MR-VP BROTH

- For in vitro use only - Catalogue No. TM85

Our MR-VP Broth is used for the differentiation of bacteria (especially *Enterobacteriacae*) by means of the methyl red and Voges-Proskauer tests.

The methyl red (MR) test is a quantitative test based on the use of the pH indicator, methyl red, to determine the amount of acid produced by an organism from glucose fermentation in MR-VP broth. A positive test reaction is indicted by a red color reaction when the methyl red reagent is added; this indicates that the test bacteria produced sufficient acid to overcome the phosphate buffering system and can maintain an acidic environment in the MR-VP Broth. For accurate methyl red test results a minimum incubation time of 48 hours is required; and a 3 to 5 day incubation period is recommended.

The VP test is credited to Voges and Proskauer since they were the first bacteriologists to observe a red color change on culture media after treatment with potassium hydroxide. Barritt was the first individual to use both potassium hydroxide and α-naphthol for the VP test; an alternate, rapid VP test was reported by Barry and Feeney using an additional reagent, creatine.

The first reagent added, α-naphthol, catalyzes the conversion of acetoin to diacetyl in the presence of oxygen. Diacetyl can then react with guanidine-containing compounds such as arginine in the presence of α-naphthol to form a pinkish-red end product. The resultant red color change is indicative of a positive VP test.

The second reagent, potassium hydroxide, absorbs carbon dioxide present in the medium and acts as an oxidizing agent thereby hastening the critical reaction that converts acetoin to diacetyl.

Formulation per Litre of Medium

Pancreatic digest of casein .....3.5 g
Peptic digest of animal tissue .....3.5 g
Dextrose .....5.0 g
Potassium phosphate.....5.0 g

pH 6.8 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature prior to inoculation.
2. Using a direct inoculum from a pure, overnight culture, inoculate the MR-VP broth with the test organism.
3. Incubate aerobically at 35°C.
4. After 24 hours, remove 1-mL of the broth to a separate tube for VP testing. (The remainder of the broth should be re-incubated for an additional 1-3 days for the methyl red test)
5. Allow VP reagents to warm to room temperature prior to use.
6. Add 0.6 mL (9 drops) of α-Naphthol Reagent (Cat# RA45-10) to the allotted portion of MR-VP broth and gently mix.
7. Add 0.2 mL (3 drops) of 40% Potassium Hydroxide (Cat# RP89-25). Shake the tube gently for 30 seconds to aerate the suspension. The broth must be exposed to oxygen for a color reaction to occur.
8. In some instances the color reactions are immediate, but allow tubes to stand for 15 minutes before making a final interpretation.
9. After a minimum of 48 hours of incubation, remove 1-mL of the MR-VP to perform the methyl red test. (Please note that 48 hours is the minimum incubation time for this test; if desired MR-VP tubes made be incubated for 3 to 5 days before the test is performed)
10. Add 2 drops of Methyl Red Reagent to the 1-mL of MR-VP broth and shake gently. Interpret color result immediately.

Interpretation of Results

Methyl Red Test:

MR Positive (+): Pink or red color at the surface of the medium

MR Negative (–): Yellow color at the surface of the medium

Voges-Proskauer Test:

VP Positive (+): Pink or red color at the surface of the medium

VP Negative (–): No change; yellow or copper color at the surface of the medium

Fig 1. Typical MR-VP results for *Enterobacteriaceae*

Result	Genus*
MR+ VP–	*Citrobacter* *Edwardsiella* *Escherichia* *Morganella* *Proteus* *Providencia* *Salmonella* *Shigella* *Yersenia*
MR– VP+	*Enterobacter* *Erwinia* *Hafnia* *Klebsiella* *Serrratia*

* Note certain species within this genera may react differently or give variable results. Consult appropriate texts for specific reactions.

Quality Control

After checking the medium for correct pH, color, depth, and sterility, the following organisms are used to determine the performance of the completed medium.

Organism	MR	VP
Enterobacter aerogenes ATCC 12453	– (yellow)	+ (red)
Escherichia coli ATCC 25922	+ (red)	– (no change)

Storage and Shelf Life

Our MR-VP Broth should be stored away from direct light in an upright position at 4 to 8°C. Under these conditions, this medium has a shelf life of 12 weeks from the date of manufacture.

Ordering Information

Cat#	Description	Format
TM85-02	MR-VP Broth 2-mL [13x100-mm Screw Cap Tube]	10/pkg
TM85-06	MR-VP Broth 6-mL [16x100-mm Screw Cap Tube]	10/pkg
RM65-25	Methyl Red Reagent 25-mL	Each
RA45-10	Alpha Naphthol Reagent 10-mL	Each
RP89-25	Potassium Hydroxide [40%] 25-mL	Each

References

1. Voges D, Proskauer B. Beitrag zur ernahrungsphysiologie and zur differential diagnose der bakterien der hamorrhagischen septicemia. Z Hyg 1898; 28:20-32.
2. Clark WM, Lubs HA. The differentiation of bacteria of the colon-aerogenes family by the use of indicators. J Infect Dis 1915; 17:160-73.
3. Barritt MM. The intensification of the Voges-Proskauer reaction by the addition of α-naphthol. J Pathol Bacteriol 1936; 42:441-54.
4. Eddy BP. The Voges-Proskauer reaction and its significance: a review. J Appl Bacteriol 1961; 24:27-41.
5. Barry AL, Feeney KL. Two quick methods for Voges-Proskauer test. Appl Micro 1967; 15:1138-41.
6. MacFaddin JF. Media for isolation-cultivation-maintenance of medical bacteria, Vol I. Baltimore: Williams & Wilkins, 1985.
7. MacFaddin, JF. Biochemical Tests for the Identification of Medical Bacteria, 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.

Original: March 2002
Revised / Reviewed: January 2014