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Instructions for Use

METHYL RED-VOGES PROSKAUER (MR-VP) BROTH

Cat. no. Z117	Methyl Red Test Reagent, Tube with Dropper, 15ml	1 vial
Cat. no. K37	Methyl Red-Voges Proskauer (MR-VP) Broth, 13x100mm Tube, 5ml	20 tubes/box
Cat. no. K237	Methyl Red-Voges Proskauer (MR-VP) Broth, 13x100mm Tube, 2ml	20 tubes/box

INTENDED USE

Hardy Diagnostics MR-VP Broth is recommended for use in the performance of the Voges-Proskauer and Methyl Red tests as an aid in the identification of enteric gram-negative bacilli.

SUMMARY

Voges-Proskauer (VP) Test

Certain bacteria produce neutral-reacting end products when cultivated in specific media. Particular enteric bacteria that ferment glucose, further metabolize pyruvic acid to form acetyl-methyl carbinol (acetoin). This end product, in the presence of atmospheric oxygen and 40% potassium hydroxide is converted to diacetyl. Diacetyl, under the catalytic action of alpha-naphthol and creatine, is converted into a red complex.[5] This is a positive Voges-Proskauer (VP) test reaction. The VP test is used primarily to separate *Escherichia coli* (VP-negative) from the *Klebsiella-Enterobacter* groups (VP-positive).

Voges and Proskauer, in 1898, first observed the production of a red color after the addition of potassium hydroxide to cultures grown on specific media.[6] Harden later revealed that the development of the red color was a result of acetyl-methyl carbinol (acetoin) production.[7] In 1936, Barrit made the test more sensitive by adding alpha-naphthol to the medium before adding potassium hydroxide.[8]

Methyl Red (MR) Test

Clark and Lubs, in 1915, discovered the ability of coliforms to produce and maintain acid products when cultivated in specific media.[9] They found that cultures of *Escherichia coli* produced a red color upon addition of methyl red. This color development was a result of strong acid production from dextrose-fermentation.

The Methyl Red (MR) test is based on the use of methyl red, a pH indicator, to detect acidity when an organism ferments glucose.[10] Because all *Enterobacteriaceae* ferment glucose, acidic metabolic byproducts are initially formed. However, with further incubation (2-5 days), MR-positive organisms continue to produce more acids. The increased acidity overcomes the phosphate buffer, thus resulting in a low pH and development of a red color.

MR-negative organisms, such as *Klebsiella pneumoniae* and *Enterobacter aerogenes*, further metabolize the fermentation products by decarboxylation. The result is an alkaline reaction (no red color) due to the production of acetoin which neutralizes the pH and results in no red color development.

Clark and Lubs developed MR-VP Broth which allowed both the MR and VP tests to be performed from the same

inoculated medium by aliquoting portions to different tubes.

FORMULA

Ingredients per liter of deionized water:*

Dipeptone	7.0gm
Dextrose	5.0gm
Potassium Phosphate	5.0gm

Final pH 6.9 +/- 0.2 at 25ºC.

* Adjusted and/or supplemented as required to meet performance criteria.

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC away from direct light. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on specimen collection.[2-5]

Method of Use:

1. Prior to inoculation, allow medium to equilibrate to room temperature.

2. Using organisms taken from an 18-24 hour pure culture, lightly inoculate the medium.

3. Incubate aerobically at 35ºC for 24 hours.

4. Following 24 hours of incubation, aliquot 1ml of the broth to a clean test tube.

5. Reincubate the remaining broth for an additional 24 hours.

VOGES-PROSKAUER (VP) TEST
6a. To the aliquot (step 4 above), add 0.6ml of 5% alpha-naphthol. Next add 0.2ml of 40% KOH.

7a. Gently shake the tube to expose the medium to atmospheric oxygen.

8a. Allow the tube to remain undisturbed for 10-15 minutes.

9a. Observe the medium for a pink-red color development. The test may be read for up to, **but not beyond** , one hour following addition of the reagents.

Note: If test reactions are negative (no red color produced) or questionable, the test can be repeated using the reincubated broth (without reagents) from step 5 above. Reincubation and repeat testing can be performed for up to 5 days.

METHYL RED (MR) TEST
6b. Following 48 hours of incubation (step 5 above), aliquot 2.5ml of the broth to a clean test tube.

