Bismuth Sulphite Agar

Intended Use
Bismuth Sulphite Agar is a highly selective medium used for isolation of *Salmonella* species, particularly *S. typhi* from clinical and non-clinical specimens.

Summary
Bismuth Sulphite Agar is a modification of the original Wilson and Blair selective medium. It is recommended by various associations for the isolation and preliminary identification of *Salmonella typhi* and other *Salmonella* from pathological materials, food, sewage, water supplies, etc. More positive isolates of *S. typhi* were obtained on this medium compared to Endo Agar, Eosin Methylene Blue Agar and Deoxycholate Agar. It is recommended by USP and IP for use in microbial limit testing. For food testing, this medium is specified for the isolation of pathogenic bacteria from raw and pasteurized milk, cheese products, dry dairy products, cultured milk and butter. It is also included in the Bacteriological Analytical Manual for food testing.

Principle
Beef extract and peptone provide nitrogen, growth factors and trace elements. Dextrose is the energy source. Disodium hydrogen phosphate is the buffer. Bismuth sulphite and brilliant green are complimentary in inhibiting Gram-positive bacteria and intestinal Gram-negative bacteria (coliform group) while allowing *Salmonella* to grow luxuriantly. This inhibitory action permits the use of a much larger inoculum than possible with other media employed for similar purposes. The use of larger inocula greatly increases the possibility of recovering the intestinal pathogens. Ferrous sulphate detects H_2S production. *S. typhi S. enteritidis* and *S. typhimurium*, typically grow as black colonies with surrounding metallic sheen resulting from H_2S production and reduction of sulphite to black ferric sulphide. *S. paratyphi* A produces light green colonies. *Shigella* species are mostly inhibited on this medium.

Formula*

Ingredients	**g/L**
Beef Extract	5.0
Peptone	10.0
Dextrose	5.0
Disodium Hydrogen Phosphate	4.0
Ferrous Sulphate	0.3
Bismuth Sulphate	8.0
Brilliant Green Indicator	0.025
Agar	20.0
Final pH (at 25°C)	7.7 ± 0.2

*Adjusted to suit performance parameters

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2ºC-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of specimen
Clinical samples - Faeces, Urine, Blood and other Pathological Material;
Food samples; Water samples

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 52.33 g of the powder in 1000 mL purified / distilled water and mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to dissolve the powder completely.
3. DO NOT AUTOCLAVE.
4. Disperse the precipitate evenly while dispensing (the sensitivity of the medium depends mainly upon uniform dispersion of freshly precipitated bismuth sulphite in the final gel) and use the medium the same day it is prepared.

Quality Control

Dehydrated Appearance: Light yellow to greenish yellow coloured, homogenous, free flowing powder.
Prepared Appearance: White to off white coloured, opaque gel with or without flocculent precipitate forms in petridishes.
Cultural Response: Cultural characteristics observed after an incubation of 24-48 hours at 30°C-35°C.

Organisms (ATCC)	**Growth**	**Colour of Colony**
Enterococcus faecalis (29212)	Complete Inhibition	-
Salmonella enterica subsp. *enterica* serovar *Typhi* (NTCC 786)	Good	Black with metallic sheen
Escherichia coli (25922)	Partial Inhibition	Brown
Salmonella enterica subsp. *enterica* serovar *Typhimurium* (14028)	Good	Black with metallic sheen
Salmonella enterica subsp. *enterica* serovar *Abony* (6017)	Good	Black with metallic sheen

Interpretation of Results

1. The typical discrete *S. typhi* colonies are black and often surrounded by a black or brownish black zone, which may be several times the size of the colony.
2. In reflected light, preferably daylight, the zone exhibits a distinctly characteristic metallic sheen. In heavy growth areas the organism frequently appears as small light green colonies. This emphasizes the importance of inoculating plates by the four-quadrant method so that some areas are sparsely populated to give discrete colonies.
3. Other strains of *Salmonella* produce black to green colonies with little or no darkening of the surrounding medium.
4. Generally, *Shigella* species other than *S. flexneri* and *S. sonnei* are inhibited. These two, do grow on this medium to produce brown to green raised colonies with depressed centers but show a crater like appearance.
5. *E. coli* is partially inhibited on this medium. If at all present, it produces small brown or greenish glistening colonies. The colour however, is confined to the colony itself and shows no metallic sheen.
6. *Enlerobacter* colonies if present exhibit a silvery sheen, appreciably lighter in colour than that produced by *S. typhi*.
7. To isolate *S. typhi* for agglutination or fermentation studies, pick characteristic colonies from this medium and subculture on MacConkey Agar. The purified colonies from MacConkey Agar may then be inoculated in differential tubed media like TSI Agar.
8. All cultures that give reactions consistent with *Salmonella* species on this medium should be confirmed biochemically as *Salmonella* species before any serological testing is performed,

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions/Limitations

1. DO NOTAUTOCLAVE or overheat as it may destroy the selectivity of the medium.
2. Prepared plates should not be stored forl onger than two days at 2°C-8°C; after which time the dye oxidizes to give a green medium that can be inhibitory to some Salmonellae.

3. The medium may be inhibitory to some strains of Salmonellae and therefore should not be used as the sole selective medium for these organisms. *S. sendai*, *S. berta*, *S. gallinarum*, *S. abortus-equi* and *S. cholerae~suis* are markedly inhibited.
1. 4, It is important to streak for well isolated colonies. The typical colonial characteristics will not develop if the growth is too heavy or confluent; *S. typhi* colonies will appear light green in these circumstances and may thus be misinterpreted as negative growth for *S. typhi*.
4. *S. typhi* and *S. arizonae* are the only enteric organisms to exhibit typical brown zones on the medium. However, *S. arizonae* is usually inhibited on this medium.
5. Some members of the coliform group that produce H_2S may grow on this medium, giving colonies similar to *S. typhi*. However, they may be differentiated because they produce gas from lactose in differential media, for example, Triple Sugar Iron Agar. *Proteus* species may be differentiated on the basis of urea hydrolysis in Urea Broth or on Urea Agar Base.
6. Colonies on this medium may be contaminated with other viable organisms; therefore, isolated colonies should be subcultured to a less selective medium like MacConkey Agar.
7. All plates should be incubated fora total of48 hours to allow growth of all typhoid strains.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Wilson and Blair, 1926. J. Pathol. Barieriol. 29:310.
2. Wilson and Blair. 1931.J. Hyg. 31:138.
3. US Pharmacopeial Convention, Inc. 2001. The United States Pharmacopoeia 25INF 20-2002. The US Pharmacopeial Convention, Inc; Rockville, Md.
4. IP, 1996, Ministry of Health and Family Welfare, Govt. of India, Vol. 2.
5. US Food and Drug Adm; 1998, Bacteriological Analytical Manual, 8th Ed; Rev. AAOAC, International, Gaithersburg, Md.
6. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201020180100	Dehydrated Culture Media	100 g
201020180500	Dehydrated Culture Media	500 g
203020470100	Bottle Media	100 mL