

Oxidase Test

Subject: Microbiology

Stage: 3st

Lab: 3

Lecturer: M.Sc. Duaa Abdul Abbas Negam



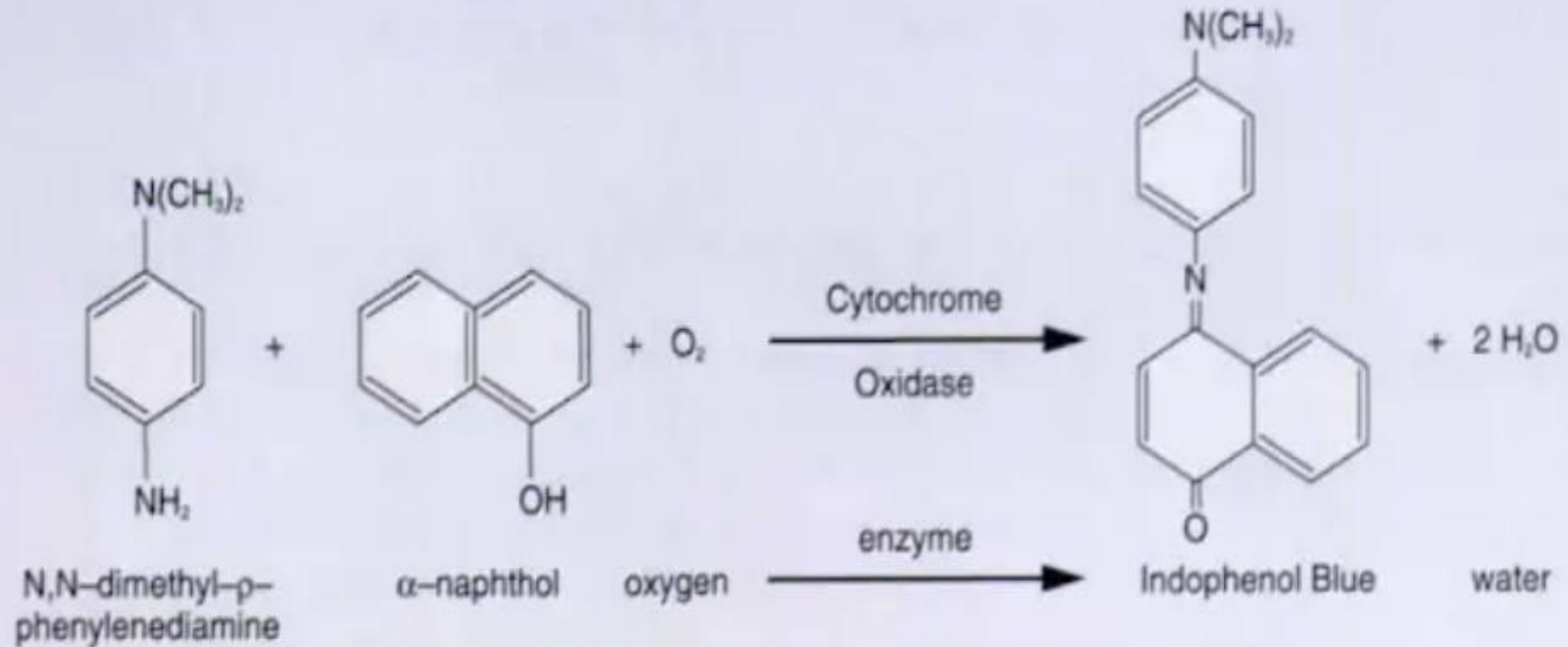
Positive

Negative

➤ Principle of Oxidase Test:

- The oxidase test is used to identify bacteria that produce cytochrome c oxidase, an enzyme of the bacterial electron transport chain.
- When present, the cytochrome c oxidase oxidizes the reagent (tetramethyl-p-phenylenediamine dihydrochloride) to indophenols, a purple or dark blue color end product.
- When the enzyme is not present, the reagent remains reduced and is colorless.

