

# **Catalase Test :**

**Principle, Procedure and Uses  
In the Microbiology Laboratory**

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# 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.

# What is Catalase Test?

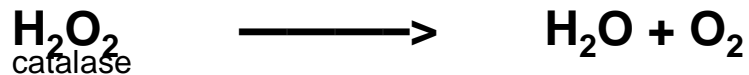
- The enzyme neutralizes the bactericidal effects of hydrogen peroxide, and its concentration in bacteria has been correlated with the pathogenicity of the organism.
- The catalase test has been extensively used over the years as it allows the differentiation of catalase-positive organisms like staphylococci from catalase-negative species like streptococci.
- The catalase test is useful in the presumptive characterization of most bacteria.
- Under the aerobic condition, 3% H<sub>2</sub>O<sub>2</sub> is used, whereas 15% H<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> for *Neisseria*
- 15% H<sub>2</sub>O<sub>2</sub> for anaerobes
- 3% H<sub>2</sub>O<sub>2</sub> 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 semiquantitative catalase for the identification of *Mycobacterium tuberculosis*
- the capillary tube and coverslip method.

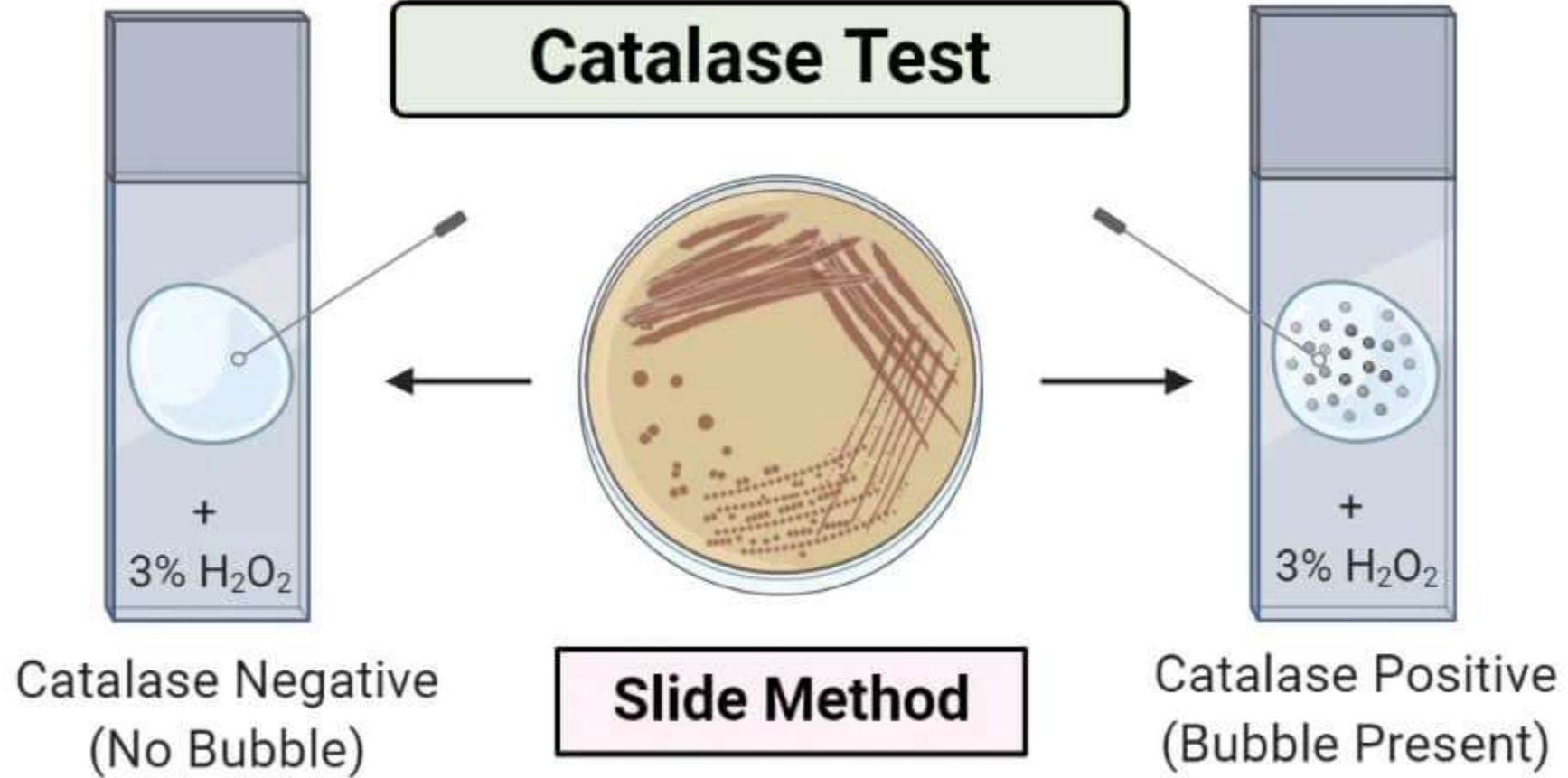
The most popular method of catalase test in clinical bacteriology is the slide or drop catalase method as it requires a small number of organisms and works on a relatively uncomplicated technique.



