M-Brilliant Green Broth

Intended Use
M-Brilliant Green Broth is used as a selective differential medium for primary screening of Salmonellae in polluted water using membrane filter technique.

Summary
Salmonella belongs to the family *Enterobacteriaceae.* They are Gram-negative, facultative anaerobic, nonsporulating, rod. These organisms are difficult to differentiate biochemically from *Escherichia coli*. Geldreich and Jeter developed membrane screening technique. Kabler and Clark applied M-Brilliant Green Broth for primary screening of *Salmonella* in polluted water. This selective differential medium is a modification of Brilliant Green Agar without agar in double strength. *Salmonella* are unable to ferment either lactose or saccharose in the medium. This allows identification of accompanying weakly lactose-positive or lactose negative, but saccharose positive microorganisms. In this technique, suitable and known quantity of water is passed through membrane filter and this filter is then kept on an absorbent pad saturated with M-Tetrathionate Broth. It is then incubated in humid atmosphere for 3 hours at 35°C and then the membrane is transferred to another absorbent pad saturated with M-Brilliant Green Broth and the incubation is continued for 15 more hours at 35°C. After the total of 18 hours incubation, the membrane is transferred to a fresh pad soaked in urease test reagent (20 g urea, 0.16 g bromothymol blue, 0.2 g phenol red, all components in 1 litre of distilled water). Urease test reagent is recommended for use in the membrane filter technique for screening of *Salmonella*. Urease test reaction is recorded after 20 minutes.

Principle
Proteose peptone and yeast extract in the medium are sources of carbon, nitrogen, vitamins and minerals. Lactose and saccharose are the carbon and energy sources. Sodium chloride provides essential ions. Phenol red is the pH indicator. Brilliant green inhibits Gram-positive and most of the Gram-negative bacteria except *Salmonella*.

Formula*

Ingredients	**g/L**
Proteose Peptone	20.0
Yeast Extract	6.0
Lactose	20.0
Saccharose	20.0
Sodium Chloride	10.0
Phenol Red	0.16
Brilliant Green	0.025
Final pH (at 25°C)	6.9 ± 0.2

*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
Water and Waste 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 76.19 g of the powder in 1000 mL purified / distilled water.
2. Heat if necessary to dissolve the powder completely. DO NOT AUTOCLAVE.
3. Cool to 35°C and saturate sterile absorbent cotton pad with 2 mL of the broth.
4. The medium should be used within 24 hours of rehydration.

Quality Control

Dehydrated Appearance: Beige to greenish-beige, homogeneous coloured, free flowing powder.
Prepared Appearance: Greenish brown coloured, clear to slightly opalescent solution without any precipitate.
Cultural Response: Cultural characteristics observed after incubation at 35°C-37°C for 18-24 hours on membrane filter in humid atmosphere.

Organism (ATCC)	Growth	Colour of Colony (on membrane filter)
Escherichia coli (25922)	Partial Inhibition	Yellowish green
Salmonella Typhi (6539)	Good	Reddish pink
Salmonella enterica subsp. *enterica* serovar *Typhimurium* (14028)	Good	Pinkish white
Staphylococcus aureus subsp. *aureus* (25923)	Inhibited	-

Result and Interpretation

1. Purple colonies that are urease positive and lactose and saccharose negative, are probably of *Proteus* species.
2. Yellow colonies that are urease negative and lactose or saccharose positive are coliforms.
3. Pink to red colonies that are urease negative and lactose and saccharose negative are probably enteric pathogens.
4. Since the urease colour reaction will eventually diffuse over the entire membrane surface, it is recommended that selection of red or pink colonies, for further subculture and serological tests to be done within 15-30 minutes after diffusion of reagent.

Performance and Evaluation

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

1. Directions
2. Storage
3. Expiry

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

1. Geldreich E. E. and Jeter M. L., 1952, Bact. Proc. SAB, Boston, P.33.
2. Kabler P. W. and Clark H. F., 1952, American J. Publ. Hlth., 42:390.
3. Kauffmann F., 1935, Z. Hyg. Infektionskr., 117:26.
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201130020100	Dehydrated Culture Media	100 g
201130020500	Dehydrated Culture Media	500 g