



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 BG Agar is recommended 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. Mix thoroughly.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, beige and may have a green tint.

Prepared Appearance: Prepared medium is orange-brown and may have a green tint, and trace to slightly hazy to slightly opalescent.

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

| Microorganism | Approx. Inoculum (CFU) | Expected Results | |
|--------------------------------------------|------------------------|--------------------------------|----------------|
| | | Growth | Reactions |
| <i>Escherichia coli</i> ATCC® 25922 | 1000 | Partial to complete inhibition | Green colonies |
| <i>Salmonella choleraesuis</i> ATCC® 13076 | 10 - 300 | Fair to good | Pink colonies |
| <i>Salmonella typhi</i> ATCC® 19430 | 1000 | None to poor | Pink colonies |
| <i>Salmonella typhimurium</i> ATCC® 14028 | 10 - 300 | Fair to good | Pink colonies |
| <i>Staphylococcus aureus</i> ATCC® 25923 | 1000 | Inhibited | --- |

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

Test Procedure

Inoculate fecal specimens 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.

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. 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 BG Agar as red colonies.⁸
4. Other prepared media should be used along with Brilliant Green Agar when testing for intestinal pathogens.

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. Tenover (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 trypticized casein, brom thymol blue, brom cresol purple, phenol red and brilliant green for bacteriological nutrient media. Br. J. Exp. Pathol. **6**:291.
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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 (517)372-9200 or fax us at (517)372-2006.