



## CRITERION™ BISMUTH SULFITE AGAR

<a href="#">Cat. no. C5210</a>	CRITERION™ Bismuth Sulfite Agar	104gm
<a href="#">Cat. no. C5211</a>	CRITERION™ Bismuth Sulfite Agar	500gm
<a href="#">Cat. no. C5212</a>	CRITERION™ Bismuth Sulfite Agar	2kg
<a href="#">Cat. no. C5213</a>	CRITERION™ Bismuth Sulfite Agar	10kg
Cat. no. C5214	CRITERION™ Bismuth Sulfite Agar	50kg

### INTENDED USE

Hardy Diagnostics CRITERION™ Bismuth Sulfite Agar is a highly selective and differential medium. It is recommended for the isolation of *Salmonella* species, especially *S. typhi*, from food and clinical specimens.

This dehydrated culture medium is a raw material intended to be used in the making of prepared media products, which will require further processing, additional ingredients, or supplements.

### SUMMARY

Salmonellosis continues to be an important public health issue in the United States and worldwide. Despite efforts to control the occurrence of *Salmonella* in domesticated animals, *Salmonella* are primary pathogens of many animals (e.g., poultry, cows, pigs, reptiles, etc.) and are the principal source of non-typhoidal salmonellosis in humans.<sup>(12)</sup> Human infections with *Salmonella* are most commonly caused by ingestion of fecally contaminated food, water, or milk. Improper handling of poultry products is many times the cause of *Salmonella*-related gastroenteritis, with about one half of salmonellosis epidemics caused by contaminated poultry and poultry products. Non-typhoidal *Salmonella* usually causes an intestinal infection accompanied by diarrhea, fever, and abdominal cramps. Typically, it is mild and self-limiting, often lasting one week or longer. All ages are affected, with the highest incidence being in infants.<sup>(2,5,12)</sup>

Typhoid fever, caused by *S. typhi*, is a serious bloodstream infection common in developing countries. It is rare in the United States and most reported cases are related to foreign travel. Typhoid fever typically presents sustained, debilitating high fever and headache, without diarrhea. There is a long, and highly variable incubation period (1 to 6 weeks). It is transmitted by person to person contact or by fecally contaminated food and water.<sup>(2)</sup> Humans are the only known reservoir for *S. typhi*.<sup>(5)</sup>

In food testing, Bismuth Sulfite Agar is specified as one of the highly selective medias to for isolation of *Salmonella* species from dairy products. Raw milk has been associated with human outbreaks involving *Salmonella*. *Salmonella* infection in dairy cattle are common and outbreaks of bovine salmonellosis have been reported. Dairy cattle can acquire *Salmonella* infection from a variety of sources, including contaminated feed or water. *Salmonella* is incapable of surviving pasteurization. Therefore, its presence in pasteurized milk is usually caused by improper processing or post pasteurization contamination.<sup>(12)</sup>

Bismuth Sulfite Agar is selective due to the presence of inhibitors, and is differential on the basis of hydrogen sulfide production.<sup>(13)</sup> Beef extract and peptones provide nitrogen, vitamins and minerals. Dextrose is an energy source. Bismuth sulfite and brilliant green are selective agents, inhibiting most commensal gram-positive and gram-negative organisms other than *Salmonella* species and some *Shigella* species. Ferrous sulfate is an indicator for hydrogen sulfide

production, which occurs when the H<sub>2</sub> S produced by *Salmonella* reacts with the iron salt. This reaction causes a black or green metallic colony and brown or black precipitate.<sup>(2)</sup>

## FORMULA

Gram weight per liter:	52.0gm/L
Pancreatic Digest of Casein	5.0gm
Peptic Digest of Animal Tissue	5.0gm
Beef Extract	5.0gm
Dextrose	5.0gm
Disodium Phosphate	4.0gm
Bismuth Sulfite Indicator	8.0gm
Ferrous Sulfate	0.3gm
Brilliant Green	0.025gm
Agar	20.0gm

Final pH 7.5 +/- 0.2 at 25°C.

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

## STORAGE AND SHELF LIFE

Store the sealed bottle(s) containing dehydrated culture medium at 2-30°C. Dehydrated culture medium is very hygroscopic. Keep lid tightly sealed. Protect dehydrated culture media from moisture and light. The dehydrated culture media should be discarded if it is not free-flowing or if the color has changed from its original light greenish beige.

Store the prepared culture media at 2-8°C.

The expiration dating on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended quality control incubation times.

Refer to the document "[Storage](#)" for more information.

## PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at [www.cdc.gov/ncidod/dhqp/gl\\_isolation.html](http://www.cdc.gov/ncidod/dhqp/gl_isolation.html).

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

Refer to the document [SDS Search](#) instructions on the Hardy Diagnostics' website for more information.

## METHOD OF PREPARATION FOR DEHYDRATED CULTURE MEDIA

1. Suspend 52.0gm of the dehydrated culture media in 1 liter of distilled or deionized water. Stir to mix thoroughly.
2. Heat to boiling to dissolve completely, approximately 1 minute. Do not overheat.
3. Do not autoclave.
4. Cool to 45-50°C.
5. Mix thoroughly before pouring into petri plates. Use poured plates the same day.

## PROCEDURE AND INTERPRETATION OF RESULTS

For information on procedures and interpretation of results, consult listed references.

## LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification.

Some formulations may require a settling period before pH testing of the prepared medium. If the pH is tested immediately after preparation and is out of specification, retest the medium after 24 hours to obtain final pH results.

Incubation of Bismuth Sulfite Agar plates at higher temperatures (i.e., 43°C.) can result in small, atypical *Salmonella* colonies. In some instances, higher temperatures will also reduce method sensitivity as well as show significantly lower recoveries.<sup>(6)</sup>

Typical *S. typhi* colonies usually develop within 24 hours, however, all plates should be incubated for 48 hours to allow growth of all typhoid strains.<sup>(13)</sup>

Bismuth Sulfite Agar plates should not be stored refrigerated (2-8°C.) for longer than 2 days. After 3 days of storage there is a reduction of media selectivity, decreasing the number of *Salmonella* recovered. It is recommended that Bismuth Sulfite Agar plates be used on the day prepared.<sup>(13)</sup>

Most *Shigella* species are usually inhibited on Bismuth Sulfite Agar; however, *S. flexneri* and *S. sonnei* may exhibit growth.<sup>(13)</sup>

*S. typhi* and *S. arizonae* are the only enteric organisms to exhibit typical brown zones in the medium; although, *S. arizonae* is usually inhibited. Other members of the Enterobacteriaceae do not produce brown zones.<sup>(13)</sup>

It is important to streak for well isolated colonies; in heavy growth areas *S. typhi* appears light green and could be interpreted as negative growth for *S. typhi*.<sup>(13)</sup>

Colonies on Bismuth Sulfite Agar may be contaminated with other viable organisms; therefore, isolated colonies should be subcultured onto a less selective medium (i.e., MacConkey Agar).<sup>(13)</sup>

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as autoclaves, incinerators, and incubators, etc., are not provided.

## QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificates of Analysis (CofA). The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Salmonella enterica</i> ATCC® 14028	A	24-48hr	35°C	Aerobic	Growth; colonies are black or greenish-gray, may have sheen, no zones or halo effect
<i>Escherichia coli</i> ATCC® 25922	B	24-48hr	35°C	Aerobic	Partial to complete inhibition; brown-green colonies
<i>Enterococcus faecalis</i> ATCC® 29212	B	24-48hr	35°C	Aerobic	Partial to complete inhibition

\* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

## USER QUALITY CONTROL

Users of dehydrated culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificates of analysis (CofA) available from Hardy Diagnostics [Certificates of Analysis](#) website. In addition, refer to the following document "[Finished Product Quality Control Procedures](#)," for more information on QC or see reference(s) for more specific information.

## PHYSICAL APPEARANCE

CRITERION™ Bismuth Sulfite Agar powder should appear homogeneous, free-flowing, and light greenish-beige in color. The prepared media should appear opaque, with a flocculent precipitate, and gray-green in color.

## REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.
2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
6. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.
7. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*. AOAC, Arlington, VA.  
<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm>.
8. Atlas, R.M. 1997. *Handbook of microbiological media*, 2nd ed. CRC Press, Inc., Boca Raton, Florida.
9. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*, Lipincott Williams & Wilkins, Philadelphia, PA.

10. Wilson, James W. and E.M.M'V. Blair. 1926. A combination of Bismuth and Sodium Sulphite affording an enrichment and selective medium for the Typhoid-Paratyphoid groups of bacteria. *The Journal of Pathology and Bacteriology*; 29:310-311.
11. Hajna, A.A. and S.R. Damon. 1956. New enrichment and plating media for the isolation of *Salmonella* and *Shigella* organisms. *Applied Microbiology*. 4:341-345.
12. Marshall, R.T., Ph.D. 1993. *Standard Methods for the Examination of Dairy Products*, 16th ed. American Public Health Association, Washington, D.C.
13. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

ATCC is a registered trademark of the American Type Culture Collection.

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