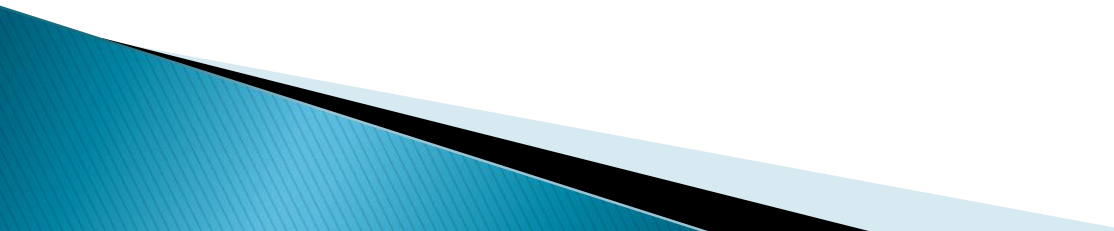


Biochemical reaction

- ▶ Bacteria **vary** in their metabolic & enzymatic activities.
 - ▶ Used in **identification** of different genera & species of bacteria.
 - ▶ Biochemical reactions are done on bacteria grown in **pure culture**.
- 

Biochemical Reactions

- 1. Sugar fermentation**
- 2. Oxidase test**
- 3. Catalase test**
- 4-coagulase test**
- 5-Nitrate reduction test**
- 6-ureas test**
- 7-indol test**

1. Sugar fermentation

Aim: To determine the ability of microbes to ferment carbohydrates with the production of an acid and/or gas.

Sugar media are composed of:

