

IMViC Series

The identification of enteric (intestinal) bacteria is of prime importance in determining certain food-borne and waterborne diseases. Many of the bacteria found in the intestines of humans and other mammals belong to the family *Enterobacteriaceae*. These bacteria are short, Gram-negative, non-endospore forming rods. They can be subdivided into lactose fermenters and nonfermenters. Examples include pathogens (*Salmonella* and *Shigella*, both lactose nonfermenters); occasional pathogens (*Klebsiella* and *Escherichia*, both lactose fermenters, and *Proteus*, a lactose nonfermenter); and normal intestinal microbiota (*Enterobacter*, lactose fermenter). The differentiation and identification of these enteric bacteria can be accomplished by using the **IMViC series of tests (Indole, Methyl Red, Voges-Proskauer, and Citrate**; the “i” is for ease of pronunciation).

Indole Production (Fig. 6-42, 6-43 and 6-44, p. 60 of the Atlas)

The amino acid **tryptophan** is found in nearly all proteins. Bacteria that contain the enzyme **tryptophanase** can hydrolyze tryptophan to its metabolic products namely, indole, pyruvic acid, and ammonia. The bacteria use the pyruvic acid and ammonia to satisfy nutritional needs; indole is not used and accumulates in the medium. The presence of indole can be detected by addition of **Kovac’s reagent**. Kovac’s reagent reacts with the indole, producing a bright red compound on the surface of the medium. Bacteria producing a red layer following addition of Kovac’s reagent are **indole positive**; the absence of a red color indicates tryptophan was not hydrolyzed and the bacteria are **indole negative**. The test may be performed in solid agar media (such as SIM agar) or in liquid media containing “tryptone” a digested protein product containing high levels of tryptophan (tryptone broth or tryptic soy broth). For this experiment we will use the liquid tryptone broth medium.

Methyl Red Test (Fig. 6-59 and 6-60, p. 67 of the Atlas)

All enteric bacteria catabolize glucose for their energy needs; however, the end products vary depending on the enzyme pathways present in the bacteria. The pH indicator **methyl red** detects a pH change to the acid range as a result of acidic end products such as lactic, acetic, and formic acids. This test is of value in distinguishing between *Escherichia coli* (a mixed acid fermenter) and *Enterobacter aerogenes* (a butanediol fermenter). **Mixed acid fermenters** such as *E. coli* produce a mixture of fermentations acids and thus acidify the medium. **Butanediol fermenters** such as *Enterobacter aerogenes* form butanediol, acetoin, and few organic acids. Consult Fig. A-5 and Table A-6, p. 207-208 of the Atlas for various types of fermentation reactions. The pH of the medium does not fall as low as during mixed acid fermentation. At a pH of 4 the methyl red indicator turns red – a **positive methyl red test**. At a pH of 6, the indicator turns yellow – a **negative methyl red test**.

Voges-Proskauer Test (Fig. 6-94, 6-95 and 6-96, p. 80-81 of the Atlas)

The Voges-Proskauer test (named after Daniel Voges, German physician, and Bernhard Proskauer, German hygienist, in the early twentieth century) identifies bacteria that ferment glucose, leading to **2-3-butanediol** accumulation in the medium. The addition of 40% KOH and a 5% solution of alpha-naphthol in absolute ethanol (Barritt’s reagent) will detect the presence of **acetoin** – a precursor metabolite in the fermentative synthesis of 2,3-butanediol (Fig. A-5, p. 207 of the Atlas). In the presence of the reagents and acetoin, a cherry-red color develops. Development of a red color in the culture medium within **15-20 minutes** following the addition of KOH and Barritt’s reagent represents a **positive VP test**; absence of a red color

is a **negative VP test**. The broth medium (MR-VP Broth) for the methyl red test and the VP test is the same, although traditionally we use a lower volume of the broth for the VP test. For the methyl red and VP test we will inoculate separate tubes each containing different volumes of the MR_VP medium (larger volume for the methyl red test, smaller volume in a screw-capped test tube for the VP test).

Citrate Utilization Test (Fig. 6-20 and 6-21 p. 51-52 of the Atlas)

The **citrate utilization test** determines the ability of bacteria to use citrate as a sole carbon source for their energy needs. This ability depends on the presence of a **citrate permease** that facilitates transport of citrate into the bacterium. Once inside the bacterium, citrate is converted to pyruvic acid and CO₂. Simmons citrate agar slants contain sodium citrate as the carbon source, NH₄⁺ as a nitrogen source, and the pH indicator bromothymol blue. This test is done on slants since O₂ is necessary for citrate utilization. When bacteria oxidize citrate, they remove it from the medium and liberate CO₂. CO₂ combines with sodium (supplied by sodium citrate) and water to form sodium carbonate – an alkaline product. This raises the pH turns the pH indicator to a blue color and represents a **positive citrate test**; absence of a color change is a **negative citrate test**. Citrate-negative cultures will also show no growth on the medium.

Period 1

Inoculate each test organism into separate tryptone broth (for Indole test), MR-VP broth tubes (large volume for the methyl red test; small volume for the VP test), and onto citrate agar slants. Repeat the IMViC series for each of the test organisms. Label each of the tubes clearly indicating the type of medium and the organism along with your group or table identification and date. Incubate the tubes at 35 °C for 24-48 hours. (Additional incubation time may be needed for slow fermenters with the MR and VP tests)

Period 2

Indole production test (Fig. 6-44, p. 60 of the Atlas)

Add 0.5 ml (about 10 drops) of Kovac's reagent to the tryptone broth tubes. Shake the tube gently. A deep red color quickly develops in the presence of indole. Negative reactions remain colorless or light yellow. The test shown in the Atlas was done with an SIM agar tube, but similar coloration will be seen with the liquid medium)

Methyl Red (MR) test (Fig. 6-60, p. 67 of the Atlas)

Add 0.2 ml (about 4-5 drops) of methyl red indicator solution. Carefully note any color change (a red color is positive for acidic fermentation products, ~ pH 4). If no color develops acid formation due to fermentation is either very weak (~ pH 6) or is not occurring.

Voges-Proskauer (VP) test (Fig. 6-96, p. 80-81 of the Atlas)

While wearing disposable gloves add 0.6 ml (~ 15 drops) of Barritt's reagent A (KOH) and 0.2 ml (~ 5 drops) of Barritt's solution B (α-naphthol in ethanol) to the tube. Cap the tube tightly and shake vigorously to aerate. Positive reactions can occur quickly or may take up to 20 minutes as indicated by the formation of a red color.

Citrate Utilization test (Fig. 6-21 p. 51-52 of the Atlas)

No additional reagent is needed. Examine the slant cultures for the presence or absence of growth and for any change in color from green to blue. The development of a deep blue color is a positive citrate utilization test.

For each test organism record the results of the **IMViC series** in this order:

1. **I**ndole production + or –
2. **M**R - Methyl Red (acid formation) + or –
3. **V**P – Voges Proskauer (acetoin formation) + or –
4. **C**itrate Utilization + or –