# 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<sub>2</sub>O<sub>2</sub> 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.

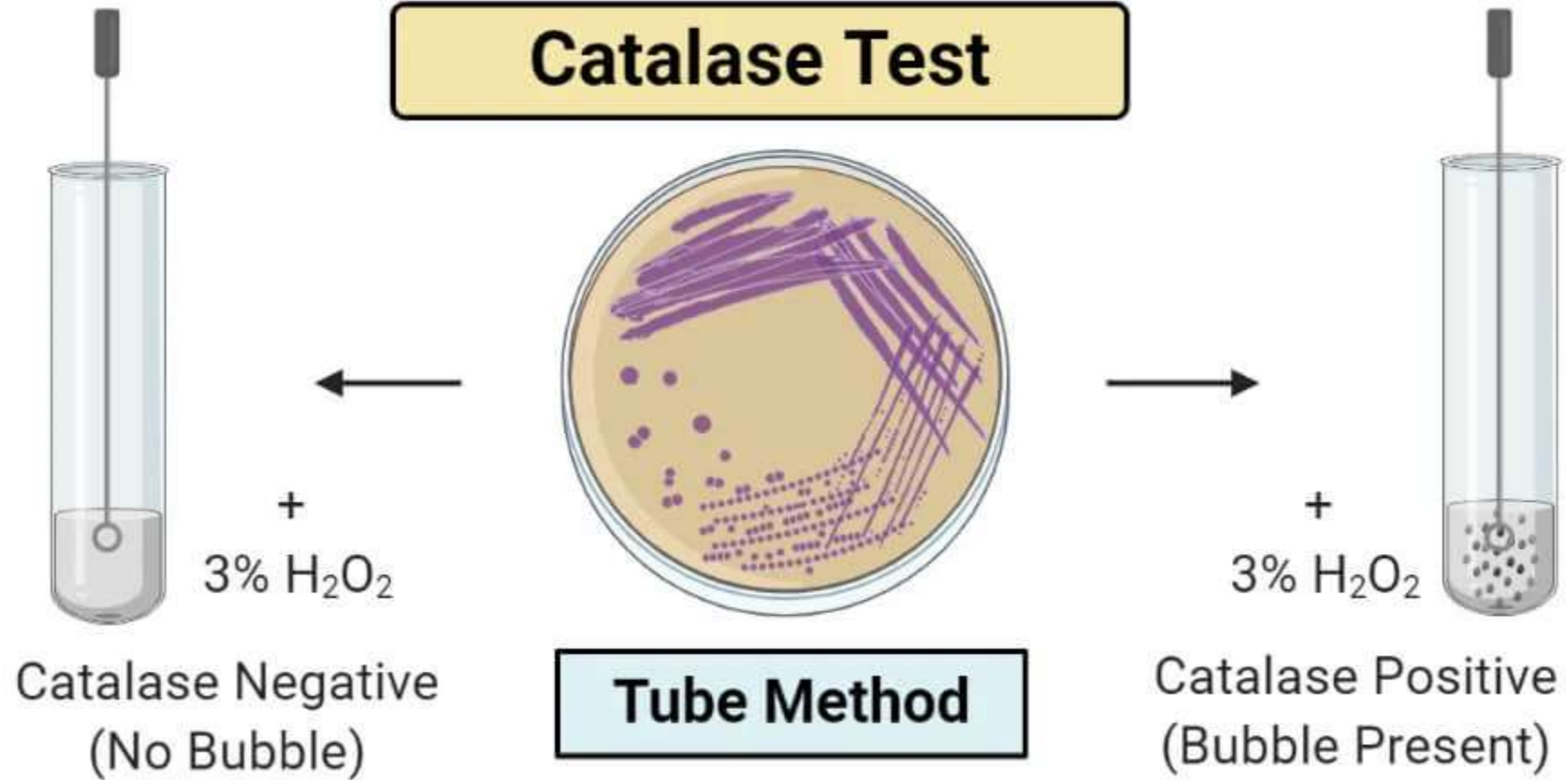
# Catalase Test



## 2. Tube Method

- About 4 to 5 drops of 3% H<sub>2</sub>O<sub>2</sub> 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



# Quality Control

As a form of quality control, the following organisms can be used for positive and negative results:

1. *Staphylococcus aureus*: Catalase **positive**.
2. *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 of 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.

# Limitations of Catalase Test

- 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<sub>2</sub>O<sub>2</sub> is extremely caustic to the skin. If contact occurs, wash immediately with 70% ethyl alcohol, not water.