

SS Agar • Salmonella Shigella Agar

Intended Use

SS Agar and Salmonella Shigella Agar are moderately selective and differential media for the isolation of pathogenic enteric bacilli, especially those belonging to the genus *Salmonella*. This formulation is not recommended for the primary isolation of *Shigella*.

Summary and Explanation

The culture media that 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. SS Agar and Salmonella Shigella Agar are examples of media used in the plating of samples for the detection of enteric pathogens that contain bile salt mixtures. This formulation is essentially a modification of the Desoxycholate-Citrate Agar described by Leifson.¹

Principles of the Procedure

SS Agar and Salmonella Shigella Agar are designated as moderately selective media based upon the degree of inhibition of gram-positive microorganisms that they inhibit due to their content of bile salts, brilliant green and citrates. Differentiation of enteric organisms is achieved by the incorporation of lactose in the medium. Organisms that 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.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ SS Agar

Dehydrated Appearance:	Very light buff to pink, free-flowing, homogeneous.
Solution:	6.0% solution, soluble in purified water upon boiling. Solution is red-orange, very slightly to slightly opalescent.
Prepared Appearance:	Red-orange, slightly opalescent.
Reaction of 6.0% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Difco™ SS Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	H ₂ S
<i>Enterococcus faecalis</i>	29212	10 ³ -2 10 ³	Partial inhibition	Colorless	–
<i>Escherichia coli</i>	25922	10 ³ -2 10 ³	Partial inhibition	Pink to red	–
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10 ² -10 ³	Good	Colorless	+
<i>Shigella flexneri</i>	12022	10 ² -10 ³	Fair to good	Colorless	–

Identity Specifications

BBL™ Salmonella Shigella Agar

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material, may contain many small tan flecks.
Solution:	6.0% solution, soluble in purified water upon boiling. Solution is medium, tan-orange to tan-red, clear to moderately hazy.
Prepared Appearance:	Medium, tan-orange to tan-red, clear to moderately hazy.
Reaction of 6.0% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

BBL™ Salmonella Shigella Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR	H ₂ S
<i>Enterococcus faecalis</i>	29212	10 ⁴ -10 ⁵	Complete inhibition	–	–
<i>Escherichia coli</i>	25922	10 ⁴ -10 ⁵	Partial to complete inhibition	Pink to red	–
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10 ³ -10 ⁴	Good	Colorless	+
<i>Shigella flexneri</i>	12022	10 ³ -10 ⁴	Good	Colorless	–

Formulae

Difco™ SS Agar

Approximate Formula* Per Liter

Beef Extract.....	5.0	g
Proteose Peptone.....	5.0	g
Lactose.....	10.0	g
Bile Salts No. 3.....	8.5	g
Sodium Citrate.....	8.5	g
Sodium Thiosulfate.....	8.5	g
Ferric Citrate.....	1.0	g
Agar.....	13.5	g
Brilliant Green.....	0.33	mg
Neutral Red.....	25.0	mg