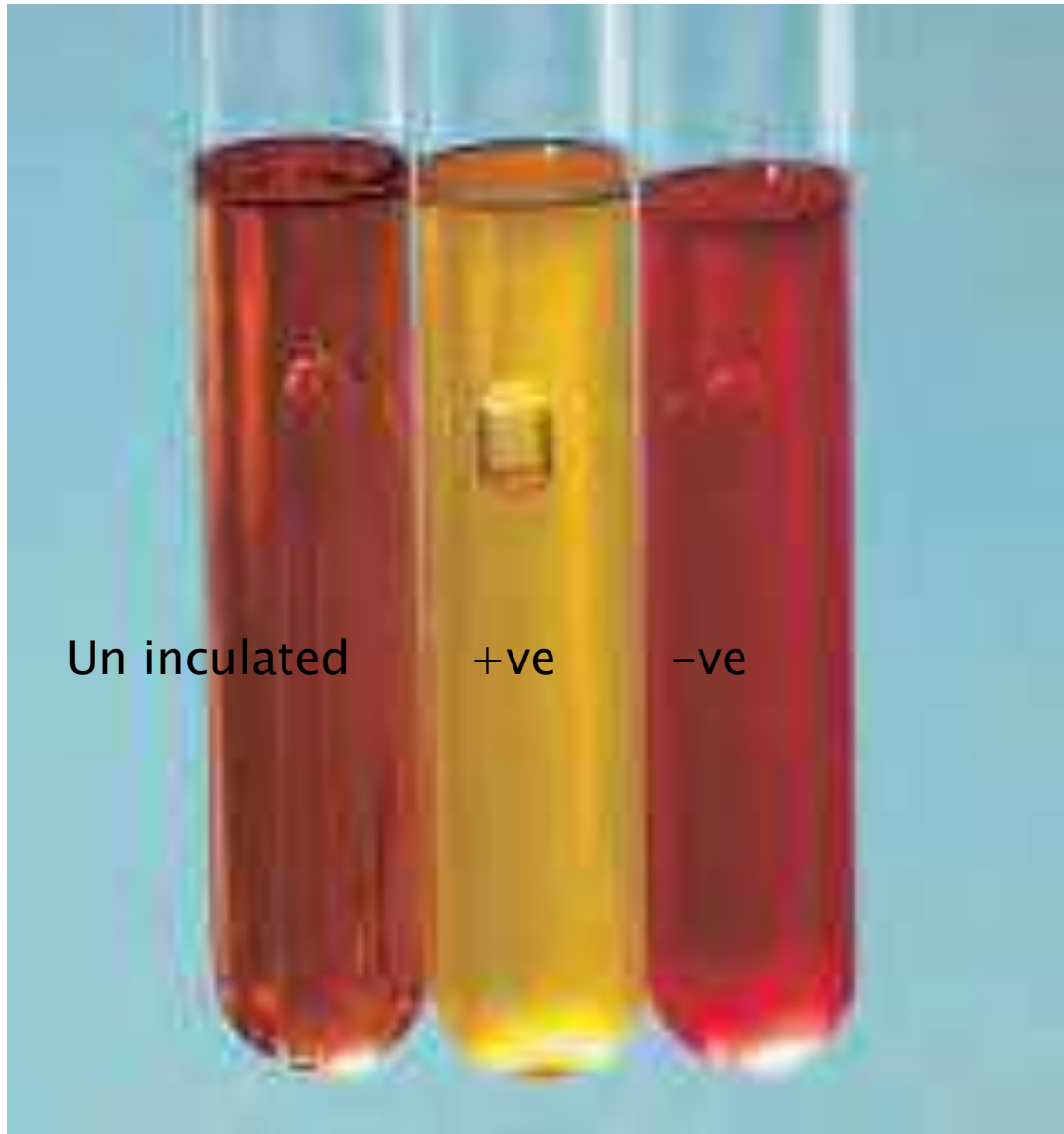
Peptone water

1% test sugar

Andrade's indicator

A small inverted (Durham's tube) tube

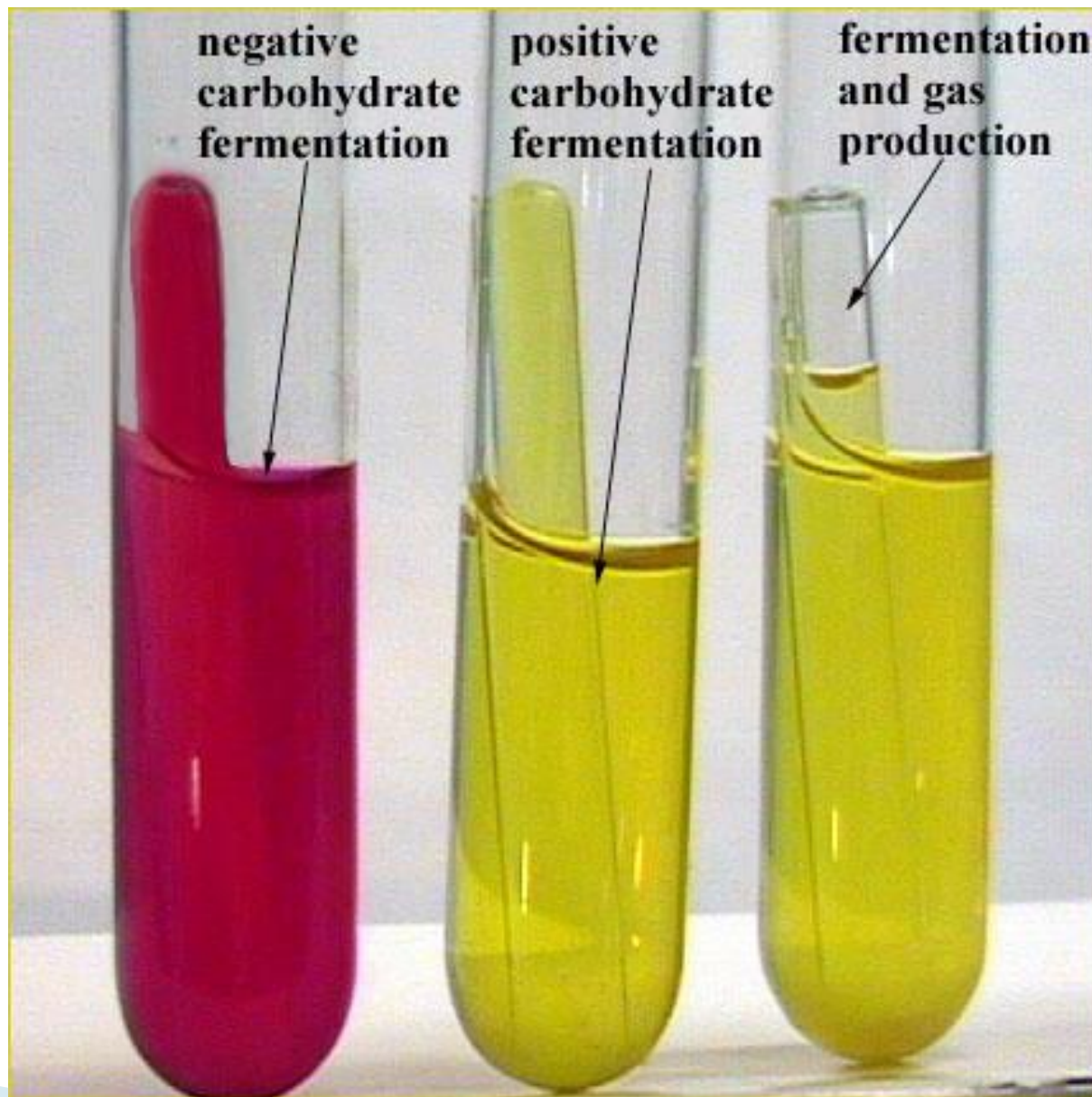
➤ Sugar fermentation can be indicated by change color of the medium from red to yellow color.