MECHANISM OF THE CYTOCHROME OXIDASE REACTION



Mechanism of the Cytochrome Oxidase Reaction

➤ Uses of Oxidase Test:

- To determine the ability of bacteria to synthesize the **cytochrome c oxidase enzyme**.
- Differentiation of **Neisseria spp.** (oxidase positive cocci) from **Staphylococcus spp.** and **Streptococcus spp.** (oxidase negative cocci)
- Differentiation of **Enterobacteriaceae** from other Gram-negative bacilli.
- Differentiation of **Pseudomonas aeruginosa** from Enterobacteriaceae.

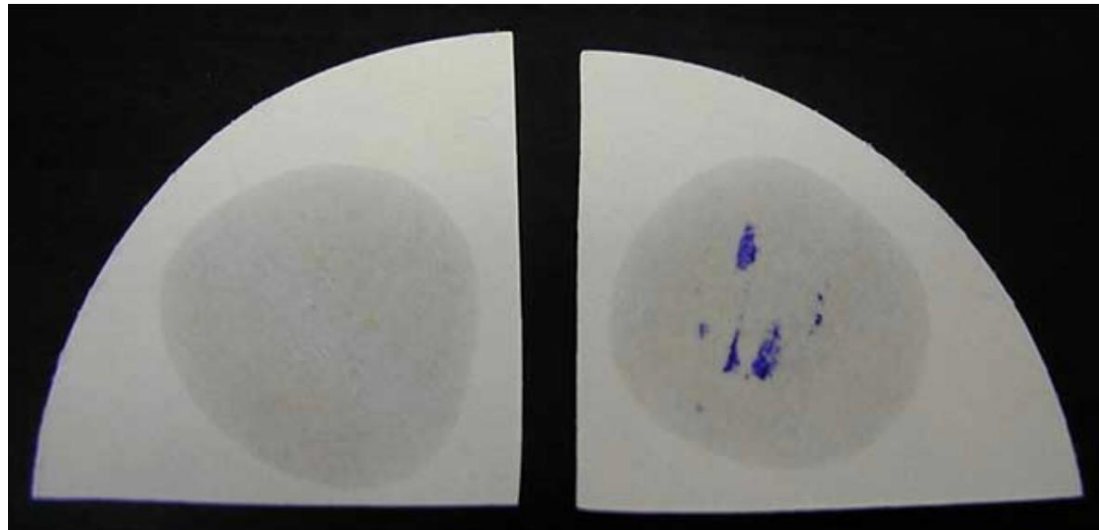
➤ **Procedure of Oxidase Test:**

- There are many method variations to the oxidase test.
- **These include**, the filter paper test, direct plate method, swab method, impregnated Disc/Strip (Oxidase Disc) method and tube method.

➤ **Filter Paper Method:**

1. Filter paper is soaked with a little freshly made 1% solution of the reagent.
2. Using a sterile inoculating loop, pick up a well-isolated colony of test bacteria from a fresh (18 to 24 hours old) culture and make a smear on the reagent-soaked filter paper piece.

