

## BGBLB/Broth

(Bile Green Brilliant Lactose Broth)

**356-4054**  
**357-8024**

### DEFINITION

Selective broth used as presumptive medium and for confirmation in the enumeration of coliforms and thermotolerant coliforms in food products and waters (MPN - Most Probable Number - technique).

### STANDARDS

#### FOOD MICROBIOLOGY

- **NF ISO 4831 (October 2006):** Microbiology Horizontal method for the detection and enumeration of coliforms - Most Probable Number technique
- **NF ISO 4832 (July 2006):** Microbiology - Horizontal method for the enumeration of coliforms - Colony count technique
- **FIL 73A (1985):** Milk and milk products - Enumeration of coliforms - Colony count at 30°C and Most Probable Number techniques

#### WATER

- **NF T90-413 (October 1985):** Water testing - Detection and enumeration of coliforms and thermotolerant coliforms – General method by inoculation in liquid medium (MPN)
- **NF T90-461/A2 (May 2007):** Water quality - Microbiology - Quality control of culture media

### PRINCIPLE

The principle of the medium relies on the ability of coliforms to ferment lactose with production of gas. This can be seen by using a Durham bell jar. The combination of bile and brilliant green partially or totally inhibits all microorganisms other than coliforms.

### PRESENTATION

- **Ready to use (single-concentration)**  
10 ml x 25 tubes **code 357-8024**
- **Dehydrated**  
500 g **code 356-4054**

### STORAGE

- Ready to use: +15-25°C, in a dark place
- Dehydrated: +15-25°C, in carefully sealed bottles in a cool, dry place

- Expiration date and batch number are shown on the package.

### THEORETICAL FORMULA

#### Single-concentration medium

Peptone	10 g
Dehydrated bovine bile	20 g
Lactose	10 g
Brilliant green	13.3 mg
Distilled water	1,000 ml

Final pH (25°C) = 7.2 ± 0.2

### OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- Distilled water

### EQUIPMENT REQUIRED (NOT SUPPLIED)

(non-exhaustive)

- Scales
- Sterile weighing bags
- Grinder
- Mixer-homogeniser
- Test tubes (16 x 160 mm and 20 x 200 mm) with autoclave-proof stoppers and Durham bell jar
- Sterile pipettes (1 ml, 10 ml...)
- Thermostatically-controlled incubator or incubation room, precise to ±1°C
- Autoclave
- All usual laboratory equipment

### PREPARATION OF DEHYDRATED MEDIUM

**Always shake before use.**

#### Single concentration medium

Dissolve 40 g of powder in 1 litre of distilled water. Heat gently if necessary and agitate until completely dissolved. Dispense 10 ml of medium per tube (16 x 160 mm) containing a Durham bell jar. Sterilise in autoclave at 115°C (± 1°C) for 20 minutes.

#### Reconstitution ratio: 40 g/l

**500 g of powder make 12.5 litres of single concentration medium.**

*NB:*

- *Double concentration medium:*  
Dissolve 80 g of powder in 1 litre of distilled water. Utilisation of 20 x 200 mm tubes.
- *Triple concentration medium:*  
Dissolve 120 g of powder in 1 litre of distilled water. Utilisation of 20 x 200 mm tubes.

## PROTOCOL

### • Preparation of samples

According to standards for the product concerned.

### • Inoculation and incubation

Use the BGBLB medium as:

#### 1. Presumptive medium

According to standards:

- Inoculate 3 tubes of single concentration BGBLB medium with 1 ml of stock solution and/or 1 ml of each of its decimal solutions. Incubate at 30°C ( $\pm 1^\circ\text{C}$ ) for 48 hours ( $\pm 2$  hr).
- Inoculate 3 tubes of double or triple concentration BGBLB with 10 ml of stock solution and/or 10 ml of each of its decimal solutions. Incubate at 37°C ( $\pm 1^\circ\text{C}$ ) for 48 hours ( $\pm 2$  h).

#### 2. Confirmation medium

Using a positive presumptive medium<sup>(1)</sup>, collect a drop by means of an inoculating loop or Pasteur pipette (previously flame sterilised) and inoculate the single concentration BLBBG broth. Incubate:

- (according to standards) at 30°C or 35°C or 37°C ( $\pm 1^\circ\text{C}$ ) for 24 hours ( $\pm 2$  hr) and/or for 48 hours ( $\pm 2$  hr) for the detection of coliforms.
- at 44°C ( $\pm 0,5^\circ\text{C}$ ) for 24 hours ( $\pm 2$  hr) and/or 48 hours ( $\pm 2$  hr) for the detection of thermotolerant coliforms.

*NB: To detect the indole production, parallel inoculation (according to the standards) of tubes of indole-free peptone water (codes 355-4180, 356 4334) or Schubert broth (codes 355-5694, 356-9634) or Tryptophane broth (code 355-4194) should be carried out.*

(1) Lauryl Sodium Sulphate broth or BLBGB or BCP-Lactose broth (codes 355-3414/356-4044).

See corresponding Technical Sheet(s)

## READING AND INTERPRETATION

### • Reading

After the incubation period, tubes presenting turbidity and gas emission (1/3 of total tube volume) can be considered positive.

### • Expression of results / Calculation

Using MPN tables, express the result according to the specific standards.

## PRECAUTIONS

- Prior to inoculation, eliminate all air present in the Durham bell jar.
- Comply with Good Laboratory Practice.

## QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

## PERFORMANCES/QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Gas production after 48 hr at 30°C
<i>Escherichia coli</i> RIVM WR1 *	+
<i>Escherichia coli</i> ATCC 25922	+
<i>Citrobacter freundii</i> ATCC 43864	+
<i>Enterococcus faecalis</i> ATCC 19433	-
STRAINS	Gas production after 48 hr at 44°C
<i>Escherichia coli</i> RIVM WR1 *	+

\* RIVM WR1 is equivalent to NCTC 13167

## KEY WORDS

BLBGB/Coliforms/*Escherichia coli*/Thermotolerant/Food products/Water/Enumeration/Brilliant Green/Lactose/Durham bell jar/Gas/MPN (Most Probable Number)/Broth