Technical Information

MR-VP Medium (Glucose Phosphate Broth)

Product Code: DM 1070

Application: - BYE Agar is a simplified medium developed for the cultivation of *Mycoplasma ,* or Pleuropneumonia like organisms and L-forms of bacteria.

Composition**

Ingredients	Gms / Litre
Buffered 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

Principle & Interpretation

Methyl Red and Voges-Proskauer test among the various tests are the two test 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- aerogens group. Both the tests are based on the detection of specific breakdown products of carbohydrate metabolism.

All members of *Enterobacteriaceae* by definition are 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, of 2-5 days, MR - positive organisms continue to produce acids, resulting in a low pH (acidic) that overcomes the phosphate buffering system and maintains 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 decreases 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 [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 [10], OMeara [11] Levine, et al [12] and Voughn et al [8].

Werkmans Test [10]: 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 [11]: 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 [12] modified OMeara technique by dissolving the creatine in a concentrated solution of potassium hydroxide Voughn, Mitchell and Levine [8] recommended the method of Barritt [13] as, addition of 1 ml of Barritt Reagent B 40% potassium hydroxide) and 3 ml of Barritt Reagent A (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 *E.coli* from the *Klebsiella - Enterobacter* groups. 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 [7].

Methodology

Suspend 17 grams of powder media in 1000 ml of distilled water. Shake well & heat to dissolve the medium completely. Distribute in test tubes in 10 ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Quality Control

Physical Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured clear solution without any precipitate

Reaction
Reaction of 1.7% w/v aqueous solutions at 25°C. pH : 6.9±0.2

pH range 6.70-7.10

Cultural Response/ characteristices
DM 1070: Cultural characteristics observed after an incubation at 30-32°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	MR Test	VP Test
Escherichia coli ATCC 25922	50-100	luxuriant	positive reaction, bright red colour	negative reaction
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	negative reaction	positive reaction, eosin pink/ red colour with in 2-5 minutes
Klebsiella pneumoniae ATCC 23357	50-100	luxuriant	negative reaction	positive reaction, eosin pink/ red colour with in 2-5 minutes

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

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6. MacFaddin J. F., 2000, Biochemical tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.
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9. Ruchhoft C. C., Kallas J. G., Chinn B. and Coulter E. W., 1931, J.Bacteriol., 22 : 125.
10. Werkman C. H., 1930, J. Bact., 20: 121.
11. OMeara R. A. Q., 1931, J. Path. Bacteriol., 34 : 401.
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13. Ewing W. H., 1986, Edwards and Ewings Identification of Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc., New York.

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