Bismuth Sulfite Agar (NCM0086)

Intended Use
Bismuth Sulfite Agar is used for the selective isolation of *Salmonella* spp. In a laboratory setting. Bismuth Sulfite Agar is not intended for use in the diagnosis of disease or other conditions in humans and conforms to FDA/BAM formulation.

Description
Salmonellosis can result from consumption of raw, undercooked, or improperly processed foods contaminated with *Salmonella*. U. S. federal guidelines require various poultry products to be routinely monitored before distribution for human consumption.

Bismuth Sulfite Agar is a modification of Wilson and Blair formula. The typhoid organism grows luxuriantly on the medium, forming characteristic black colonies. Gram-positive bacteria and coliforms are inhibited on Bismuth Sulfite Agar. The inhibitory action of Bismuth Sulfite Agar permits the use of a large inoculum, increasing the possibility of recovering pathogens that may be present in small numbers. Bismuth Sulfite Agar is generally accepted for routine detection of most *Salmonella* spp. Bismuth Sulfite Agar is used for the isolation of *S. typhi* and other *Salmonella* spp. from food. Bismuth Sulfite Agar is a standard methods medium for industrial applications and the laboratory environment.

Typical Formulation

Enzymatic Digest of Casein	5.0 g/L
Enzymatic Digest of Animal Tissue	5.0 g/L
Beef Extract	5.0 g/L
Dextrose	5.0 g/L
Disodium Phosphate	4.0 g/L
Ferrous Sulfate	0.3 g/L
Bismuth Sulfite Indicator	8.0 g/L
Brilliant Green	0.025 g/L
Agar	20.0 g/L

Final pH: 7.7 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution
1. Refer to SDS

Preparation
1. Suspend 52 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute.
3. Mix thoroughly to obtain a uniform suspension prior to dispensing.
4. Freshly prepared Bismuth Sulfite Agar is very selective, therefore suitable for heavily contaminated samples. However, it is not recommended to store prepared Bismuth Sulfite Agar at 4°C for longer than 2 days; after 3 days of storage the medium may change to a green color with a reduction in selectivity of competing microorganisms and an increased potential for the inhibition of *Salmonella* colonies.

Test Procedure
For isolation of *Salmonella typhi* and other *Salmonella* spp. consult appropriate references.

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Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige to greenish-beige.

Prepared Appearance: Prepared medium is opaque and may have a few dark particles suspended in medium, and light greenish-yellow to light/medium greenish beige.

Expected Cultural Response: Cultural response on Bismuth Sulfite Agar at 35 ± 2°C after 40 - 48 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reactions
Enterococcus faecalis ATCC® 29212	≥1000	Completely inhibited	--
Escherichia coli ATCC® 25922	≥1000	Partially to completely inhibited	Pale green to olive-green colonies
Escherichia coli ATCC® 8739	≥1000	Partially to completely inhibited	Pale green to olive-green colonies
Salmonella typhimurium ATCC® 14028	100-300	Fair to excellent	Olive-black to black colonies ± metallic sheen
Shigella flexneri ATCC® 12022	≥1000	Partially to completely inhibited	Green to brown colonies
Salmonella enterica ATCC® 13076	100-300	Fair to excellent	Green to olive-green colonies ± metallic sheen

The organisms listed are the minimum that should be used for quality control testing.

Results

Typical *S. typhi* surface colonies are olive-green to black, surrounded by black or brown-black zone with or without a metallic sheen. This zone may be several times the size of the colony. Other strains of *Salmonella* produce black to green colonies with little or no darkening of surrounding medium. *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.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container.

Limitations of the Procedures

1. Atypical *S. typhi* appears light green and may be misinterpreted as non-*Salmonella spp.*
2. *S. typhi* and *S. arizonae* are the only enteric organisms to exhibit typical brown zones on the medium. However, *S. arizonae* is usually inhibited. 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.
3. Do not autoclave medium. Heating medium for a long period may destroy selectivity properties.
4. Do not expose Bismuth Sulfite Agar to sunlight.

Storage

Dehydrated culture media: 2-30ºC away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

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