Product :
METHYL RED VOGES PROSKAUER BROTH (MRVP Broth)

Also known as
CLARKS LUBS MEDIUM

Specification
Classic liquid medium used for differential tests (Voges-Proskauer and Methyl Red) in Enterobacteriaceae according to ISO and FIL-IDF standards.

Formula * in g/L
Peptone..... 7.0
Dextrose..... 5.0
Buffer phosphate..... 5.0

Final pH 6.9 ±0,2 at 25 ºC
* Adjusted and /or supplemented as required to meet performance criteria

Directions
Dissolve 17 g of powder into 1 L of distilled water, heating if necessary. Dispense in tubes and sterilize in the autoclave at 121ºC for 15 minutes.

Description
This classical Lubs and Clark medium is used to perform Methyl Red (MR) and Voges-Proskauer (VP) tests, and together with Indol and Citrate tests allows the differentiation of coliforms. These reactions are as follows:

Methyl Red test
Amongst the Enterobacteriaceae, *E. coli* ferments glucose by the mixed acid pathway, accumulating acidic substances, which produces a large decrease in the initial pH. This change is detected by the methyl red indicator, that is yellow above pH 5,1 and red below pH 4,4.

Voges-Proskauer test
Enterobacteria of *Klebsiella-Enterobacter* biotype ferment glucose by the 2,3-butanediol pathway. Although acidic substances are produced in this way, at the end products are mostly neutral or alkaline. Due to this, incubation must be extended to 3 days. After this period, the methyl red reaction is negative. Nonetheless, Voges-Proskauer test is complementary to Methyl Red test. It shows the 2,3-butanediol and acetone production, (substances hard to identify in the mixed acid pathway). It takes advantage of the fact that these two products, in an alkaline environment, oxidize to diacetyl, which reacts with guanidine and produces coloured compounds.

Technique

There are several techniques used to carry out these tests. An example of one is as follows:
The medium is inoculated with a pure culture of the microorganism to study and incubated at 30ºC for at least 3 days or for a maximum of 5 days. Just before reading, the culture is separated in two aliquots, one for each test.

Methyl Red test:
Add 4-5 drops of Methyl Red Solution to the culture, and shake in order to homogenize. The test is considered positive if it turns red and negative if it remains yellow.
- Positive (turning red): E.coli, Edwarsiella, Shigella, Salmonella, Citrobacter, Proteus, Klebsiella ozoenae, Klebsiella rhinoscleromatis, Yersinia.
- Negative (turning yellow): Enterobacter, Hafnia, Serratia, Klebsiella pneumoniae.
- With Erwinia, this reaction is very variable.

Voges-Proskauer test:
Add to the medium Barrit's Reagent until it becomes a milky in appearance. Then, add O'Meara's Reagent until this milky appearance disappears. Shake vigourously. The test is positive if medium turns a pink-violet colour, beginning at the top of the tube. If the test is negative, it remains the same colour. Relative amounts of each reagent depend on initial volumes of medium. Never incubate above 30ºC.
- Positive (pink-red): Enterobacter, Hafnia, Klebsiella pneumoniae, Serratia.
- Negative (no colour change): Escherichia, Edwarsiella, Citrobacter, Salmonella, Shigella, Yersinia, Klebsiella ozonae, Klebsiella rhinoscleromatis.
- With Proteus and Erwinia spp, this reaction is meaningless as it can be very variable.

Voges-Proskauer test may be performed more rapidly, using very small volumes of medium and large inocula. This allows short incubations (18-20 hours), and also, reading may be accelerated by heating the culture almost to boiling after adding the reagents. However, false results are more probable using this method.

Quality control

Incubation temperature: 28-30ºC **Incubation time:** 24-48-72 h

Inoculum: ≥ 10^3 CFU (specificity) according to ISO 11133:2014/Amd 1:2018 & Adm 2:2021.

Microorganism	Growth	Remarks
Citrobacter freundii ATCC® 8090	Good	VP (-) RM (+)
Enterobacter aerogenes ATCC® 13048	Good	VP (+) RM (-)
Escherichia coli ATCC® 25922	Good	VP (-) RM (+)
Escherichia coli ATCC® 8739	Good	VP (-) RM (+)
Salmonella typhimurium ATCC® 14028	Good	VP (-) RM (+)
Serratia marcenses ATCC® 13880	Good	VP (+) RM (±)

References

· ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
· BARRIT, M. (1936) The intensification of the Voges Proskauerreaction by the adition of alpha-naphtol. J. Path. Basct. 42:441-452.
· CLARK, W. & H. LUBS (1915) The differentiation of bacteria of the colon-aerogenes family by the use of indicators. J. Infect. Dis. 17:160-173.
· FDA (Food and Drug Adminstrations) (1998) Bacteriological Analytical Manual. 8th ed. Revision A. AOAC International. Gaithersburg. MD. USA.
· FIL-IDF Standard 181 (1998) Dried milk products. Enumeration of Bacillus cereus. MPN technique.
· ISO Standard 6579-1 (2017) Microbiology of food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1 : Detection of Salmonella spp.
· ISO Standard 6585 (2001) Milk and milk products - Detection of Salmonella.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· MOLLÄNDER, R., J. BÖHMANN & B. GREWIG (1982) Die Verstörkung der Voges-Proskauer Reaktion duch fumarat. Zbt. Bakt. Hyg. I Abt. Orig. A 252:316-323.
· O'MEARA, R. (1931) A simple, delicate and rapid method of detecting the formation of acetyl-methyl-carbinol by bacteria fermenting carbohydrates. J. Pathol. Bact 34:401-406.
· PASCUAL ANDERSON. Mª.Rº. (1992) Microbiología Alimentaria. Díaz de Santos, S.A. Madrid.
· SCHWEIZERISCHES Lebensmittelbuch (2005) 5th ed. Chap. 56. Bundesamt für Gesundheit. Direktionsbereich Verbraucherschutz. Berna.
· VOGES & PROSKAUER (1898) Beitrag zur Ernährungsphysiologic und zur Differentialdiagnose der hämorrhagischen Septicäemia. Z. Hyg 28:20-32.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).