Catalase and Oxidase Test

Dr. Md. Abdus Shabur Talukder

PhD (Japan)

What is Catalase Test?

The catalase test is a biochemical test for aerobic organisms that detects the production of catalase enzyme in the organism.

- Catalase enzyme is a common enzyme that is found in all living beings that survive in oxygen and catalyzes the decomposition of hydrogen peroxide, releasing water and oxygen.
- Catalase is an essential enzyme in pathogenic organisms as it protects the organism from oxidative damage from the reactive oxygen species (ROS).

•The enzyme neutralizes the bactericidal effects of hydrogen peroxide, and its concentration in bacteria has been correlated with the pathogenicity of the organism.

•The catalase test has been extensively used over the years as it allows the differentiation of **catalase-positive organisms like staphylococci from catalase-negative species like streptococci**.

•The catalase test is useful in the presumptive characterization of most bacteria.

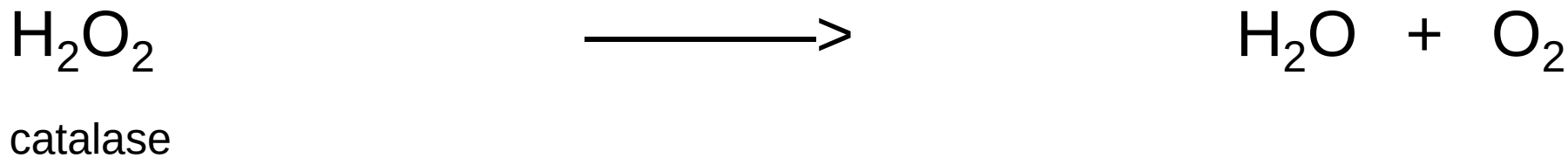
•Under the aerobic condition, 3% H_2O_2 is used, whereas 15% H_2O_2 is used under anaerobic conditions.

Objectives of Catalase Test

- To detect the ability of organisms to produce the **catalase enzyme**.
- To differentiate catalase-positive organisms like ***micrococci*** and ***staphylococci*** from catalase-negative organisms like ***streptococci***.

Principle of Catalase Test

- The metabolic activity of **aerobic and facultative anaerobic** microorganisms produce toxic by-products like **hydrogen peroxide and superoxide radical (O_2^-).**
- These products are **toxic to the organisms and might even result in cell lysis** if not broken down. In the case of pathogenic organisms, different mechanisms are found that break down these products to **non-toxic substances.**
- Bacteria capable of synthesizing the enzyme catalase hydrolyze **hydrogen peroxide into water and gaseous oxygen**, which results in the liberation of **gas bubbles.**



- The production of catalase thus protects the organism against the **lethal effect of hydrogen peroxide accumulated at the end of the aerobic metabolism.**
- The presence of the catalase enzyme can be demonstrated by adding **hydrogen peroxide to the bacterial inoculum**, which results in the rapid liberation of **oxygen bubbles**. The lack of enzyme is demonstrated by the **absence of such bubbles.**

Microorganism Tested

- **Young** (18 hours old or less, if possible) colonies of bacteria growing on agar media, preferably **Blood Agar**.
- **For anaerobes**, the colonies should be exposed to air for 30 minutes prior to testing.

Reagents and Supplies Used

Hydrogen peroxide reagent

- 30% H_2O_2 for *Neisseria*
- 15% H_2O_2 for anaerobes
- 3% H_2O_2 for other bacteria (purchase or dilute 30% 1:10 in deionized water prior to use)

Supplies

- Glass slide
- Sterile wooden or glass sticks or platinum loops or wires

Procedure of Catalase Test

- There are more than one method or procedure variations for the catalase test. These methods include the **slide or drop catalase test**, the **tube method**, the heat-stable catalase used for the differentiation of ***Mycobacterium* species**, the semi quantitative catalase for the identification of ***Mycobacterium tuberculosis***, and the capillary tube and coverslip method. The most popular method of catalase test in clinical bacteriology is the **slide or drop catalase method** as it requires a small number of organisms and works on a relatively uncomplicated technique.

1. Slide Method

•A microscope slide is placed inside a petri dish. The use of a petri dish is optional and is used to limit catalase aerosols, which might carry viable bacterial cells.

