BRILLIANT GREEN AGAR (7117)

Intended Use
Brilliant Green Agar is used for the selective isolation of *Salmonella* spp.

Product Summary and Explanation
Salmonellosis continues to be an important public health problem worldwide. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness. Typhoid fever, caused by *Salmonella typhi*, is characterized by fever, headache, diarrhea, and abdominal pain, and can result in fatal respiratory, hepatic, and or neurological damage.[1] Infection can result from consumption of raw, undercooked, or improperly processed foods contaminated with *Salmonella*. U. S. federal guidelines require various poultry products routinely monitored before distribution for human consumption.

Kristensen, Lester, and Jurgens first described the use of Brilliant Green Agar as a primary plating medium for the isolation of *Salmonella*.[2] The report described the medium as useful for the differentiation of "paratyphoid B" from other intestinal gram-negative bacilli.[2] Kaufmann modified their formula and used Brilliant Green Agar in addition to Tetrathionate Broth for the isolation of *Salmonella* from stool specimens.[3]

Brilliant Green Agar is recommended for use in testing clinical specimens.[1,4] The outstanding selectivity of this medium permits use of moderately heavy inocula, which should be evenly distributed over the surface. Brilliant Green Agar is used in the microbial limits test.[5] Brilliant Green Agar supplemented with novobiocin is used in food testing.[6]

Principles of the Procedure
Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue provide sources of nitrogen, amino acids, and carbon. Yeast Extract supplies vitamins required for organism growth. Sodium Chloride maintains the osmotic balance of the medium. Lactose and Sucrose are the carbohydrates in the medium. Brilliant Green inhibits gram-positive bacteria and most gram-negative bacilli other than *Salmonella* spp. Phenol Red is the pH indicator and turns the medium yellow with the formation of acid when lactose and/or sucrose is fermented. Agar is the solidifying agent.

Formula / Liter
Yeast Extract..... 3 g
Enzymatic Digest of Casein ..... 5 g
Enzymatic Digest of Animal Tissue..... 5 g
Sodium Chloride ..... 5 g
Lactose ..... 10 g
Sucrose..... 10 g
Brilliant Green ..... 0.0125 g
Phenol Red ..... 0.08 g
Agar ..... 20 g
Final pH: 6.9 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions
1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 58 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing, and beige with a green tint.

Prepared Appearance: Prepared medium is brown-green, slightly opalescent, and may be trace to slightly hazy.

Expected Cultural Response: Cultural response on Brilliant Green Agar at 35°C after 18 - 24 hours incubation.

Microorganism	Response	Reactions
Escherichia coli ATCC® 25922	partial inhibition	green colonies
Salmonella choleraesuis ATCC® 13076	growth	pink colonies
Salmonella typhi ATCC® 19430	partial inhibition	pink colonies
Salmonella typhimurium ATCC® 14028	growth	pink colonies
Staphylococcus aureus ATCC® 25923	inhibited	---

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

Test Procedure

For isolation of *Salmonella* from clinical specimens, inoculate fecal specimens and rectal swabs on the first quadrant of Brilliant Green Agar and streak for isolation. Incubate plates at 35°C. Examine plates after 18 – 24 hours for colonies with characteristic morphologies associated with *Salmonella* spp. Refer to appropriate references or standard methods for other applications.

Results

Typical *Salmonella* spp. colonies are opaque and pink. The few lactose and/or sucrose fermenting Organisms that grow are readily differentiated due to formation of green colonies. Brilliant Green Agar is not suitable for the isolation of *S. typhi* or *Shigella* spp., although some strains of *S. typhi* may grow.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. 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.

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 when stored as directed.

Limitations of the Procedure

1. Colonies of *Salmonella* spp. can be red, pink, or white depending on length of incubation and strain.[7]
2. Medium is normally orange-brown, however after incubation it can turn bright red and return to normal color at room temperature.[7]
3. Taylor showed that slow lactose fermenters, *Proteus*, *Citrobacter*, and *Pseudomonas* may grow on Brilliant Green Agar as red colonies.[8]
4. Other primary plating medium such as MacConkey Agar should be used when testing for intestinal pathogens. Fluid enrichments, such as Selenite Broth, should be used with Brilliant Green.

Packaging

Brilliant Green Agar	**Code No.**	**7117A**	**500 g**
		7117B	**2 kg**
		7117C	**10 kg**

References

1. **P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
2. **Kristensen, M., V. Lester, and A. Jurgens.** 1925. On the use of trypsinized casein, brom thymol blue, brom cresol purple, phenol red and brilliant green for bacteriological nutrient media. Br. J. Exp. Pathol. **6**:291.
3. **Kauffmann, F.** 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren Fur Salmonellabacillen. Z. Hyg. Infektionskr. **117**:26.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
5. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville. MD.
6. **Federal Register.** 1993. Chicken disease caused by *Salmonella enteritidis*; proposed rule. Fed. Regist. **58**:41048-41061.
7. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, MD.
8. **Taylor, W. I.** 1965. Isolation of shigellae. I. Xylose lysine agars: New media for isolation of enteric pathogens. Am J. Clin. Pathol. **44**:471.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.