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HARDY DIAGNOSTICS
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Instructions for Use

BRILLIANT GREEN (BG) SULFA AGAR

Cat. no. G87	BG Sulfa Agar, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics BG Sulfa Agar is a selective medium recommended for the isolation of *Salmonella* spp., other than *S. typhi* and *S. paratyphi*, from food following an enrichment procedure.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

In 1925, Kristensen et al. described the first use of Brilliant Green Agar as a primary plating medium for the isolation of *Salmonella* spp.[8] Kauffmann later modified the formula for use in conjunction with tetrathionate broth.[6] In 1955, Osborne and Stokes described the sulfa-modification to further enhance the selective properties of the medium; they found that whole egg and egg yolk products neutralize traditional brilliant green medium and considerably reduced its effectiveness in selecting for *Salmonella*.[11] Osborne and Stokes showed that a small amount of sulfapyridine added to the medium restored its selective properties against *Escherichia coli* and *Proteus* spp., thereby improving *Salmonella* isolation.

Hardy Diagnostics BG Sulfa Agar incorporates phenol red as the pH indicator and brilliant green as an inhibitory agent against gram-positive microorganisms and gram-negative bacilli. Sulfapyridine is a sulfonamide antibiotic effective against gram-positive and gram-negative microorganisms. Casein peptone, digest of animal tissue and yeast extract provide nitrogen, vitamins, and essential minerals for growth. Organisms that ferment lactose and/or sucrose exhibit yellow to yellow-green colonies surrounded by a yellow-green zone. *Salmonella* appears as red to pink-white colonies surrounded by a red zone in the medium. The red coloration indicates the lack of lactose or sucrose utilization.

FORMULA

Ingredients per liter of deionized water:*

Lactose	10.0gm
Sucrose	10.0gm
Sodium Chloride	5.0gm
Pancreatic Digest of Casein	5.0gm
Peptic Digest of Animal Tissue	5.0gm
Yeast Extract	3.0gm
Phenol Red	0.08gm

Sulfapyridine	1.0gm
Brilliant Green	12.5mg
Agar	20.0gm

Final pH 6.9 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date 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 incubation times as stated below.

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." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

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 M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[1-3,5,10,12] Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the specimen should be inoculated onto an appropriate transport medium in order to maintain viability of the organisms.

General Method:

1. Allow the medium to warm to room temperature prior to inoculation.

2. Inoculate the medium from a previously inoculated selective enrichment broth (e.g. Tetrathionate Broth with Brilliant Green (Cat. no. K164) using the four quadrant technique to produce isolated colonies. A heavy inoculum should be used since the medium is quite inhibitory.[3] Alternatively, if a swab is being used, roll the swab over a small area near the edge of the plate and proceed with the streak method as outlined to obtain isolated colonies. In addition, it

is recommended that a non-selective medium (e.g. MacConkey Agar, Cat. no. G35) be streaked in parallel to increase the recovery of gram-negative microorganisms and to characterize other organisms present in the sample.

3. Incubate plates aerobically at 35ºC. for up to 48 hours and examine for typical colony morphology.

Procedure for Testing Food Samples:[2,4]

1. Allow the medium to warm to room temperature as above. Weigh out 25gm of the food sample to be tested into a sterile blender jar. Add 225ml of Nutrient Broth (Cat. no. U322) and blend to mix well. Incubate the sample for 18-24 hours at 35ºC.

2. Transfer 1ml of the incubated nutrient broth to a tube of Tetrathionate Broth with Brilliant Green (Cat. no. K164) or Selenite Cysteine Broth (Cat. no. K69). Incubate both broths for 18-24 hours at 35ºC.

3. Transfer a loopful (10µl) of each broth to separate plates of BG Sulfa Agar and Bismuth Sulfite Agar (Cat. no. C5211) and incubate plates aerobically at 35ºC.

4. Examine plates at 18-24 hours for typical *Salmonella* colonial morphology. If growth is not observed, reincubate the plates for an additional 24 hours.

