

Buffered Glucose Broth (MR-VP Medium), Granulated

GM070S

Buffered Glucose Broth (MR-VP Medium), granulated is recommended for studying Methyl Red and Voges-Proskauer tests to differentiate amongst coli-aerogenes group.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Dextrose	5.000
Dipotassium phosphate	5.000
Final pH (at 25°C)	7.5±0.1

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 15 grams in 1000 ml of distilled water. Distribute in test tubes in 3 ml amounts or as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Clark and Lubs (1) found that the addition of methyl red to cultures of *Escherichia coli* resulted in a red colour due to high acidity produced during dextrose fermentation. Voges-Proskauer (2) reported red colouration after addition of potassium hydroxide to specific culture media with organisms grown in it. The investigators developed MR-VP Broth which enables both tests to be performed in same medium in different tubes. The red colour produced by the addition of potassium hydroxide to cultures is due to the ability of organisms to produce a neutral product acetoin (acetyl methyl carbinol) from dextrose (3). The acetoin is oxidized in the presence of oxygen and alkali to produce diacetyl which reacts with creatine to produce a red colour. This formulation is in accordance with standards as BIS (5) and ISO committee (8) recommended for the detection of coli-aerogenes group. A slightly modified medium i.e GM070S/M070S is recommended by BIS (4,6,7) for the detection of *E. coli*, *Vibrio parahaemolyticus* and *Bacillus cereus* responsible for food poisoning. To test *V.parahaemolyticus* for VP, addition of 2-3% Sodium chloride to the medium is required.

The Methyl Red (MR) test is performed after maximum of 5 days of incubation at 30°C (9) and Voges-Proskauer test (VP) cultures are incubated at 30°C for 24-48 hours (10). Various other tests have been suggested by Werkman (11), OMeara (12) Levine, Epstein and Voughn (13) and Voughn, Mitchell and Levine (9). Werkmans Test (8): Add 2 drops of a 2% solution of ferric chloride to 50 ml culture and 5 ml of 10% sodium hydroxide. Shake the tube to mix well. Stable copper colour developing in a few minutes is positive reaction. OMeara Test (8): Add of 25 mg of solid creatine to 5ml culture and then add 5 ml concentrated (40%) sodium hydroxide. Red colour development in a few minutes after shaking the tube well, is a positive reaction. Levine, Epstein and Voughn (13) modified OMeara technique by dissolving the creatine in a concentrated solution of potassium hydroxide. Voughn, Mitchell and Levine (9) recommended the method of Barritt (14) as, addition of 1 ml of 40% potassium hydroxide and 3 ml of 5% a - naphthol in absolute ethanol to 5 ml culture. Positive test is indicated by eosine pink colour within 2-5 minutes.

Quality Control

Appearance

Cream coloured granular medium

Colour and Clarity of prepared medium

Light yellow coloured clear solution without any precipitate.

Reaction

Reaction of 1.5% w/v aqueous solution at 25°C. pH : 7.5±0.1

pH

7.40-7.60

Cultural Response

Cultural characteristics observed after an incubation at 30°C for 48 hours .

Organism	Inoculum (CFU)	Growth	MR Test	VP Test
Cultural Response <i>Bacillus cereus</i> ATCC 10876	50-100	luxuriant	Negative reaction, yellow colour	Positive reaction, eosin pink / red colour within 2-5 minutes
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	Negative reaction, yellow colour	Positive reaction, eosin pink / red colour within 2-5 minutes
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	Positive reaction , bright red colour	Negative reaction, no colour change
<i>Klebsiella pneumoniae</i> ATCC 23357	50-100	luxuriant	Negative reaction, yellow colour	Positive reaction, eosin pink / red colour within 2-5 minutes
<i>Salmonella</i> Typhi ATCC 6539	50-100	luxuriant	Positive reaction , bright red colour	Negative reaction, no colour change
<i>Vibrio parahaemolyticus</i> ATCC 17802	50-100	poor	Negative reaction, yellow colour	Negative reaction, no colour change

Storage and Shelf Life

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

Reference

- 1.Clark and Lubs, 1915, J. Inf. Dis., 17 : 160.
- 2.Voges and Proskauer, 1898, Zeit, Hyg., 28 : 20. ,,
3. MacFaddin, J.F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
- 4.Bureau of Indian Standards, IS : 5887 (Part I) 1976, reaffirmed 1986.
- 5.Bureau of Indian Standards, IS : 5887 (Part - III) 1976.
- 6.Bureau of Indian Standards, IS : 5887 (Part IV) 1976.
- 7.Bureau of Indian Standards, IS : 5887 (Part - V) 1976, reaffirmed 1986.
- 8.International Organization for Standardization (ISO), 1993, Draft ISO/DIS 6597.
- 9.Vaughn, Mitchell and Levine, 1939, J. Am. Water Works Association, 31:993.
10. Kallas, Chinn and Coulter, 1931, J. Bact., 22 : 125.
11. Werkman, 1930, J. Bact., 20 : 121.
12. OMeara, 1931, J. Path. Bacteriol., 34 : 401.
13. Levine, Epstein and Voughn, 1934, Am. J. of Publ. Health, 24 : 505.
14. Barritt, 1936, J. Path. Bacteriol., 42 : 441.

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