Bismuth Sulfite Agar

Intended Use

Bismuth Sulfite Agar is a highly selective medium used for isolating *Salmonella* spp., particularly *Salmonella* Typhi, from food and clinical specimens.

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 nontyphi *Salmonella* often causes mild, self-limiting illness.[1] Typhoid fever, caused by *S.* Typhi, is characterized by fever, headache, diarrhea and abdominal pain, and can produce fatal respiratory, hepatic, splenic and/or neurological damage. These illnesses result from consumption of raw, undercooked or improperly processed foods contaminated with *Salmonella*. Many cases of *Salmonella*-related gastroenteritis are due to improper handling of poultry products. United States federal guidelines require various poultry products to be routinely monitored before distribution for human consumption but contaminated food samples often elude monitoring.

Bismuth Sulfite Agar is a modification of the Wilson and Blair[2-4] formula. Wilson[5,6] and Wilson and Blair[2-4] clearly showed the superiority of Bismuth Sulfite medium for isolation of *S.* Typhi. Cope and Kasper[7] increased their positive findings of typhoid from 1.2 to 16.8% among food handlers and from 8.4 to 17.5% among contacts with Bismuth Sulfite Agar.

Employing this medium in the routine laboratory examination of fecal and urine specimens, these same authors[8] obtained 40% more positive isolations of *S.* Typhi than were obtained on Endo medium. Gunther and Tuft,[9] employing various media in a comparative way for the isolation of typhoid from stool and urine specimens, found Bismuth Sulfite Agar most productive. On Bismuth Sulfite Agar, they obtained 38.4% more positives than on Endo Agar, 33% more positives than on Eosin Methylene Blue Agar, and 80% more positives than on the Desoxycholate media. These workers found Bismuth Sulfite Agar to be superior to Wilson's original medium. Bismuth Sulfite Agar was stable, sensitive and easier to prepare. Green and Beard,[10] using Bismuth Sulfite Agar, claimed that this medium successfully inhibited sewage organisms. The value of Bismuth Sulfite Agar as a plating medium after enrichment has been demonstrated by Hajna and Perry.[11]

Since these earlier references to the use of Bismuth Sulfite Agar, this medium has been generally accepted as routine for the detection of most *Salmonella*. The value of the medium is demonstrated by the many references to the use of Bismuth Sulfite Agar in scientific publications, laboratory manuals and texts.

For food testing, the use of Bismuth Sulfite Agar is specified for the isolation of pathogenic bacteria from raw and pasteurized milk, cheese products, dry dairy products, cultured milks and butter.[1,12-14] The use of Bismuth Sulfite Agar is also recommended for use in testing clinical specimens.[15,16] In addition, Bismuth Sulfite Agar is valuable when investigating outbreaks of *Salmonella* spp., especially *S.* Typhi.[17-19]

Bismuth Sulfite Agar is used for the isolation of *S.* Typhi and other *Salmonella* from food, feces, urine, sewage and other infectious materials. The typhoid organism grows luxuriantly on the medium, forming characteristic black colonies, while gram-positive bacteria and members of the coliform group are inhibited. This inhibitory action of Bismuth Sulfite Agar toward gram-positive and coliform organisms permits the use of a much larger inoculum than possible with other media employed for similar purposes in the past. The use of larger inocula greatly increases the possibility of recovering the pathogens, especially when they are present in relatively small numbers. Small numbers of organisms may be encountered in the early course of the disease or in the checking of carriers and releases.

Principles of the Procedure

In Bismuth Sulfite Agar, beef extract and peptone provide nitrogen, vitamins and minerals. Dextrose is an energy source. Disodium phosphate is a buffering agent. Bismuth sulfite indicator and brilliant green are complementary in inhibiting gram-positive bacteria and members of the coliform group, while allowing *Salmonella* to grow luxuriantly. Ferrous sulfate is included for detection of H_2S production. When H_2S is present, the iron in the formula is precipitated, giving positive cultures the characteristic brown to black color with metallic sheen. Agar is the solidifying agent.

Formula

Difco™ Bismuth Sulfite Agar

Approximate Formula* Per Liter		
Beef Extract	5.0	g
Peptone	10.0	g
Dextrose	5.0	g
Disodium Phosphate	4.0	g
Ferrous Sulfate	0.3	g
Bismuth Sulfite Indicator	8.0	g
Agar	20.0	g
Brilliant Green	25.0	mg

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

Directions for Preparation from Dehydrated Product

1. Suspend 52 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. DO NOT AUTOCLAVE.
3. Evenly disperse the precipitate when dispensing. Use the medium the same day it is prepared.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

Identity Specifications

Difco™ Bismuth Sulfite Agar

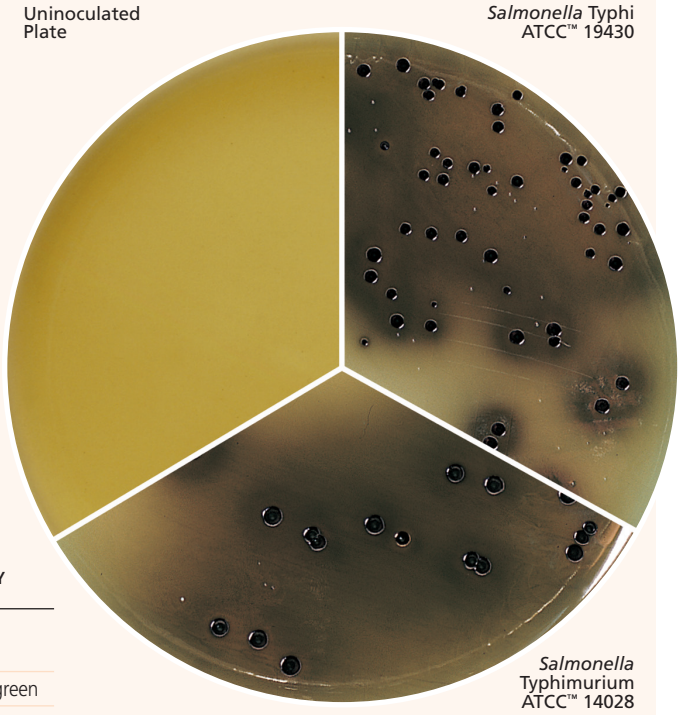
Dehydrated Appearance:	Light beige to light green, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in purified water upon boiling. Solution is light green, opaque with a flocculent precipitate that can be dispersed by swirling contents of flask.
Prepared Appearance:	Light gray-green to medium green, opaque with a flocculent precipitate.
Reaction of 5.2% Solution at 25°C:	pH 7.7 ± 0.2