BBL™ Salmonella Shigella Agar

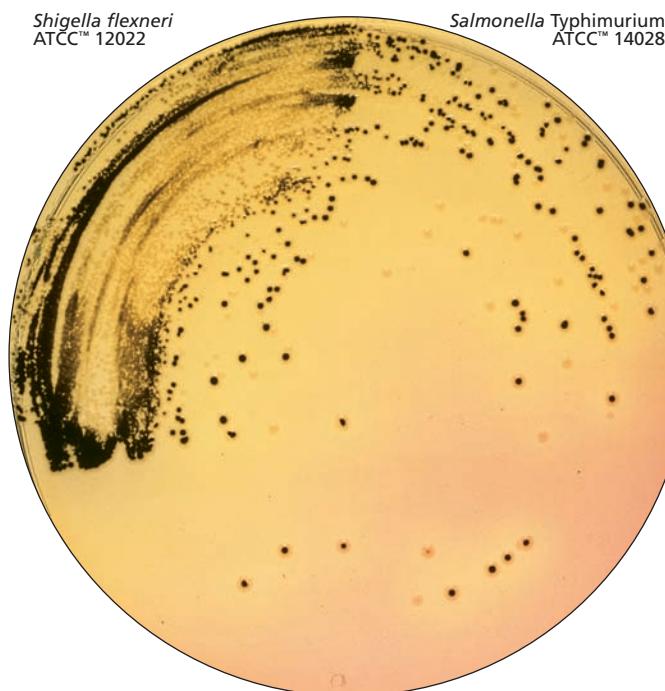
Approximate Formula* Per Liter

Beef Extract.....	5.0	g
Pancreatic Digest of Casein.....	2.5	g
Peptic Digest of Animal Tissue.....	2.5	g
Lactose.....	10.0	g
Bile Salts.....	8.5	g
Sodium Citrate.....	8.5	g
Sodium Thiosulfate.....	8.5	g
Ferric Citrate.....	1.0	g
Agar.....	13.5	g
Brilliant Green.....	0.33	mg
Neutral Red.....	25.0	mg

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

Directions for Preparation from Dehydrated Product

1. Suspend 60 g of the powder in 1 L of purified water. Mix thoroughly.



Limitation of the Procedure

Due to the relatively high level of selectivity, some *Shigella* strains may not grow on SS Agar and Salmonella Shigella Agar and, therefore, these media are not recommended for the primary isolation of *Shigella*.^{1,2} Media recommended for the isolation of *Shigella* are Hektoen Enteric and XLD agars.³

2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Cool the medium to approximately 45-50°C and pour into Petri dishes.
4. Allow the plates to dry for approximately 2 hours with the covers partially removed.
5. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Use standard procedures to obtain isolated colonies from specimens. 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. Incubate plates, protected from light, at 35 ± 2°C for 18-24 hours. If negative after 24 hours, reincubate an additional 24 hours.

Expected Results

Typical colonial morphology on Salmonella Shigella Agar is as follows:

<i>Escherichia coli</i>	Slight growth, pink or red
<i>Enterobacter/Klebsiella</i>	Slight growth, pink
<i>Proteus</i>	Colorless, usually with black center
<i>Salmonella</i>	Colorless, usually with black center
<i>Shigella</i>	Colorless
<i>Pseudomonas</i>	Irregular, slight growth
Gram-positive bacteria.....	No growth

References

1. Leifson. 1935. J. Pathol. Bacteriol. 40:581.
2. Taylor and Harris. 1965. Am. J. Clin. Pathol. 44:476.
3. Pollock and Dahlgren. 1974. Appl. Microbiol. 27:197.

Availability

Difco™ SS Agar

BS12	CMPH2	COMPF	MCM9
Cat. No.	274500	Dehydrated – 500 g	
	212118	Dehydrated – 2 kg	
	274300	Dehydrated – 10 kg	

BBL™ Salmonella Shigella Agar

BS12	CMPH2	COMPF	MCM9
Cat. No.	211596	Dehydrated – 100 g	
	211597	Dehydrated – 500 g	
	211600	Dehydrated – 5 lb (2.3 kg)	
	293306	Dehydrated – 25 lb (11.3 kg)	

United States and Canada

Cat. No.	221181	Prepared Plates – Pkg. of 20*
	221279	Prepared Plates – Ctn. of 100*

Europe

Cat. No.	254047	Prepared Plates – Pkg. of 20*
	254085	Prepared Plates – Ctn. of 120*

Japan

Cat. No.	251181	Prepared Plates – Pkg. of 20*
	251279	Prepared Plates – Ctn. of 100*
	251134	Prepared Plates – Ctn. of 200*
	251826	Prepared I Plate™ Dishes – Ctn. of 200*

BBL™ Salmonella Shigella Agar//Hektoen Enteric Agar

Cat. No.	297426	Prepared I Plate™ Dishes – Pkg. of 20*
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*Store at 2-8°C.