BG Sulfa Agar SBG Sulfa Enrichment

Intended Use

BG Sulfa Agar is used for isolating Salmonella.

SBG Sulfa Enrichment is used for enriching *Salmonella* prior to isolation procedures.

Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of *Salmonella* in domesticated animals. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness. The illness results from consumption of raw, undercooked or improperly processed foods contaminated with *Salmonella*. Many of these cases of *Salmonella*-related gastroenteritis are due to improper handling of poultry products. Various poultry products are routinely monitored for *Salmonella* before their distribution for human consumption, but in many instances, contaminated food samples elude detection.

BG (Brilliant Green) Sulfa Agar is a highly selective medium. Osborne and Stokes¹ added 0.1% sodium sulfapyridine to Brilliant Green Agar to enhance the selective properties of this medium for *Salmonella*. This formula is recommended as a selective isolation medium for *Salmonella* following enrichment.² It is also recommended for direct inoculation with primary specimens for *Salmonella* isolation.

For food testing, BG Sulfa Agar has been used for detection of *Salmonella* in low and high moisture foods.^{3,4} It has also been used for detecting *Salmonella* in feeds and feed ingredients.⁵ This medium is recommended when testing foods for *Salmonella* following USDA guidelines.⁶⁻⁸

SBG (Selenite Brilliant Green) Sulfa Enrichment is prepared according to the formula described by Stokes and Osborne.⁹ The researchers found that whole egg and egg yolk reduced the selective properties of selenite brilliant green enrichment.¹ They also found that the addition of sulfapyridine (SBG Sulfa Enrichment) restored these selective properties.¹

SBG Sulfa Enrichment is a selective enrichment for the isolation of *Salmonella* species, especially from egg products. The shell and the contents of the egg at the time of oviposition are generally sterile or harbor very few microorganisms. Contamination of the shell occurs afterwards from nesting material, floor litter and avian fecal matter.¹⁰⁻¹²

Principles of the Procedure

In BG Sulfa Agar, peptone and yeast extract provide nitrogen, vitamins and minerals. Lactose and sucrose are the sources of carbohydrates in the medium. Brilliant green and sodium pyridine are complementary in inhibiting gram-positive bacteria and most gram-negative bacilli other than *Salmonella* spp. Phenol red is the pH indicator that turns the medium a yellow color with the formation of acid when lactose and/or sucrose is fermented. Agar is the solidifying agent.

Peptone provides the nitrogen, minerals and amino acids in SBG Sulfa Enrichment. Yeast extract is the vitamin source. D-Mannitol is the carbon source to stimulate organism growth. The phosphates act as buffers in the enrichment. Sodium taurocholate, sodium selenite and brilliant green are the selective agents. The selective agents are used to inhibit gram-positive organisms and enteric bacteria other than *Salmonella*. Sodium sulfapyridine is added to increase selectivity.

Formulae

Difco[™] BG Sulfa Agar

Approximate Formula* Per Liter g Proteose Peptone No. 3......10.0 q q Saccharose 10.0 q Sodium Sulfapyridine1.0 g Sodium Chloride5.0 q q Brilliant Green 12.5 mg Phenol Red.....0.08 g

Difco[™] SBG Sulfa Enrichment

Approximate Formula* Per Liter		
Yeast Extract	5.0	g
Peptone	5.0	g
D-Mannitol	5.0	g
Sodium Taurocholate	1.0	g
Sodium Sulfapyridine	0.5	g
Sodium Selenite	4.0	g
Dipotassium Phosphate	2.65	g
Monopotassium Phosphate	1.02	g
Brilliant Green	5.0 m	١ġ
*Adjusted and/or supplemented as required to meet performance criteria.		0





User Quality Control

<i>Identity Specifications</i> Difco™ BG Sulfa Agar					
Dehydrated Appearance:	Pink, free flowing, homogeneous.				
Solution:	5.9% solution, soluble in purified water upon boiling. Solution is very dark amber, very slightly to slightly opalescent.				
Prepared Appearance:	Orange-brown to dark reddish-amber, slightly opalescent.				
Reaction of 5.9% Solution at 25°C:	pH 6.9 ± 0.2				