3. Observe for color change and note the time required for change in color for up to 60 seconds.

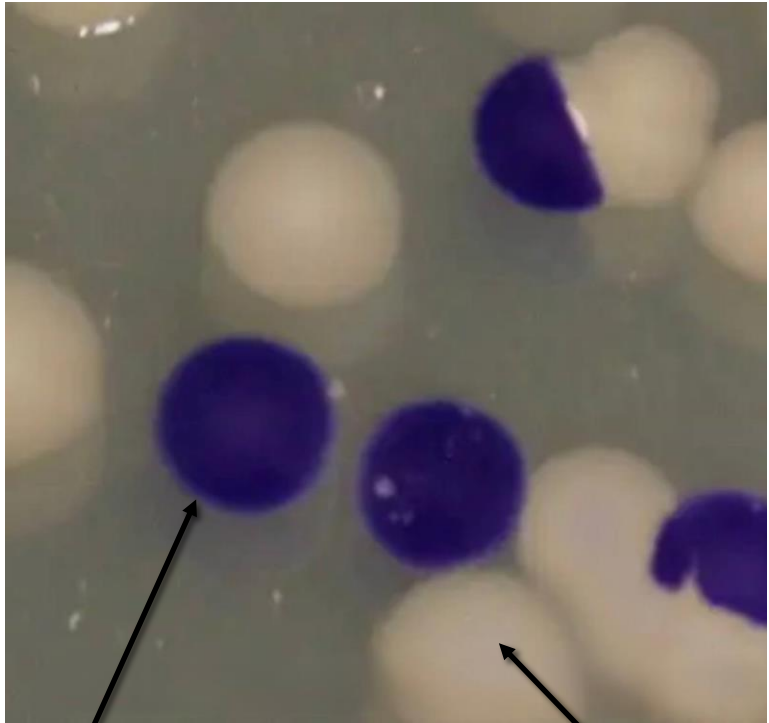


Negative

Positive

- **Oxidase-positive:** *Pseudomonas aeruginosa*
- **Oxidase-negative:** *Escherichia coli*

➤ **Direct plate method:**

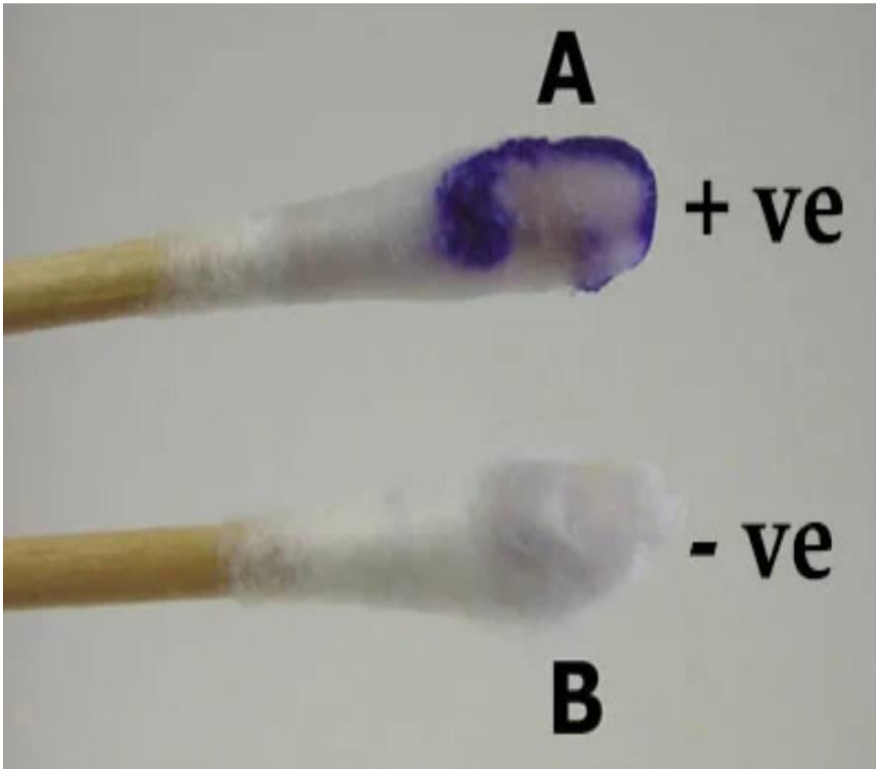


Positive

Negative

- **Oxidase-positive:** *Vibrio cholerae* showing purple colonies.
- **Oxidase-negative:** *Escherichia coli* with lack of color change.

