Core Microbiology Skills

How to Perform a Catalase Test

Today, we're going to be learning how to do a catalase test. The catalase test is one of several basic microbiological tests used to differentiate bacterial species. How does it do that? Well, bacteria that contain the catalase enzyme can hydrolyze hydrogen peroxide into water and oxygen, and this will result in visible gas bubbles. It is especially effective in differentiating Staphylococcus species, which are catalase positive, and Streptococcus species, which are catalase negative. It is also used as part of the rule out and refer protocol for sentinel laboratories in the identification of biothreat agents such as Bacillus anthracis.

In this training program, you will learn the proper techniques in performing two methods of catalase tests: the slide method and the tube method. For these procedures, you will need the following: personal protective equipment, a three percent solution of hydrogen peroxide (H2O2); a three percent solution is commonly used to perform this test with most aerobic bacteria. However, the higher concentrations, such as 10 percent, 15 percent, or 30 percent solutions of hydrogen peroxide may be used when performing the test on other bacteria.

Warning: A 30 percent solution of hydrogen peroxide is caustic. If skin contact occurs, wash immediately with 70 percent ethyl alcohol — not water. You will also need sterile wood applicator sticks or plastic loops. Avoid using wire loops because the metal within the loop could interfere with the reaction and cause a false positive. You'll also need pipettes, a biohazardous waste container, and young colonies of bacteria on agar media. Preferably, these colonies should be 18 to 24 hours old, and blood or chocolate agar media can be used. If you are performing the slide method, two clean glass slides and two petri dishes will be needed to contain aerosols produced by the catalase reaction. If you are performing the tube method, two 12 x 75 millimeter test tubes with caps and a test tube rack will be needed. These tests are usually performed on the laboratory bench. Because these tests produce aerosols that can carry infective bacteria, this test can be performed inside a petri dish, tube, or inside a biosafety cabinet.

The slide method: We are doing both a positive and negative test for demonstration purposes only. Place each slide, one for the positive reaction and one for the negative reaction, onto the bench top, label them, and place them in separate uncovered petri dishes. Using a sterile wooden stick or plastic loop, touch the center of an 18- to 24-hour-isolated colony from the positive control plate and smear a small area on the glass slide. Then discard the stick or loop into a sharps container. Remember, if you're using colonies grown on a blood agar plate, be careful not to pick up any red blood cells with the colony. Red blood cells contain catalase and can provide a false positive result. Repeat the process with your negative control.

Next, using a dropper, add one or two drops of H2O2 solution to the smeared colony on the positive control slide and immediately place the cover onto the petri dish. Against your bench top or a dark background, look for the formation of bubbles immediately upon the addition of the H2O2. You may need to use a magnifying lens or other visual aid. An immediate formation of bubbles indicates a positive reaction. A weak positive has one to two bubbles. Discard your pipette into a biohazardous

waste container. To verify the accuracy of your reagents, next, test the negative control slide. Add one drop of H2O2 solution to the smeared colony on the negative control slide and close the petri dish. No formation of bubbles within 20 seconds indicates a negative reaction.

The Tube Method: To perform the tube method, first, label the two 12 x 75 millimeter test tubes positive and negative and place them in a test tube rack. The tube will contain the aerosols. Then add four to five drops of H2O2 solution to each of the 12 x 75 millimeter test tubes. Discard your pipette into a biohazardous waste container. Use the sterile applicator stick or plastic loop and pick up a small amount of an 18- to 24-hour-old isolated colony from the positive control. Place and leave the applicator stick or plastic loop in the tube. Place the tube against the bench top or a dark background and look for the immediate formation of bubbles, which indicates a positive reaction. A weak positive has one to two bubbles. Repeat the process for the negative control. No formation of bubbles within 20 seconds indicates a negative reaction.

An alternate method is to use a tube with a cap. Add a few drops of the H2O2 to the tube Transfer colony material from the plate to the wall of the tube just above the level of H2O2. Cap the tube. Then tilt the tube until the H2O2 washes over the colony material on the wall and observe for evidence of bubbling.

So as you can see, the catalase test is a rapid, straightforward test that when done properly, can differentiate between Staphylococcus species, which is catalase positive, and the Streptococcus species, which is catalase negative. It is also used as part of the rule out or refer protocol for sentinel laboratories in the identification of biothreat agents such as Bacillus anthracis.

Link to video job aid: https://reach.cdc.gov/jobaid/how-perform-catalase-test.