MR-VP HiVeg™ Medium (Glucose Phosphate HiVeg™ Broth) (Buffered Glucose HiVeg™ Broth) MV070

Intended Use:

Recommended for the performance of the Methyl Red and Voges-Proskauer tests in differentiation of the coli-aerogenes group from clinical and non clinical samples.

Composition**

Ingredients	Gms / Litre
Buffered HiVeg™ Peptone	7.000
Dextrose (Glucose)	5.000
Dipotassium phosphate	5.000
Final pH (at 25°C)	6.9±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 17.0 grams in 1000 ml of purified / distilled water. Heat if necessary to dissolve the medium completely. Distribute in test tubes in 10 ml amounts and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Methyl Red and Voges-Proskauer test are among the two various tests used in the biochemical identification of bacterial species. These tests were originally studied by Voges, Proskauer (17) and subsequently by Clark and Lubs (5) to differentiate between members of the coli- aerogens group. Both the tests are based on the detection of specific breakdown products of carbohydrate metabolism.

All members of *Enterobacteriaceae* are, by definition, glucose fermenters. In MR-VP Broth, after 18-24 hours of incubation, fermentation produces acidic metabolic by products. Therefore initially all enterics will give a positive MR reaction if tested (3,4,7). However, after further incubation, required by the test procedure (2-5 days), MR - positive organisms continue to produce acids, resulting in a low pH (acidic) that overcomes the phosphate buffering system and maintains an acidic environment in the medium (pH 4.2 or less). MR-negative organisms further metabolize the initial fermentation products by decarboxylation to produce neutral acetyl methyl carbinol (acetoin), which results in decreased acidity in the medium and raises the pH towards neutrality (pH 6.0 or above) (11). In the presence of atmospheric oxygen and alkali, the neutral end products, acetoin and 2, 3-butanediol, are oxidized to diacetyl, which react with creatine to produce a red colour. MR-VP HiVeg™ Medium is prepared by completely replacing animal based peptones with vegetable peptones to avoid BSE/TSE risks associated with animal peptones. The Methyl Red (MR) test is performed after 5 days of incubation at 30°C (16). The Voges-Proskauer test (VP) cultures are incubated at 30°C for 24-48 hours (14). Various test procedures have been suggested for performing the VP test by Werkman (19), OMeara (13) Levine, et al (10) and Voughn et al (16).

Werkmans Test (19): Add 2 drops of a 2% solution of ferric chloride to 50 ml culture and 5 ml of 10% sodium hydroxide. Shake the tube to mix well. Stable copper colour developing in a few minutes is positive reaction. OMeara Test (14): Add 25 mg of solid creatine to 5 ml culture and then add 5 ml concentrated (40%) sodium hydroxide. Red colour development in a few minutes after shaking the tube well is a positive reaction. Levine, Epstein and Voughn (10) modified OMeara technique by dissolving the creatine in a concentrated solution of potassium hydroxide (R031, OMeara Reagent). Voughn, Mitchell and Levine (16) recommended the method of Barritt (7) as, addition of 1 ml of Barritt Reagent B (R030 - 40% potassium hydroxide) and 3 ml of Barritt Reagent A (R029 - 5% a-naphthol in absolute ethanol) to 5 ml culture. Positive test is indicated by eosin pink colour within 2-5 minutes.

Type of specimen

Isolated Microorganism from Clinical samples, food and dairy samples, water samples

Specimen Collection and Handling

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).
For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,15,18).
For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(2)
After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. The MR and VP tests should not be relied upon as the only means of differentiating *E.coli* from the *Klebsiella-Enterobacter* groups.
2. Also occasionally a known acetoin-positive organism fails to give a positive VP reaction. To overcome this possibility, gently heat the culture containing the VP reagents (12).

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light yellow coloured clear solution without any precipitate

Reaction
Reaction of 1.7% w/v aqueous solution at 25°C. pH : 6.9±0.2

pH
6.70-7.10

Cultural Response
Cultural characteristics observed after an incubation at 30-32°C for 18-48 hours.

Organism	Inoculum (CFU)	Growth	MR Test	VP Test
Klebsiella pneumoniae ATCC 23357	50-100	luxuriant	negative reaction	positive reaction, eosin pink / red colour within 2-5 minutes
Escherichia coli ATCC 25922 (00013*)	50-100	luxuriant	positive reaction, bright red colour	negative reaction
#Klebsiella aerogenes ATCC 13048 (00175*)	50-100	luxuriant	negative reaction	positive reaction, eosin pink / red colour within 2-5 minutes

Key : *Corresponding WDCM numbers.
- Formerly known as *Enterobacter aerogenes*

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

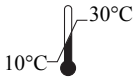
Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Barry A. L., Bernsohn K. L., Adams A. B., Thrup L. D., Appl. Microbiol., 1970, 20 (6), 866-870.
4. Branson D., Methods in Clinical Bacteriology, Springerfield, IL: Charles C Thomas, 1972, 32-33.
5. Clark W. M. and Lubs H. K., 1915, J. Infect. Dis., 17:160.
6. Cowan S. T., Cowan and Stuls Manual for the Identification of Medical Bacteria, 2nd Ed., Cambridge, Cambridge University Press, 1974, 37,48.
7. Ewing W. H., 1986, Edwards and Ewings Identification of Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc., New York.
8. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
9. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
10. Levine M., Epstein S. S. and Voughn R. H., 1934, Am. J. Publ. Health, 24: 505.
11. MacFaddin J. F., 2000, Biochemical tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.
12. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
13. OMeara R. A. Q., 1931, J. Path. Bacteriol., 34 : 401.
14. Ruchhoft C. C., Kallas J. G., Chinn B. and Coulter E. W., 1931, J.Bacteriol., 22 : 125.
15. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
16. Vaughn R. H., Mitchell N. B. and Levine M., 1939, J. Am. Water Works Association, 31:993.
17. Voges O. and Proskauer B., 1898, Z. Hyg. Infektionskr., 28:20.
18. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed.,APHA Inc., Washington, D.C.
19. Werkman C. H., 1930, J. Bact., 20: 121.

Revision : 03/ 2018

IVD — In vitro diagnostic medical device

CE Marking



Storage temperature

Do not use if package is damaged

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