MR -VP Medium (Glucose Phosphate Broth or Buffered Glucose Broth)

Intended Use
MR-VP Medium also known as Glucose Phosphate Broth/Buffered Glucose Broth is used for the differentiation of coli-aerogenes group by means of the Methyl Red and Voges Proskauer reactions.

Summary
Clark and Lubs found that the addition of methyl red to cultures of *E. coli* resulted in a red colour due to the high acidity produced during dextrose fermentation. Voges-Proskauer reported red colouration after the addition of potassium hydroxide to specific culture media with organisms in it. Thus, the investigators developed MR-VP Medium, which enables both tests to be performed in the same medium in different tubes. ISO has recommended this medium for the detection of coli-aerogenes group. MRVP Medium is included in the Bacteriological Analytical Manual for food and cosmetics testing and is also recommended by APHA for the examination of foods and milk.

Principle
Methyl red positive organisms produce high levels of acid during fermentation of dextrose, overcoming the phosphate buffering system and produce a red colour on the addition of methyl red pH indicator. In Voges-Proskauer test, the red colour produced by the addition of potassium hydroxide to cultures of certain microbial species is due to their ability to produce a neutral end product, acetoin (acetylmethylcarbinol), from the fermentation of dextrose. The acetoin is oxidized in the presence of oxygen and alkali to produce diacetyl, which reacts with creatine to give a red colour, which is a positive VP test.

Formula*

Ingredients	**g/L**
Buffered Peptone	7.0
Dextrose	5.0
Dipotassium Phosphate	5.0
Final pH (at 25°C)	6.9 ± 0.2

*Adjusted to suit performance parameters.

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Type of Specimen
Food and Dairy samples; Clinical samples; Water samples

Specimen Collection and Handling
Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions
1. Suspend 17.00 g of the powder in 1000 mL of purified / distilled water.
2. Mix thoroughly.
3. If necessary, heat to dissolve the powder completely.
4. Dispense 10 mL amounts in test tubes.
5. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.

Quality Control

Dehydrated Appearance: Cream coloured, homogenous, free flowing powder
Prepared Appearance: Light yellow to amber coloured, clear solution without any precipitate.
Cultural Response: Cultural characteristics observed after an incubation of 18-48 hours at 30°C-35°C. After incubation add 2-3 drops of Methyl Red Indicator for MR Test and for VP Test add 1-2 drops of Creatine, 2-3 drops of Barritt Reagent A and 1-2 drops of Barritt Reagent B.

Organism (ATCC)	**Growth**	**MR Test**	**VP Test**
Klebsiella aerogenes (13048)	Good	- (Yellow)	+ (Red)
Escherichia coli (25922)	Good	+ (Red)	- (Yellow)
Klebsiella pneumoniae subsp. *pneumoniae* (10031)	Good	- (Yellow)	+ (Red)

Interpretation of Results

1. **Methyl Red Test:**
Positive test - Red colour at the surface of the medium.
Negative test - Yellow colour at the surface of the medium.
2. **Voges-Proskauer Test:**
Positive test - Development of a distinct red colour within 5 minutes.
Negative test - Appearance of a yellow colour or copper like colour on the surface of the medium.
3. Certain species within *Enterobacteriaceae* genera may react differently or give variable results.

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions / Limitations

1. While adding the VP reagents to the medium, it is important that the a-naphthol be added first and the KOH added second. A change in the order may produce invalid test results.
2. False positive VP results may occur if VP tests are read beyond one hour following the addition of reagents.
3. Results of the MR and VP tests need to be used in conjunction with other biochemical tests to differentiate genus and species within the *Enterobacteriaceae.*
4. A precipitate may form in the potassium hydroxide solution. The precipitate has not been shown to reduce the effectiveness of the reagent.
5. Most members of the family *Enterobacteriaceae* give either a positive MR test or a positive VP test. However, certain organisms such as *Hafnia alvei* and *Proteus mirabilis* may give a positive result for both.
6. Incubation time for the methyl red test cannot be shortened by increasing the concentration of dextrose in the medium or by heavily inoculating the broth.
7. Incubate MR negative tests for more than 48 hours and test again.
8. Read the VP test at 48 hours. Increased incubation may produce acid conditions in the broth that will interfere with reading the results.
9. Due to the possible presence of acetoin, diacetyl or related substances in certain raw materials, the use of media, low in these substances (MR-VP Medium) is recommended for this test.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. US Food and Drug Adm; 1998, Bacteriological Analytical Manual, 8th Ed; Rev. A, AOAC, International, Gaithersburg, Md.
2. Downes and Ito (ed.) 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th edition, APHA Washington DC.
3. H. Wehr and J. Frank, 2004, Std. Methods for The Examination of Dairy Products, 17th Edition; APHA, Washington, DC.
4. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product description	Pack Size
201130640100	Dehydrated Culture Media	100 g
201130640500	Dehydrated Culture Media	500 g