Indole Nitrite Medium (Trypticase™ Nitrate Broth)

Intended Use

Indole Nitrite Medium is used for the identification of microorganisms by means of the nitrate reduction and indole tests.

Summary and Explanation

Indole Nitrite Medium was developed to serve the dual role of detecting indole production and nitrate reduction of a wide range of microorganisms. Due to its nutritive content, the medium will support the growth of aerobes, microaerophiles and facultative and obligate anaerobes.

Indole Nitrite Medium can be used for nitrite tests with members of the *Enterobacteriaceae* but is not recommended for the indole test with these organisms since they reduce nitrate to nitrite, which prevents the detection of indole.[1] Tryptophan (Trypticase™) 1% Solution is the medium of choice for indole test with enteric bacilli.

User Quality Control

Identity Specifications

BBL™ Indole Nitrite Medium (Trypticase™ Nitrate Broth)

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	2.5% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, trace hazy to hazy.
Prepared Appearance:	Light to medium, yellow to tan, trace hazy to hazy.
Reaction of 2.5% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

BBL™ Indole Nitrite Medium (Trypticase™ Nitrate Broth)

Prepare the medium per label directions. Inoculate with fresh broth cultures diluted 1:10 and incubate at 35 ± 2°C for 2 days under appropriate atmospheric conditions.

ORGANISM	ATCC™	RECOVERY	NITRATE	INDOLE
Clostridium perfringens	13124	Good	+	–
Clostridium bifermentans	17836	Good	–	+
Escherichia coli	25922	Good	+	+

Principles of the Procedure

The casein peptone contains tryptophan, which is attacked by certain microorganisms, resulting in the production of indole, detectable by the addition of chemical reagents to 18- to 48-hour cultures. Potassium nitrate serves as the substrate for determining the ability of microorganisms to reduce nitrates to nitrites.

Formula

BBL™ Indole Nitrite Medium (Trypticase™ Nitrate Broth)

Approximate Formula* Per Liter

Pancreatic Digest of Casein	20.0	g
Disodium Phosphate	2.0	g
Dextrose	1.0	g
Agar	1.0	g
Potassium Nitrate	1.0	g

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

1. Suspend 25 g of the powder in 1 L of purified water. Add 2 g of agar for use as a motility medium. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense in regular test tubes, filing them half full. Autoclave at 121°C for 15 minutes.
4. If the medium is more than 2 days old at the time of use, boil and cool prior to use.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate the tubes with pure cultures of the organisms being evaluated. Replicate tubes should be inoculated if it is desired to test for the presence of indole or nitrites after incubation User for various lengths of time. Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere. The caps of tubes inoculated with obligate anaerobes should be tightened during incubation.

1. Indole Test
   The test for indole may be performed as soon as heavy growth has taken place, usually after 18 to 48 hours of incubation. The test may be performed by any suitable method, such as with Kovacs' reagent (add 0.5 mL, Cat. No. 261185) or Ehrlich's reagent employing *p*-dimethylaminobenzaldehyde.[2] Testing for indole may be made after 24 hours of incubation; if negative, the test should be repeated on another culture incubated for 48 hours.
2. Nitrite Test
   The test for nitrites may be performed at several intervals during the incubation process if replicate tubes were inoculated. The presence of nitrites may be detected by any of several methods.[2] Addition of approximately 5 drops each of sulfanilic acid (Cat. No. 261197) and N, N-dimethyl-1-naphthylamine (Cat. No. 261198) reagents permits the detection of nitrites. If prior tests are negative, a final test should be conducted at 48 hours of incubation.

Expected Results

1. Indole Test
   The production of a pink to red color following addition of the reagent is a positive test for indole formation.
2. Nitrite Test
   A pink to red color develops, after addition of the reagents, if nitrite is present, and indicates that nitrate reduction has occurred. Since some organisms further reduce nitrite to ammonia, add a small amount of zinc dust (Cat. No. 261207) to tubes exhibiting no color. A pink color in this part of the test indicates no nitrate reduction. A colorless reaction indicates that nitrates have been completely reduced.

Consult appropriate references for an explanation of the reactions involved and expected results with specific microorganisms.[3,4]

Limitations of the Procedure

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.

References

1. Smith, Rogers and Bettge. 1972. Appl. Microbiol. *43*:423.
2. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
3. Forbes, Sahm and Weissfeld. 2007. Bailey & Scott's diagnostic microbiology, 12th ed. Mosby, Inc., St. Louis, Mo.
4. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Indole Nitrite Medium (Trypticase™ Nitrate Broth)

BAM

Cat. No.	211299	Dehydrated – 500 g
	221655	Prepared Tubes – Pkg. of 10*

Difco™/BBL™ Indole Reagent

Cat. No.	261185	Droppers, 0.5 mL – Ctn. of 50

Difco™/BBL™ Nitrate A Reagent

Cat. No.	261197	Droppers, 0.5 mL – Ctn. of 50

Difco™/BBL™ Nitrate B Reagent

Cat. No.	261198	Droppers, 0.5 mL – Ctn. of 50

Difco™/BBL™ Nitrate C Reagent

Cat. No.	261207	Droppers, 1 g – Ctn. of 50

**Store at 2-8°C.*