Principles of the Procedure

Peptones, yeast extract and dextrose provide amino acids and other nitrogenous and carbonaceous substances, vitamins and minerals essential for bacterial metabolism. Motility can be read because of the semi-solid consistency of the medium. Organisms that possess the enzyme "tryptophanase" degrade the amino acid tryptophan to indolepyruvic acid, from which indole can be formed through deamination.[3] When ornithine decarboxylase is present, the ornithine is decarboxylated to putrescine which causes a rise in the pH and corresponding color change of the bromcresol purple from yellow to purple.

Formula

Difco™ MIO Medium

Approximate Formula* Per Liter		
Yeast Extract	3.0	g
Peptone	10.0	g
Tryptone	10.0	g
L-Ornithine HCl	5.0	g
Dextrose	1.0	g
Agar	2.0	g
Bromcresol Purple	0.02	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 31 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

To prepare the stored medium for use in motility studies, loosen caps, heat the medium to boiling and cool to room temperature prior to inoculation. Inoculate tubes of medium by a single stab to 1/4 inch from the bottom of the tube using growth from a primary isolation plate or other pure culture. Incubate all tubes for 18-24 hours at 35 ± 2°C in an aerobic atmosphere.

Expected Results

Read motility and decarboxylase activity prior to the addition of the reagent for the detection of indole production.

1. Motility is indicated by growth extending from the line of inoculation. Nonmotile organisms grow only along the line of inoculation.
2. Decarboxylation of ornithine is indicated by the development of a turbid purple to a faded yellow-purple color. A negative reaction is indicated by a yellow color.
3. Indole production is indicated by the formation of a pink to red color after the addition of three or four drops of Kovacs' reagent to the surface of the medium and gentle shaking. A negative reaction is indicated by the development of a yellow color.

Refer to appropriate texts for typical reactions produced by various members of the *Enterobacteriaceae*.[4-6]

References

1. Ederer and Clark. 1970. Appl. Microbiol. 2:849.
2. Oberhofer and Hajkowski. 1970. Am. J. Clin. Pathol. *54*:720.
3. MacFaddin. 2000. Biochemical tests for identification of medical bacteria, 3rd ed. Lippincott Williams & Wilkins, Baltimore, Md.
4. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., New York, N.Y.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
6. Farmer. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

Difco™ MIO Medium

BAM

Cat. No.		
273520	Dehydrated – 500 g	

BBL™ Motility Indole Ornithine Medium

BAM

Cat. No.		
221517	Prepared Deeps (K Tubes), 5 mL – Pkg. of 10*	
221518	Prepared Deeps (K Tubes), 5 mL – Ctn. of 100*	

**Store at 2-8°C.*

MR-VP Medium • MR-VP Broth

Intended Use

MR-VP Medium and MR-VP Broth (Methyl Red-Voges Proskauer Medium/Broth, also known as Buffered Peptone-Glucose Broth) are used for the differentiation of bacteria by means of the methyl red and Voges-Proskauer reactions.

Summary and Explanation

Voges and Proskauer, in the latter part of the 19th century, reported the initial observations regarding the production of a red color after the addition of potassium hydroxide to specific culture media in which various organisms had grown.[1]

Clark and Lubs,[2] in 1915, found that the addition of methyl red to cultures of *Escherichia coli* resulted in a red color due to the high acidity produced during the fermentation of dextrose. The smaller amount of acid produced by *Klebsiella pneumoniae* and *Enterobacter aerogenes* is converted to acetoin resulting in an alkaline reaction (negative methyl red test).

In the Voges-Proskauer test, Reagent A (5% [w/v] alpha-naphthol in absolute alcohol) contains a catalyst enhancing the formation of specific metabolic products that form a red complex upon the addition of Reagent B (40% [w/v] potassium hydroxide in purified water).