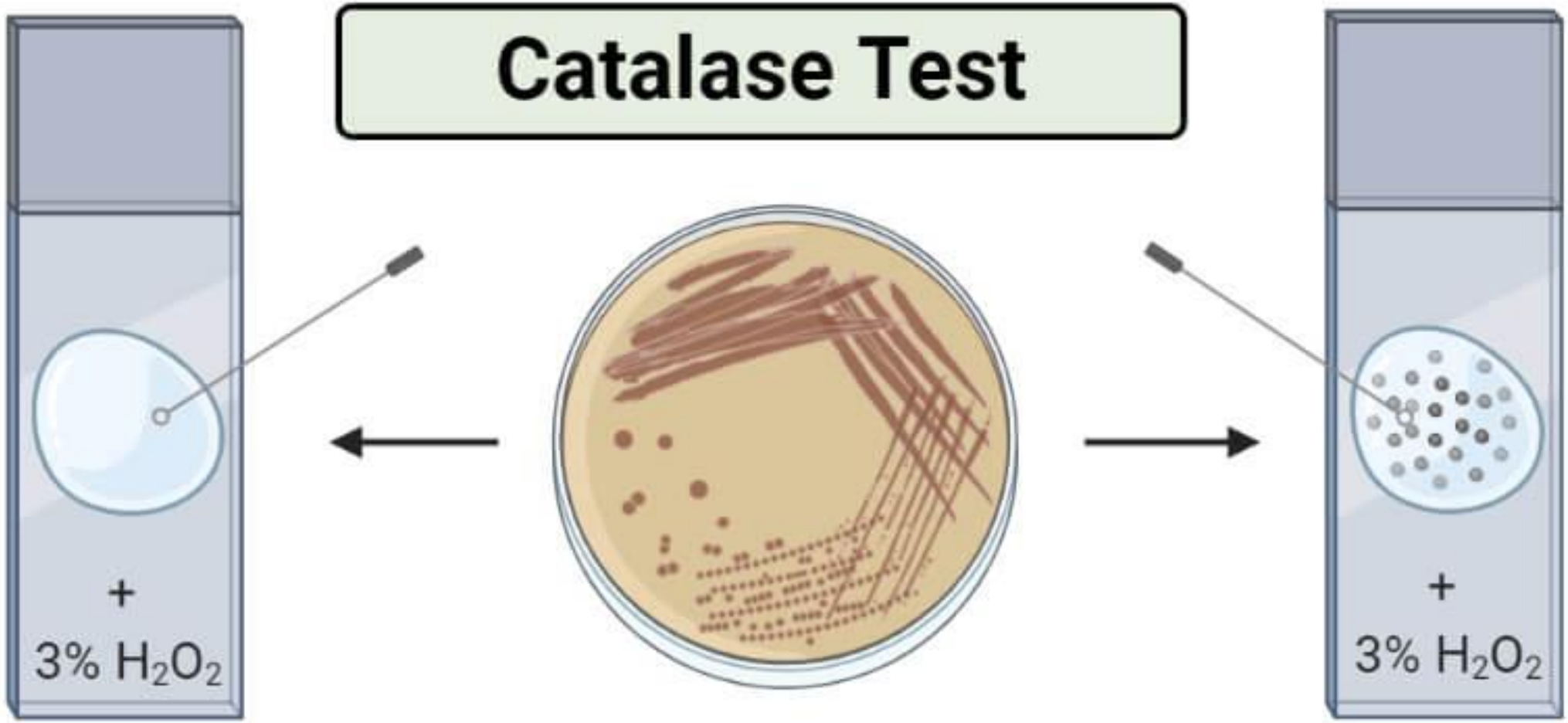
•A small amount of organism is collected from a well-isolated 18- to 24-hour colony with a sterile inoculating loop or wooden applicator stick and placed onto the microscope slide.

•However, no agar must be picked up with the colony, especially when the culture is picked up from blood agar.

•A drop of **3% H_2O_2 onto the organism on the microscope slide** by using a dropper or Pasteur pipette.

•The formation of **bubbles is observed** against a dark background to enhance readability.

