

## BISMUTH SULFITE AGAR (7113)

### 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.<sup>7</sup>

### Product Summary and Explanation

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.<sup>1-3</sup> 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.<sup>4-8</sup>

### Principles of the Procedure

Enzymatic Digest of Casein, Enzymatic Digest of Animal Tissue, and Beef Extract provide sources of nitrogen, carbon, and vitamins required for organism growth. Dextrose is the carbohydrate present in Bismuth Sulfite Agar. Disodium Phosphate is the buffering agent. Bismuth Sulfite Indicator and Brilliant Green are complementary, inhibiting Gram-positive bacteria and coliforms, allowing *Salmonella* spp. to grow. Ferrous Sulfate is used for H<sub>2</sub>S production. When H<sub>2</sub>S is present, the iron in the formula is precipitated, and positive cultures produce the characteristic brown to black color with metallic sheen. Agar is the solidifying agent.

### Formula / Liter

Enzymatic Digest of Casein.....	5 g
Enzymatic Digest of Animal Tissue .....	5 g
Beef Extract .....	5 g
Dextrose.....	5 g
Disodium Phosphate.....	4 g
Ferrous Sulfate .....	0.3 g
Bismuth Sulfite Indicator .....	8 g
Brilliant Green .....	0.025 g
Agar .....	20 g

Final pH: 7.5 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

### Precautions

1. For Laboratory Use Only.
2. IRRITANT. Irritating to eyes, skin, and respiratory system. May cause sensitization by inhalation.

### Directions

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.<sup>10</sup> 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.<sup>9</sup>

### **Quality Control Specifications**

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

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

**Expected Cultural Response:** Cultural response on Bismuth Sulfite Agar at  $35 \pm 2^\circ\text{C}$  after 40 - 48 hours incubation.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reactions
<i>Enterococcus faecalis</i> ATCC® 29212	$\geq 1000$	Completely inhibited	---
<i>Escherichia coli</i> ATCC® 8739	$\geq 1000$	Partially to completely inhibited	Pale green to olive-green colonies
<i>Escherichia coli</i> ATCC® 25922	$\geq 1000$	Partially to completely inhibited	Pale green to olive-green colonies
<i>Salmonella typhimurium</i> ATCC® 14028	100 - 300	Fair to excellent	Olive-green to black colonies $\pm$ metallic sheen
<i>Shigella flexneri</i> ATCC® 12022	$\geq 1000$	Partially to completely inhibited	Pale green to olive-green colonies

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

### **Test Procedure**

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

### **Results**

Typical *S. typhi* surface colonies are 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.

### **Storage**

Store sealed bottle containing the dehydrated medium at  $2 - 30^\circ\text{C}$ . Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

### **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 Procedure**

1. *S. typhi* appears light green and may be misinterpreted as negative for *S. typhi*.<sup>9</sup>
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.<sup>9</sup> 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.<sup>9</sup>
3. Do not autoclave medium. Heating medium for a long period may destroy selectivity properties.
4. Do not expose Bismuth Sulfite Agar to sunlight.<sup>9</sup>

### **Packaging**

Bismuth Sulfite Agar	Code No.	7113A	500 g
		7113B	2 kg
		7113C	10 kg

## References

1. **Wilson, W. J., and E. M. Blair.** 1926. A combination of bismuth and sodium sulphite affording an enrichment and selective medium for the typhoid-paratyphoid groups of bacteria. *J. Pathol. Bacteriol.* **29**:310.
2. **Wilson, W. J., and E. M. Blair.** 1927. Use of a glucose bismuth sulphite iron medium for the isolation of *B. typhosus* and *B. proteus*. *J. Hyg.* **26**:374-391.
3. **Wilson, W. J., and E. M. Blair.** 1931. Further experience of the bismuth sulphite media in the isolation of *B. typhosus* and *B. proteus*. *J. Hyg.* **31**:138-161.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
5. **Vanderzant, C., and D.F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3<sup>rd</sup> ed. American Public Health Association, Washington, D.C.
6. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23<sup>rd</sup> ed. The United States Pharmacopeial Convention, Rockville, MD.
7. **[www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm](http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalmanualBAM/default.htm)**
8. **Cunniff, P. (ed.).** Official methods of analysis of AOAC International, 16<sup>th</sup> ed. AOAC International, Arlington, VA.
9. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, MD.
10. **Cook, G.T.** 1952. *J. Path. Bact.* **64**:559.

## Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.