BRILLIANT GREEN AGAR MEDIA

PRODUCT:

Plated Media:

Brilliant Green Agar	P1177
Brilliant Green Sulfa Agar	P1179

PURPOSE:

Brilliant Green Agar is a highly selective medium designed for the isolation of *Salmonella* species (except *S. typhi* and *S. paratyphi*) from infectious material such as fecal specimens, dairy products, and food. Brilliant Green with Novobiocin is recommended for testing in food for *Salmonella*.

PRINCIPLE:

Kristensen et al.[2] first described the use of Brilliant Green Agar as a primary plating medium for the isolation of *Salmonella.* Later, the formula was modified by Kauffmann[1] who used the medium in conjunction with an enrichment, tetrathionate broth.

The selective agent, brilliant green, is incorporated into the medium to inhibit gram-positive organisms and most gram-negative bacilli. Animal digests and yeast extract provide nitrogen, vitamins, and minerals. Lactose and sucrose provide carbohydrates for fermentation. Sodium chloride maintains the osmotic balance in the media, and agar is the solidifying agent. Inhibition of gram-positive organisms and most gram-negative bacilli other than *Salmonella* is complete even with a moderately heavy inoculum.

Brilliant Green Sulfa Agar is a highly selective medium. Osborne and Stokes[5] added 0.1% sodium sulfapyridine to Brilliant Green Agar to enhance the selective properties of this medium for *Salmonella*. This formula is recommended as a selective isolation medium for *Salmonella* following enrichment.

FORMULA:

Approximate, per liter deionized filtered water.

(1) Brilliant Green Agar Media:

Peptic Digest of Animal Tissue	5.0 g
Pancreatic Digest of Casein	5.0
Yeast Extract	3.0
Lactose	10.0
Sucrose	10.0
Sodium Chloride	5.0
Agar	20.0
Brilliant Green	12.5 mg
Phenol Red	80.0

Final pH 6.9 ± 0.2 at 25°C

(2) Brilliant Green Sulfa Agar:

Same as **(1)** with the addition of 0.08 g of Sulfadiazine

PRECAUTIONS:*

For *in vitro* diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt, store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, or discoloration), or if the expiration date has passed.

Discoloration of the medium will occur if exposed to light for long periods.

Limitations: Processing delays, in excess of 2-3 hours (see “Specimen Collection”) of an unpreserved stool specimen, greatly jeopardizes the recovery of many salmonellas. These organisms are very susceptible to the acidic changes which occur with a drop in temperature of the feces.[6]

Colonies of *Salmonella* species may vary in color from red to pink-white depending on the length of incubation and the strain.[3]

Colony morphology is a presumptive aid only and does not provide information for speciation. Further biochemical and serological tests must be performed to complete the identification.

Other nonlactose-fermenting or slow lactose-fermenting colonies may grow on the agar and mimic the enteric pathogens.[7]

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

The medium is normally reddish-brown to green. Upon incubation, the medium can become bright red, but returns to normal color at room temperature.

This medium is highly selective. It is recommended to inoculate a less selective medium such as MacConkey, Hektoen, or other suitable media, as well as an enrichment broth, for best results in isolating all enteric pathogens.

PROCEDURE:*

Specimen Collection: Information on specimen collection is found in standard reference material. In general, specimens should be protected from extremes of heat and cold and delivered to the laboratory within 2-3 hours. If there is a delay, suitable transport media such as Cary-Blair or Enteric Pathogen Transport must be used to maintain the viability of the organisms.

Method of Use: Prior to inoculation, the medium should be brought to room temperature. Inoculate the infectious material in such a manner as to facilitate isolation of individual colonies. Four-quadrant streaking is recommended for maximum isolation. Incubate aerobically at 35°C and examine plates at 18-24 hours.

Interpretation: Typically, *Salmonella* appear as red to pink-white colonies surrounded by a brilliant red zone in the medium. Lactose or sucrose fermenters, if not inhibited completely, will grow as yellow to greenish-yellow colonies surrounded by a yellow-green zone. Other non-lactose fermenters can also appear as red-pink-white-colored colonies surrounded by red zones in the medium. Organisms producing this effect are possible enteric pathogens and should have further biochemical and/or serological tests performed.

Materials Required but Not Provided: Transport media and standard microbiological supplies and equipment such as those products commonly used in a microbiological laboratory are not provided.

QUALITY CONTROL:*

Brilliant Green agar and Brilliant Green Sulfa agar media:

Microorganisms Used (ATCC #):	**Expected Results:**
Salmonella typhimurium (14028)	Growth; reddish-pink colonies with red diffusion, (black centers observed on BG Sulfa medium)
Shigella flexeneri (12022)	Inhibition, partial (colorless colonies) to complete
Escherichia coli (25922)	Inhibition, partial (yellow colonies) to complete
Enterococcus faecalis (29212)	Inhibition

User Quality Control: Check for signs of contamination and deterioration. Brilliant green media should appear slightly opalescent and reddish-brown in color.

BIBLIOGRAPHY:

1. Kauffmann, F., *Z. Hyg. Infektionskr.*, 117:26, 1935.
2. Kristensen, M., V. Lester, and A. Jurgens, *Br. J. Exp. Pathol.*, 6:291, 1925.
3. MacFadden, J.F. 1985. *Media for Isolation-Cultivation-Identification-Maintenance of Medicinal Bacteria*, Vol. 1 Williams & Wilkins, Baltimore, MD.
4. Miller, R.G. and C.R. Tate. 1990. *Modification of brilliant green agar by adding sodium novobiocin to increase selectivity for Salmonella*. Maryland Poultryman. **4**:7-11
5. Osborn and Stokes. 1955. Appl. Microbiol. 3:295.
6. Sack, R. B., et al., *Cumitech 12*, American Society for Microbiology, Washington, D. C., 1980.
7. Taylor, W. I., *Am. J. Clin. Pathol.*, 44:471, 1965.
8. Murray, P.R., et al., *Manual of Clinical Microbiology, 9th ed.*, American Society for Microbiology, Washington, D. C., 2007.

*For more detailed information, consult appropriate references.

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