TMV 324 - GLUCOSE PHOSPHATE BROTH (BUFFERED GLUCOSE BROTH) (MR-VP MEDIUM) (VEG.)

INTENDED USE

Used in differentiation of bacteria by MR-VP test.

PRODUCT SUMMARY AND EXPLANATION

Buffered Glucose Veg 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 and subsequently by Clark and Lubs 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. 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). 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. The Voges-Proskauer test (VP) cultures are incubated at 30°C for 24-48 hours. Various test procedures have been suggested for performing the VP test by Werkman, OMeara 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 (OMeara Reagent). Voughn, Mitchell and Levine recommended the method of Barritt 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 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.

COMPOSITION

Ingredients	Gms / Ltr
Buffered peptone (veg.)	7.000
Dextrose	5.000
Dipotassium phosphate	5.000

PRINCIPLE

This medium contains Veg. buffered peptone water, which supports growth of organisms. Dextrose serves as immediate carbon source. Dipotassium phosphates provide buffering capability.

INSTRUCTION FOR USE

- Dissolve 17 grams in 1000 ml of distilled water.
- Heat if necessary to dissolve the medium completely.
- Distribute in test tubes in 10 ml amounts and sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Cream to yellow homogeneous free flowing powder.
Appearance of prepared medium : Light yellow coloured clear solution.
pH (at 25°C) : 6.9±0.2

INTERPRETATION

Cultural characteristics observed after an incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	MR Test	VP Test	Incubation Temperature	Incubation Period
Escherichia coli	25922	50-100	Luxuriant	Positive reaction, bright red colour	Negative reaction, yellow colour	30°C	48 Hours
Enterobacter aerogenes	13048	50-100	Luxuriant	Negative reaction, yellow colour	Positive reaction, eosin pink / red colour within 2-5 minutes	30°C	48 Hours
Klebsiella pneumoniae	23357	50-100	Luxuriant	Negative reaction, yellow colour	Positive reaction, eosin pink / red colour within 2-5 minutes	30°C	48 Hours

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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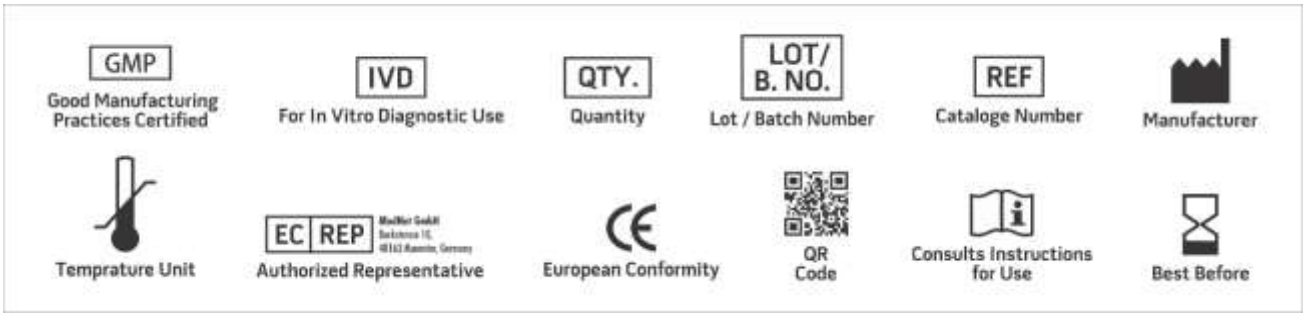

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**

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