

Oxidase Biochemical Assay

Tests for the presence of the enzyme indophenol oxidase.

Intended Use:

The oxidase test is based on the production of an enzyme called indophenols oxidase. This enzyme oxidizes a redox dye (present in the reagent) which results in a color change of yellow to dark purple.

Principle:

Indophenol oxidase, in the presence of atmospheric oxygen, oxidizes the phenylenediamine oxidase reagent to form a dark purple compound, indophenol.

Test Procedure:

1. Have you instructor or IA crush the ampule inside the dropper.
2. Tap bottom on tabletop a few times. Then invert for convenient drop-by-drop dispensing of reagent
3. Preparation for testing:
 - a. Colonies to be tested must be isolated from other colonies
 - b. The use of fresh isolates (18-24 hr cultures) is recommended for routine testing.
 - c. If refrigerated, cultures must be allowed to reach room temperature prior to testing
4. Performing the test – Filter Paper Method
 - a. Add a few drops of oxidase test reagent to a strip of filter paper (Whatman No. 1 or equivalent).
 - b. Streak a loopful of bacteria onto the reagent-saturated paper with a platinum loop or wooden applicator stick. Use of steel or nichrome loops may cause false-positive reactions

Results:

Positive reactions turn the bacteria violet to purple immediately, or up to 30 seconds. Negative reactions remain colorless or turn light pink/light purple after 30 seconds. Delayed reactions should be ignored.

Limitations:

- Allow up to 30 seconds for a positive reaction.
- Any delayed reactions should be considered negative.
- Do not add excess reagent, at it may cause the reaction to fade on oxides-positive organisms.
- Steel loop, nichrome loop, and wire loop containing iron may give a false-positive reaction. A platinum loop or wooden applicator stick is recommended.