Catalase Test



Catalase Negative
(No Bubble)

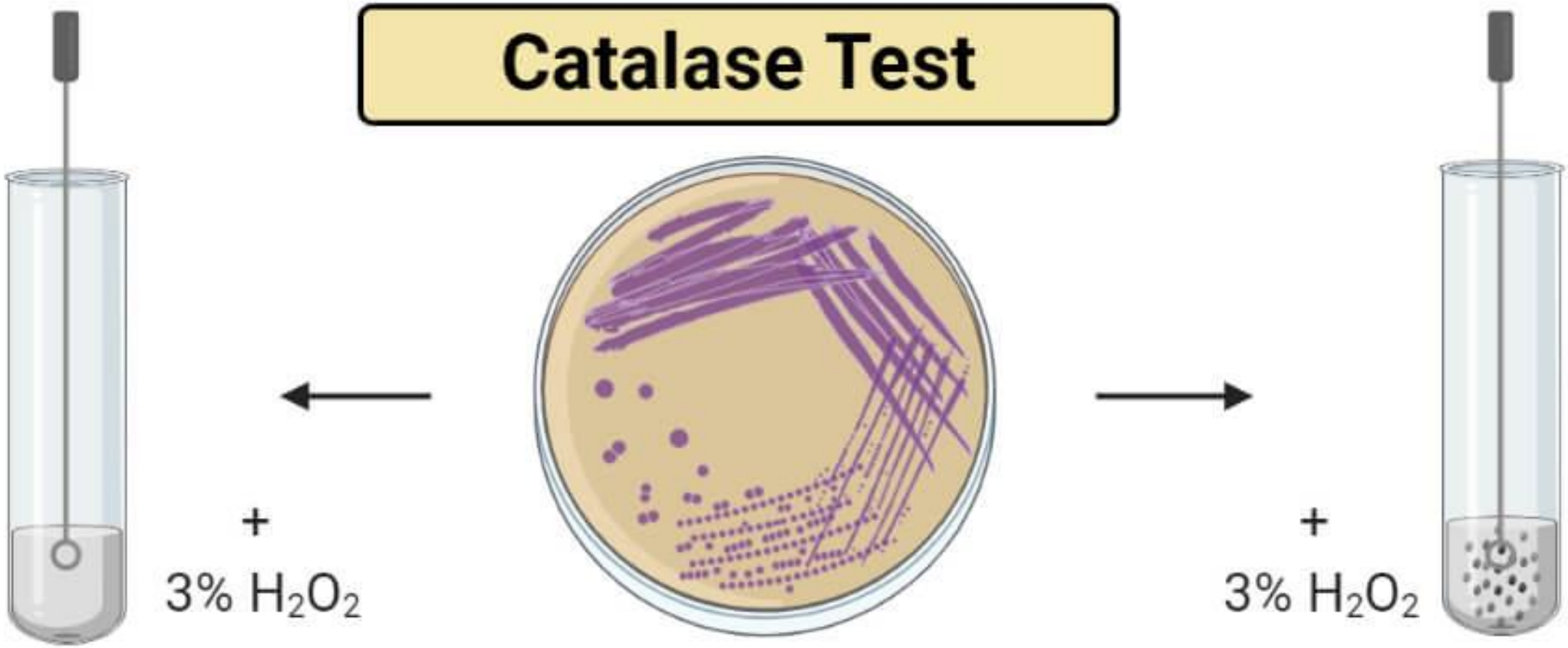
Slide Method

Catalase Positive
(Bubble Present)

2. Tube Method

- About 4 to 5 drops of 3% H_2O_2 are added to a test tube.
- Using a wooden applicator stick, a small amount of organisms from a well-isolated 18- to 24-hour colony is collected and placed into the test tube.
- The tube is placed against a dark background and observed for immediate bubbles.

Catalase Test



Catalase Negative
(No Bubble)

Tube Method

Catalase Positive
(Bubble Present)

Quality Control

- As a form of quality control, the following organisms can be used for positive and negative results:
- ***Staphylococcus aureus*: Catalase positive.**
- ***Streptococcus pyogenes*: Catalase-negative**.

