**Methyl Red Voges Proskauer Broth (MRVP Broth) (Clarks Lubs Medium)**

**Specification**
Classical liquid medium for differential tests (Voges Proskauer and Methyl Red) on *Enterobacteria* according to ISO standards 6579 and 6585 and FIL – IDF 93 standard.

**Formula** (in g/liter)
- Peptone: 7.0
- Dextrose: 5.0
- Potassium phosphate: 5.0
- Final pH: 7.0 ± 0.2

**Directions**
Dissolve 17 g of powder in 1 liter of distilled water, heating up only if necessary. Dispense into tubes and sterilize by autoclaving at 121°C for 15 minutes.

**Description**
The classical Clark and Lubs medium is used to perform the tests of Methyl Red and Voges Proskauer. As Indole and Citrate tests (IMVIC) this medium allows the differentiation within the *coli form* group of bacteria. The fundamentals of these reactions are as follows:

**Methyl Red Test** (M.R. test)
Among the *Enterobacteriaceae*, the *E. coli* biotype ferments glucose by the mixed acid pathway, accumulating acid, which reduces the initial pH. It can be detected by the methyl red indicator, which turns yellow above the pH 5.1 and becomes red below pH 4.4.

**Voges Proskauer Test** (V.P. test)
*Enterobacteria of Klebsiella-Enterobacter* biotype ferment the glucose by the 2-3-butanediol pathway. Although acids are produced, neutral or alkaline products are also formed and at the end the reaction is neutral or alkaline. Therefore, the incubation must be extended up to 3 days. After this period, the methyl red reaction is negative.

Nonetheless, the Voges Proskauer test is complementary to the Methyl Red test in some ways. It shows 2-3-butanediol and acetoin production, these substances are difficult to find in the mixed acid pathway. It exploits the fact that these two products, in alkaline medium, oxidize themselves to diacetil, which reacts with guanidine and produces visibly coloured compounds.

**Technique**
There are several techniques to carry out these tests. One of them is as follows:
The tube with medium is inoculated with the microorganism to be studied and incubated at 30°C for at least 3 days and up to 5 days maximum. Just before reading, the culture is separated in two portions, one for each test.

1) **Methyl Red Test.**
Add 4-5 drops of Methyl Red Reagent to the culture, and shake the tube in order to homogenize. Observe for the colour development in the medium. The test is considered positive if it turns to red and negative if it remains yellow.

**Positive** (red colouration): *E. coli, Edwardsiella, Shigella, Salmonella, Citrobacter, Proteus, Klebsiella ozoenae*, *Klebsiella rhinoscleromatis, Yersinia*.

**Negative** (yellow colouration): *Enterobacter, Hafnia, Serratia, Klebsiella pneumoniae*.
With *Erwinia*, this reaction has no significance since it offers variable reactions.
2) Voges Proskauer Test
Add Barrit's Reagent to the medium (Ref. 06-027) until it gets a milky appearance and then add O'Meara's Reagent (Ref. 06-006) until the milky appearance disappears. Shake vigorously.

The test is **positive** if the medium acquires a pink-violet colour, forming at the top of the tube. If the test is **negative**, there is no colour formation. Relative amounts of each reagent depend on initial volumes of the medium. Never incubate above 30°C.

**Positive** (pink-intense red): *Enterobacter*, *Hafnia*, *Klebsiella pneumoniae*, *Serratia*.

**Negative** (no colour change): *Escherichia*, *Edwardsiella*, *Citrobacter*, *Salmonella*, *Shigella*, *Yersinia*, *Klebsiella ozonae*, *Klebsiella rhinoscleromatis*.

With *Proteus* and *Erwinia* types, this reaction has no significance since they offer variable reactions.

The Voges Proskauer test may be performed in a quicker way, using very little volumes of medium and massive inoculum. This allows readings with short Incubation times (18-20 hours). Also the readings may be accelerated by gently heating the culture almost to the boiling point after adding the reagents.

However, erroneous results are more likely by using this method.

We refer to the FIL-IDF Standard for the specific technique of MRVP Modified Broth.

**References**

VOGES, O., B. PROSKAUER (1898) Beitrag zur Ernährungsphysiologie und zur Differentialdiagnose der hämorrhagischen Septicaemie. Z. Hyg.


O’MEARA, R. (1931) A simple delicate and rapid method of detecting the formation of acetylcarbinol by bacteria fermenting carbohydrates.

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