MR-VP HiVeg™ Medium (Buffered Glucose HiVeg™ Broth / Glucose Phosphate HiVeg™ Broth), Granulated

GMV070

MR-VP HiVeg™ Medium (Buffered Glucose HiVeg Broth/ Glucose Phosphate HiVeg™ Broth), granulated is recommended for the performance of the Methyl Red and Voges-Proskauer tests in differentiation of the coli-aerogenes group.

Composition**

Ingredients	Gms / Litre
Buffered HiVeg peptone	7.000
Dextrose	5.000
Dipotassium phosphate	5.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 17 grams in 1000 ml of distilled water. Heat, if necessary to disslove the medium completely. Distribute in test tubes in 10 ml amounts or as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Buffered Glucose HiVeg Broth is prepared by using vegetable peptones in place of animal peptones which is free from BSE/ TSE risks. Methyl Red and Voges-Proskauer test are among the two various tests used in the biochemical identification of bacterial species. These tests were originally studied by Voges, Proskauer (1) and subsequently by Clark and Lubs (2) to differentiate between members of the coli- aerogenes group. Both the tests are based on the detection of specific breakdown products of carbohydrate metabolism. All members of *Enterobacteriaceae* are, by definition, glucose fermenters. In MR-VP Broth, after 18-24 hours of incubation, fermentation produces acidic metabolic byproducts. Therefore initially all enterics will give a positive MR reaction if tested (3-5). However, after further incubation, required by the test procedure (2-5 days), MR – positive organisms continue to produce acids, resulting in a low pH (acidic) that overcomes the phosphate buffering system and maintain an acidic environment in the medium (pH 4.2 or less). MR-negative organisms further metabolize the initial fermentation products by decarboxylation to produce neutral acetyl methylcarbinol (acetoin), which results in decreased acidity in the medium and raises the pH towards neutrality (pH 6.0 or above) (6). In the presence of atmospheric oxygen and alkali, the neutral end products, acetoin and 2, 3-butanediol, are oxidized to diacetyl, which react with creatine to produce a red colour. The Methyl Red (MR) test is performed after 5 days of incubation at 30°C (7,8). The Voges-Proskauer test (VP) cultures are incubated at 30°C for 24-48 hours (9). Various test procedures have been suggested for performing the VP test by Werkman, OMeara (7) Levine, et al and Voughn et al . Werkmans Test: 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: Add 25 mg of solid creatine to 5 ml 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 modified OMeara technique by dissolving the creatine in a concentrated solution of potassium hydroxide (R031, OMeara Reagent). Voughn, Mitchell and Levine recommended the method of Barritt as, addition of 1 ml of Barritt Reagent B (R030 - 40% potassium hydroxide) and 3 ml of Barritt Reagent A (R029 - 5% a-naphthol in absolute ethanol) to 5 ml culture. Positive test is indicated by eosin pink colour within 2-5 minutes. The MR and VP tests should not be relied upon as the only means of differentiating from the groups. Also occasionally a known acetoin-positive organism fails to give a positive VP reaction. To overcome this possibility, gently heat the culture containing the VP reagents.

Quality Control

Appearance

Cream to yellow coloured granular medium

Prepared Medium

Light yellow clear solution in tubes

Reaction
Reaction of 1.7% 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 30°C for 48 hours.

Cultural Response

Organism	Growth	MR Test	VP Test
Cultural Response			
Enterobacter aerogenes ATCC 13048	luxuriant	Negative reaction, yellow colour	Positive reaction, eosin pink / red colour within 2-5 minutes
Escherichia coli ATCC 25922	luxuriant	Positive reaction, bright red colour	Negative reaction, no colour change
Klebsiella pneumoniae ATCC 23357	luxuriant	Negative reaction, yellow colour	Positive reaction, eosin pink / red colour within 2-5 minutes

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.Voges. and Proskauer. 1989. Zeit, Hyg, 28.
2.Clark. and Lubs. 1915. J. Inf. Dis, 17.
3.MacFaddin, J. F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria vol. 1. Baltimore: Williams and Wilkins.
4.International Organization for Standardization (ISO), 1993, Draft ISO/DIS 6597.
5.Vaughn., Mitchell. and Levine. 1939. J. Am. Water Works Association, 31.
6.Kallas., Chinn. and Coulter. 1931. J. Bact, 22.
7.O'Meara. 1931. J. Path. Bacteriol, 34.
8.Werkman. 1930. J. Bact., 20.
9.Levine. 1934. Am. J. Publ. Health, 24.

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