Un inoculated

+ve

-ve



TSI

Aim: To differentiate among and between the members of *Enterobacteraceae* family

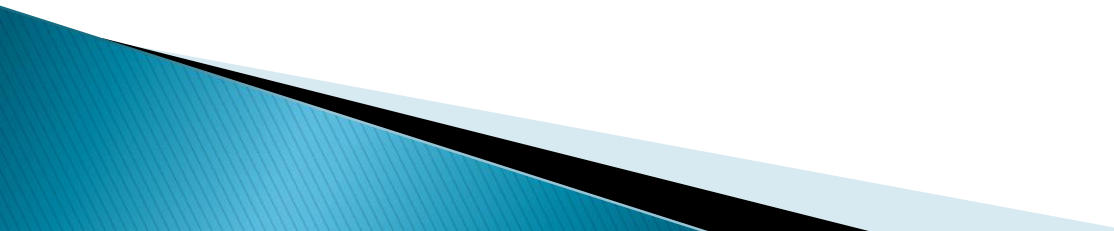
Media consist of

- The pH indicator, **phenol red**, is also incorporated into the medium to detect acid production from carbohydrate fermentation.
- slants contain a 1% concentration of **lactose** and **sucrose** and 0.1% **glucose**.
- **ferrous sulfate** make H₂S indicator.

- ▶ The un inoculated medium is red in colour due to presence of phenol red.

....The indicator is red at alkaline pH and yellow at acidic pH, at neutral pH it remains red.

The following reactions may occur in the TSI tube

- ▶ **Yellow butt (A) and red slant (K)** due to the fermentation of glucose as s. variable lactose fermenter.
 - ▶ **Yellow butt (A) and slant (A)** due to the fermentation of lactose, sucrose & glucose .
 - ▶ **Gas formation** noted by splitting of the agar.
 - ▶ **formation (H_2S)** seen by blackening of the agar.
- 

Yellow butt (A) and red slant (K)

- salmonella pullorum gallinarum.
- Para typhoid.
- ✓ In case of salmonella arizona if slow lactose fermentation due to s.Arizona variable in lactose fermentation.

Yellow butt (A) and slant (A)

- ▶ **E.coli.**
- ▶ In case of salmonella arizona if rapid lactose fermentation due to s.Arizona variable in lactose fermentation

Gas formation

- ▶ E.coli.

