27: Oxidase Test

Objectives

- Test for the enzyme oxidase on your unknown isolates

The oxidase test is a key test to differentiate between the families of *Pseudomonadaceae* (ox +) and *Enterobacteriaceae* (ox -), and is useful for speciation and identification of many other bacteria, those that have to use oxygen as the final electron acceptor in aerobic respiration. The enzyme cytochrome oxidase is involved with the reduction of oxygen at the end of the electron transport chain.

There may be different types of oxidase enzymes produced by bacteria. The colorless redox reagent, tetramethyl-p-phenylenediamine dihydrochloride (or dimethyl can be used) used in the test will detect the presence of the enzyme oxidase and, reacting with oxygen, turn a color. The oxidase reagent contains a chromogenic reducing agent, a compound that changes color when it becomes oxidized, so it acts as an artificial electron acceptor for the enzyme oxidase. The oxidized reagent forms the colored compound indophenol blue.

**IMPORTANT POINTS**

- We keep the oxidase reagent either frozen or unopened in dropper bottles until needed. If old reagent is sitting out on the bench and is PURPLE, ask for a new tube from the instructor.
- Use a young culture, preferably less than 24 hrs old.
- Use a culture growing on an agar plate or agar slant.
- Use FRESH reagent, less than a couple of hours old.
- Pick your inoculum, not with a metal loop (reagent may react with the metal), but with a wooden stick.
- Read the reaction within 20 seconds (NOT after), usually it will change in less than 15 seconds. The oxygen will change the reagent color as time passes, so it must be read quickly.
MATERIALS NEEDED

- oxidase reagent
- wooden rods

THE PROCEDURE

1. Pick a **good-sized** amount of inoculum (already incubated and grown) from a plate culture or slant culture and place it on a piece of filter paper FIRST.
2. Add **one drop** of the reagent (if it is dark blue, it is old and should not be used). OR you can drop the reagent directly onto the slant or plate, but that might damage your culture.
3. TIME the reaction: a positive reaction will occur within 20 seconds. DO NOT READ the reaction after 30 seconds.

INTERPRETATION

![Oxidase neg. Oxidase pos.](image)

The reagent acts as an artificial electron acceptor for the enzyme oxidase and is oxidized to form the colored compound Wurster’s blue. Wurster’s blue is a purple compound that is readily visible and signifies a positive reaction. A positive reaction will usually occur within 10-15 seconds, and will be a **bluish-purple** color that progressively becomes more purple.

QUESTIONS

1. Why do you have to read this reaction within 30 seconds?
2. Why does the oxidase reagent need to be fresh?

Contributors

- Jackie Reynolds, Professor of Biology (Richland College)