

MR-VP Medium

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

REF: BS.1/MVP01.010.001

10 Tubes

CLINICAL SIGNIFICANCE

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] alpha-naphthol 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.

METHOD 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.

MEDIA COMPOSITION

Item	Formula per liter of medium
Buffered peptone	7 gm
Dipotassium phosphate	5 gm
Dextrose	5 gm

pH after sterilization 6.9 $\pm$ 0.2

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses.).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment.

For further information, refer to the MR-VP Medium materialsafety data sheet.

STORAGE AND STABILITY

BioScien MR-VP Medium tube should be stored on receipt store between 2-8°C. Use before expiry date on the label. Product performance is best if used within stated expiry period.

Directions

Using a light inoculum, inoculate tubes of MR-VP media with 18- to 24-hour pure cultures. Incubate tubes aerobically at 35 $\pm$ 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.
3. Most members of the family Enterobacteriaceae give either a positive MR test or a positive VP test. However, certain organisms such as *Hafnia alvei* and *Proteus mirabilis* may give a positive result for both tests.
4. Incubation time for the Methyl Red test cannot be shortened by increasing the dextrose concentration in the medium or by heavily inoculating the broth.
5. Incubate MR-negative tests for more than 48 hours and test again.
6. Read the VP test at 48 hours. Increased incubation may produce acid conditions in the broth that will interfere with reading the results.
7. VP reagents must be added in the order and the amounts specified or a weak-positive or false-negative reaction may occur. A weak-positive reaction may be masked by a copper-like color which may form due to the reaction of KOH and α -naphthol.
8. Read the VP test within 1 hour of adding the reagents. The KOH and α -naphthol may react to form a copper-like color, causing a potential false-positive interpretation.
9. Due to the possible presence of acetoin, diacetyl or related substances in certain raw materials,⁸ the use of media low in these substances (such as MR-VP media) is recommended for this test.

Deterioration

The color of **BioScien** MR-VP Medium tube is Light amber, clear solution in sterile tubes. If there are any physical changes or signs of deterioration (shrinking, cracking, or discoloration), and contaminations for hydrated media, discard the medium.

SPECIMEN

Pharmaceutical samples, Cosmetic samples

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Loop
- Incubator

PERFORMANCE CHARACTERISTICS

Cultural characteristics observed after 48-72 hours at 28-30 °C.

Microorganism	Methyl Red	Voges-Proskauer
<i>Enterobacter aerogenes</i> ATCC 13048	Yellow	Red
<i>Escherichia coli</i> ATCC 25922	Red	No change
<i>Klebsiella pneumoniae</i> subsp. <i>Pneumoniae</i> ATCC 23357	Yellow	Red

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control still out of range please contact **BioScien** technical support.

REFERENCES

1. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
2. Sabouraud K., 1892, Ann. Dermatol. Syphilol, 3:1061.
3. Carlier, G., (1948). Brit. J. Derm. Syph. 60,61.
4. American Type Culture Collection, Manassas, Va., U.S.A.
5. U.S. Pharmacopeia, (1985). 21st Revision. U.S. Pharmacopeial Convention, Inc., Rockville, Maryland.

SYMBOLS IN PRODUCT LABELLING			
	For in-vitro diagnostic use		Number of <n> test in the pack
	Batch Code/Lot number		Caution
	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		