University of AL-Mustansiriyah
College of Sciences
Biology Department
Bacterial Taxonomy

Muhammad Tawfeeq
Suhad Abbas Abid

Lab. 3: Biochemical tests

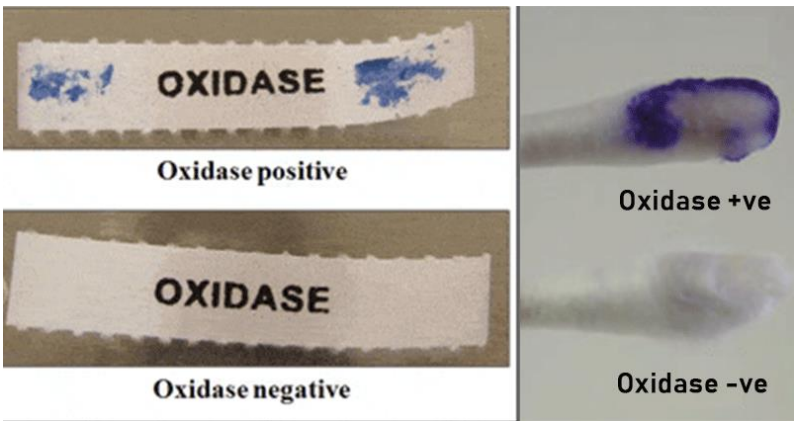
1. Oxidase test

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)** to **(indophenols)** purple color end product. When the enzyme is not present, the reagent remains reduced and is colorless.

Procedure

Filter paper method: Place a piece of filter paper in petri dish and add 3 drops of freshly prepared oxidase reagent. Using a sterile glass rod, remove a colony of test organism from a culture plate and smear it on the filter paper.

The results: Oxidase positive organisms give blue color within 5-10 seconds, and in oxidase negative organisms, color does not change.



► Precaution to be taken while performing oxidase test:

1. Do not use Nickel-base wires containing chromium and iron to pick not colony and make smear as this may give false positive results.

2. Read the results within 10 seconds, timing is critical.

3. The oxidase test must be performed from blood agar or another medium without a fermentable sugar because the acid produced by the sugar fermenting colonies will inhibit the oxidase reaction.

► Bacterial genera characterized as oxidase **positive** include *Neisseria* and *Pseudomonas*. Genera of the *Enterobateraceae* family are characterized as oxidase **negative**.

2. Catalase test

This test demonstrates the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide (H_2O_2). It is used to differentiate those bacteria that produces an enzyme catalase, such as *staphylococci*, from non-catalase producing bacteria such as *streptococci*. Normally 3% H_2O_2 is used for the routine culture.

The enzyme catalase mediates the breakdown of hydrogen peroxide into oxygen and water. The presence of the enzyme in a bacterial isolate is evident when a small inoculum is introduced into hydrogen peroxide, and the rapid elaboration of oxygen bubbles occurs. The lack of catalase is evident by a lack of or weak bubble production. The culture should not be more than 24 hours old.

$$2H_2O_2 \xrightarrow[\text{catalase}]{} 2H_2O + O_2 \text{ (gas bubbles)}$$

Bacteria thereby protect themselves from the lethal effect of Hydrogen peroxide which is accumulated as an end product of aerobic carbohydrate metabolism.

Procedure:

a- Tube method

1. Pour 1-2 ml of hydrogen peroxide solution into a test tube.

2. Using a sterile wooden stick or a glass rod, take several colonies of the 18 to 24 hours test organism and immerse in the hydrogen peroxide solution.

3. Observe for immediate bubbling.

b- Slide method

1. Use a loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean, dry glass slide.
2. Place drop of 3% H_2O_2 in the glass slide.
3. Observe for the evolution of oxygen bubbles.

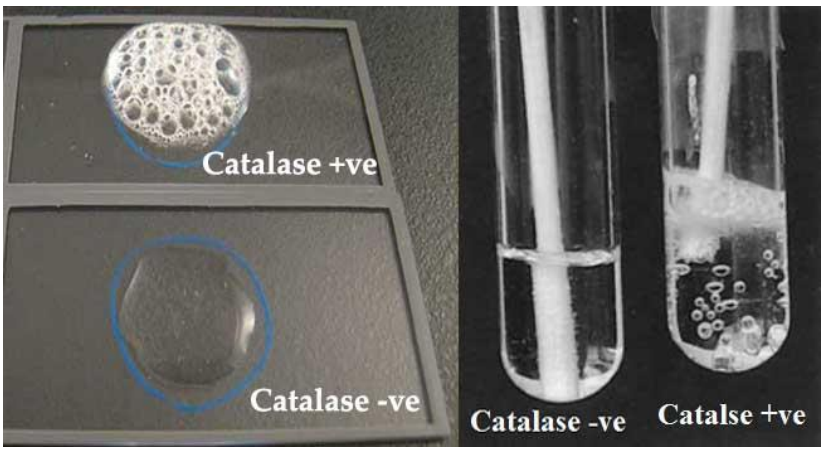
The results:

Positive: many bubbles produced, active bubbling

Examples: *Staphylococci*, *Micrococci*, *Listeria*, *Corynebacterium diphtheria*, *Burkholderia cepacia*, *Nocardia*, *Pseudomonas*, *Mycobacterium tuberculosis*, *Cryptococcus*, *Rhodococcus equi*, and the family *Enterobacteriaceae* (*Citrobacter*, *E. coli*, *Enterobacter*, *Klebsiella*, *Shigella*, *Yersinia*, *Proteus*, *Salmonella*, *Serratia*).

Negative: No or very few bubbles produced.

Examples: *Sterptococcus* and *Enterococcus* spp.



► precaution to be taken while performing catalase test:

1. The test organisms should not be taken from blood agar culture. Red blood cells contain catalase and their presence will give a false positive test.

2. Culture should be 18 to 24 hours old.

3. Hydrogen peroxide must be fresh as it is very unstable.

4. Iron wire loop should not be used.

5. Some bacteria produce a peroxidase that catalyzes a breakdown of hydrogen peroxide causing the reaction to be weakly positive; (a few bubbles elaborated slowly). This should not be confused with a truly positive reaction.

6. Do not add organism to reagent, particularly if iron-containing inoculating loops are used. Iron containing loops will cause false positive test results if exposed to hydrogen peroxide.

3. Coagulase test

Coagulase is an enzyme produced by *Staphylococcus aureus* that converts (soluble) fibrin. Other *Staphylococci* do not produce coagulase; thus this test can distinguish *S. aureus* from the other *Staphylococci*. Coagulase test is performing by two methods:

a- Slide coagulase test is done to detect bound coagulase or clumping factor.

b- Tube coagulase test is done detect free coagulase.

Procedure:

1. Emulsify a staphylococcal colony in a drop of water on a clean and grease free glass slide with a minimum of spreading.

2. Make similar suspension of control positive and negative strains to confirm the proper reactivity of plasma.

3. Dip a flamed and cooled straight inoculating wire into the undiluted plasma at room temperature, withdraw, and stir the adhering traces of plasma (not a loopful) into the staphylococcal suspension on the slide. Flame the wire and repeat for the control suspension.

4. Read as positive a coarse clumping of cocci visible to naked eye within 10 seconds. Read as negative the absence of clumping or any reaction taking more than 10 seconds to develop.

The results:

1. Coagulase positive: macroscopic clumping in 10 seconds or less in coagulase plasma drop and no clumping in saline or water drop.

2. Coagulase negative: no clumping in either drop.