M-Brilliant Green Broth

M1102

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

Composition**

Ingredients	Gms / Litre
Proteose peptone	20.000
Yeast extract	6.000
Lactose	20.000
Saccharose	20.000
Sodium chloride	10.000
Phenol red	0.160
Brilliant green	0.025
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 76.19 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. DO NOT AUTOCLAVE. Cool to 35°C and saturate sterile absorbent cotton pad with 2 ml of the broth. The medium should be used within 24 hours of rehydration.

Principle And Interpretation

Salmonella is a gram-negative, facultatively anaerobic, non-sporulating, rod in the family *Enterobacteriaceae* . They are widely distributed in animals affecting mainly the stomach and the intestines. These organisms are difficult to differentiate biochemically from *Escherichia coli* . Geldreich and Jeter (1) developed membrane screening technique. Kabler and Clark (2) 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 (3). *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.

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* .

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 (M1115). 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 grams urea, 0.16 grams bromothymol blue, 0.2 grams 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. Purple colonies that are urease positive and lactose and saccharose negative, are probably of *Proteus* species. Yellow colonies that are urease negative and lactose or saccharose positive are coliforms. Pink to red colonies that are urease negative and lactose and saccharose negative are probably enteric pathogens. 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.

Quality Control

Appearance

Light yellow to pink homogeneous free flowing powder

Colour and Clarity of prepared medium
Greenish brown coloured clear to slightly opalescent solution

Reaction
Reaction of 7.62% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

Cultural Response
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours on membrane filter in humid atmosphere.

Cultural Response

Organism	Inoculum (CFU)	Growth	Colour of colony (on membrane filter)
Escherichia coli ATCC 25922	50-100	none-poor	yellowish green
Salmonella Typhi ATCC 6539	50-100	poor-fair	reddish pink
Salmonella Typhimurium ATCC 14028	50-100	good-luxuriant	pinkish white
Salmonella Enteritidis ATCC 13076	50-100	good-luxuriant	pinkish white
Staphylococcus aureus ATCC 25923	>=10^3	inhibited	-

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label.

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.

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