

User Quality Control

Identity Specifications

Difco™ ISP Medium 1

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	0.8% solution, soluble in purified water upon boiling. Solution is light amber, clear to very slightly opalescent.
Prepared Appearance:	Light amber, clear to very slightly opalescent.

Reaction of 0.8%
Solution at 25°C: pH 7.0 ± 0.2

Difco™ ISP Medium 2

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in purified water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Appearance:	Light to medium amber, slightly opalescent.

Reaction of 3.8%
Solution at 25°C: pH 7.2 ± 0.2

Difco™ ISP Medium 4

Dehydrated Appearance:	White to light beige, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in purified water upon boiling. Solution is white to off-white, opaque with precipitate.
Prepared Appearance:	White to off-white, opaque, may have a precipitate.

Reaction of 3.7%
Solution at 25°C: pH 7.2 ± 0.2

Cultural Response

Difco™ ISP Medium 1, ISP Medium 2 or ISP Medium 4

Prepare the medium per label directions. Inoculate tubes of prepared ISP Medium 1 with the test organisms and incubate at 30 ± 2°C for up to 96 hours. Inoculate prepared ISP Medium 2 and ISP Medium 4 with the test organisms by placing approximately 0.1 mL of inoculum near the edge of the plate. Five parallel streaks across the plate are made from this 0.1 mL of inoculum, followed by four perpendicular streaks. Incubate inoculated plates at 30 ± 2°C for 48-96 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Streptomyces albus</i>	3004	10 ² -10 ³	Good
<i>Streptomyces lavendulae</i>	8664	10 ² -10 ³	Good

Directions for Preparation from Dehydrated Product

- Suspend the powder in 1 L of purified water:
 - Difco™ ISP Medium 1 – 8 g;
 - Difco™ ISP Medium 2 – 38 g;
 - Difco™ ISP Medium 4 – 37 g.
 Mix thoroughly.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Autoclave at 121°C for 15 minutes.
- Mix thoroughly while dispensing ISP Medium 4.
- Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For details on the use of these media for characterization of *Streptomyces* species, consult the reference.¹ For a complete discussion on the isolation and maintenance of *Streptomyces* species refer to appropriate references.^{2,3}

Expected Results

Refer to appropriate references and procedures for results.

References

- Shirling and Gottlieb. 1966. *Int. J. Syst. Bacteriol.* 16:313.
- Isenberg (ed.). 1992. *Clinical microbiology procedures handbook*, vol. 1. American Society for Microbiology, Washington, D.C.
- Murray, Baron, Pfaller, Tenover and Tenover (ed.). 1999. *Manual of clinical microbiology*, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ ISP Medium 1

Cat. No. 276910 Dehydrated – 500 g

Difco™ ISP Medium 2

Cat. No. 277010 Dehydrated – 500 g

Difco™ ISP Medium 4

Cat. No. 277210 Dehydrated – 500 g

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.

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, filling 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

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 Betge. 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. 1998. *Bailey & Scott's diagnostic microbiology*, 10th ed. Mosby, Inc., St. Louis, Mo.
4. Murray, Baron, Pfaller, Tenover and Tenover (ed.). 1999. *Manual of clinical microbiology*, 7th ed. American Society for Microbiology, Washington, D.C.

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
<i>Clostridium perfringens</i>	13124	Good	+	-
<i>Clostridium bifermentans</i>	17836	Good	-	+
<i>Escherichia coli</i>	25922	Good	+	+

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, 0.5 mL – Ctn. of 50

*Store at 2-8°C.

Infusion Broth

Intended Use

Infusion Broth is a general purpose medium that supports the growth of a wide variety of microorganisms.

Summary and Explanation

Infusion Broth is one of many liquid media that support the growth of fastidious as well as nonfastidious microbial species. It may be used in the conventional manner for a variety of purposes. Blood or serum may be added aseptically after the medium is sterilized.

With the addition of 65 g/L of sodium chloride, the medium can be used to differentiate enterococci from other streptococci. The enterococci grow in the presence of the high salt content, while pyogenic, viridans and lactic streptococci are unable to grow.

Principles of the Procedure

Infusion Broth consists of heart muscle infusion, casein peptone and yeast extract that are sources of organic nitrogen, carbon, sulfur, vitamins and trace substances. Sodium chloride provides osmotic equilibrium.

User Quality Control

Identify Specifications

BBL™ Infusion Broth

Dehydrated Appearance:	Fine, homogenous, free of extraneous material.
Solution:	2.5% solution, soluble in purified water. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 2.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

BBL™ Infusion Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 7 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
<i>Bacteroides fragilis</i>	25285	≤ 10 ³	Growth
<i>Enterococcus faecalis</i>	29212	≤ 10 ³	Growth
<i>Micrococcus luteus</i>	9341	≤ 10 ³	Growth
<i>Neisseria meningitidis</i>	13090	≤ 10 ³	Growth
<i>Streptococcus pneumoniae</i>	6305	≤ 10 ³	Growth
<i>Streptococcus pyogenes</i>	19615	≤ 10 ³	Growth

Formula

BBL™ Infusion Broth

Approximate Formula* Per Liter

Heart Muscle, Infusion from (solids)	2.0	g
Pancreatic Digest of Casein	13.0	g
Yeast Extract	5.0	g
Sodium Chloride	5.0	g

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

Directions for Preparation from Dehydrated Product

1. Dissolve 25 g of the powder in 1 L of purified water.
2. Autoclave at 121°C for 15 minutes.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Inoculate and incubate the medium as appropriate for the specimen or the known or unknown organism being cultured.^{1,2}

Expected Results

Growth in the tubes is indicated by the presence of turbidity compared to an uninoculated control.

If growth appears, cultures should be examined by a Gram stain and subcultured onto appropriate media; e.g., a BBL™ Trypticase™ Soy Agar with 5% Sheep Blood and/or Chocolate II Agar, LEMB Agar or MacConkey II Agar plates. If anaerobes are suspected, subcultures should be incubated anaerobically, as in BBL™ GasPak™ or BBL™ GasPak™ EZ anaerobic systems, or an alternative anaerobic system.

References

1. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
2. Murray, Baron, Pfaller, Tenover and Tenover (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Infusion Broth

Cat. No. 211305 Dehydrated – 25 lb (11.3 kg)

