

Directions for Preparation from Dehydrated Product

1. Suspend 52 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. DO NOT AUTOCLAVE.
3. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

Meat and Meat Products

1. Weigh 25 g of the sample into a sterile blender jar and add 225 mL of Buffered Peptone Water. Macerate for a sufficient time to give 15,000-20,000 revolutions.
2. Aseptically transfer the contents of the blender jar to a 500 mL flask. Incubate at $37 \pm 0.1^\circ\text{C}$ for 16-20 hours.
3. Transfer 10 mL samples to 100 mL Muller Kauffmann Tetrathionate Broth.
4. Incubate the Muller Kauffmann Tetrathionate Broth at $42-43^\circ\text{C}$.

Sewage Polluted Natural Water

This procedure is applicable to the isolation of *Salmonella* spp. other than *S. typhi*.

1. Inoculate 25 mL aliquots of the sample into 25 mL of double strength Buffered Peptone Water and incubate at 37°C for 18 hours.
2. Transfer 1 mL samples into 10 mL of Muller Kauffmann Tetrathionate Broth.
3. Incubate at 43°C for 48 hours.

Brilliant Green Bile Agar

Intended Use

Brilliant Green Bile Agar is used for isolating, differentiating and enumerating coliform bacteria.

Summary and Explanation

Noble and Tonney¹ described Brilliant Green Bile Agar for determining the relative density of coliform bacteria in water and sewage. The medium is particularly useful in selectively isolating *Salmonella* spp. from other coliform bacteria.

Principles of the Procedure

Brilliant Green Bile Agar contains peptone as a source of carbon, nitrogen, vitamins and minerals. Lactose is a fermentable carbohydrate. Oxgall (bile) and brilliant green inhibit gram-positive bacteria and most gram-negative bacteria except coliforms. Basic fuchsin and eriochlorine are pH indicators. Monopotassium phosphate is a buffering agent. Agar is the solidifying agent.

Differentiation of the coliforms is based on fermentation of lactose. Bacteria that ferment lactose produce acid and, in the

Subculture

1. Subculture from the broth at 18-24 hours and at 48 hours onto Brilliant Green Agar Modified.
2. Examine for typical colonies of *Salmonella* after overnight incubation at 37°C .

Expected Results

Salmonella will produce red colonies.

Limitations of the Procedure

1. Organisms other than *Salmonella* spp., such as *Morganella morganii* and some *Enterobacteriaceae*, may grow on the medium.
2. Confirmatory tests, such as fermentation reactions and seroagglutination, should be carried out on all presumptive *Salmonella* spp.

References

1. Guinee and Kampelmacher. 1962. *Antonie van Leeuwenhoek* 28:417.
2. Heard, Jenner and Linton. 1969. *Br. Vet. J.* 125:635.
3. H. M. S. O. 1982. Methods for the isolation and identification of salmonellae (other than *Salmonella typhi*) from water and associated materials.
4. International Organisation for Standardization. 1974. Draft International Standard ISO/DIS 3565. Geneva, Switzerland.
5. British Poultry Meat Society. 1982. A manual of recommended methods for the microbiological examination of poultry and poultry products.
6. Harvey and Price. 1976. *J. Hyg. Camb.* 77:333.

Availability

Difco™ Brilliant Green Agar Modified

ISO

Cat. No. 218801 Dehydrated – 500 g

Europe

Cat. No. 254490 Prepared Plates – Pkg. of 20*

*Store at $2-8^\circ\text{C}$.

presence of basic fuchsin, form deep red colonies with a pink halo. Bacteria that do not ferment lactose form colorless to faint pink colonies. Coliform bacteria typically ferment lactose, producing deep red colonies, while *Salmonella* spp., which do not ferment lactose, produce colorless to faint pink colonies.

Formula

Difco™ Brilliant Green Bile Agar

Approximate Formula* Per Liter

Peptone	8.25 g
Lactose	1.9 g
Oxgall	2.95 mg
Sodium Sulfite	205.0 mg
Ferric Chloride	29.5 mg
Monopotassium Phosphate	15.3 mg
Agar	10.15 g
Erioglaucine	64.9 mg
Basic Fuchsin	77.6 mg
Brilliant Green	29.5 µg

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

User Quality Control

Identity Specifications
Difco™ Brilliant Green Bile Agar
 Dehydrated Appearance: Light purple, free-flowing, homogeneous.
 Solution: 2.06% solution, soluble in purified water upon boiling. Solution is bluish-purple, slightly opalescent.
 Prepared Appearance: Bluish-purple, slightly opalescent.
 Reaction of 2.06% Solution at 25°C: pH 6.9 ± 0.2

Cultural Response
Difco™ Brilliant Green Bile Agar
 Prepare the medium per label directions. Inoculate using the pour plate technique and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	COLONY COLOR
<i>Enterobacter aerogenes</i>	13048	10 ² -10 ³	Good	Pink
<i>Escherichia coli</i>	25922	10 ² -10 ³	Good	Deep red with bile precipitate
<i>Salmonella choleraesuis</i> subsp. <i>choleraesuis</i> serotype Typhimurium	14028	10 ² -10 ³	Good	Colorless to light pink
<i>Staphylococcus aureus</i>	25923	10 ³ -2 × 10 ³	Marked to complete inhibition	–

Directions for Preparation from Dehydrated Product

- Suspend 20.6 g of the powder in 1 L of purified water. Mix thoroughly.
- Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
- Autoclave at 121°C for 15 minutes.
- Test samples of the finished product for performance using stable, typical control cultures.

Procedure

See appropriate references for specific procedures.^{2,3}

Expected Results

Refer to appropriate references and procedures for results.^{2,3}

Limitation of the Procedure

The medium is sensitive to light, particularly direct sunlight, which produces a decrease in the productivity of the medium and a change in color from deep blue to purple or red. The medium should be prepared just prior to use and, when necessary to store the medium, it should be kept in the dark.

References

- Nobel and Toney, 1935. J. Am. Water Works Assoc. 27:108.
- Clesceri, Greenberg and Eaton (ed.). 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
- Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ Brilliant Green Bile Agar
 Cat. No. 214100 Dehydrated – 500 g

Brilliant Green Bile Broth 2%

Intended Use

Brilliant Green Bile Broth 2% (Brilliant Green Lactose Bile Broth) is used for the detection of coliform organisms in foods, dairy products, water and wastewater, as well as in other materials of sanitary importance.

Summary and Explanation

Brilliant Green Bile Broth 2% is formulated according to the American Public Health Association (APHA)¹ specifications for use in the confirmation of presumptive tests for coliforms.

Principles of the Procedure

Brilliant Green Bile Broth 2% contains two inhibitors of both gram-positive and selected gram-negative organisms; i.e., oxgall and brilliant green dye. Organisms, primarily coliforms, which are resistant to the action of the inhibitors and which ferment the lactose, are able to replicate in this medium. Fermentation is detected by gas production.