INTERPRETATION OF RESULTS

Typical *Salmonella* colonies on BG Sulfa Agar appear as red to pink-white with red zones. The red coloration of the medium indicates that lactose or sucrose was not utilized.

Lactose or sucrose fermenting microorganisms not completely inhibited by the medium will show as yellow to yellow-green colonies with a yellow-green or green zone.

Escherichia coli may be partially inhibited and present as yellow to yellow-green colonies with a green halo.

Shigella spp. may exhibit partial to complete inhibition with colorless colonies.

Other non-lactose fermenting microorganisms may mimic enteric pathogens and present as red to pink-white colonies surrounded by red zones. Further biochemical testing is needed to fully identify these strains.[14]

LIMITATIONS

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

Salmonella typhi, *S. paratyphi* and *Shigella* spp. do not grow adequately on this medium, limiting its effectiveness as a screening tool for stool cultures.

The medium is highly selective. Inoculating a less selective medium, such as MacConkey, Hektoen, or other suitable media in conjunction with an enrichment broth, is recommended for best results in isolating all enteric pathogens.

Colonies of other non-lactose fermenting or slow lactose fermenting species, such as *Proteus* or *Pseudomonas*, may mimic the growth of enteric pathogens.[14]

The normal coloration of this medium is brownish-orange, yet it may become more red to bright red upon incubation. The medium will return to its normal color when allowed to equilibrate to room temperature.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media,

incinerators, and incubators, etc., as well as serological and biochemical reagents, 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 Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Salmonella enterica ATCC® 14028**	A	18-48hr	35°C	Aerobic	Growth; red to pink-white colonies with red zones
Escherichia coli ATCC® 25922	B	18-48hr	35°C	Aerobic	Partial inhibition; yellow to yellow-green colonies
Enterococcus faecalis ATCC® 29212**	B	18-48hr	35°C	Aerobic	Inhibited; no color change
Proteus mirabilis ATCC® 12453**	B	18-48hr	35°C	Aerobic	Inhibited; no color change

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

USER QUALITY CONTROL

End users of commercially prepared 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 certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

BG Sulfa Agar should appear slightly opalescent, and brownish-orange 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. D'Aoust, J.Y. and A.M. Sewell. 1986. Slow Rehydration for Detection of *Salmonella* spp. in Feeds and Feed Ingredients. *Appl. and Environ. Micro.* ; Vol. 51, No. 6, p. 1220-1223.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology* , C.V. Mosby Company, St. Louis, MO.

4. Health Protection Branch. 1978. *Methods for the Isolation and Identification of Salmonella from Foods.* Acceptable (HPB) method MFA-20. Health and Welfare Canada, Ottawa.

5. Isenberg, H.D. *Clinical Microbiology Procedures Handbook* , Vol. I, II & III. American Society for Microbiology, Washington, D.C.

6. Kauffmann, F. 1935. *Z. Hyg. Infektionskr.* ; 117:26.

7. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology* , J.B. Lippincott, Philadelphia, PA.

8. Kristensen, M., et al. 1925. *Br. J. Exp. Pathol.* ; 6:291.

9. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria* , Vol. I. Williams & Wilkins, Baltimore, MD.

10. Jorgensen., et al. *Manual of Clinical Microbiology* , American Society for Microbiology, Washington, D.C.

11. Osborne, W.W. and J.L. Stokes. 1955. A Modified Selenite Brilliant-Green Medium for the Isolation of *Salmonella* from Egg Products. *Appl. Microbiol.* ; 3:295.

12. *Quality Assurance for Commercially Prepared Microbiological Culture Media* , M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

13. Sack, R.B., et al. 1980. *Cumitech 12;* American Society for Microbiology, Washington, D.C.

14. Taylor, W.I. 1965. *Am. J. Clin. Pathol.* ; 44:471.

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HDQA 2207D [D]