

TECHNICAL DATA SHEET

BRILLIANT GREEN BILE BROTH (BGBB)

CONFIRMATION OF COLIFORMS

1 INTENDED USE

Brilliant Green Bile Broth (BGBB) is used for the confirmation of coliforms and thermotolerant coliforms in food products, water used for watering food products and for effluent / run-off water testing. It can also be used as an enumeration media for coliforms in frozen dairy and ice cream products.

The typical composition of the media responds to that defined in the food microbiology standards NF ISO 4831 and NF ISO 4832. It also conforms to the standards used for water control NF T90-413 and PR NF T90-413.

2 HISTORY

Research and development of a culture medium inhibiting microorganisms other than coliform bacteria has long interested bacteriologists. In 1926, Dunham and Schoenlein studied the proportions of bile and brilliant green which could give good results. Jordan showed that this medium was better than Lactose Broth for the detection of coliform bacteria in water. For the control of milk pasteurization, MacCraday and Langevin satisfactorily used Brilliant Green Bile Broth for the detection of coliform bacteria. Mackenzie verified that the brilliant green concentration was sufficient to effectively inhibit the culture of lactose-fermenting anaerobes, in particular *Clostridium perfringens*.

3 PRINCIPLES

The simultaneous presence of ox bile and brilliant green inhibit almost all Gram-positive organisms and Gram-negative bacteria other than coliforms.

The brilliant green concentration was specifically determined in order to prevent the growth of lactose-fermenting anaerobes at 44°C, which avoids false positives.

Development of coliform bacteria is shown by turbidity and gas production in the Durham tubes, as a result of lactose fermentation.

4 TYPICAL COMPOSITION

The composition can be adjusted to obtain optimal performance.

For 1 liter of media :

- Tryptone	10,0 g
- Bacteriological ox bile	20,0 g
- Lactose.....	10,0 g
- Brilliant green	13,3 mg

pH of the ready-to-use media at 25 °C : 7,2 ± 0,2.

5 PREPARATION

- Dissolve 40.0 g of dehydrated medium (BK002) in 1 liter of distilled or deionized water.
- Stir slowly until complete dissolution.
- Dispense in appropriately-sized tubes containing a Durham tube.
- Sterilize in an autoclave at 121°C for 15 minutes.
- After cooling, the Durham tubes should not contain trapped air.

✓ **Reconstitution :**
40,0 g/L

✓ **Sterilization :**
15 min at 121 °C

Note :

Double concentration broth can be used for certain specific applications.
Suspend 80,0 g of dehydrated media (BK002) in 1 liter of distilled or demineralized water.

6 INSTRUCTIONS FOR USE

Confirmation of coliforms (ISO standard 4831) :

- Inoculate a loop from Laurylsulfate Tryptose broth (simple & double concentration) in tubes of BGGB prepared previously or ready-to-use (BM011).
- Incubate for 24 ± 2 hours at 30 ± 1 °C or at 37 ± 1 °C, according to the protocol being followed.
- If the production of gas is not observed at this stage, continue to incubation another 48 ± 2 hours.

✓ **Inoculation :**
1 loop

✓ **Incubation :**
24 to 48 h at 30 or 37°C

Confirmation of coliforms (ISO standard 4832) :

- Take 5 characteristic colonies from VRBL agar and inoculate each of them into previously made tubes of BGGB or into ready-to-use tubes (BM011).
- Incubate for 24 ± 2 hours at 30 + 1°C or at 37 ± 1 °C, according to the protocol being followed.

✓ **Inoculation :**
Inoculate 5 colonies

✓ **Incubation :**
24 h at 30 or 37 °C

Confirmation of coliforms in water (NF standard T90-413) :

- From presumptive media (Lactose broth or Laurylsulfate simple or double concentration), inoculate a loop of culture to be confirmed into previously made tubes of BGGB or into ready-to-use tubes (BM011).
- Incubate for 48 hours at 37 ± 1 °C for coliforms or at 44 ± 1 °C in a water bath for thermotolerant coliforms.

✓ **Inoculation :**
1 loop

✓ **Incubation :**
48 h at 37 or 44 °C

7 RESULTS

Lactose fermentation, which results in the production of gas in the Durham tubes (volume at least equal to 1/10 of the tube volume) in less than 48 hours, indicates the presence of coliform bacteria.

8 QUALITY CONTROL

Dehydrated media : greenish powder, free-flowing and homogeneous.

Prepared media : green solution, limpid.

Typical culture response after 24-48 hours of incubation at 30 °C (NF EN ISO 11133) :

Microorganisms		Growth	Gas production (Durham tube)
<i>Escherichia coli</i>	WDCM 00012	Good, score 2	≥ 5 mm
<i>Citrobacter freundii</i>	WDCM 00006	Good, score 2	≥ 5 mm
<i>Enterococcus faecalis</i>	WDCM 00087	Partially inhibited, score 0-1	Negative

Typical culture response after 48 hours of incubation at 37 °C (FD T 90-461) :

Microorganisms		Growth	Gas production (Durham tube)
<i>Escherichia coli</i>	WDCM 00179	Good, score 2	≥ 5 mm
<i>Enterococcus faecalis</i>	WDCM 00176	Partially inhibited, score 0-1	Negative

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.

Ready-to-use media, simple concentration, in tubes containing Durham tubes : 2-8 °C.

The expiration dates are indicated on the labels.

Prepared media in tubes^(*) : 90 days at 2-25 °C, shielded from light.

^(*)Benchmark value, determined in standard conditions of preparation, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :

500 g bottle BK002HA

Ready-to-use media (simple concentration) in tubes with Durham tubes :

50 x 10 mL tubes BM01108

11 BIBLIOGRAPHY

Dunham, H.G. and Schoenlein , H.W.. 1926. Stain Technology, **1** : 129.

Jordan. 1927. Journal of American Water Works Association, **18** : 337.

McCrary and Langevin. 1932. Journal of Dairy Science, **15** : 321.

Mackenzie, E.F.W., Taylor, W.E., and Gilbert, W.E.. 1948. Recent experiments in the rapid identification of *Bacterium coli* type I. Journal of General Microbiology, **2** : 197-204.

Rodier, J. 1984. L'analyse de l'eau. Dénombrement des coliformes, coliformes fécaux, et *Escherichia coli* présumés. Dunod 7ème Ed., 793-798.

NF T 90-413. Octobre 1985. Essais des eaux. Recherche et dénombrement des coliformes et des coliformes thermotolérants. Méthode générale par ensemencement en milieu liquide (NPP).

PR NF T 90-413. Mars 2012. Essais des eaux. Recherche et dénombrement des coliformes et des coliformes thermotolérants. Méthode générale par ensemencement en milieu liquide (NPP).

Journal Officiel du 21 Septembre 1968. Méthodes officielles de prélèvement et d'analyse bactériologiques des glaces et crèmes glacées (Arrêté du 30 Août 1968).

NF ISO 4831. Octobre 2006. Microbiologie des aliments. Méthode horizontale pour la recherche et le dénombrement des coliformes. Technique du nombre le plus probable.

NF ISO 4832. Juillet 2006. Microbiologie des aliments. Méthode horizontale pour le dénombrement des coliformes. Méthode par comptage des colonies.

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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