METHYL RED-VOGES-PROSKAUER (MR-VP) MEDIA

PRODUCT:

Tube Media:[a]

MR-VP Broth, item no. T6981, T3335

[a]see catalog for ordering options

PURPOSE:

MR-VP media are designed to test the method by which an organism ferments glucose on the basis of the by-products that are produced in the fermentation process.

PRINCIPLE:

Clark and Lubs[1] were the first to note the ability of certain organisms to produce stable acid products when cultivated in certain media. When an organism ferments glucose and produces pyruvic acid, two alternative pathways for metabolism may be utilized by the bacteria. Organisms utilizing mixed-acid fermentation during the metabolism of pyruvic acid produce a sufficiently acid pH (below 4.4) to overcome the buffer system in the MR-VP. When methyl red indicator is added after 48-72 hours of incubation, a red color is produced in the medium. Organisms capable of utilizing this pathway are referred to as methyl red positive.

Voges and Proskauer[5] were the first to describe a color reaction produced by certain bacteria after the fermentation of glucose, when the medium was treated with potassium hydroxide and exposed to air. It was noted that some organisms may further metabolize the pyruvic acid to acetyl methyl carbinol (acetoin). Bacteria that use this pathway produce lesser quantities of acid and therefore will not produce a color change when the methyl red reagent is added. Acetyl methyl carbinol is a neutral-reacting end product, raising the pH of the medium to 6.0 or greater. In the presence of oxygen and 40% KOH, acetoin is converted to diacetyl. When alpha-naphthol is added, a red color complex is formed.

FORMULAS:

Approximate, per liter deionized filtered water.

(1) MR-VP Broth:

Peptic Digest of Animal Tissue	3.5 g
Pancreatic Digest of Casein	3.5
Dextrose	5.0
Potassium Phosphate	5.0

Final pH 6.9 ± 0.2 at 25°C

PRECAUTIONS:*

For in vitro diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, evaporation, or discoloration), or if the expiration date has passed.

Limitations: Methyl red should not be interpreted unless the medium has been incubated for 48 hours. If the MR test is performed too early, results may be equivocal or falsely positive. MR-negative organisms will not have had a sufficient time to metabolize the acid by-products, and may, therefore, appear MR-positive after 18-24 hours of incubation. An exception should be made for MR-VP Agar, which may be tested after 24 hours of incubation.

Avoid testing an extremely turbid broth inoculum; bacterial growth is inhibited if the inoculum is excessively large. With each logarithmic decrease in inoculum size, there is an increase in the time required for the MR-positive organisms to accumulate enough acidic products to overcome the buffer system.

Occasionally, a known acetoin-positive organism may not give a positive VP reaction. To overcome this phenomenon, gently heat the culture containing the VP reagents. A VP-positive organism will demonstrate a positive test (red color) after heating.

The MR-VP tests should not be relied upon as the only means of identification for the differentiation of genera within the family *Enterobacteriaceae*. Citrate and indole tests must be performed in conjunction with the MR-VP tests. It is possible for some organisms to destroy acetoin, making the MR-VP invalid for identification purposes.

VP-positive organisms are not necessarily MR-negative. Certain organisms such as *Enterobacter hafniae* and *Proteus mirabilis* may give both a positive MR and VP reaction, although the VP may be delayed.

The order in which the VP reagents are added is important. First add the alpha-naphthol; then add the KOH. A reversal in the order of the reagents may give a weak-positive or false-negative result. Excess KOH may mask a weak VP-positive reaction due to a copper-like color which is formed by a reaction with alpha-naphthol alone. A VP test may be held for up to one hour for the detection of small amounts of acetoin. Exposure to the reagents for over an hour may lead to false-positive results.

PROCEDURE:*

Specimen Collection: Not applicable since these media are not for primary isolation. These media are used in characterizing pure cultures. Isolated organisms, established isolation techniques, and tests for purity are necessary before inoculating these media. Direct inoculation of specimens will produce erroneous results. Information on specimen collection may be found in standard reference texts.

Method of Use: Prior to inoculation, the medium should be brought to room temperature. Lightly inoculate the culture medium with organisms taken from a pure 18- to 24-hour culture (colony or broth culture such as tryptone). Incubate aerobically at 35°C for 24 hours. For the VP broth test, remove a 1.0-ml aliquot. To the aliquot first add 0.6 ml of alpha-naphthol; next add 0.2 ml of 40% KOH. Shake the tube gently to expose the medium to atmospheric oxygen. Allow the tube to stand 10-15 minutes before interpreting the color result. If the test results are negative or questionable, the remaining broth (without reagents) may be reincubated and retested for up to 5 days. Reincubate the remaining broth another 24 hours for the MR test, remove a 2.5-ml aliquot and add 5 drops of methyl red indicator. Interpret the result immediately. For the rapid test, inoculate 0.5 ml of MR-VP broth or agar with one colony. Incubate at 35°C for 18 hours, then add 1 drop of methyl red reagent and read the reaction.

Interpretation:

MR positive:	Culture will remain red after the addition of methyl red indicator at the surface.
MR negative:	A yellow color will occur at the surface of the medium.
VP positive:	After addition of reagents, a pinkish-red color will remain at the broth surface (acetoin is present).
VP negative:	Yellow color appears at broth surface. If a copper-like color appears with the naphthol reagent, the test is still negative.

Materials Required but Not Provided: Standard microbiological supplies and equipment such as loops, needles, incubator, incinerator, inoculation system, and are not provided.

QUALITY CONTROL:*

Microorganisms Used (ATCC#):	**Expected Results:** MR	VP
Escherichia coli (25922)	(+)	(-)
Enterobacter aerogenes (13048)	(-)	(+)

Key: See “Interpretation”

User Quality Control: Check for signs of contamination and deterioration.

BIBLIOGRAPHY:

1. Clark, W. M., and H. A. Lubs, *J. Inf. Dis.*, 17:160-173, 1915.
2. Koneman, E. W., et al., *Color Atlas and Textbook of Diagnostic Microbiology*, 3rd ed., J. B. Lippincott, Philadelphia, 1988.
3. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington, D. C., 1985.
4. MacFaddin, J. F., *Biochemical Tests for Identification of Medical Bacteria*, 2nd ed., Williams and Wilkins, Baltimore, 1980.
5. Voges, O., and B. Proskauer, *Zeit. Hyg.*, 28:20-32, 1898.

*For more detailed information, consult appropriate references and/or details in the preface of the PML Technical Manual.

PML MICROBIOLOGICALS, INC.
DATA #505

Revision Date: January 2001