Cultural Response

Difco™ Bismuth Sulfite Agar

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
Enterococcus faecalis	29212	10^3	Marked to complete inhibition	–
Escherichia coli	25922	10^3	Partial inhibition	Brown to green
Salmonella enterica subsp. *enterica* serotype Typhi	19430	10^2-10^3	Good	Black with sheen
Salmonella enterica subsp. *enterica* serotype Typhimurium	14028	10^2-10^3	Good	Black or greenish-gray, may or may not have sheen



Procedure

For isolation of *Salmonella* spp. from food, samples are enriched and selectively enriched. Streak 10 µL of selective enrichment broth onto Bismuth Sulfite Agar. Incubate plates for 24-48 hours at 35°C. Examine plates for the presence of *Salmonella* spp. Refer to appropriate references for the complete procedure when testing food samples.[1,12-14]

For isolation of *Salmonella* spp. from clinical specimens, inoculate fecal specimens and rectal swabs onto a small area of one quadrant of the Bismuth Sulfite Agar plate and streak for isolation. This will permit the development of discrete colonies. Incubate plates at 35°C. Examine at 24 hours and again at 48 hours for colonies resembling *Salmonella* spp.

For additional information about specimen preparation and inoculation of clinical specimens, consult appropriate references.[15-19]

Expected Results

The typical discrete *S.* Typhi surface colony is black and surrounded by a black or brownish-black zone which may be several times the size of the colony. By reflected light, preferably daylight, this zone exhibits a distinctly characteristic metallic sheen. Plates heavily seeded with *S.* Typhi may not show this reaction except near the margin of the mass inoculation. In these heavy growth areas, this organism frequently appears as small light green colonies. This fact emphasizes the importance of inoculating plates so that some areas are sparsely populated with discrete *S.* Typhi colonies. Other strains of *Salmonella* produce black to green colonies with little or no darkening of the surrounding medium.

Generally, *Shigella* spp. other than *S. flexneri* and *S. sonnei* are inhibited. *S. flexneri* and *S. sonnei* strains that do grow on this medium produce brown to green, raised colonies with depressed centers and exhibit a crater-like appearance.

Escherichia coli is partially inhibited. Occasionally a strain will be encountered that will grow as small brown or greenish glistening colonies. This color is confined entirely to the colony itself and shows no metallic sheen. A few strains of *Enterobacter aerogenes* may develop on this medium, forming raised, mucoid colonies. *Enterobacter* colonies may exhibit a silvery sheen, appreciably lighter in color than that produced by *S.* Typhi. Some members of the coliform group that produce hydrogen sulfide may grow on the medium, giving colonies similar in appearance to *S.* Typhi. These coliforms may be readily differentiated because they produce gas from lactose in differential media, for example, Kligler Iron Agar or Triple Sugar Iron Agar. The hydrolysis of urea, demonstrated in Urea Broth or on Urea Agar Base, may be used to identify *Proteus* sp.

To isolate *S.* Typhi for agglutination or fermentation studies, pick characteristic black colonies from Bismuth Sulfite Agar and subculture them on MacConkey Agar. The purified colonies from MacConkey Agar may then be picked to differential tube media such as Kligler Iron Agar, Triple Sugar Iron Agar or other satisfactory differential media for partial identification. All cultures that give reactions consistent with *Salmonella* spp. on these media should be confirmed biochemically as *Salmonella* spp. before any serological testing is performed. Agglutination tests may be performed from the fresh growth on the differential tube media or from the growth on nutrient agar slants inoculated from the differential media. The growth on the differential tube media may also be used for inoculating carbohydrate media for fermentation studies.

Limitations of the Procedure

1. It is important to streak for well-isolated colonies. In heavy growth areas, *S.* Typhi appears light green and may be misinterpreted as negative growth for *S.* Typhi.[20]
2. *S.* Typhi and *S. arizonae* are the only enteric organisms to exhibit typical brown zones on the medium. Brown zones are not produced by other members of the *Enterobacteriaceae*. However, *S. arizonae* is usually inhibited.[20]
3. Colonies on Bismuth Sulfite Agar may be contaminated with other viable organisms; therefore, isolated colonies should be subcultured to a less selective medium (e.g., MacConkey Agar).[20]
4. Typical *S.* Typhi colonies usually develop within 24 hours; however, all plates should be incubated for a total of 48 hours to allow growth of all typhoid strains.[20]
5. DO NOT AUTOCLAVE. Heating this medium for a period longer than necessary to just dissolve the ingredients destroys its selectivity.

References

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Availability

Difco™ Bismuth Sulfite Agar

AOAC BAM CCAM COMPF SMD SMWW

Cat. No. 273300 Dehydrated – 500 g

Mexico

Cat. No. 252612 Prepared Plates – Pkg. of 10*

**Store at 2-8°C.*