



## $\alpha$ -Naphthylamine Solution

R009

### Intended Use

$\alpha$ -Naphthylamine Solution is used for determination of nitrate reduction by bacterial strains along with Sulphanilic acid (R015)

### \*Composition\*

#### Ingredients

$\alpha$ -Naphthylamine	5.0gm
5N Acetic acid	1000.0ml

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Inoculate growth from an 18 - 24 hours pure culture into Nitrate HiVeg Broth, (MV439). Incubate at 35°C for 12 to 24 hours. Very rarely prolonged incubation upto 5 days may be required. Add 0.5 ml alpha -naphthylamine along with 0.5 ml sulphanilic acid (R015).

### Principle And Interpretation

The  $\alpha$ -Naphthylamine solution and Sulphanilic acid is used to determine nitrate reduction by members of Enterobacteriaceae. The reduction of nitrates ( $\text{NO}_3$ ) leads to the formation of nitrites ( $\text{NO}_2$ ) and may progress to the liberation of nitrogen gas. The nitrate reductase producing organisms reduce nitrate to nitrite which reacts with sulphanilic acid to form a diazonium salt. This salt reacts with  $\alpha$ -naphthylamine to form a red coloured, water soluble azo dye which results in the visualization of pink-red colour. A distinct red colour formation within 1-2 minutes indicates reduction of nitrate to nitrite.

### Type of specimen

1. The specimen is any isolated colony on primary or subculture plates.

### Specimen Collection and Handling

1. For clinical samples follow appropriate techniques for handling specimens as per established guidelines (1,2).
  2. For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,5).
  3. For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(4)
- After use, contaminated materials must be sterilized by autoclaving before discarding.

### Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

### Limitations

1. 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.
2. Nitrate Broth and Nitrate Reagents A and B are not recommended for use in determining nitrate utilization by *Mycobacterium* spp.
3. 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.
4. 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.

