

## Biochemical Tests

Biochemical tests are the tests which performed on different bacteria for their identification on the basis of their biochemical activities towards different biochemical compounds.

### Some Common Biochemical Tests

All species of bacteria possess a unique set of metabolic activities which are controlled by bacterial enzymes. These bacterial enzymes determine the type of test suitable in recognition of structural differences and metabolic activities.

- **Catalase Test**

Many aerobic bacteria produce an enzyme known as **catalase** that detoxifies **hydrogen peroxide**. The positivity of test is determined by gas (O<sub>2</sub>) formation in form of bubbles.

#### Procedure

1. Obtain a glass slide and a bottle of **hydrogen peroxide**.
2. Using a sterilized inoculating loop, smear a small amount of bacteria onto the dry slide.
3. Place a drop of **hydrogen peroxide** on top of the bacteria.
4. Look for bubbles immediately:

#### Results

- **bubbles** = catalase positive
- no bubbles = catalase negative

- **Oxidase Test**

Bacteria which function on aerobic respiration often possess **cytochrome C** and a **cytochrome C oxidase**. The test uses oxidase reagent applied filter paper, timely **color change** indicates the positivity of result.

1. Obtain an oxidase test strip and an empty petri plate.
2. Place the oxidase test strips face up on the half empty petri plate.
3. Lightly moisten the oxidase test strip with a small drop of water. **Do not over-moisten the strip!**
4. Using a sterile swab or sterile loop, obtain a **large** amount of bacteria from a petri plate.
5. Rub the bacteria on the swab or sterile loop onto the moistened region of the oxidase test strip.
6. Start a timer for **30 seconds**.
7. Watch for **purple color** to develop on the oxidase test strip within **30 seconds**.

#### Results

- **purple** or **bluish** color change in less than **30 seconds** is oxidase positive
- **no color change** within **30 seconds** is oxidase negative (color change after 30 seconds is considered oxidase negative)

- **Indole Test**

**Coliform bacteria** possess an enzyme **tryptophanase** which can hydrolyze the amino acid( **tryptophan**) to form **indole**, **ammonia** and pyruvic acid. The timely color change during the test determines the success of the test. And its one of a group of tests called **IMVic** tests commonly used to identify a bacterial species especially coliforms.

**I : indol**

**M: methyl red**

**V: voges prosakaur test**

**(i): just to form a word**

**C: citrate test**

This test will identify the presented strain is able to convert tryptophan to indol or not

Requirements:

- **Peptone broth** ( a nutrient enriched with Amino acid tryptophan)
- Bacterial sample (*Escherichia coli*)(coliforms)
- **Kovac's reagent**

Procedure:

1. Prepare peptone broth which is enriched with amino acid tryptophane
2. using sterile wire, inoculate the broth with the given samples of organism and label the tubes with name of organism
3. Incubate the tubes at 37°C for 24-48 hours.
4. After proper incubation, add 4-8 drops of kovac's reagent to the tube touching the wall of glass tube
5. Roll each tube between your palms to mix the reagent through the culture.
6. Let stand for a while and observe for the development of cherry red color ring at the surface of media.

Results

- Indole test positive: **cherry red color ( *E. coli* )**
- Indole test negative: no red color