MR-VP BROTH

INTENDED USE

MR-VP Broth (Methyl Red-Voges Proskauer Medium/Broth, also known as Buffered Peptone-Glucose Broth) is used for the differentiation of bacteria by means of the methyl red and Voges-Proskauer reactions.

SUMMARY AND EXPLANATION

Voges and Proskauer, in the latter part of the 19th century, reported the initial observations regarding the production of a red color after the addition of potassium hydroxide to specific culture media in which various organisms had grown.[1]

Clark and Lubs,[2] in 1915, found that the addition of methyl red to cultures of *Escherichia coli* resulted in a red color due to the high acidity produced during the fermentation of dextrose. The smaller amount of acid produced by *Klebsiella pneumoniae* and *Enterobacter aerogenes* is converted to acetoin resulting in an alkaline reaction (negative methyl red test).

In the Voges-Proskauer test, Reagent A (5% [w/v] alphanaphthol in absolute alcohol) contains a catalyst enhancing the formation of specific metabolic products that form a red complex upon the addition of Reagent B (40% [w/v] potassium hydroxide in purified water).

MR-VP Medium/Broth was developed to enable both the MR and the VP tests to be performed in the same medium, although in different tubes or on aliquots from the same tube.

PRINCIPLE

Methyl red-positive organisms produce high levels of acid during fermentation of dextrose, overcome the phosphate buffer system and produce a red color upon the addition of the methyl red pH indicator.

In the Voges-Proskauer test, the red color produced by the addition of potassium hydroxide to cultures of certain microbial species is due to the ability of the organisms to produce a neutral end product, acetoin (acetylmethylcarbinol), from the fermentation of dextrose.[3] The acetoin is oxidized in the presence of oxygen and alkali to produce a red color.[3] This is a positive Voges-Proskauer reaction.

REAGENTS (FORMULA)

Pancreatic Digest of Casein	3.5	g
Peptic Digest of Animal Tissue ...	3.5	g
Potassium Phosphate	5.0	g
Dextrose	5.0	g
Deionized Water	1000.0	ml

PROCEDURE

Using a light inoculum, inoculate tubes of MR-VP media with 18- to 24-hour pure cultures. Incubate tubes aerobically at 35 ± 2°C for a minimum of 48 hours but preferably for 5 days.

Prepare the methyl red indicator by dissolving 0.1 g of methyl red in 300 mL of 95% ethyl alcohol. Add sufficient purified water to make 500 mL.

After the appropriate incubation period, aseptically remove aliquots (1 mL for the VP test) of the medium and conduct the following tests:

1. Methyl Red Test – Add 5 drops of methyl red indicator to an aliquot of the broth. Interpret the color result immediately.

2. Voges-Proskauer Test – Empty the contents (15 drops) from the reagent A dropper and 5 drops from the reagent B dropper into 1 mL of broth culture. Shake well after the addition of each reagent to aerate the sample.

EXPECTED RESULTS

1. Methyl Red Test

    a. Positive – red color at surface of the medium.

    b. Negative – yellow color at surface of the medium.

2. Voges-Proskauer Test

    A positive reaction is indicated by the development of a distinct red color which occurs within 5 minutes.

Certain species within *Enterobacteriaceae* genera may react differently or give variable results. Consult appropriate texts for reactions of specific species.[3-6]

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Escherichia coli ATCC 25922	35 ± 2°C for 40-48 hours	Growth, MR (+), VP (-)
Enterbacter aerogenes ATCC 12022	35 ± 2°C for 40-48 hours	Growth, MR (-). VP (+)

ATCC® is a registered trademark of American Type Culture Collection.

BIBLIOGRAPHY

1. Voges and Proskauer. 1898. Z. Hyg. 28:20.

2. Clark and Lubs. 1915. J. Infect. Dis. 17:160.

3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore, Md.

4. Ewing. 1986. Edwards and Ewing's identification of Enterobacteriaceae, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.

5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.

6. Murray, Baron, Jorgensen, Landry and Pfaller (ed.). 2007. Manual of clinical microbiology, 9th ed. American Society for Microbiology, Washington, D.C.

CBSA life

155-196 Innovation Drive, Winnipeg, MB, R3T 2N2, Canada

Phone: +1 (204) 269-2255 Email: info@cbsalife.com Website: https://cbsalife.com