

Principles of the Procedure

SFP Agar Base contains peptones as sources of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins, which stimulate bacterial growth. Ferric ammonium citrate and sodium sulfite are H₂S indicators. Clostridia reduce sulfite to sulfide, which reacts with iron to form a black iron sulfide precipitate. Antimicrobial Vial P contains polymyxin B and Antimicrobial Vial K contains kanamycin; both are inhibitors to organisms other than *Clostridium* spp. Egg Yolk Enrichment 50% provides egg yolk lecithin, which some clostridia hydrolyze. Agar is the solidifying agent.

Formulae

Difco™ SFP Agar Base

Approximate Formula* Per Liter	
Yeast Extract	5.0 g
Proteose Peptone No. 3	7.5 g
Pancreatic Digest of Casein	7.5 g
Soytone	5.0 g
Ferric Ammonium Citrate	1.0 g
Sodium Bisulfite	1.0 g
Agar	20.0 g

Difco™ Egg Yolk Enrichment 50%

Sterile concentrated egg yolk emulsion.

Difco™ Antimicrobial Vial K

25 mg Kanamycin per 10 mL vial.

Difco™ Antimicrobial Vial P

30,000 units Polymyxin B per 10 mL vial.

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

Directions for Preparation from Dehydrated Product

Difco™ SFP Agar Base

Base Layer:

- Suspend 47 g of the powder in 900 mL of purified water. Mix thoroughly.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Autoclave at 121°C for 15 minutes. Cool to 50°C.
- Add 100 mL Egg Yolk Enrichment 50%, 10 mL of rehydrated Antimicrobial Vial P (30,000 units polymyxin B sulfate) and 4.8 mL rehydrated Antimicrobial Vial K (12 mg kanamycin). Mix thoroughly.

Cover Layer:

- Suspend 47 g of the powder in 1 L of purified water.
- Prepare as above, except omit Egg Yolk Enrichment 50%.
- Test samples of the finished product for performance using stable, typical control cultures.

Difco™ Antimicrobial Vial K (Kanamycin)

- To rehydrate, aseptically add 10 mL sterile purified water per vial.
- Rotate in an end-over-end motion to dissolve the contents completely.

Difco™ Antimicrobial Vial P (Polymyxin B)

- To rehydrate, aseptically add 10 mL of sterile purified water per vial.
- Rotate in an end-over-end motion to dissolve the contents completely.

Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

References

- Shahidi and Ferguson. 1971. *Appl. Microbiol.* 21:500.
- Labbe. 2001. *In* Downes and Ito (ed.). *Compendium of methods for the microbiological examination of foods*, 4th ed. American Public Health Association, Washington, D.C.
- Rhodehamel and Harmon. 1995. *FDA bacteriological analytical manual*, 8th ed. AOAC International, Gaithersburg, Md.
- Andrews. 2000. *In* Horwitz (ed.), *Official methods of analysis of AOAC International*, 17th ed. AOAC International, Gaithersburg, Md.
- MacFaddin. 1985. *Media for isolation-cultivation-identification-maintenance of medical bacteria*, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ SFP Agar Base

AOAC BAM COMPF ISO

Cat. No. 281110 Dehydrated – 500 g

Difco™ Antimicrobial Vial K

Cat. No. 233391 Vial – 6 × 10 mL*

Difco™ Antimicrobial Vial P

Cat. No. 232681 Vial – 6 × 10 mL*

Difco™ Egg Yolk Enrichment 50%

AOAC BAM COMPF

Cat. No. 233471 Bottle – 12 × 10 mL*

233472 Bottle – 6 × 100 mL*

*Store at 2-8°C.

SIM Medium

Intended Use

SIM Medium is used to differentiate enteric bacilli on the basis of sulfide production, indole formation and motility.

Summary and Explanation

Hydrogen sulfide production, indole formation and motility are distinguishing characteristics which aid in the identification of the *Enterobacteriaceae*, especially *Salmonella* and *Shigella*. SIM Medium, therefore, is useful in the process of identification of enteric pathogens.

Principles of the Procedure

The ingredients in SIM Medium enable the determination of three activities by which enteric bacteria can be differentiated. Sodium thiosulfate and ferrous ammonium sulfate are indicators of hydrogen sulfide production. The ferrous ammonium sulfate reacts with H₂S gas to produce ferrous sulfide, a black precipitate.¹ The casein peptone is rich in tryptophan, which is attacked by certain microorganisms resulting in the production of indole. The indole is detected by the addition of chemical

User Quality Control

Identity Specifications

BBL™ SIM Medium

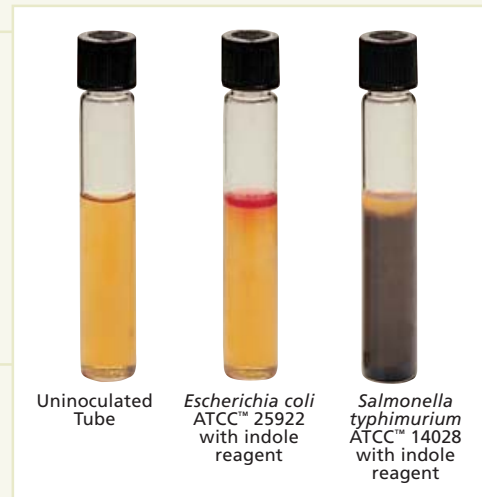
Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	3.0% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Light to medium, yellow to tan, clear to slightly hazy.
Reaction of 3.0% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

BBL™ SIM Medium

Prepare the medium per label directions. Stab inoculate using heavy inocula of fresh cultures and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	RECOVERY	MOTILITY	H ₂ S	INDOLE
<i>Escherichia coli</i>	25922	Good	+	-	+
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	13311	Good	+	+	-
<i>Shigella flexneri</i>	9199	Good	-	-	-



Uninoculated Tube

Escherichia coli ATCC™ 25922 with indole reagent

Salmonella typhimurium ATCC™ 14028 with indole reagent

reagents following the incubation period. Motility detection is possible due to the semisolid nature of the medium. Growth radiating out from the central stab line indicates that the test organism is motile.

Formula

BBL™ SIM Medium

Approximate Formula* Per Liter	
Pancreatic Digest of Casein	20.0 g
Peptic Digest of Animal Tissue	6.1 g
Ferrous Ammonium Sulfate	0.2 g
Sodium Thiosulfate	0.2 g
Agar	3.5 g

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

Directions for Preparation from Dehydrated Product

1. Suspend 30 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Dispense and autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Loosen caps, boil and cool before use. Using growth from a pure culture, stab an inoculating needle two-thirds of the distance to the bottom in the center of the tube. Incubate tubes with loosened caps for 18-24 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Following incubation, observe for motility (diffuse growth outward from the stab line or turbidity throughout the medium) and for H₂S production (blackening along the stab line). To detect indole production, add three or four drops of Kovacs' reagent² and observe for a red color (positive reaction).

Consult appropriate references for activities of specific microorganisms.²⁻⁴

References

1. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.
2. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
3. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
4. Farmer. 1999. In Murray, Baron, Pfaller, Tenover and Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ SIM Medium

BAM	
Cat. No.	211578 Dehydrated – 500 g
	221010 Prepared Tubes – Pkg. of 10
	221011 Prepared Tubes – Ctn. of 100