

MR VP Medium

Used for the differentiation of group *Escherichia-Enterobacter*
(Methyl Red and Voges-Proskauer reactions)

Formula in grams per liter:

Peptone Mixture	7,00	Dextrose	5,00
Potassium Phosphate	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. If needed, heat slightly to dissolve completely. Dispense in tubes and sterilize at 121°C (15 lbs sp) for 15 minutes.

Uses:

For the differentiation of the enteric gram negative bacilli, especially the *Escherichia Enterobacter* group. MR-VP Medium is used as an aid in the differentiation of enteric gram negative bacilli on the basis of methyl red and acetylmethylcarbinol (Voges Proskauer) reactions of the *Escherichia/Enterobacter* group.

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 to neutral or alkaline, such as *Enterobacter*.

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 oxidation of acetylmethylcarbinol to diacetyl which reacted with the peptone of the medium to give a red color. *Enterobacter* oxidizes the acetylmethylcarbinol and gives the red coloration, in contrast to *Escherichia coli* which does not.

Method:

Methyl red test:

Add 5 drops of a 0.4% solution of methyl red to 5 ml. of a culture incubated 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:

To 5 ml. of medium inoculated and incubated up to 5 days, 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 35-37° C. It is important that the reagents be added in sequence. A positive test is indicated by 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 Tests:

Microorganisms	Growth	MR	VP
<i>Enterobacter aerogenes</i> ATCC 13048	Good	- (yellow)	+ (red)
<i>Escherichia coli</i> ATCC 25922	Good	+ (red)	- (w/o change)
<i>Klebsiella pneumoniae</i> ATCC 23357	Good	+	-

