Laboratory Session 3

1. Staining

E. coli, Ps. aeruginosa, and H. influenzae are identified through **Gram staining**.

Note: All three are Gram-negative rods (pink/red color under the microscope).

2. Lactose Fermentation on Endo Agar

Endo Agar is a diagnostic and differential medium used to differentiate Enterobacteriaceae based on their ability to ferment lactose.

Procedure: The microbial culture is inoculated onto Endo Agar (in a Petri dish) and incubated in a thermostat at **37°C for 18–24 hours**.

Interpretation: Bacteria are identified based on the color and appearance of the colonies.

Result	Visual Appearance	Interpretation	Example
Lactose-Positive (+)	Dark red colonies with a metallic (golden/greenish) sheen.	The bacterium ferments lactose.	*Escherichia coli*
Lactose-Negative (-)	Colorless, transparent, or pale pink colonies.	The bacterium does not ferment lactose.	*Salmonella / Shigella*

3. Oxidase Test

This test is used to identify bacteria based on the presence or absence of **Cytochrome C oxidase**. The lack of Cytochrome C distinguishes the *Enterobacteriaceae* family from other Gram-negative bacteria. It is also used to differentiate *Pseudomonas aeruginosa* from other non-fermenting bacteria.

Procedure:

1. Take a colony from the Petri dish.
2. Rub it onto a piece of filter paper that has been moistened with **oxidase reagent**.
3. Observe for a color change within **10 seconds**.

Interpretation:

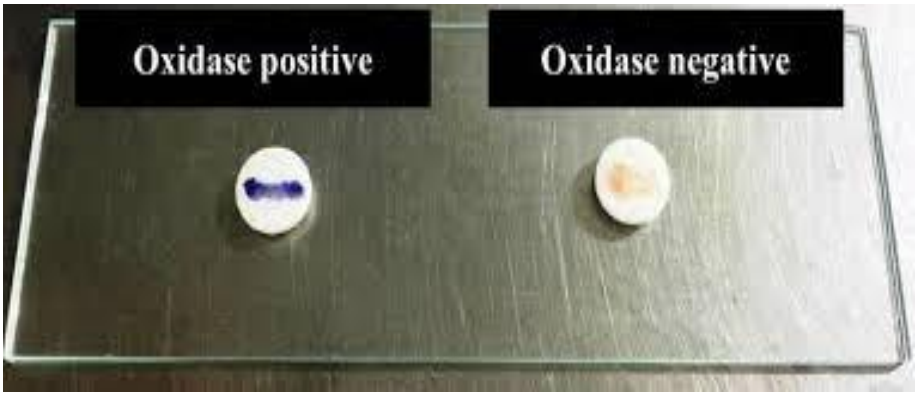
Positive Result: The paper turns **dark blue or purple** within 10 seconds.

Negative Result: No color change occurs.

Examples for Comparison:

Positive (Ox+): *Pseudomonas aeruginosa*

Negative (Ox-): *Escherichia coli*



4. Indole Test

The Indole test is widely used in bacteriology to determine a bacterium's ability to break down the amino acid **tryptophan** using the enzyme **tryptophanase**. This test is critical for identifying members of the *Enterobacteriaceae* family (for example, *E. coli*). Bacteria that produce the enzyme tryptophanase hydrolyze tryptophan, resulting in the production of indole.

Procedure: Indole detection is performed using **Kovacs'** or **Ehrlich's reagent**, which reacts with indole to form a red or cherry-red ring.

To perform the test, you will need:

Culture Medium: Tryptophan-rich broth (e.g., Peptone Water or Tryptone Broth).

Reagent: Kovacs' reagent.

Culture: A pure culture of the bacteria being studied (18–24 hour growth).

Steps:

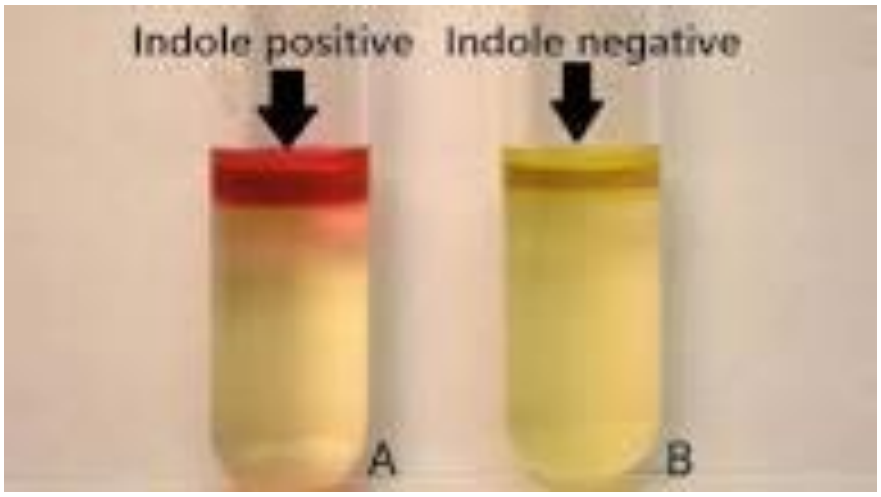
1. Inoculate the tryptophan broth with the bacteria using a sterile loop.
2. Close the tube cap (loosely/non-hermetically).
3. Place the tube in a thermostat at **35–37°C** for 24 to 48 hours.

