

Test Kit for MR-VP

Intended Use

Test Kit for MR-VP is used for detection of acid and acetoin production and for differentiation of *Enterobacteriaceae*.

Summary

In general, different bacteria convert dextrose and glucose to pyruvate using different metabolic pathways. Some of these pathways produce unstable acidic products, which quickly convert to neutral compounds. Some organisms use the butylene glycol pathway, which produces neutral end products including acetoin and/or 2,3-butanediol. Other organisms use the mixed acid pathway, which produces stable acidic end products such as lactic, formic and acetic acid. A medium like MR-VP Broth is used to determine the pathway a given organism uses to ferment glucose; to this end reagents like Methyl Red Indicator or Barritt Reagent A and Barritt Reagent B are added along with Creatine to detect end products or intermediates produced by a given fermentation pathway.

Principle

The MR-VP test includes detection of acid and acetoin production respectively and is mainly used for the differentiation of *Enterobacteriaceae*.

Methyl Red test detects the production of sufficient acid during the fermentation of glucose and the maintenance of conditions such that the pH of an old culture is sustained below 4.4 as shown by a change in colour of the Methyl Red Indicator. Methyl red positive organisms produce high levels of acid during fermentation of dextrose, overcome the phosphate buffer system and produce a red color upon addition of Methyl Red Indicator.

Voges Proskauer test detects the production of acetylmethylcarbinol (acetoin), a natural product formed from pyruvic acid in the course of glucose fermentation. The red colour produced by the addition of Barritt Reagent A and Barritt Reagent B to cultures is due to the ability of organism to produce a neutral product acetoin. The acetoin is oxidized in the presence of oxygen and alkali to produce diacetyl, which reacts in the presence of Creatine to produce a red colour.

Reagent

The Microexpress® Rapid Test Kit for MR-VP is a reagent set for laboratory use only.

The Microexpress® Rapid Test Kit for MR-VP comprises of:

1. 5 vials containing 1 mL medium each for MR test.
2. 5 vials containing 0.2 mL medium each for VP test.

Additional Material Required

0.9% Saline, micropipettes, culture media, activated 2% glutaraldehyde solution, sterile test tube, incubator/water bath at 35°C-37°C.

Directions

Preparation of Inoculum

1. Isolate the organism to be identified on Brain Heart Infusion Agar (BHI).
2. Pick up a single well-isolated colony and streak on to BHI agar slant for enrichment and incubate at 35°C-37°C for 18-24 hours.
3. Observe for good growth.
4. Wash the growth with 2-3 mL sterile saline.
5. Match the turbidity of this suspension to McFarland Standard Number 0.5.

Inoculation of Vials for Methyl Red Test

1. Bring the medium/vial (MR) to room temperature.
2. Inoculate the vial with 100 µL of the above prepared inoculum.
3. Incubate at 35°C-37°C for 18-24 hours.
4. Observe for growth.
5. Add 2-3 drops of Methyl Red Indicator.
6. Observe for colour change.

Inoculation of Vials for Voges Proskauer Test

1. Bring the medium/vial (VP) to room temperature.
2. Inoculate the vial with 100 µL of the above prepared inoculum.
3. Incubate at 35°C-37°C for 18-24 hours.
4. Observe for growth.
5. Add 1-2 drops of Creatine, 2-3 drops of Barritt Reagent A, and 1-2 drops of Barritt Reagent B while shaking at intervals to ensure aeration.
6. Observe for colour change.

Quality Control

Appearance of the Medium: Clear, yellow medium.

Cultural Response: Vials are inoculated with 100 µL culture suspension of following organisms, incubated for 18-24 hours and results observed by adding 2-3 drops of Methyl Red Indicator for MR Test and for VP Test 1-2 drops of creatine, 2-3 drops of Barritt Reagent A and 1-2 drops of Barritt Reagent B.

Organism (ATCC)	Results	
	Reaction in MR Test	Reaction in VP Test
<i>Escherichia coli</i> (25922)	+	-
<i>Klebsiella aerogenes</i> (13048)	-	+

Key: + = Pink to red colour, - = No change in colour

Interpretation of Results

1. Development of pink to red colour indicates a positive MR and VP test.
2. No change in colour denotes a negative test.

Remarks

1. The Microexpress® Test Kit for MR-VP is an *In vitro* diagnostic kit for laboratory and professional use only. Not for medicinal use.
2. The Microexpress® Test Kit for MR-VP cannot be used directly on clinical specimens.
3. Do not use damaged or leaking kits. Avoid contact of reagents with skin and eyes.
4. Clinical samples and microbial cultures should be considered as pathogenic biohazard and handled accordingly. Good laboratory practices and hazard precautions must be observed at all times.
5. Always use pure culture and a heavy inoculum for testing.
6. The test is an aid to identification and is not a confirmatory test. Complete identification should include determination of Gram reaction, morphology, and other biochemical and serological tests.
7. The KOH and alpha-naphthol may react to form a copper like colour, causing a potential false positive reaction.
8. VP reagents must be added in the order and the amounts specified or a weak-positive or false-negative reaction may occur. A weak positive reaction may be masked by a copper like colour. which may form due to the reaction of KOH and alpha naphthol.
9. Certain species within *Enterobacteriaceae* may react differently or give variable results.

Storage and Stability

1. Store the Microexpress® Test Kit for MR-VP at 2°C-8°C away from bright light.
2. Stability of the Microexpress® Test Kit for MR-VP is as per the expiry date mentioned on the label.

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. Practical Medical Microbiology, Mackie & McCartney, 13th edition 1969, Edited by J. G. Coffee, J. P. Duguid.
2. Two quick methods for Voges-Proskauer test by A. L. Barry and K. L. Feeney, Applied Microbiology, Sept. 1967, p.: 1138-1141.
3. Clarke P.H. And S.T. Cowan. Biochemical Methods For Bacteriology, J. Gen. Microbiol., 1952, Vol. 6: 187-197.

4. Improved 18-hour Methyl Red Test, A.L. Barry, *et. al.*, Applied Microbiology, Vol. 20, No. 6, Dec. 1970, p: 866-870.
5. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat. No.	Product Description	Pack Size
203200400001	Ready Prepared Kit	1 Kit (10Tests)

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.
