Biochemical Media Laboratory I & II

Biochemical tests are used to detect the presence of specific enzymes, which will allow you to distinguish between various microorganisms.

The IMViC tests are used to distinguish between members of Enterobacteriaceae, such as *Escherichia coli* and *Enterobacter aerogenes*. Each of the capital letters in “IMViC” stands for a different test. “I” is for indole, “M” is for methyl red, “V” is for Vogues-Proskauer, and “C” is for citrate. The pattern obtained from the reactions can be used to point to specific organisms. For example, + + - - or pos, pos, neg, neg is the pattern that describes *Escherichia coli*. The pattern - - + + or neg, neg, pos, pos refers to the reactions seen with *Enterobacter aerogenes*.

The **indole test** determines whether or not a microbe has the ability to break down the amino acid tryptophan into indole, pyruvic acid, and ammonia. In order for this to be possible, the microbe must produce an enzyme called tryptophanase. You will test this by inoculating your microbe into casitone broth. This media contains casein, which is a milk protein rich in tryptophan. Following incubation, add 5 drops of Kovacs test reagent to the tube. Kovacs reagent contains para-dimethylamino benzaldehyde, which interacts with indole to produce a red compound. So, if indole is present, a ruby red color will form at the top of the test tube. The red color is a positive result.

Both the Methyl Red and Vogues-Proskauer tests utilize the same MR-VP broth, which is composed of peptones, a phosphate buffer, and glucose. The **methyl red test** tests for the ability of a microbe to perform mixed-acid fermentation. Organisms that perform mixed-acid fermentation produce enough acid to overcome the buffering capacity of the broth, so a decrease in pH results. Following incubation, add 5 drops of methyl red to your tube. Methyl red turns red below a pH of 4.4 and yellow above a pH of 6. A positive result for this test is a red color after the addition of the pH indicator. A yellow or orange color indicates a negative result.

The **Vogues-Proskauer** test detects organisms that utilize the butylene glycol fermentation pathway and produce a product called acetyl methyl carbinol (acetoin). If acetoin is produced, it can be detected by a color change produced by molecular oxygen and the addition of two reagents, VP A – 5% α-naphthol and VP B – 40% KOH. These reagents are added AFTER incubation. Acetoin is oxidized to diacetyl in the presence of KOH, and the α-naphthol reacts with diacetyl and other components of the media to produce a red color. This usually appears as a red band at the top of the broth 15 to 30 minutes following the addition of the reagents. A copper color indicates a negative result. The addition of reagents MUST occur while using the ventilation hood.

The **citrate test** is used to determine if a microbe can use citrate as its sole source of carbon. If the microbe produces the enzyme citrase, it can break down citrate into oxaloacetic acid and acetic acid. The oxaloacetic acid is then hydrolyzed into pyruvic acid and CO₂. CO₂ reacts with components of the medium to produce an alkaline compound. The alkaline pH of the media turns the pH indicator (bromthymol blue) from green to a deep Prussian blue. Blue represents the positive result, green indicates a negative result.
Phenol Red (PR) carbohydrate fermentation broth tests include the PR glucose, PR sucrose, PR lactose, PR maltose, and the PR mannitol fermentation tests. The PR sugar broth contains peptones, a single carbohydrate, a pH indicator (phenol red), and an inverted durham tube to capture gas. Phenol red is yellow at an acid pH and become red and then fuchsia as pH becomes more alkaline. If the microbe is capable of fermenting the sugar, acidic byproducts are formed, pH falls, and the broth turns yellow. If gas is produced as a consequence of the fermentation, an air bubble will be present in the durham tube or the tube will be floating at the top of the broth. Gas will not be produced in the absence of sugar fermentation.

The tryptic nitrate broth test determines whether the microbe produces nitrate reductase, an enzyme that allows the microbe to reduce nitrate to nitrite. Following incubation, add 5 drops of Nitrate A (sulfanilic acid) and 5 drops of Nitrate B (dimethyl –β-naphthylamine) to the broth. If nitrite is present a red color will appear in the media after approximately 5 minutes. If a red color does not appear there are two possible conclusions. First, the organism did not reduce nitrate to nitrite. The second possibility is that the organism did indeed reduce nitrate to nitrite and the nitrite left the test tube in the form of a gaseous byproduct (such as N2, or NH3 or NO). This would be a positive test for nitrate reduction. To determine which of these two possibilities occurred add a pinch of powdered zinc to the broth. Zinc will actually catalyze the conversion of nitrate to nitrite. If the organism never in the first place reduced nitrate to nitrite, nitrate should still be in the broth. Addition of zinc will produce nitrite from the unused nitrate and the media (reagents A and B already added) will turn red after approximately 5 minutes. At this point of the procedure, this result represents a negative result, indicating that the organism does not have the ability to reduce nitrate to nitrite. If instead a red color does not develop after approximately 5 minutes after adding the zinc, this indicates that the organism did indeed reduce nitrate to nitrite and the nitrite left the tube in the form of a gas. This is a positive result.

The urea agar slant is used to determine if the microbe can produce the enzyme urease and hydrolyze urea producing alkaline products. Among other things, the agar contains buffers, urea, and the pH indicator phenol red. If the microbe makes urease, alkaline products will be formed, pH will increase, and the agar turns fuchsia. Lack of this color change should be interpreted as a negative result.

The triple sugar iron (TSI) agar contains peptones, phenol red, iron, glucose, sucrose, and lactose. TSI has 1/10 the amount of glucose as lactose and sucrose. We want to know if the organism can ferment any or all of the three sugars present in the media. If sugar fermentation occurs, pH decreases and phenol red turns the agar yellow. Sugar fermentation may or may not be accompanied by gas production. Gas production can be recognized by the appearance of bubbles or cracks within the media. A yellow butt indicates that the microbe is capable of glucose fermentation only. The glucose is used up in the media after 10 hours, and the aerobic areas of the slant revert to an alkaline state (red). If the microbe has the ability to ferment lactose and/or sucrose in addition to glucose, both the butt and slant will be yellow.
TSI slants are also used to determine if an organism can produce H₂S (hydrogen sulfide) from the reduction of sulfur. When H₂S is produced, it reacts with the iron in the media to form FeS (iron sulfide), which is a black precipitate. The black precipitate is a positive result for H₂S production. Sulfur reduction only occurs in an acidic environment, so some sugar fermentation must have occurred. If the black precipitate hides the color of the butt, look at the color of the top of the slant in order to determine what sugars were fermented.

Sources:

Professor Scott Rose “Biochemical media” handout

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http://www.austincc.edu/microbugz/phenol_red_broth.php

http://www.austincc.edu/microbugz/urease_test.php

http://www.austincc.edu/microbugz/triple_sugar_iron_agar.php