

BIOCHEMICAL TEST FOR DISEASE DIAGNOSIS

Various biochemical test are used for the identification of bacteria based on the character of a particular bacteria to give its positive and negative result for a particular biochemical test.

1. The Indole Test

The test organism is inoculated into tryptone broth, a rich source of the amino acid tryptophan. Indole positive bacteria such as *Escherichia coli* produce tryptophanase, an enzyme that cleaves tryptophan, producing indole and other products. When Kovac's reagent (p-dimethylaminobenzaldehyde) is added to a broth with indole in it, a dark pink color develops. The indole test must be read by 48 hours of incubation because the indole can be further degraded if prolonged incubation occurs. The acidic pH produced by *Escherichia coli* limits its growth.

2. The Methyl Red and Voges-Proskauer Tests

The methyl red (MR) and Voges-Proskauer (VP) tests are read from a single inoculated tube of MR-VP broth. After 24-48 hours of incubation the MR-VP broth is split into two tubes. One tube is used for the MR test; the other is used for the VP test. Media contains glucose and peptone. All enterics oxidize glucose for energy; however the end products vary depending on bacterial enzymes. Both the MR and VP tests are used to determine what end products result when the test organism degrades glucose. *E. coli* is one of the bacteria that produces acids, causing the pH to drop below 4.4. When the pH indicator methyl red is added to this acidic broth it will be cherry red (a positive MR test). *Klebsiella* and *Enterobacter* produce more neutral products from glucose (e.g. ethyl alcohol, acetyl methyl carbinol). In this neutral pH the growth of the bacteria is not inhibited. The bacteria thus begin to attack the peptone in the broth, causing the pH to rise above 6.2. At this pH, methyl red indicator is a yellow color (a negative MR test).

The reagents used for the VP test are Barritt's A (alpha-naphthol) and Barritt's B (potassium hydroxide). When these reagents are added to a broth in which acetyl methyl carbinol is present, they turn a pink-burgundy color (a positive VP test). This color may take 20 to 30 minutes to develop.

3. Catalase Test

Catalase is the enzyme that breaks hydrogen peroxide (H_2O_2) into H_2O and O_2 . Hydrogen peroxide is often used as a topical disinfectant in wounds, and the bubbling that is seen is due to the evolution of O_2 gas. H_2O_2 is a potent oxidizing agent that can wreak havoc in a cell; because of this, any cell that uses O_2 or can live in the presence of O_2 must have a way to get rid of the peroxide. One of those ways is to make catalase.

4. The Citrate Test

The citrate test utilizes Simmon's citrate media to determine if a bacterium can grow utilizing citrate as its sole carbon and energy source. Simmon's media contains

bromthymol blue, a pH indicator with a range of 6.0 to 7.6. Bromthymol blue is yellow at acidic pH's (around 6), and gradually changes to blue at more alkaline pH's (around 7.6). Uninoculated Simmon's citrate agar has a pH of 6.9, so it is an intermediate green color. Growth of bacteria in the media leads to development of a Prussian blue color (positive citrate). Enterobacter and Klebsiella are citrate positive while E.coli is negative.

5. Oxidase Test

The oxidase test identifies organisms that produce the enzyme cytochrome oxidase. Cytochrome oxidase participates in the electron transport chain by transferring electrons from a donor molecule to oxygen. The oxidase reagent contains a chromogenic reducing agent, which is a compound that changes color when it becomes oxidized. If the test organism produces cytochrome oxidase, the oxidase reagent will turn blue or purple within 15 seconds.

6. Urease Test

Urease broth is a differential medium that tests the ability of an organism to produce an exoenzyme, called urease that hydrolyzes urea to ammonia and carbon dioxide. The broth contains two pH buffers, urea, a very small amount of nutrients for the bacteria, and the pH indicator phenol red. Phenol red turns yellow in an acidic environment and fuchsia in an alkaline environment. If the urea in the broth is degraded and ammonia is produced, an alkaline environment is created, and the media turns pink. Many enterics can hydrolyze urea; however, only a few can degrade urea rapidly. These are known as "rapid urease-positive" organisms. Members of the genus *Proteus* are included among these organisms. Urea broth is formulated to test for rapid urease-positive organisms. The restrictive amount of nutrients coupled with the use of pH buffers prevent all but rapid urease-positive organisms from producing enough ammonia to turn the phenol red pink.

7. Phenol Red Broth

Phenol Red Broth is a general-purpose differential test medium typically used to differentiate gram negative enteric bacteria. It contains peptone, phenol red (a pH indicator), a Durham tube, and one carbohydrate. Three different kinds of phenol red broths. One contains glucose; one contains lactose, and the last contains sucrose. The objective of the exercise is to determine which organisms can utilize each sugar. Phenol red is a pH indicator which turns yellow below a pH of 6.8 and fuchsia above a pH of 7.4. If the organism is able to utilize the carbohydrate, an acid by-product is created, which turns the media yellow. If the organism is unable to utilize the carbohydrate but does use the peptone, the by-product is ammonia, which raises the pH of the media and turns it fuchsia. When the organism is able to use the carbohydrate, a gas by-product may be produced. If it is, an air bubble will be trapped inside the Durham tube. If the organism is unable to utilize the carbohydrate, gas will not be produced, and no air bubble will be formed.

8. Triple Sugar Iron Agar

Triple sugar iron agar (TSI) is a differential medium that contains lactose, sucrose, a small amount of glucose (dextrose), ferrous sulfate, and the pH indicator phenol red. It is used to differentiate enterics based on the ability to reduce sulfur and ferment carbohydrates. As with the phenol red fermentation broths, if an organism can ferment any of the three sugars present in the medium, the medium will turn yellow. If an organism can only ferment dextrose, the small amount of dextrose in the medium is used by the organism within the first ten hours of incubation. After that time, the reaction that produced acid reverts in the aerobic areas of the slant, and the medium in those areas turns red, indicating alkaline conditions. The anaerobic areas of the slant, such as the butt, will not revert to an alkaline state, and they will remain yellow. This happens with *Salmonella* and *Shigella*

9. Coagulase Test

The coagulase test identifies whether an organism produces the exoenzyme coagulase, which causes the fibrin of blood plasma to clot. Organisms that produce catalase can form protective barriers of fibrin around themselves, making themselves highly resistant to phagocytosis, other immune responses, and some other antimicrobial agents. The coagulase slide test is used to identify the presence of bound coagulase or clumping factor, which is attached to the cell walls of the bacteria. Bound coagulase reacts with the fibrinogen in plasma, causing the fibrinogen to precipitate. This causes the cells to agglutinate, or clump together, which creates the "lumpy" look of a positive coagulase slide test. You may need to place the slide over a light box to observe the clumping of cells in the plasma. The coagulase tube test has been set up as a demo for you to observe in class. This version of the coagulase test is used to identify the presence of either bound coagulase or free coagulase, which is an extracellular enzyme. Free coagulase reacts with a component of plasma called coagulase-reacting factor. The result is to cause the plasma to coagulate. In the demo, the coagulase plasma has been inoculated with *Staphylococcus aureus* and *Staphylococcus epidermidis* and allowed to incubate at 37°C for 24 hours. *Staphylococcus aureus* produces free coagulase; *Staphylococcus epidermidis* does not. The coagulase test is useful for differentiating potentially pathogenic *Staphylococci* such as *Staphylococcus aureus* from other Gram positive, catalase-positive cocci.

Results: