



QUALITY CONTROL PROCEDURES

I INTRODUCTION

Salmonella Shigella Agar is a moderately selective and differential medium for the isolation, cultivation and differentiation of gram-negative enteric microorganisms isolated from both clinical and nonclinical specimens.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with dilutions of the cultures listed below.
 - a. Streak the plates for isolation. Use 18- to 24-h broth cultures of *Enterococcus faecalis* and *Escherichia coli* diluted to yield 10⁴-10⁵ CFU/plate. For the remaining organisms, use 18- to 24-h broth cultures diluted to yield 10³-10⁴ CFU/plate.
 - b. Incubate plates at 35 ± 2°C in an aerobic atmosphere.
 - c. Include **Trypticase™** Soy Agar with 5% Sheep Blood (TSA II) plates as nonselective controls for all organisms.
2. Examine plates after 18–24 h for growth, colony size, pigmentation and selectivity.
3. Expected Results

CLSI Organisms	ATCC™	Recovery	Colony Color
* <i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	Growth	Colonies colorless with or without black centers
* <i>Shigella flexneri</i>	12022	Growth	Colorless colonies
* <i>Enterococcus faecalis</i>	29212	Inhibition (complete)	
* <i>Escherichia coli</i>	25922	Inhibition (partial to complete)	Colonies pink to rose-red with precipitate

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.2 ± 0.2.
4. Note the firmness of plates during the inoculation procedure.
5. Incubate uninoculated representative plates aerobically at 35 ± 2°C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Salmonella Shigella Agar (SS Agar) is a differentially selective medium for the isolation of pathogenic enteric bacilli, especially those belonging to the genus *Salmonella*. This medium is not recommended for the primary isolation of *Shigella*.

V SUMMARY AND EXPLANATION

The culture media which have been developed for the selection and differentiation of enteric microorganisms from clinical and nonclinical materials inhibit the growth of gram-positive species to a varying degree due to the presence of either pure bile salts, mixtures of bile salts or dyes. One of the formulations currently used in the plating of samples for the detection of enteric pathogens is Salmonella Shigella Agar, which is an example of the media containing bile salt mixtures. This medium is essentially a modification of the Desoxycholate-Citrate Agar described by Leifson.¹

VI PRINCIPLES OF THE PROCEDURE

Salmonella Shigella Agar is designated as a moderately selective medium based upon the degree of inhibition of gram-positive microorganisms which it inhibits due to its content of bile salts, brilliant green and citrates.

Differentiation of enteric organisms is achieved by the incorporation of lactose in the medium. Organisms which ferment lactose produce acid which, in the presence of the neutral red indicator, results in the formation of red colonies. Lactose-nonfermenters form colorless colonies. The latter group contains the majority of the intestinal pathogens, including *Salmonella* and *Shigella*.

The sodium thiosulfate and ferric citrate enable the detection of hydrogen sulfide production as evidenced by colonies with black centers.

VII REAGENTS

Salmonella Shigella Agar

Approximate Formula* Per Liter Purified Water

Beef Extract	5.0 g	Sodium Thiosulfate.....	8.5 g
Pancreatic Digest of Casein	2.5 g	Ferric Citrate	1.0 g
Peptic Digest of Animal Tissue	2.5 g	Neutral Red.....	0.025 g
Lactose	10.0 g	Agar.....	13.5 g
Bile Salts.....	8.5 g	Brilliant Green.....	0.330 mg
Sodium Citrate.....	8.5 g		

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

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

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"²⁻⁵ and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2–8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8°C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking 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.^{6,7} Specimens should be obtained before antimicrobial therapy has been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Salmonella Shigella Agar

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

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. A nonselective medium should also be streaked to increase the chance of recovery when the population of gram-negative organisms is low and to provide an indication of other organisms present in the specimen.

Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area.

Incubate plates, protected from light, at 35 ± 2°C for 18–24 h 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 (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After incubation most plates will show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. Better isolation is obtained due to the inhibitory action of the medium.

Typical colonial morphology on Salmonella Shigella Agar is as follows:

E. coliSlight growth, pink or red
Enterobacter/KlebsiellaSlight growth, pink
ProteusColorless, usually with black center
Salmonella.....Colorless, usually with black center
ShigellaColorless
PseudomonasIrregular, slight growth
Gram-positive bacteria.....No growth

XI LIMITATIONS OF THE PROCEDURE

Due to the relatively high level of selectivity, some *Shigella* strains may not grow on SS Agar and, therefore, the medium is not recommended for the primary isolation of *Shigella*. Media recommended for the isolation of *Shigella* are Hektoen Enteric and XLD agars.

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.⁶⁻¹¹

A single medium is rarely adequate for detecting all organisms of potential significance in a specimen. Cultures of specimens grown on selective media should, therefore, be compared with specimens cultured on nonselective media to obtain additional information and help ensure recovery of potential pathogens.

XII AVAILABILITY

Cat. No.	Description
221181	BBL™ Salmonella Shigella Agar, Pkg. of 20 plates
221279	BBL™ Salmonella Shigella Agar, Ctn. of 100 plates

XIII REFERENCES

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