

## Indole Nitrate Broth

LQ081

For identification of microorganisms by means of nitrate reduction and indole production.

### Composition\*\*

Ingredients	Gms / Litre
Tryptone	20.000
Disodium phosphate	2.000
Dextrose	1.000
Potassium nitrate	1.000
Agar	1.000
Final pH ( at 25°C)	7.2±0.2

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

### Directions

Label the ready to use LQ081 bottle. Inoculate the sample and Incubate at specified temperature and time.

### Principle And Interpretation

Indole Nitrate Medium (Tryptone Nitrate Medium), due to the nutritive content, supports growth of aerobes, microaerophiles, and facultative as well as obligate anaerobes. It serves a dual purpose of detecting indole production and nitrate reduction in a wide range of microorganisms. Casein enzymic hydrolysate contains tryptophan, which is acted upon by certain microorganisms, resulting in the production of indole. Potassium nitrate acts as the substrate for determining nitrate reduction by microorganisms. Duplicate tubes of Indole Nitrate Medium may be inoculated and tested for the presence of nitrates or indole after incubation for various lengths of time. Nitrate test is performed by addition of 0.5 ml each of Sulphanilic Acid (R015) and alpha -Naphthylamine (R009). The development of pink colour indicates nitrate reduction. The colour develops due to presence of nitrite generated from reduction of nitrate. When nitrate is further reduced to ammonia, no colour develops. Add a pinch of zinc dust to the tube. The formation of pink colour after addition of zinc dust indicates that nitrate is not reduced. Indole production can be tested by the addition of Kovacs Reagent (R008) or Ehrlich reagent (R005) (1,2). The formation of a deep red colour in the reagent layer after gentle agitation indicates positive indole test. Indole Nitrate Medium is not recommended for indole test in coliform and other enteric bacteria, as they reduce nitrate to nitrite, which prevents the detection of indole (3). Indole Nitrite Medium should not be used for detecting indole production by members of the *Enterobacteriaceae*. The tubed medium should be boiled for 2 minutes and cooled, without agitation, before use.

### Quality Control

#### Appearance

Sterile, slightly opalescent, Indole Nitrate broth in bottles.

#### Colour

Light amber coloured medium.

#### Quantity of Medium

5 ml of medium in bottles.

#### Reaction

7.00- 7.40

#### Cultural response

Cultural characteristics was observed after incubation at 35-37°C for 18-48 hours.

#### Sterility Check

Passes release criteria.

#### Cultural Response

Organism	Growth	Indole	Nitrate reduction	Growth under anaerobic conditions
<b>Cultural response</b>				
<i>Escherichia coli</i> ATCC 25922	Luxuriant	Positive	Positive	
<i>Staphylococcus aureus</i> ATCC 25923	Luxuriant	Negative	Positive	
<i>Clostridium perfringens</i> ATCC 12924		Negative	Positive	Luxuriant
<i>Clostridium sporogenes</i> ATCC 11437		Negative	Negative	Luxuriant
<i>Clostridium sordellii</i> ATCC 9714		Positive	Negative	Luxuriant

## Storage and Shelf Life

Store between 15-25°C. Use before expiry date on the label.

## Reference

1. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore. 2. Murray P. R., Baron J. H., Pfaller M. A., Tenover J. C. and Tenover F. C., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C. 3. Smith R. F., Rogers R. R., and Bettge C. L., 1972, Appl. Microbiol., 23:423.

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