

Methyl Red and Voges-Proskauer (MR-VP)

This information is an abbreviated version of what is presented in the DIFCO and BBL Manual.

Intended Use:

MR-VP medium/broth is two tests in one, the Methyl Red Test and the Voges-Proskauer test. It is very useful in separating members of the family Enterobacteriaceae and some Streptococcus. Most members of the Enterobacteriaceae give either a positive MR or a positive VP test. Several species are positive for both, such as *Proteus mirabilis*.

Principle:

MR-VP is a buffered Peptone-Glucose broth. Organisms that ferment dextrose will release acid into the broth. Adding Methyl Red, an indicator dye which turns red at pH 4.4 and yellow at pH 6.2, to the inoculated MR-VP medium indicates if the bacteria fermented dextrose. The Voges and Proskauer test was originally developed in 1898 by two German associates of Robert Koch. (Pioneers in Medical Laboratory Science. Retrieved 06/09/04 <http://www.hoslink.com/PIONEERS.htm>) Some bacteria can be distinguished on the basis of their production of acetoin, a neutral end product, after incubation in buffered pepton-glucose media. The addition of alpha-naphthol and KOH solutions will result in a pink-red color within a few minutes.

Test Procedure:

1. Lightly inoculate the tube from a single colony, preferably an 18-24 hour culture.
2. Slightly loosen the cap and incubate the tubes at 35-37°C for 48 hours.
3. After incubation, use a sterile pipette to remove two - 1mL aliquots and place into two small tubes.
 - One tube is for the methyl red test and the other for the Voges-Proskauer test.
 - You do not want to contaminate your original broth tube in case you have to do further incubation.
4. Add 5 drops of methyl red to one tube. Read the result immediately. Do NOT mix the tube.
5. For the Voges-Proskauer test add 15 drops of Voges-Proskauer A reagent. Mix well to aerate the sample. Oxygen is needed to complete the reaction.
6. Add 5 drops of Voges-Proskauer B to the tube and mix well to aerate the sample.
7. Read the results within 5-15 minutes.

Results:

Methyl Red - A red color at the surface is considered a positive result. A negative test is indicated by a yellow color at the surface.

Voges-Proskauer - A positive test is indicated by a pink-red color developing within 5 minutes.

Limitations of Procedure:

- Other tests are needed to definitively identify the Enterobacteriaceae.
- The VP test should be done at 48 hours. Longer incubation times could result in false positives.
- The VP reagents must be added in the order listed and with mixing to avoid weak-positive or false-negative results.
- The broth must be incubated for a minimum of 48 hours for the MR test. Negative MR tests should be incubated for an additional 48 hours.