

OXIDASE TEST

Principle and Purpose

Oxidase enzymes are key components of the electron transport system and have a critical role in aerobic respiration. In particular, cytochrome c oxidase, which is usually only present in aerobic organisms, uses molecular oxygen as an electron acceptor generating water as a consequence of the reduction-oxidation reaction. The oxidase test, as conducted within this exercise, will detect the activity of cytochrome oxidase or indophenol oxidase.

Not all microbes possess these enzymes. The presence or absence of oxidases can be used to distinguish between various species of cocci as well as Gram-negative bacilli. Among the cocci, *Micrococcus*, *Neisseria* and *Moraxella* are oxidase positive, whereas most species of *Staphylococcus*, *Streptococcus*, and *Enterococcus* are oxidase negative. An example of an oxidase negative bacillus is *Pseudomonas*, whereas enterobacteria are generally oxidase positive.

In the present exercise, students will use a commercial product, OxiSticks™ Oxidase Swabs (Fig. 1; Hardy Diagnostics), which are impregnated with N,N,N',N'-tetramethyl-*p*-phenylenediamine dihydrochloride (TMPD). If present in a test organism, cytochrome c oxidase or indophenol oxidase will be reduced by this reagent, i.e., accept an electron from TMPD. The TMPD then forms a product that is dark purple/blue to maroon in color within 30 seconds.



Figure 1. Oxidase Swab Test. The development of a blue/purple color within 10-20 seconds is indicative of a positive oxidase reaction.

Learning Objectives

Upon completion of this exercise, a student should be able to:

- Understand the underlying mechanism of the oxidase test;
- Properly conduct the oxidase test; and
- Accurately interpret the results of this test.

Materials Required

The following materials are necessary to successfully conduct this exercise:

Organisms - The following organisms should be provided as 18-36 hour-old TSA Petri dish (preferred) or slant cultures:

- *Enterococcus faecalis* (ATCC 19433) [abbreviated as *E. faecalis*]
- *Micrococcus luteus* (ATCC 4698) [abbreviated as *M. luteus*]
- *Moraxella catarrhalis* (ATCC 23246) [abbreviated as *M. catarrhalis*]
- *Staphylococcus aureus* (ATCC 25923) [abbreviated as *S. aureus*]

Materials

- OxiSticks™ Oxidase Swabs (Cat. No. Z193; Hardy Diagnostics, Santa Maria, CA; https://catalog.hardydiagnostics.com/cp_prod/Content/hugo/OxiStripsOxisticks.htm),

Procedure

There are several means of conducting the oxidase test, including the direct application oxidase reagent to colonies on an agar plate and the transfer of bacteria to a paper strip impregnated with the reagent. However, this exercise describes the use of a commercially available swab impregnated with the oxidase reagent, TMPD, that can detect the presence of the oxidase.

- 1) Remove an OxiSticks™ Oxidase swab from its container without touching the tip.
- 2) Use the swab to carefully, yet quickly collect 3-4 well isolated colonies from an agar plate culture (or growth from an agar slant culture) of *S. aureus*.

Interpretation of Results: The appearance of a blue/purple to maroon color on the swab within 30 seconds indicates a positive oxidase test. Any color development appearing after 30 seconds of inoculation should be disregarded.

Record any observations on the data report sheet attached to this document.

- 3) Repeat steps 1 and 2 for colonies from an agar plate culture (or growth from an agar slant culture) of *E. faecalis*.
- 4) Repeat steps 1 and 2 for colonies from an agar plate culture (or growth from an agar slant culture) of *M. catarrhalis*.
- 5) Repeat steps 1 and 2 for colonies from an agar plate culture (or growth from an agar slant culture) of *M. luteus*.

Disposal/Recycling of Materials

Place all used OxiSticks™ Oxidase swabs in the bench-top disposal bin.

Student Name: _____

OXIDASE TEST OF SELECTED COCCI

Record the color of the oxidase reaction on the test swab for each bacterial species tested and indicate if the test is positive or negative for the presence of oxidase.

Color Reaction of Selected Cocci to Oxidase Swab Test

<i>Moraxella catarrhalis</i>		<i>Enterococcus faecalis</i>		<i>Staphylococcus aureus</i>		<i>Micrococcus luteus</i>	
Color Reaction	Oxidase Positive?	Color Reaction	Oxidase Positive?	Color Reaction	Oxidase Positive?	Color Reaction	Oxidase Positive?

Discussion Questions

1. Why should the use of a nichrome- or iron-containing loop/wire be avoided as an inoculating device when performing the oxidase test?
2. Do obligate anaerobes require a functional oxidase? Why or why not?
3. Do facultative anaerobes require a functional oxidase? Why or why not?