PRODUCT SPECIFICATION SHEET

Buffered Glucose Broth / Glucose Phosphate Broth (MR-VP Medium) (DM142)

Intended use
MR-VP Medium (Glucose Phosphate Broth/Buffered Glucose Broth) (DM142) is used for performing the Methyl Red and Voges Proskauer tests in differentiation of coli-aerogenes group.

Product Summary and Explanation
This glucose-phosphate medium is recommended for the Methyl-red and Voges-Proskauer tests, for the differentiation of the coli-aerogenes group[1].In 1915, Clark and Lubs[2]demonstrated that the colon-aerogenes family of bacteria could be divided in to two groups based on their action in a peptone and dextrose medium. When tested with 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 product is known as the Methyl Red (MR) test. This test distinguishes those organisms able to form large amounts of acid from glucose so that the pH falls below 4.4 produce low pH level. Voges and Proskauer[3]described a red fluorescent coloration which appeared after the addition of potassium hydroxide to cultures of certain organisms in glucose medium. The coloration was shown to be due to the oxidation of the acetylmethyl-carbinol producing diacetyl which reacts with the peptone of the medium to give a red color[4,5].This is known as (VP) test. The MR and VP test appear in the identification of scheme for the Enterobacteriaceae.[6]which are important isolates in clinical microbiology, as well as in the microbiology of foods and dairy products.

Principles of the Procedure
All members of the 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 butylenes glycol 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.[7] In the VP test, acetoin is oxidized in the presence of oxygen and potassium hydroxide (KOH) to diacetyl, which produces a red color.[8]

Formula / Liter

Ingredients	Gms / Litre
Buffered peptone	7.00
Dextrose	5.00
Dipotassium hydrogen phospate	5.00
Final pH: 6.9 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 17 grams in 1000 ml of distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Distribute in test tubes in 10 ml amounts.
4. Sterilize by autoclaving at 15 lbs pressure (121°C) for15 minutes

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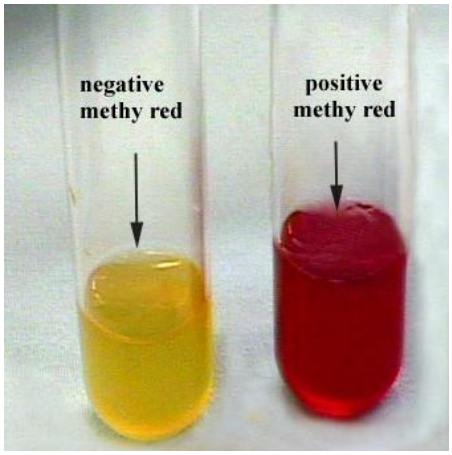
Quality Control Specifications

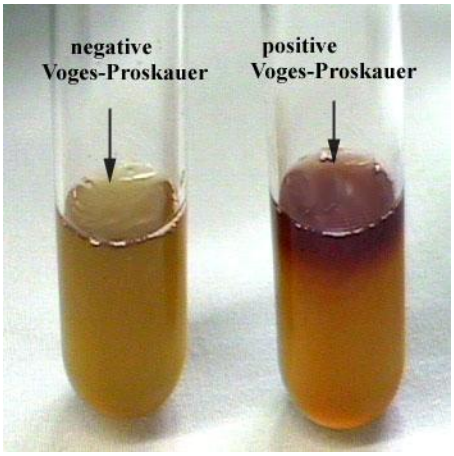
Dehydrated Appearance	Cream to yellow colored, homogeneous, free flowing powder
Prepared Medium	Light yellow coloured clear solution without any precipitate
Reaction of 1.7% Solution	pH : 6.9 ± 0.2
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 30-32°C for18-48 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	MR Test	VP Test
1.	*Escherichia coli ATCC 25922*	50 -100	Luxuriant	Positive reaction, bright red colour	Negative reaction
2.	*Enterobacter aerogenes ATCC 13048*	50 -100	Luxuriant	Negative reaction	Positive reaction, eosin pink/red colour within 2-5 minutes
3.	*Klebsiella pneumoniae ATCC 23357*	50-100	Luxuriant	Negative reaction	Positive reaction, eosin pink / red colour within 2-5 minutes

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





Test Procedure

1. Inoculate MR-VP Medium with growth from single colony.
2. Incubate at 35± 2°C for 48 hours.
3. Test as follows

Methyl Red Test

1. Transfer 2.5 ml of the MR-VP Medium culture to a tube (13x100mm).
2. Add 5 drops of methyl red indicator and observe for a colour change.

VP Test

1. Transfer 2.5 ml of the MR-VP Medium culture to a tube (13x100mm).
2. Add 0.3 ml (6 drops) of Voges-Proskauer Reagent A (5% a-naphthol).
3. Add 0.1 ml (2 drops) of Voges-Proskauer Reagent B (40% KOH).
4. Gently agitate the tube and let stand for 10 to 15 minutes.
5. Observe for color change.

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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 reagent; repeat test after an additional 18 to 24 hours incubation.

Voges-Proskauer (VP) Test

Positive: Red color.

Negative: No red color.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

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

Limitations of the Procedure

1. The MR-VP reactions are only part of the tests required to identify organisms.
2. Each laboratory should standardize on the inoculums density, volume of broth and the test container size.
3. MRtest require a minimum incubation of 48 hours before the pH indicator is added.
4. When using Barritt's reagent add a-naphthol first and KOH second; do not reverse this order.
5. Vaughn et al. [7]warned of false positive VP reactions if the completed tests are left standing for over an hour.

Packaging

Product Name : MR-VP Medium (Glucose Phosphate Broth)

Product Code : DM142

Available Pack sizes : 100gm / 500gm

References

1. DHSS Report 71 (1982) 'The bacteriological Examination of Drinking Water Supplies' HMSO London.
2. Clark, W.M., and H.A.Lubs. 1915. The differntion of bacteria of the colon-aerogenes family by the use of indicators. J. infect. Dis. 17:160-173.
3. Voges O. and Proskauer B. (1898) Z. f. Hyg. 28.20-22.
4. Harden A. and Walpole G.S. (1906) Proc. Roy. Soc. B. 77.399
5. Harden A. and Norris D. (1911) Physiol.42.332.
6. Farmer ,J.J., III. 1995. Enterobacteriaceae: Introduction and identifaction , p.438-499. In P.R. Murray, E.J. Baron,M.A. Pfaller, F.C. Tenover, and R> H. Yolken (ed), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washigton, D.C.
7. Vaughn R.,Mitchell N. B. and Levine M. (1939) J. Amer. Water works Asso.31.993-1DHSS Report

Further Information

For further information please contact your local MICROMASTER Representative.

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