

MR-VP MEDIUM

CAT N°: 1512

For the differentiation of the *Escherichia-Enterobacter* group (Methyl Red and Voges-Proskauer reactions) from clinical samples

FORMULA IN g/l

Peptone Mixture	7.00	Potassium Phosphate	5.00
Dextrose	5.00		

Final pH 6.9 ± 0.2 at 25°C

PREPARATION

Suspend 17 grams of the medium in one liter of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Dispense into appropriate containers and sterilize in autoclave at 121°C for 15 minutes. The prepared medium should be stored at 2-8°C. The color is clear amber.

The dehydrated medium should be homogeneous, free-flowing and light beige in color. If there are any physical changes, discard the medium.

USES

MR-VP MEDIUM is used in the differentiation of enteric Gram-negative bacilli on the basis of the methyl red and acetylmethylcarbinol (Voges-Proskauer) reactions of the *Escherichia-Enterobacter* group.

Casein peptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Potassium phosphate acts as a buffer system.

In 1915, Clark and Lubs used methyl red as an indicator of acidity in the cultures of the Coli-Enterobacter group. This test is now known as the methyl red test and serves to distinguish between those microorganisms that produce and maintain a high concentration of acid from those that initially produce a small amount of acid and are capable of later attacking those same acids, turning the medium neutral or alkaline, such as *Enterobacter* species.

Voges and Proskauer described in 1898 a fluorescent red coloration that appeared in certain cultures upon adding drops of KOH solution. Later it was supposed that this reaction was due to the oxidation of acetylmethylcarbinol to diacetyl that reacted with the peptone of the medium to give a red color. *Enterobacter* bacteria oxidize the acetylmethylcarbinol and give a red coloration, in contrast to *Escherichia coli* that does not.

Methyl Red test (MR):

Add 5 drops of a 0.4% solution of methyl red to 5 ml of an already incubated tube at a temperature of 35 ± 2°C for 3 to 5 days. A positive reaction will give a red color, and a negative a yellow color. The reaction is immediate.

Voges-Proskauer test (VP):

To a 5 ml of up to 5 days inoculated and incubated medium, add 0.6 ml of 5% alpha-naphthol in absolute ethanol and 0.2 ml of 40% sodium hydroxide and shake from time to time over a 15-minute period. The tube may be held at room temperature or incubated at 35 ± 2°C. It is important to add the reagents in sequence. A positive test is indicated by the development of a faint pink to red color. The test should not be read after one hour because negative VP cultures may develop a copper color after that time.

MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of $35 \pm 2^{\circ}\text{C}$ and observed after 24 - 48 hours or after 5 days. Determine the Methyl Red and Voges-Proskauer test reactions.

Microorganisms	Growth	MR	VP
<i>Enterobacter aerogenes</i> ATCC 13048	Good	- (yellow)	+ (red)
<i>Escherichia coli</i> ATCC 25922	Good	+ (red)	- (without change)

BIBLIOGRAPHY

Clark and Lubs. J.: Inf. Dis. 17:160. 1955.

Ewing. Enterobacteriaceae. USPHS.

Edwards and Ewing. Identification of Enterobacteriaceae Burgess Publ. Co. Minneapolis, Minn., 1962.

Voges, O., and B. Proskauer. 1898. Z. Hyg. 28: 20-22.

Association of Official Analytical Chemists. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

