrite detection strips - Thin long strip with the reaction pad at one end.
R2 Zinc dust reagent - Free flowing fine greyish coloured powder.

Interpretation of results

Observation of colour change	**Result**	
Directly with Nitrite Detection Strip	**With Nitrite Detection Strip**	**On Addition of Zinc Dust**
Pale pink/deep red/violet	-	Nitrate Positive Strain
No colour change	No colour change	Nitrate Positive Strain
No colour change	Pale pink/deep red/violet	Nitrate Negative Strain

Quality control

Organisms (ATCC)	**Reaction with Nitrite Detection Strip**
Klebsiella aerogenes (13048)	+
Escherichia coli (25922)	+
Listeria monocytogenes (19117)	-

Key:
+ = Pink colour
- = No colour change

Precautions/limitations

1. The Nitrite Detection Kit is an in vitro diagnostic kit for laboratory and professional use only. Not for medicinal use.
2. The Nitrite Detection Kit cannot be used directly on clinical specimens. Only pure cultures should be used to obtain optimum results.
3. At times, the organism may give contradictory results because of mutation or media used for isolation, cultivation and maintenance. Results are prominent when fresh and enriched culture is used. The test should be performed within 30 minutes of removing the cultures from the incubator. Prolonged exposure of the culture at room temperature may result in diminished enzyme activity.
4. Ensure that only a pinch of Zinc Dust is added. Excess zinc dust if added, may result in rapid reduction of nitrate beyond nitrite to nitrogenous gases and thus nitrite may not be detected.
5. Clinical samples and microbial cultures should be considered as pathogenic biohazard and handled accordingly. Good laboratory practices and hazard precautions must be observed at all times.
6. The test is an aid to identification and is not a confirmatory test. Complete identification should include determination of gram reaction, morphology, and other biochemical and serological tests.
7. Do not use damaged or leaking kits. Avoid contact of reagents with skin and eyes.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

References

1. Practical Medical Microbiology, Mackie & McCartney, 13th edition 1989, Edited by J. G. Collee, J. P. Duguid.
2. Diagnostic Microbiology, Bailey & Scott, 9th Edition, Mosby1994.
3. Clarke P.H. And S.T. Cowan, Biochemical Methods for Bacteriology, J. Gen. Microbiol., 1952, Vol. 6: 187-197.
4. An Improved Reagent For Mycobacterial Nitrate Reductase Tests, Nancy G. Warren, et.al. Journal Of Clinical Microbiology, Sept. 1983, Vol. 18, No. 3, P: 546-549.
5. Nonliquid Reagent For Detecting Nitrate Reduction, Anno S. Lampe, Journal Of Clinical Microbiology, Oct. 1981, Vol. 14, No. 4, P: 452-454.
6. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product	Pack Size
204140200001	Nitrite Detection Kit	1 Kit (25 Tests)

Disclaimer
Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.