## 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.

PROCEDURE

a. Place a small amount of growth from your culture onto a clean microscope slide. If using colonies from a blood agar plate, be very careful not to scrape up any of the blood agar—blood cells are catalase positive and any contaminating agar could give a false positive.

b. Add a few drops of $H_2O_2$ onto the smear. If needed, mix with a toothpick. DO NOT use a metal loop or needle with $H_2O_2$; it will give a false positive and degrade the metal.

c. A positive result is the rapid evolution of $O_2$ as evidenced by bubbling.

d. A negative result is no bubbles or only a few scattered bubbles.

e. Dispose of your slide in the biohazard glass disposal container. Dispose of any toothpicks in the Pipet Keeper.

## OXIDASE TEST

Basically, this is a test to see if an organism is an aerobe. It is a check for the presence of the electron transport chain that is the final phase of aerobic respiration. Normally, oxygen is the final electron acceptor for this system. In the oxidase test, an artificial final electron acceptor (N,N,N',N'-tetramethyl phenylenediamine dihydrochloride) is used in the place of oxygen. This acceptor is a chemical that changes color to a dark blue/purple when it takes the electron from the last element (cytochrome oxidase) in the electron transport chain.

PROCEDURE

SHARE OPEN DROPPER BOTTLES. OPEN A NEW REAGENT DROPPER ONLY IF THERE ARE NONE OPEN YET!

a. To open an new reagent dispenser: Hold reagent dropper upright and point tip away from yourself. Grasp the middle with thumb and forefinger and squeeze gently to crush the glass ampule inside the dropper. Tap the bottom on the tabletop a few times. Invert the ampule and squeeze gently for drop-by-drop dispensing.

b. With a sterile swab, obtain a small amount of organism from an agar slant or plate.

c. Place one drop of reagent onto the culture on the swab.

d. Positive reactions turn the bacteria violet to purple immediately or within 10 to 30 seconds. Delayed reactions should be ignored.