
MR-VP BROTH

INTENDED USE

Remel MR-VP Broth is a liquid medium recommended for use in qualitative procedures for the performance of the Methyl Red (MR) and Voges-Proskauer (VP) tests as an aid in the identification of enteric gram-negative bacilli.

SUMMARY AND EXPLANATION

In 1898, Voges and Proskauer reported the initial observation regarding the production of a red color after the addition of potassium hydroxide to cultures grown on an appropriate medium.¹ In 1936, Barritt made the Voges-Proskauer test more sensitive by adding alpha naphthol to the medium prior to adding potassium hydroxide.² The methyl red test was first described in 1915 by Clark and Lubs, who found it useful in differentiating between the coli-aerogenes group of enteric bacteria.³ These investigators developed MR-VP Broth to allow both tests to be performed using the same medium, after aliquoting to different tubes.

PRINCIPLE

The MR test is based on the use of a pH indicator, methyl red, to determine the hydrogen ion concentration (pH) present when an organism ferments glucose.⁴ All members of the *Enterobacteriaceae* ferment glucose, resulting in the production of acid. Initially all enterics are MR-positive.⁵ However, after further incubation (2-5 days), the MR-positive organisms continue to produce more acids, overcoming the phosphate buffer and resulting in a low pH and a red color development. The MR-negative organism further metabolizes the fermentation products by decarboxylation, producing acetylmethylcarbinol (acetoin) which results in a neutral pH. The VP test is based on the detection of acetylmethylcarbinol derived from glucose metabolism and is used primarily to separate *Escherichia coli* (VP negative) from the *Klebsiella-Enterobacter* groups (VP positive).⁴ Acetylmethylcarbinol is a precursor to 2,3-butanediol production. In the presence of atmospheric oxygen and alkali, acetylmethylcarbinol and 2,3-butanediol are oxidized to diacetyl which is the reactant for the pink-red color produced in the VP test.⁶

REAGENTS (CLASSICAL FORMULA)*

Dextrose	5.0 g	Casein Peptone	3.5 g
Dipotassium Phosphate	5.0 g	Meat Peptone	3.5 g
		Demineralized Water	1000.0 ml

pH 6.9 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

1. Inoculate MR-VP Broth using a light inoculum removed from a pure 18-24 hour culture.
2. Incubate in ambient at 33-37°C for a minimum of 48 hours. Prolonged incubation up to 5 days may be required for the MR test and up to 10 days for the VP test.⁴
3. Following incubation, aseptically remove 1 ml aliquots of the medium to perform the following tests.

Methyl Red Test:

1. Add 1-2 drops Methyl Red (REF R21236) to a 1 ml aliquot of MR-VP Broth.
2. Observe immediately for a red color development.

Voges-Proskauer Test (Barritt Method^{2,7}):

1. Add 0.6 ml of 5% Alpha Naphthol (VP A) (REF R21200) to a 1 ml aliquot of MR-VP Broth.
2. Add 0.2 ml of 40% Potassium Hydroxide (VP B) (REF R21281) to the broth.
3. Shake the tube gently for 30 seconds to 1 minute to expose the medium to atmospheric oxygen so as to oxidize the acetylmethylcarbinol.
4. Observe for a pink-red color development within 15 minutes.

INTERPRETATION OF THE TEST

Methyl Red Test:

Positive Test - Red color development on surface of medium
Negative Test - Yellow color on surface of medium

Voges-Proskauer Test:

Positive Test - Pink-red color development on surface of medium
Negative Test - Yellow color on surface of medium

QUALITY CONTROL

All lot numbers of MR-VP Broth have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL

Escherichia coli ATCC® 25922
Enterobacter aerogenes ATCC® 13048

INCUBATION

Ambient, 48 h @ 33-37°C
Ambient, 48 h @ 33-37°C

RESULTS

MR (+) VP (-)
MR (-) VP (+)

(Continued on back)

LIMITATIONS

1. Do not attempt to interpret the MR result with less than 48 hours of incubation.⁷ If the MR test is performed too early, the results may be equivocal or falsely positive since MR-negative organisms may not have had sufficient time to metabolize the initial acidic products from glucose fermentation.⁴
2. Avoid testing an extremely turbid broth-inoculum mixture. Bacterial growth is inhibited if the inoculum exceeds the maximum cell concentration of about 10^9 viable cells/ml.⁴
3. The following variables should be standardized to obtain optimal and reproducible results: (a) the inoculum density, (b) the total volume of broth, and (c) the size of the test tube used. An orange color reaction often occurs when too large a volume of broth is used.⁴
4. Occasionally a known acetylmethylcarbinol-positive organism may fail to give a positive VP test and it may be necessary to gently heat the culture containing the VP reagents.⁴
5. The order and amount of VP reagents added must be followed exactly since reversal of the order or an excess of potassium hydroxide may cause false results.⁷

BIBLIOGRAPHY

1. Voges, O. and B. Proskauer. 1898. Z. Hyg. 28:20-32.
2. Barritt, M.M. 1936. J. Pathol. Bacteriol. 42:441-454.
3. Clark, W.M. and H.A. Lubs. 1915. J. Infect. Dis. 17:160-173.
4. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.
5. Cowan, S.T. and K.G. Steel. 1966. Manual for the Identification of Medical Bacteria. Cambridge Univ. Press, Cambridge, UK.
6. Tittsler, R.P. 1938. J. Bacteriol. 35:157.
7. MacFaddin, J.F. 2000. Biochemical Tests for Identification of Medical Bacteria. 3rd ed. Lippincott Williams & Wilkins, Philadelphia, PA.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

ATCC® is a registered trademark of American Type Culture Collection.
IFU 61430, Revised March 30, 2011

Printed in U.S.A.

remel

12076 Santa Fe Drive, Lenexa, KS 66215, USA
General Information: (800) 255-6730 Website: www.remel.com Email: remel@remel.com
Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128