Core Microbiology Skills

How to Perform an Oxidase Test

In this training program, you will learn the proper technique to perform the oxidase test. This test is one of several tests used to identify Gram-negative bacteria. The oxidase test is based on the production of the enzyme indophenol oxidase or cytochrome oxidase. Indophenol oxidase oxidizes the phenylenediamine oxidase reagent to form a dark purple compound, indophenol blue. This is usually only present in aerobic organisms. You have a positive reaction if the paper develops a deep blue color within 10 to 30 seconds. A weak reaction may occur in 30 to 60 seconds. This indicates that the indophenol oxidase has oxidized the phenylenediamine oxidase reagent to form indophenol blue.

For this procedure, you will need the following materials and equipment: personal protective equipment, a timer or clock, a sharps container, and a petri dish. You will also need filter paper. Do not use the filter paper once it begins to darken. And you'll need sterile wooden sticks. You can also use platinum loops, plastic loops, or platinum non-nichrome wires. For the reagent, we will be using an oxidase solution based on the formulation by Kovacs.

Procedure

There are multiple methods used in the clinical laboratory for performing the oxidase test. In this video, we will only show the filter paper method. Please note: you should always refer to the reagent manufacturer's instructions. This procedure is made up of three steps: preparing the filter paper, testing the colony, and interpreting the results.

First, place a piece of filter paper in a petri dish. Next, moisten the filter paper with one to two drops of the oxidase reagent. Always check that the reagent has not expired. To test the colony, pick an isolated colony with a stick, wire, or loop and smear it onto the reagent-dampened filter paper. Place the stick or plastic loop into the sharps container. If a wire or platinum loop is used, then sterilize it according to your protocol. Set the timer and observe for no more than 60 seconds.

You have a positive reaction if the paper develops a deep blue color instantly or within 10 to 60 seconds. A strong positive will develop a blue purple color within 30 seconds. And a weak positive will develop a blue purple color within 30 to 60 seconds. If the paper does not show any color change within 60 seconds, you have a negative reaction. Do not read any results past 60 seconds. Remember to check the manufacturer's instructions for timing of their reagent.

In summary, the oxidase test is one of several tests used to identify Gram-negative bacteria. There are two possible results with this test. A positive reaction, which shows a blue purple color within 10 to 60 seconds, and a negative reaction, which shows no color within 60 seconds. Last, remember to dispose of all the waste into the biological waste container.

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