Testing (Adding the Reagent):

1. After incubation, add 0.5 ml (approximately 5 drops) of **Kovacs' reagent** to the tube.
2. Shake the tube gently.
3. Observe for a color change on the liquid surface after 2–5 minutes.

Table: Interpretation of Results

Result	**Visual Appearance**	**Interpretation**	**Example**
Positive (+)	A **red/cherry-colored ring** forms on the surface.	The bacterium produces tryptophanase.	*Escherichia coli*, *Proteus vulgaris*
Negative (-)	The reagent color remains on the surface (**yellow/orange**).	Indole was not produced.	*Klebsiella pneumoniae*, *Salmonella spp.*



Quality Control

To verify the accuracy of the test, the following control strains are used:

Positive Control: *Escherichia coli* ATCC 25922

Negative Control: *Klebsiella pneumoniae* ATCC 13883

5. API 20E System

API 20E is an identification system for the *Enterobacteriaceae* family and other Gram-negative rods. API (Analytical Profile Index) represents a standardized, miniaturized biochemical testing system.

Principle

The strip consists of **20 microtubes** containing dehydrated substrates. A bacterial suspension is inoculated into the strip, which reacts with the provided substrates. During incubation, metabolic end-products cause **color changes**, which are observed either directly or after the addition of specific reagents.

The numerical clusters generated from these tests are used to identify the specific bacterial strain.

Interpretation

The reactions are read using a standard interpretation chart. By totaling the scores of the positive results, a **numerical profile** (digit code) is generated. This profile is entered into an electronic database (software) to determine the genus and species of the pathogen.

6. KOH Test (The String Test)

In dermatology, the KOH test is a quick diagnostic method for fungal infections of the skin, nails, and hair. However, in **bacteriology**, the KOH test (specifically the **3% Potassium Hydroxide test**) serves an entirely different purpose. It is a rapid alternative or supplementary method to Gram staining.

Its primary goal is to differentiate bacteria into two groups: **Gram-positive** and **Gram-negative**.

Advantages over Gram Staining:

Speed: It does not require fixation, staining, or lengthy washing steps.

Accuracy with Old Cultures: Occasionally, aging Gram-positive bacteria can appear Gram-negative during staining (false result). The KOH test is more reliable in these cases because it reacts directly to the physical structure of the cell wall.

Procedure:

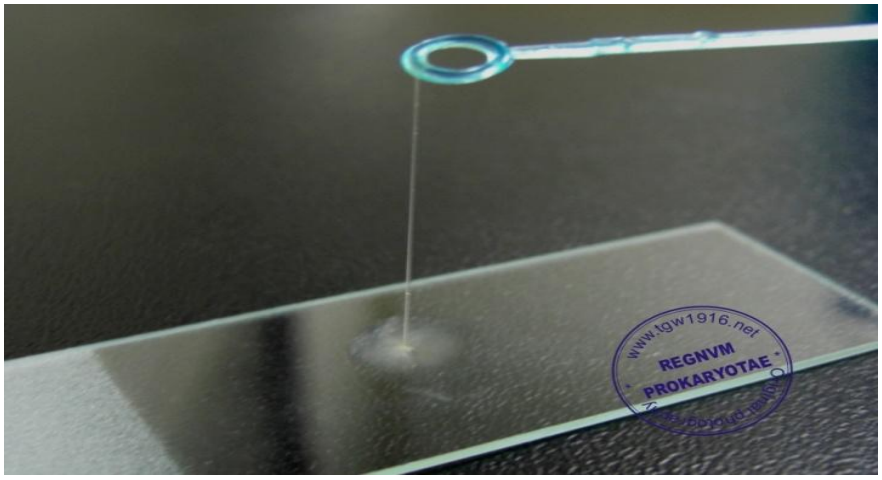
The test is very simple and takes only 60 seconds:

1. Place one drop of **3% KOH solution** on a microscope slide.
2. Using a bacteriological loop, transfer a pure culture of the bacteria into the drop.
3. Mix intensively for **30–60 seconds**.
4. Slowly lift the loop upward.

Interpretation:

Positive Reaction (Gram-negative bacteria): The solution becomes viscous, and a sticky "string" or thread forms as the loop is lifted. This happens because the KOH dissolves the thin cell wall, causing the cells to lyse and release their DNA.

Negative Reaction (Gram-positive bacteria): The consistency of the solution does not change; it remains liquid, and no "string" is formed. This is because the thick peptidoglycan layer of Gram-positive bacteria resists the KOH.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