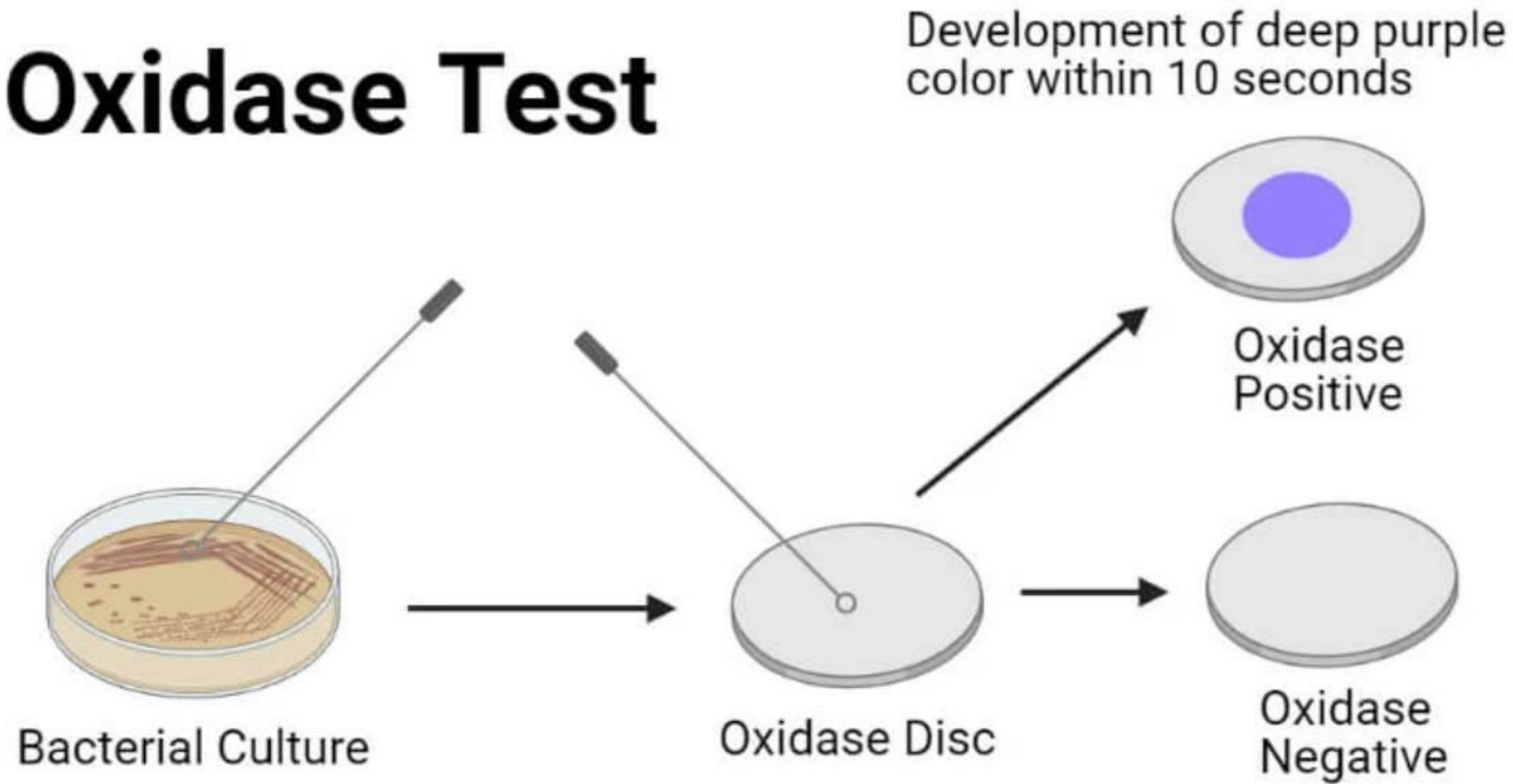
➤ **Swab method:**



- **Oxidase positive:** *Haemophilus* spp.
- **Oxidase negative:** *Streptococcus* spp.

➤ **Impregnated Disc/Strip (Oxidase Disc) Method:**

Oxidase Test



➤ **Tube method:**



Positive

Negative

- **Oxidase positive:** *Neisseria sicca*.
- **Oxidase negative:** *Staphylococcus aureus*.

➤ Result Interpretation of Oxidase Test:

➤ Positive Test:

- Development of purple to deep blue color within 10 to 30 seconds indicates a positive oxidase test.
- Development of purple to deep blue color within 30 to 60 seconds indicates a weak oxidase positive reaction or delayed oxidase positive.



➤ **Negative Test:**

- No development of purple to deep blue color within 60 seconds.