MR-VP Medium/Broth was developed to enable both the MR and the VP tests to be performed in the same medium, although in different tubes or on aliquots from the same tube.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ MR-VP Medium

Dehydrated Appearance:	Very light to light beige, free-flowing, homogeneous.
Solution:	1.7% solution, soluble in purified water. Solution is light amber, clear.
Prepared Appearance:	Light amber, clear.
Reaction of 1.7% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Difco™ MR-VP Medium

Prepare the medium per label directions. Inoculate with fresh cultures and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC™	RECOVERY	METHYL RED	VOGES-PROSKAUER
Enterbacter aerogenes	13048	Good	– (yellow)	+ (red)
Escherichia coli	25922	Good	+ (red)	– (no change)
Klebsiella pneumoniae subsp. *pneumoniae*	23357	Good	– (yellow)	+ (red)

Identity Specifications

BBL™ MR-VP Broth

Dehydrated Appearance:	Fine, homogeneous, free of extraneous material.
Solution:	1.7% solution, soluble in purified water. Solution is pale to light, yellow to tan, clear to slightly hazy.
Prepared Appearance:	Pale to light, yellow to tan, clear to slightly hazy.
Reaction of 1.7% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

BBL™ MR-VP Broth

Prepare the medium per label directions. Inoculate two sets of tubes (3 mL, Voges-Proskauer and 5 mL, Methyl Red) with fresh cultures and incubate at 35 ± 2°C for 48 hours (3 mL) and 5 days (5 mL).

ORGANISM	ATCC™	RECOVERY	METHYL RED	VOGES-PROSKAUER
Citrobacter freundii	8454	Good	+ (red)	– (no change)
Enterbacter aerogenes	13048	Good	– (yellow)	+ (red)
Escherichia coli	25922	Good	+ (red)	– (no change)
Klebsiella pneumoniae subsp. *pneumoniae*	33495	Good	– (yellow)	+ (red)

Principles of the Procedure

Methyl red-positive organisms produce high levels of acid during fermentation of dextrose, overcome the phosphate buffer system and produce a red color upon the addition of the methyl red pH indicator.

In the Voges-Proskauer test, the red color produced by the addition of potassium hydroxide to cultures of certain microbial species is due to the ability of the organisms to produce a neutral end product, acetoin (acetylmethylcarbinol), from the fermentation of dextrose.[3] The acetoin is oxidized in the presence of oxygen and alkali to produce a red color.[3] This is a positive Voges-Proskauer reaction.

Formulae

Difco™ MR-VP Medium

Approximate Formula* Per Liter

Buffered Peptone	7.0	g
Dipotassium Phosphate	5.0	g
Dextrose	5.0	g

BBL™ MR-VP Broth

Approximate Formula* Per Liter

Pancreatic Digest of Casein	3.5	g
Peptic Digest of Animal Tissue	3.5	g
Potassium Phosphate	5.0	g
Dextrose	5.0	g

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

Directions for Preparation from Dehydrated Product

1. Dissolve 17 g of the powder in 1 L of purified water. Mix thoroughly.
2. If necessary, heat slightly to dissolve.
3. Dispense and autoclave at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