Cultural Response Difco[™] BG Sulfa Agar

Prepare the medium per label directions. Inoculate and incubate at 35 \pm 2°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLOR OF COLONIES/ MEDIUM
Enterococcus faecalis	29212	10 ³ -2×10 ³	None	–/no change
Escherichia coli	25922	10 ² -3×10 ²	None to poor	Yellow-green/ Yellow-green
Proteus vulgaris	13315	10 ² -3×10 ²	None	–/no change
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Enteritidis	13076	10 ² -3×10 ²	Good	Pink-white/ red
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10 ² -3×10 ²	Good	Pink-white/ red

Directions for Preparation from Dehydrated Product Difco[™] BG Sulfa Agar

- 1. Suspend 59 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. Autoclave at 121°C for 15 minutes. Avoid overheating, which will decrease selectivity.
- 4. Test samples of the finished product for performance using stable, typical control cultures.

Difco[™] SBG Sulfa Enrichment

- 1. Suspend 24.2 g of the powder in 1 L of purified water. Mix thoroughly.
- 2. Heat with frequent agitation and boil for 5-10 minutes to completely dissolve the powder. Avoid overheating. DO NOT AUTOCLAVE.
- 3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Refer to appropriate references for specific procedures for the isolation and cultivation of *Salmonella* from meat, poultry and egg products and other foods.^{2,7,8}

Identity Specifications Difco[™] SBG Sulfa Enrichment

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.42% solution, soluble in purified water. Solution is green, opalescent, may have a precipitate.
Prepared Appearance:	Green, opalescent without significant precipitate.
Reaction of 2.42% Solution at 25°C:	рН 7.2 ± 0.2

Cultural Response Difco™ SBG Sulfa Enrichment

Prepare the enrichment per label directions. Inoculate and incubate at $35 \pm 2^{\circ}$ C for 18-24 hours. After incubation, subculture onto plates of MacConkey Agar and incubate at $35 \pm 2^{\circ}$ C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR ON MACCONKEY
Escherichia coli	25922	10 ² -3×10 ²	None to poor	Pink, if any
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Enteritidis	13076	10 ² -3×10 ²	Good	Colorless
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10 ² -3×10 ²	Good	Colorless
Shigella sonnei	9290	10 ² -3×10 ²	None to fair	Colorless

Expected Results

BG Sulfa Agar

The typical *Salmonella* colonies appear as pink-white to red opaque colonies surrounded by a brilliant red medium. The few lactose and/or sucrose fermenting organisms that grow are readily differentiated due to the formation of a yellow-green colony surrounded by an intense yellow-green zone. BG Sulfa Agar is not suitable for the isolation of *S*. Typhi or *Shigella*; however, some strains of *S*. Typhi may grow forming red colonies.

SBG Sulfa Enrichment

Examine prepared media for growth. Positive tubes should be subcultured onto prepared media for isolation and identification of bacteria.

Limitations of the Procedure

- 1. On BG Sulfa Agar colonies of *Salmonella* spp. vary from red to pink to white depending on length of incubation and strain.¹³
- 2. BG Sulfa Agar is normally orange-brown in color; however, on incubation, it turns bright red and returns to normal color at room temperature.¹³
- 3. S. Typhi does not grow adequately on BG Sulfa Agar. *Shigella* spp. do not grow on BG Sulfa Agar.¹³
- 4. Do not autoclave BG Sulfa Agar longer than 15 minutes; longer periods decrease the selectivity of the medium.



- 5. Since BG Sulfa Agar is highly selective, it is recommended that less selective media, such as MacConkey Agar, be used simultaneously.
- 6. SBG Sulfa Enrichment should be used in conjunction with a selective prepared medium for bacterial identification.

References

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Availability

Difco[™] BG Sulfa Agar

CCAM COMPF USDA

Cat. No. 271710 Dehydrated - 500 g

Difco[™] SBG Sulfa Enrichment

USDA

Cat. No. 271510 Dehydrated - 500 g

