



QUALITY CONTROL PROCEDURES

I INTRODUCTION

MR-VP Broth is a medium which aids in the differentiation of bacteria by means of the methyl red and Voges-Proskauer reactions.

II PERFORMANCE TEST PROCEDURE

- Inoculate representative samples with the cultures listed below.
 - Inoculate duplicate tubes using a 0.01 mL calibrated loop with 10⁻¹ dilutions of 18- to 24-h **Trypticase™** Soy Broth cultures.
 - Incubate tubes with loosened caps at 35 ± 2°C in an aerobic atmosphere.
- Examination of tubes for growth and determination of reactions.
 - Voges-Proskauer Reaction**—Read tubes after 18–24 h of incubation for growth and reaction. To perform the reaction proceed as follows (using one half of the duplicate tubes):
 - Add 0.6 mL of alpha-naphthol solution (see Barritt reagent, below) to each tube including uninoculated controls.
 - Add 0.2 mL potassium hydroxide solution to each tube including uninoculated controls.
 - Vortex each tube for several seconds.
 - Positive reactions occur at once or within 5 min.
 - Read for the development of a faint pink to deep red color. The appearance of a pink to red color indicates the presence of acetylmethylcarbinol.
 - Methyl Red Reaction**—Read the second half of the duplicate tubes after 5 days incubation for growth and reaction. Proceed as follows:
 - Add five drops of the methyl red indicator solution (see “Test Procedure”) to the test media (including the uninoculated controls).
 - Read the reactions immediately. The appearance of a distinct red color indicates the presence of a high degree of acidity and is regarded as a positive reaction.
 - Record reactions as follows: + = positive reaction (red color); – = negative reaction (yellow color).
- Expected Results

Organisms	ATCC™	MR Reaction	VP Reaction
* <i>Escherichia coli</i>	25922	+	–
<i>Citrobacter freundii</i>	8454	+	–
* <i>Enterobacter aerogenes</i>	13048	–	+
<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	33495	–	+

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

- Examine tubes as described under “Product Deterioration.”
- Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
- Incubate uninoculated representative tubes aerobically at 20–25°C and 30–35°C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

MR-VP Broth is used for the differentiation of bacteria by means of the methyl red and Voges-Proskauer reactions.

V 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* subsp. *pneumoniae* and *Enterobacter aerogenes* is converted to acetoin resulting in an alkaline reaction (negative methyl red test). These investigators developed MR-VP Broth which enabled both tests to be performed in the same medium, although in different tubes or on aliquots from the same tube.

VI PRINCIPLES OF THE PROCEDURE

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 diacetyl which reacts with creatine to produce a red color.³ This is a positive Voges-Proskauer reaction.

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.

VII REAGENTS

MR-VP Broth

Approximate Formula* Per Liter Purified Water

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

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–25°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

This product is not intended for use directly with specimens or mixed cultures. The organism to be tested must first be in pure culture.

IX PROCEDURE

Material Provided: MR-VP Broth

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

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

Preparation of methyl red indicator⁴

Dissolve 0.1 g methyl red in 300.0 mL 95% ethyl alcohol and add sufficient distilled water to make 500 mL.

Preparation of Voges-Proskauer reagents⁴

1. Barritt reagent

Prepare a 5% alpha-naphthol solution in absolute ethanol and a 40% potassium hydroxide (KOH) solution in distilled water.

2. O'Meara modified reagent

Dissolve 40.0 g of potassium hydroxide in 100.0 mL of distilled water. Add 0.3 g of creatine. Refrigerate the reagent between uses and discard any unused portion after 3 weeks.

User Quality Control: See "Quality Control Procedures."

Quality control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

After the appropriate incubation periods, aseptically remove aliquots of the medium and conduct the following tests.

1. Methyl Red Test—Add five drops of methyl red indicator to a 5 mL aliquot of the broth. Interpret the color result immediately.
 - a. Positive—red color at surface of the medium.
 - b. Negative—yellow color at surface of the medium.
2. Voges-Proskauer Test
 - a. Using Barritt reagent
Add 0.6 mL of alpha-naphthol solution and 0.2 mL of KOH solution to 1.0 mL of culture. Shake well after the addition of each reagent. Positive reactions occur at once or within 5 min (final reading) and are indicated by the production of a red color.
 - b. Using O'Meara modified reagent
Add 1.0 mL of reagent to 1.0 mL of culture. Allow tubes to remain at room temperature or at 37°C in an aerobic atmosphere and read results after 4 h. Tests should be aerated by shaking the tubes. Positive reactions are indicated by development of an eosin pink to red color.

Typical MR-VP results for the *Enterobacteriaceae* are as follows:

Result	Genus*
MR+ VP-	<i>Citrobacter</i> <i>Edwardsiella</i> <i>Escherichia</i> <i>Morganella</i> <i>Proteus</i> <i>Providencia</i> <i>Salmonella</i> <i>Shigella</i> <i>Yersinia</i>
MR- VP+	<i>Enterobacter</i> <i>Erwinia</i> <i>Hafnia</i> <i>Klebsiella</i> <i>Serratia</i>

*Certain species within these genera may react differently or give variable results. Consult appropriate texts for reactions of specific species.³⁻⁶

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.⁴⁻¹⁰

XII AVAILABILITY

Cat. No.	Description
221667	BBL™ MR-VP Broth, Pkg. of 10 size K tubes
221668	BBL™ MR-VP Broth, Ctn. of 100 size K tubes

XIII REFERENCES

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