Brilliant Green Bile Broth 2%

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

Summary
Brilliant Green Bile Broth 2% is formulated as per American Public Health Association specifications for use in presumptive identification and confirmation of coliforms. It is included in the Bacteriological Analytical Manual for food testing.

Principle
Peptone provides the essential nutrients. Lactose is the fermentable carbohydrate. Oxgall and brilliant green inhibit most of the Gram-positive organisms including lactose fermenting Clostridia and selected Gram-negative organisms. Coliforms, which are resistant to the action of inhibitors and which ferment lactose, can replicate in this medium. Fermentation is detected by gas production and is seen as bubbles in the inverted Durham's tubes. Production of gas in the inverted Durham's tube indicates presence of faecal coliforms, since non-faecal coliforms growing in this medium do not produce gas.

Formula*

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxgall</td>
<td>20.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>10.0</td>
</tr>
<tr>
<td>Brilliant Green</td>
<td>0.0133</td>
</tr>
<tr>
<td>Peptone</td>
<td>10.0</td>
</tr>
<tr>
<td>Final pH (at 25°C)</td>
<td>7.2 ± 0.2</td>
</tr>
</tbody>
</table>

*Adjusted to suit performance parameters

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of specimen
Food and dairy samples
Water samples

Specimen Collection and Handling
Ensure that all samples are properly labelled.
Follow appropriate techniques for handling samples as per established guidelines.
Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.
The samples must be stored and tested within the permissible time duration.
After use, contaminated materials must be sterilized by autoclaving before discarding

Directions
1. Suspend 40.01 g of the powder in 1000 mL distilled water and mix well.
2. Warm slightly to dissolve the powder completely.
3. Dispense into tubes containing inverted Durham's tubes.
4. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.
5. DO NOT AUTOCLAVE DOUBLE STRENGTH BROTH
Quality Control

**Dehydrated Appearance:** Beige to greenish-beige, homogeneous, free flowing powder.

**Prepared Appearance:** Emerald green coloured, clear solution without any precipitate.

**Growth Promotion Test:** Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP/IP and growth is observed after an incubation at 30°C-35°C for 18-48 hours.

**Growth Promoting Properties:** The test results observed are within the specified temperature and shortest period of time, inoculating ≤ 100 cfu (at 30°C-35°C for 18 hours).

**Inhibitory Properties:** No growth of the test microorganism occurs for the specified temperature and the longest period of time specified inoculating >100 cfu (at 30°C-35°C for ≥ 48 hours).

### Organisms (ATCC)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth</th>
<th>Gas</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterobacter aerogenes</em> (13048)</td>
<td>Good</td>
<td>+</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (8739)</td>
<td>Good</td>
<td>+</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (25922)</td>
<td>Good</td>
<td>+</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (6538)</td>
<td>Inhibited</td>
<td>-</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> (25923)</td>
<td>Inhibited</td>
<td>-</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> (29212)</td>
<td>Inhibited</td>
<td>-</td>
</tr>
</tbody>
</table>

**Interpretation of Results**

1. The medium becomes turbid and yellowish green in colour when bacterial growth occurs, and when accompanied by copious gas formation, it is presumptive of the presence of coli-aerogenes organisms.
2. Turbidity and gas production in the Brilliant Green Bile Broth 2% incubated at 44°C is indicative of a positive test for *E. coli*. To confirm the presence of *E. coli*, carry out indole production test at 44°C in Tryptone water.

**Performance and Evaluation**

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

**Precautions/Limitations**

1. Do not autoclave double strength broth.
2. Gram-positive spore forming organism may produce gas to give a false positive reaction if the bile / brilliant green inhibition is compromised by the food material.
3. Turbidity alone is not a positive test for coliforms.
4. It may be necessary to invert the tube prior to inoculation if bubbles are trapped in the Durham's tube.

**Warranty**

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

**Reference**

4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.
<table>
<thead>
<tr>
<th>Cat No.</th>
<th>Product description</th>
<th>Pack Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>201020320100</td>
<td>Dehydrated Culture Media</td>
<td>100 g</td>
</tr>
<tr>
<td>201020320500</td>
<td>Dehydrated Culture Media</td>
<td>500 g</td>
</tr>
</tbody>
</table>

**Disclaimer**

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.