

QUALITY CONTROL PROCEDURES

I INTRODUCTION

SIM (Sulfide Indole Motility) Medium is used for the determination of sulfide production, indole formation and motility of enteric microorganisms.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. Loosen caps, boil and cool before use.
 - b. Inoculate the tubes by stabbing a straight needle down the center of the medium to approximately one-half its depth using 10⁻¹ dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures.
 - c. Incubate with loosened caps at 35 ± 2 °C in an aerobic atmosphere.
2. Examine tubes after 18 – 24 and 42 – 48 h for growth, motility and sulfide.
3. After 48 h, test for indole production. Add 0.2 mL of Kovacs' Reagent down the inside of the tubes. Observe for the production of a pink to red color (positive reaction).
4. Expected Results

Organisms	ATCC™	H ₂ S	Indole	Motility
* <i>Escherichia coli</i>	25922	–	+	+
* <i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	13311	+	–	+
* <i>Shigella sonnei</i>	9290	–	–	–

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under "Product Deterioration."
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes at 20 – 25 °C and 30 – 35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

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

V 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.

VI 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 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.

VII REAGENTS

SIM Medium

Approximate Formula* Per Liter Purified Water

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.

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2 – 25 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.^{2,3} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: SIM Medium

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Loosen caps, boil and cool before use. Using a light inoculum of a pure culture, stab an inoculating needle one-half of the distance to the bottom in the center of the tube. Incubate tubes with loosened caps for 18 – 48 h at 35 ± 2 °C in an aerobic atmosphere.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X 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 Kovács reagent² and observe for a red color (positive reaction).

Consult appropriate references for activities of specific microorganisms.⁴⁻⁶

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.²⁻⁶

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of SIM Medium are tested for performance characteristics. Representative samples of the lot are tested with **Trypticase** Soy Broth cultures diluted 10⁻¹ of *Salmonella* Typhimurium (ATCC 13311), *Escherichia coli* (ATCC 25922) and *Shigella sonnei* (ATCC 9290), by stabbing the center of the medium to approximately one-half its depth with an inoculating needle. The tubes are incubated with loosened caps at 35 ± 2 °C and read after 18 – 24 h and 42 – 48 h for growth, motility, indole formation and sulfide production. Growth of all organisms is moderate to heavy within 48 h. Both *E. coli* and *Salmonella* Typhimurium are motile as is evidenced by the growth pattern of the organism in the medium; i.e., growth originates at the center line of inoculation and spreads evenly throughout the medium. *S. sonnei* is nonmotile; i.e., growth is evident only along the center line of inoculation. Only *Salmonella* Typhimurium is positive for hydrogen sulfide production indicated by blackening of the medium. After 48 h incubation, 0.2 mL of Kovács Reagent is added to each tube. Only *E. coli* is positive for indole formation denoted by a medium pink to dark pink color reaction in the tube.

XIII AVAILABILITY

Cat. No.	Description
221010	BBL™ SIM Medium, Pkg. of 10 size K tubes
221011	BBL™ SIM Medium, Ctn. of 100 size K tubes

XIV REFERENCES

1. MacFaddin, J.F. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore.
2. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.L. Landry and M.A. Pfaller (ed.) 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.
3. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
4. Ewing, W.H. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York.
5. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.
6. Farmer, J.J., III. 1999. *Enterobacteriaceae*: introduction and identification, p. 442-458. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

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