Result and Interpretation of Catalase Test

- The positive test is demonstrated by the immediate **appearance of bubbles.**
- The appearance of one or two bubbles represents a weak reaction.
- A negative test is represented by **no bubbles or a few bubbles after 20 s.**

Reporting result

- The catalase test separates **staphylococci (positive) from streptococci and enterococci (negative).**
- *Bacillus is* catalase-positive, and *Clostridium* spp. are catalase-negative.
- The test is useful to separate among the **fastidious Gram-negative rods.**
- ***Neisseria gonorrhoeae*** produces an enhanced elaboration of bubbles not seen with other members of the genus due to **superoxol.**

Uses of Catalase Test

- Catalase test is essential for **differentiating catalase-positive Micrococcaceae** and **Staphylococcaceae from catalase-negative Streptococcaceae.**
- The test also allows differentiating aerobic and obligate anaerobic organisms.
- The value of the test has been found in the presumptive differentiation among certain **Enterobacteriaceae.**
- This is an important test for the differentiation of **aerotolerant** strains of ***Clostridium*,** which are **catalase-negative**, from *Bacillus,* which are **catalase positive**.
- This test has been used to differentiate between genera and is also valuable in the speciation of certain **gram-positive** organisms such as ***Aerococcus***

Limitations of Catalase Test

- RBCs contain catalase, and thus, in order to avoid false-positive results, blood agar should not be picked up with the colony. If a colony is difficult to pick up or doesn't grow well, the test can be repeated from the culture on a different media.
- The test should not be tested from Mueller-Hinton agar.
- Collecting colonies with metal bacteriological loop materials might yield false-positive results; however, platinum loops do not yield false-positive results.
- Because the enzyme is present in viable cells only, colonies that are older than 24 hours should not be used.
- Older cultures may give **false-negative** results.

•Reversing the order of adding the reagent to the colony might result in **false-negative results**.

•The reagent and the colony should not be mixed.

•Some strains of S. aureus may appear catalase-negative by drop method so the test should be repeated with the tube method.

•30% H_2O_2 is extremely caustic to the skin. If contact occurs, wash immediately with 70% ethyl alcohol, not water.

Objective of Oxidase Test

To determine the ability of the organism to produce the cytochrome oxidase enzyme

Principle of Oxidase Test

- The oxidase test is designed for specifically detecting the presence of the terminal enzyme system in aerobic respiration called **cytochrome C oxidase or cytochrome a3**. Cytochrome C oxidase is the terminal or last H2 electron acceptor in an aerobic respiratory mechanism which is composed of a number of enzymes that alternatively oxidize and reduce each other by donating or accepting electrons derived from H_2.

- The ability of an organism to produce the **cytochrome C oxidase** can be determined by using the reagent **tetramethyl-p-phenylenediamine dihydrochloride** impregnated in the filter disk. The reagent serves as an artificial substrate donating electrons and thereby becoming oxidized to a deep purple compound in the presence of the enzyme **oxidase and free O2**. **Development of pink, then maroon, and finally dark purple coloration after rubbing the organism in the oxidase disc containing the reagent indicates a positive reaction.** The positive reaction involves the conversion of **colorless, reduced tetramethyl-p-phenylenediamine to oxidized form into deep purple color in presence of Cytochrome C oxidase**. No color change is indicative of the **negative test** result.

