# Identification of an Unknown Bacteria

**Theory:**

There are many biochemical tests that can be used to identify the biochemical properties or physical properties of microbes. Each individual microbe species has a result for each of these tests, and combining the results of many tests can quickly narrow down what possible groups of microbes are present—possibly even down to a single species if enough tests are done. This identification process has been streamlined for hospital and clinic laboratories, but today we will conduct each test by hand and interpret the results at the final lab.

## BIOCHEMICAL TESTS:

### SIM agar:

SIM agar is a solid media that is 3 test in one. The "**S**" stands for **sulfide** (**$H_2S$ gas**) **production**. $H_2S$ gas formation in the tube appears as a black precipitant (the SIM agar starts out as a yellow color). The "**I**" stands for **indole production**. The amino acid tryptophan that is found in the SIM agar can be converted into indole as long as the unknown organism produces the enzyme **tryptophanase**. To reveal any indole produced by the tryptophanase, some Kovac's reagent is added to the surface of the SIM agar and a cherry red color is revealed. If there is no indole present, then the yellowish Kovac's reagent fluid stays a yellow color. The "**M**" stands for **motility**. If an organism has **flagella**, it will swim away from the stab site and fill the entire tube. If there is no flagella, the bacteria only grow in the stab and on the surface of the SIM agar.

### MRVP:

The "**MR**" stands for **Methyl Red**, and the "**VP**" stands for "**Voges-Proskauer**". The MRVP tube starts out as a clear yellowish fluid. Some bacteria will produce acidic byproducts, and some will produce neutral pH byproducts as various resources (such as glucose) are fermented. To check for the production of either acidic or neutral byproducts of metabolism, we need to perform two separate tests on the MRVP tube. After a week of growth, aseptically transfer half of the MRVP tube's contents into another clean test tube. In one tube, add 5 drops of methyl red. In the other tube add 15 drops (1 vial) of VP reagent, shake it gently, and wait 15 to 30 minutes.

Methyl Red is a pH indicator dye that turns red at a pH of 4.4 or lower. It turns yellow at a pH of 6.2 or higher. Bacteria from your gut like members of Enterobacteriaceae (such as *Proteus mirabilis*) can produce a lot of acid by fermenting the glucose (dextrose) in the MRVP media. A red color at the surface is considered positive, and a yellow color is negative.

If a bacteria produces a pH neutral byproduct such as acetoin, this byproduct can be detected by adding the VP reagent and waiting a few minutes. A pink-red color on the surface is considered positive for acetoin production (especially if it show up within 5 minutes).

**Sugar Fermentation:**

Some organisms ferment various sugars rather than run those sugars through an anaerobic cycle versus a aerobic cycle. The anaerobic fermentation process produces various acids that drop the pH. The various sugar solutions only have one sugar type per tube (**glucose**, **sucrose**, or **lactose**). Simply put, each bacteria species needs specific enzymes in order to run it through an aerobic cycle or else the sugar gets fermented as it goes through the anaerobic cycle. There is also a pH indicator in the vial that starts out as a red color, but will change to yellow as the pH drops due to the fermentation of the sugar.

**Simmon's Citrate Agar**:

Citrate is a weak organic acid found in many fruits and vegetables. It can serve as a carbon and energy source for many microorganisms. The **citrate** is transported into the microorganism via the enzyme citrate permease and then cut into two pieces (**acetate** and **oxaloacetate**) by **citrate lyase**. Microbes with a full and complete TCA cycle and use the oxaloacetate via the aerobic metabolism. If the microbe does not have a complete TCA cycle, then it can usually break off a $CO_2$ from the oxaloacetate, which changes it to pyruvate and this pyruvate can then be fermented into various byproducts dropping the pH. The Simmon's Citrate Agar has a pH indicator dye in it called bromothymol blue, which will change from green to blue if the pH goes alkaline (pH > 7). Only microbes which can use the oxaloacetate in the full and complete TCA cycle via aerobic metabolism will see the blue color change.

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These are just some of the many biochemical tests available for the identification of various bacterial species. You can add to this a streak isolation on Nutrient Agar and look at colony color, and morphology, perform a Gram stain to look at cell morphology and the Gram stain result (positive or negative). Cultures onto blood agar, MSA agar, and EMB agar are also helpful results to obtain.

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## Materials:

| Amount per Student | Material |
|---|---|
| 1 | unknown bacteria (in nutrient broth test tube) |
| 1 | Nutrient Agar Plate |
| 1 | SIM Agar Tube |
| 1 | MR/VP Tube |
| 1 | Simmon's Citrate Agar Plate |
| 1 | Phenol red broth with 1% glucose |
| 1 | Phenol red broth with 1% lactose |
| 1 | Phenol red broth with 1% sucrose |

**Procedure:**

1. Use a sterile inoculating needle ("stab") to transfer unknown bacteria to the SIM tube. Be sure to "stab" strait down the middle of the agar and pull the stab strait out again.
2. Use the sterile inoculating loop and perform separate streak isolations onto the NA plate and the Simmon's citrate plate.
3. Use the sterile inoculating loop and perform separate transfers of unknown bacteria to each of the tubes: 1% glucose, 1% lactose, 1% sucrose, and the MRVP tube. Be sure to fully sterilize the inoculation loop before each tube to avoid contamination.
4. Store all plates and and tubes at room temperature. Interpret results at the next lab.