7b. Add five drops of Methyl Red reagent (Cat. no. Z117).

8b. Observe the medium for the **immediate** development of a red color.

INTERPRETATION OF RESULTS

Voges-Proskauer Test
A positive VP test is demonstrated by the development of a pink-red color on the surface of the medium 15 minutes to one hour after the addition of the reagents.

A negative VP test is demonstrated by the appearance of a yellow color on the surface of the medium. Development of a copper-like color is also interpreted as negative.

Methyl Red Test
A positive MR test is demonstrated by the development of a stable red color on the surface of the medium after the addition of methyl red indicator.

A negative MR test is demonstrated by the development of a yellow color on the surface of the medium.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

The methyl red test must not be performed unless the medium has been incubated for a minimum of 48 hours. Tests that are run too early may result in false-positive interpretation.

It is important that a light inoculum be used. If an inoculum is too heavy, bacterial growth may be inhibited and result in invalid test results.

Some organisms that are capable of producing acetyl methyl carbinol (acetoin) produce false-negative VP reactions. These false-negative VP reactions have been seen in cultures incubated for 48-72 hours or longer, organisms that exhibit small colonies (0.5mm) on agar and poor growth in MR-VP broth, and some strains of *Enterobacteriaceae* (due to the breakdown of acetoin).

Some organisms destroy acetoin, thereby rendering the MR-VP tests invalid.

Organisms that are VP-positive are not necessarily MR-negative. *Enterobacter hafnia* and *Proteus mirabilis* are examples of organisms that are both MR- and VP-positive, although the VP reaction may be delayed.

Incubation periods up to 5 days may be necessary for the methyl red test and up to 10 days for the Voges-Proskauer test.[10]

When adding the VP reagents to the medium, it is important that the alpha-naphthol be added first and the KOH added second. A change in the order may produce invalid test results.

False-positive VP results may occur if VP tests are read beyond one hour following the addition of reagents.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, alpha-naphthol (Cat. no. Z91), 40% KOH (Cat. no. Z92), Methyl Red (Cat. no. Z117), etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

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Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli ATCC® 25922	A	48hr	35°C	Aerobic	Growth; MR-positive (red), VP-negative (yellow)
Enterobacter cloacae ATCC® 23355	A	48hr	35°C	Aerobic	Growth; MR-negative (yellow), VP-positive (pink-red)

* Refer to the document "Inoculation Procedures for Media QC" for more information.

USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

MR-VP Broth should appear clear, and light amber in color.

Escherichia coli (ATCC® 25922) grown in MR-VP Broth (Cat. no. K37). Incubated aerobically for 24 hours at 35ºC. A 1mL aliquot was removed and 0.6mL of alpha-naphthol (Cat. no. Z91) and 0.2mL of 40% KOH (Cat. no. Z92) was added to the tube. No development of a pink-red color within 10-60 minutes was indicative of a negative VP reaction.

Enterobacter cloacae (ATCC® 23355) grown in MR-VP Broth (Cat. no. K37). Incubated aerobically for 24 hours at 35ºC. A 1mL aliquot was removed and 0.6mL of alpha-naphthol (Cat. no. Z91) and 0.2mL of 40% KOH (Cat. no. Z92) was added to the tube. Development of a pink-red color within 10-60 minutes was indicative of a positive VP reaction.

Escherichia coli (ATCC® 25922) grown in MR-VP Broth (Cat. no. K37). Incubated aerobically for 48 hours at 35ºC. A 2.5mL aliquot was removed and five drops of methyl red was added to the tube. The immediate development of a red color was indicative of a positive MR reaction.

Enterobacter cloacae (ATCC® 23355) grown in MR-VP Broth (Cat. no. K37). Incubated aerobically for 48 hours at 35ºC. A 2.5mL aliquot was removed and five drops of methyl red was added to the tube. No immediate development of a red color was indicative of a negative MR reaction.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

6. Voges, O. and B. Proskauer. 1898. *Zeit. Hyg.*; 28:20-32.

7. Harden, A. 1906. *Proc. Roy. Soc.*, (London); 77:424-425.

8. Edwards, P.R. and Ewing, W.H. 1955. *Identification of Enterobacteriaceae*, Burgess Publ. Co., Minneapolis, MN.

9. Clark, W.M. and Lubs, H.A. 1915. *J. Infect. Dis.*; 17:160-173.

10. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

11. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]