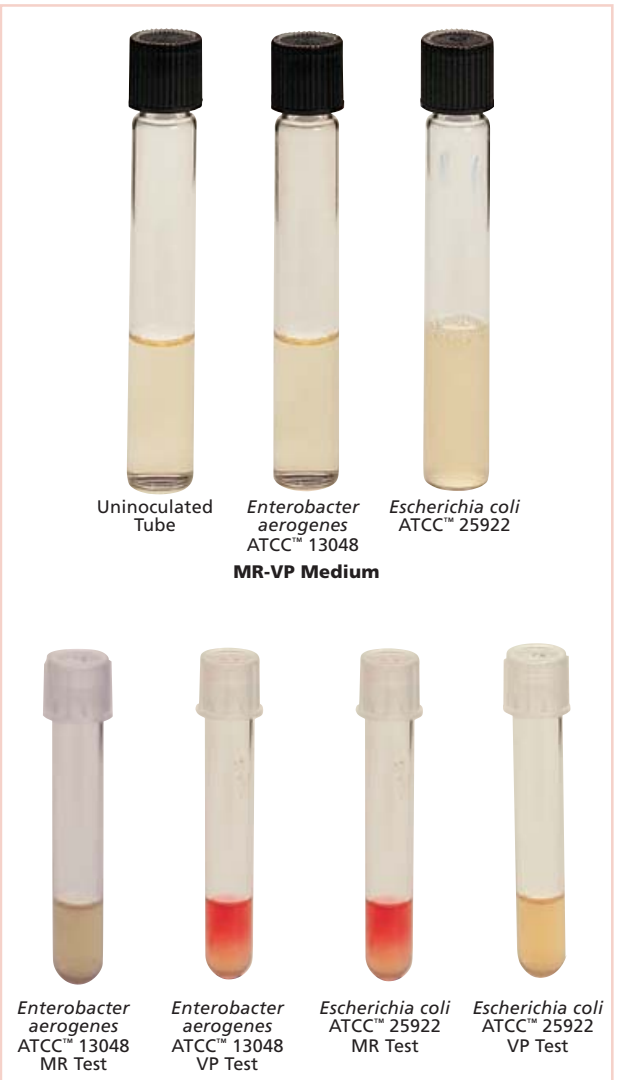
Procedure

Using a light inoculum, inoculate tubes of MR-VP media with 18- to 24-hour pure cultures. Incubate tubes aerobically at 35 ± 2°C for a minimum of 48 hours but preferably for 5 days.

Prepare the methyl red indicator by dissolving 0.1 g of methyl red in 300 mL of 95% ethyl alcohol. Add sufficient purified water to make 500 mL.

After the appropriate incubation period, aseptically remove aliquots (1 mL for the VP test) of the medium and conduct the following tests:

1. Methyl Red Test – Add 5 drops of methyl red indicator to an aliquot of the broth. Interpret the color result immediately.
2. Voges-Proskauer Test – Empty the contents (15 drops) from the reagent A dropper and 5 drops from the reagent B dropper into 1 mL of broth culture. Shake well after the addition of each reagent to aerate the sample.



Expected Results

1. Methyl Red Test
   a. Positive – red color at surface of the medium.
   b. Negative – yellow color at surface of the medium.
2. Voges-Proskauer Test
   A positive reaction is indicated by the development of a distinct red color which occurs within 5 minutes.

Certain species within *Enterobacteriaceae* genera may react differently or give variable results. Consult appropriate texts for reactions of specific species.[3-6]

Limitations of the Procedure

1. 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*.
2. A precipitate may form in the potassium hydroxide reagent solution. This precipitate has not been shown to reduce the effectiveness of the reagent.
3. 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 tests.
4. Incubation time for the Methyl Red test cannot be shortened by increasing the dextrose concentration in the medium or by heavily inoculating the broth.[7]
5. Incubate MR-negative tests for more than 48 hours and test again.
6. Read the VP test at 48 hours. Increased incubation may produce acid conditions in the broth that will interfere with reading the results.[7]
7. 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 color which may form due to the reaction of KOH and α-naphthol.[7]
8. Read the VP test within 1 hour of adding the reagents. The KOH and α-naphthol may react to form a copper-like color, causing a potential false-positive interpretation.[7]
9. Due to the possible presence of acetoin, diacetyl or related substances in certain raw materials,[8] the use of media low in these substances (such as MR-VP media) is recommended for this test.

References

1. Voges and Proskauer. 1898. Z. Hyg. *28*:20.
2. Clark and Lubs. 1915. J. Infect. Dis. *17*:160.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkins, Baltimore, Md.
4. Ewing. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, N.Y.
5. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
6. Farmer. 1999. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
7. Isenberg (ed.). 1992. Clinical microbiology procedures handbook, vol. 1 American Society for Microbiology, Washington, D.C.
8. Barritt. 1936. J. Pathol. *42*:441.

Availability

Difco™ MR-VP Medium

AOAC BAM COMPF ISO SMD SMWW USDA

Cat. No.	216300	Dehydrated – 500 g

BBL™ MR-VP Broth

AOAC BAM COMPF ISO SMD SMWW USDA

Cat. No.	211383	Dehydrated – 500 g
	221667	Prepared Tubes – Pkg. of 10
	221668	Prepared Tubes – Ctn. of 100

Difco™/BBL™ Voges-Proskauer Reagent A

Cat. No.	261192	Droppers – Ctn. of 50

Difco™/BBL™ Voges-Proskauer Reagent B

Cat. No.	261193	Droppers – Ctn. of 50