MR-VP BROTH (7237)

Intended Use
MR-VP Broth is used for the differentiation of microorganisms on the basis of acid or acetylmethyl carbinol production (MR-VP reaction).

Product Summary and Explanation
In 1915, Clark and Lubs demonstrated that colon-aerogenes family of bacteria could be divided into two groups based on their action in a peptone and dextrose medium.[1] When tested with the pH indicator methyl red, the "coli" group produced high acidity while the "aerogenes" group produced a less acid reaction. The test to detect high-acid end products is known as the Methyl Red (MR) test. The test to detect less-acid end products is based on the procedure described by Voges and Proskauer in 1898.[2] A color reaction occurs when certain cultures, incubated in a medium containing peptone and dextrose, are treated with potassium hydroxide and exposed to air. This reaction detects the formation of acetylmethylcarbinol, known as the Voges-Proskauer (VP) test.

The MR and VP tests appear in the identification scheme for *Enterobacteriaceae*, important isolates in clinical microbiology[3] and food and dairy microbiology testing.[4,5] MR-VP Broth is also known as Methyl Red-Voges-Proskauer Medium.

Principles of the Procedure
Enzymatic Digest of Casein and Enzymatic Digest of Animal Tissue are carbon, nitrogen, and vitamin sources used for general growth requirements in MR-VP Broth. Dextrose is the fermentable carbohydrate. Potassium Phosphate is a buffering agent. Members of *Enterobacteriaceae* convert glucose to pyruvate by the Embden-Meyerhof pathway. Some bacteria metabolize pyruvate by the mixed acid pathway and produce acidic end products (pH < 4.4), such as lactic, acetic, and formic acids. Other bacteria metabolize pyruvate by the butyleneglycol pathway and produce neutral end products (pH > 6.0), one of which is acetoin (acetylmethylcarbinol). In the MR test the pH indicator, methyl red, detects acidic end products.[6] In the VP test, acetoin is oxidized in the presence of oxygen and potassium hydroxide (KOH) to diacetyl, producing a red color.[6] The addition of naphthol before KOH enhances the sensitivity of the test.[6]

Formula / Liter
Enzymatic Digest of Casein ..... 3.5 g
Enzymatic Digest of Animal Tissue..... 3.5 g
Dextrose..... 5 g
Potassium Phosphate..... 5 g
Final pH: 6.9 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution
1. For Laboratory Use.

Directions
1. Dissolve 17 g of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is yellow gold to amber and clear.

Expected Cultural Response: Cultural response in MR-VP Broth after 2 days at 35°C incubation for Methyl Red test and 2 days at 35°C incubation for Voges-Proskauer test.

Microorganism	Response	Reactions	
		MR	VP
Enterobacter aerogenes ATCC® 13048	growth	negative	positive
Escherichia coli ATCC® 25922	growth	positive	negative
Proteus vulgaris ATCC® 13315	growth	positive	negative
Serratia marcescens ATCC® 8100	growth	negative	positive

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate MR-VP Broth with growth from a single colony. Incubate at 35 ± 2°C for 48 hours. Proceed with Methyl Red or Voges-Proskauer test.

Methyl Red Test

Transfer 2.5 mL of the MR-VP Broth culture to a tube (13 x 100mm). Add 5 drops of Methyl Red and observe for a color change.

Voges-Proskauer Test

Transfer 2.5 mL of the MR-VP Broth culture to a tube (13 x 100mm). Add 0.3 mL (6 drops) of Voges-Proskauer Reagent A (5% α-naphthol). Add 0.1 mL (2 drops) of Voges-Proskauer Reagent B (40% KOH). Gently agitate the tube and let stand for 10 – 15 minutes. Observe for a color change.

Results

Methyl Red (MR) Test: Positive – bright red color; Negative – yellow-orange color.
Note: If the test is negative continue to incubate the broth without added reagents, repeat the test after an additional 18 – 24 hours incubation.

Voges-Proskauer (VP) Test: Positive – red color, Negative – no red color.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitation of the Procedure

Results of the MR and VP tests need to be used in conjunction with other biochemical tests to differentiate genus and species within *Enterobacteriaceae*.

Packaging

MR-VP Broth	**Code No.**	**7237A**	**500 g**
		7237B	**2 kg**
		7237C	**10 kg**

References

1. **Clark, W. M., and H. A. Lubs.** 1915. The differentiation of bacteria of the colon-aerogenes family by the use of indicators. J. Infect. Dis. **17:**160-173.
2. **Voges, O., and B. Proskauer.** 1898. Z. Hyg. **28:**20-22.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.).** Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
4. **Vanderzant, C. and D. F. Splittstoesser (eds.).** Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **Marshall, R. T. (ed.).** Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
6. **Isenberg, H. D. (ed.).** 1994. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (410)780-5120 or fax us at (410)780-5470.