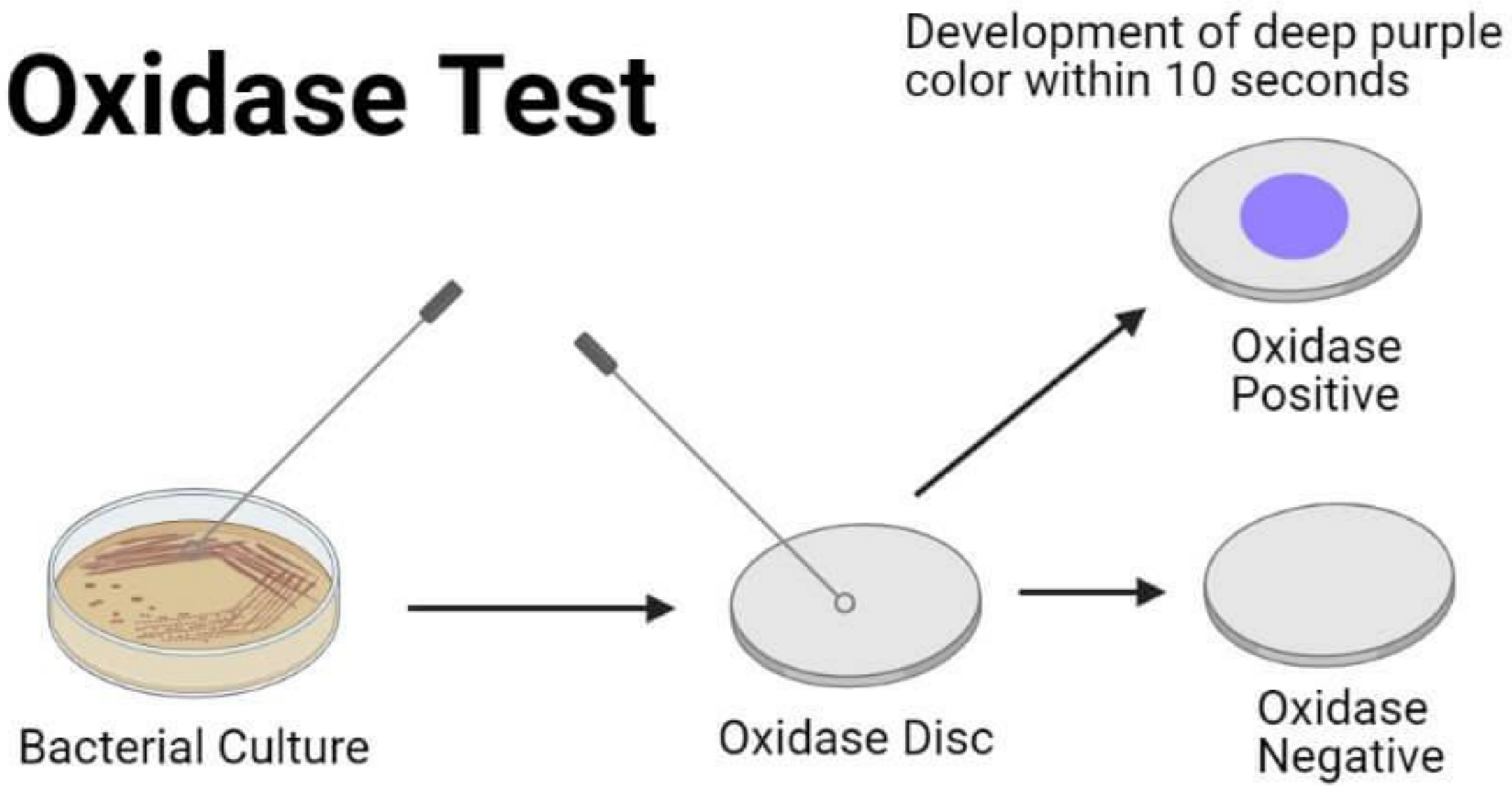
Procedure of Oxidase Test

1. Take a filter paper soaked with the substrate **tetramethyl-p-phenylenediamine dihydrochloride.**
2. Moisten the paper with sterile distilled water.
3. Pick the colony to be tested with a wooden or platinum loop and smear in the filter paper.
4. Observe inoculated area of paper for a color change to deep blue or purple within 10-30 seconds.

Or

1. Take a commercially available oxidase disc containing the reagent.
2. Pick the isolated colony to be tested and rub it in the disc.
3. Observe for color change within 10 seconds.

Result Interpretation of Oxidase Test



- **Positive test:** Development of deep purple color within 10 seconds
- **Negative test:** Absence of color

Limitations of Oxidase Test

1. The reagents used in the oxidase test have been shown to **auto-oxidize**, and hence false positive result may be obtained.

2. Nickel, steel, and other wire loops may give false-positive results, so it is important to use only **platinum or inert transfer loops**, such as **sterile wood sticks** commonly used in teaching laboratories.

3. Bacteria grown on media containing high concentrations of **glucose show inhibited oxidase activity**, so it is recommended to test colonies grown on media without excess sugar, such as nutrient agar.

Thank you