

## BRILLIANT GREEN BILE BROTH 2%

Ready to use tubes

### INTENDED USE

Ready to use tubes for the detection and confirmation of coliform bacteria in water, sewage, dairy products and foodstuffs.

### TYPICAL FORMULA (g/l)

Oxgall	20.0000
Lactose	10.0000
Peptone	10.0000
Brilliant Green	0.0133

Final pH 7.2 ± 0.2

### DESCRIPTION

Brilliant Green Bile Broth 2% is a selective medium recommended for the detection and confirmation of coliform and heat - tolerant coliform bacteria and *E.coli* in water, sewage, dairy products and foodstuffs.

The presence of the brilliant green suppresses the growth of the anaerobic lactose-fermenting bacteria (*Clostridium perfringens*) without obtaining false positives when incubated at 44°C. The brilliant green and bile salts inhibit the growth of Gram-positive microorganisms.

It is recommended by ISO 4831 for the confirmatory test of coliform bacteria in foodstuffs and by ISO 5541/2 as primary inoculation liquid medium for the detection of coliform bacteria in milk and milk products by MPN method.

FDA Bacteriological Analytical Manual reports the use of Brilliant Green Bile Broth 2% for the confirmed test of coliforms. It is recommended by APHA for the confirmatory test of faecal coliform bacteria by incubating at 44°C.

Brilliant Green Bile Broth 2% is used together with Peptone Water at 44°C for the Mackenzie test.

### TECHNIQUE

For the enumeration of coliform bacteria with the most probable number (MPN) technique proceed as following:

1. Prepare the test sample and the decimal dilution in accordance with the specific laboratory method using Maximum Recovery Diluent (Cat. N° 401691) or other suitable diluent.
2. Take three tubes of double-strength Lauryl Pepto Bios Broth and by means of a sterile pipette transfer to each tube 10ml of the test sample, if liquid or 10ml of the initial suspension in the case of other products.
3. Then take three tubes of single-strength Lauryl Pepto Bios Broth and by means of a sterile pipette transfer to each tube 1 ml of the test sample, if liquid or 10ml of the initial suspension in the case of other products.
4. Repeat the inoculation of the single strength and the double strength liquid medium for each further decimal dilution, using a fresh pipette for each dilution.
5. Incubate the tubes of double-strength Lauryl Pepto Bios Broth at 30 or 37°C for 24 +/- 2 hours
6. Incubate the tubes of single-strength Lauryl Pepto Bios Broth at 30 or 37°C for 24 +/- 2 hours and for further 24 hours if neither gas nor opacity is observed after 24 hours.
7. From each of the incubated tubes with double-strength Lauryl Pepto Bios Broth inoculate with a loop a tube of Brilliant Green Bile Broth (confirmation medium) and incubate at 30 or 37°C for 24 +/- 2 hours or, if gas formation is not observed, for 48 +/- 2 hours.
8. Carry out the same procedure for the incubated single-strength Lauryl Pepto Bios Broth showing gas formation or opacity.
9. For each dilution of incubated confirmation liquid medium count the total number of tubes in which gas formation is observed.
10. Express the results as the Most Probable Number of coliforms on the basis of gas production in the Brilliant Green Bile Broth 2% tubes after 48 hours incubation.

For the enumeration of faecal coliform bacteria with the most probable number technique proceed as following:

From each of the positive tubes of MacConkey Broth, inoculate, with a loop, a tube of Brilliant Green Bile Broth 2% and a tube of Peptone Water and incubate at 44 °C. Observe for gas production in Brilliant Green Bile Broth 2%, after 24 and 48 hours of incubation and test the indole production in Peptone Water after 24 hour of incubation. The MacConkey Broth tubes, which are positive to gas production in Brilliant Green Bile Broth 2% and are indole positive must be considered positive for faecal coliforms.

For the detection of coliform bacteria in milk and milk products, with MPN technique, proceed as following:

1. Take three tubes of double strength Brilliant Green Bile Broth 2% and transfer 10ml of the liquid test sample or 10ml of the primary dilution.
2. Take three tubes of single strength Brilliant Green Bile Broth 2% and transfer 1 ml of the liquid test sample or 1 ml of the primary dilution.
3. For each of the dilutions  $10^{-1}$  or  $10^{-2}$  (according to the circumstances) take three tubes of single strength Brilliant Green Bile Broth 2% and transfer 1ml of the respective dilution into each of these tubes.
4. Incubate the tubes of double strength broth at 30°C for 24 hours.
5. Incubate the tubes of single strength broth at 30°C for 48 hours.
6. From each of the incubated tubes of double strength broth inoculate with a loop a tube of single strength broth and incubate at 30°C for 48 hours.
7. From each of the inoculated tubes showing production of gas in the Durham tubes streak a loopful on Levine EMB Agar and incubate at 30°C for 18-24 hours. Consider as characteristic growth those colonies that are metallic, red/pink and mucoid in appearance (confirmatory test)
8. Record for each dilution the number of confirmed positive tubes.

#### PRECAUTIONS

For laboratory use only. Observe approved biohazard precautions and aseptic techniques. To be used only by adequately trained and qualified laboratory personnel. Sterilize all biohazard waste before disposal.

#### STORAGE

Store at 2-8° away from direct light - When stored as directed the tubed media remain stable until the expiry date shown on the label. Do not use beyond stated expiry date. Media should not be used if there are any signs of deterioration, discoloration or contamination.

#### REFERENCES

- . APHA (1980). Standard Methods for the Examination of Water and Wastewater. 15th edition
- . Mackenzie, E.F.W., Taylor, E.W. and Gilbert, W.E. (1948), J. Gen. Microbiol., **2**,197
- . FDA (1995) Bacteriological Analytical Manual, 8<sup>th</sup> ed. Revision A, 1998. Published by AOAC International.
- . ISO 4833 Microbiology-General guidance for the enumeration of coliforms – Most probable number technique. 1991-03-01
- . ISO 5541-2 Milk and Milk products- Enumeration of coliforms – Part 2 Most probable number technique at 30°C . 1986-12-01

#### PACKAGING

**551265 Brilliant Green Broth 2%, 20 x 10 ml ready to use fermentation tubes**