**Experiment No: 02**

**Experiment Name: Catalase Test**

**Principle:**

Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; H2O2. The catalase enzyme neutralizes the bactericidal effects of hydrogen peroxide and protects them. Anaerobes generally lack the Catalase enzyme.

Catalase mediates the breakdown of hydrogen peroxide H2O2 into oxygen and water. To find out if a particular bacterial isolate is able to produce catalase enzyme, small inoculum of bacterial isolate is mixed into hydrogen peroxide solution (3%) and is observed for the rapid elaboration of oxygen bubbles occurs.

**Apparatus:**

1. Test Tube or Slide

2. Glass rod or Wooden Stick

3. Pipette

4. Pipette Filler

5. Dropper

**Reagent:**

1. Hydrogen peroxide (H2O2)

Percentage of H2O2 used on catalase test:

1. For routine testing of aerobes, 3% hydrogen peroxide is used. 2. 15% H2O2 solution: for the identification of anaerobic bacteria

**Procedure:**

**A. Slide Test**

1. Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick

2. Place a drop of 3% H2O2 on to the slide and mix.

3. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling.

4. A negative result is no bubbles or only a few scattered bubbles. 5. Observe

**B. Tube Test**

1. Add 1ml to 2ml of 3% H2O2 (Hydrogen peroxide) to in a test tube

2. Using a wooden stick/glass rod, collect a small amount of organism from a well-isolated 18- to 24-hour colony and place into the test tube

3. Observe for immediate bubble formation (O2 + water = bubbles) at the end of the wooden stick/Glass rod.

**Results:**

**Catalase Positive reactions:** Evident by immediate effervescence (bubble formation)

**Catalase Negative reaction:** No bubble formation (no catalase enzyme to hydrolyze the hydrogen peroxide)

**Precautions:**

1. Do not use a metal loop or needle with H2O2; it will give a false positive and degrade the metal.

2. If using colonies from a blood agar plate, be very careful not to scrape up any of the blood agar as blood cells are catalase positive and any contaminating agar (carryover of red blood cells) could give a false positive.

3. Because some bacteria possess enzymes other than catalase that can decompose hydrogen peroxide, a few tiny bubbles forming after 20 to 30 seconds is not considered as positive test.