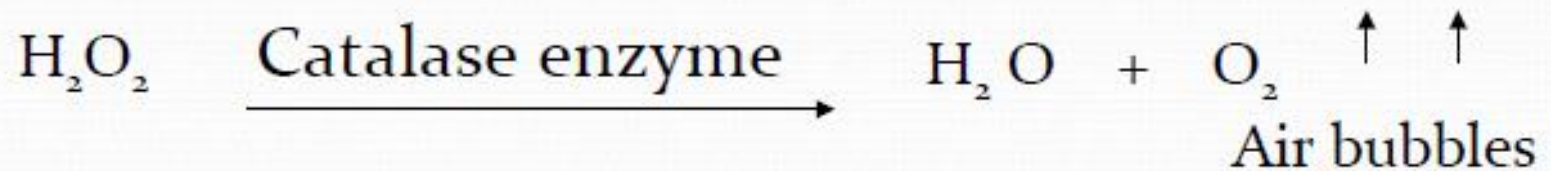
formation (H₂S)

- ▶ salmonella pullorum.
- ▶ Also may occur in salmonella gallinarum.



Catalase Test

▶ Principle:



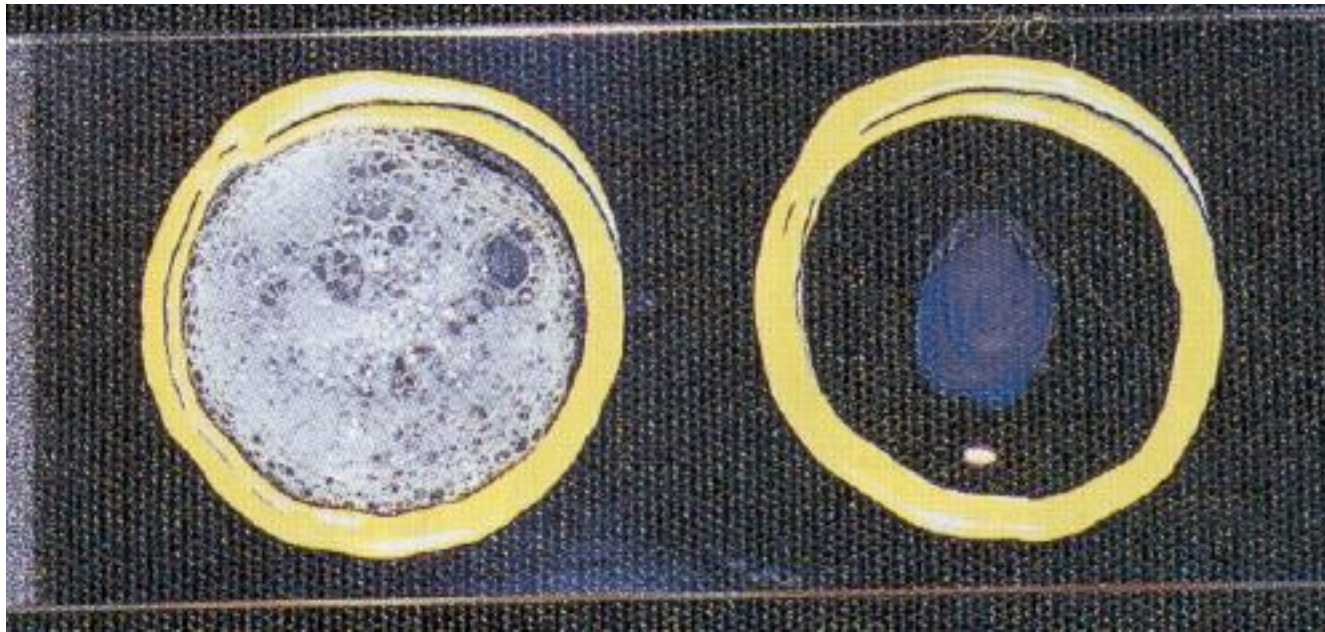
- **Positive** : rapid and sustained appearance of bubbles.
- **Negative** :lack of bubble formation.

➤ Positive result in case of;

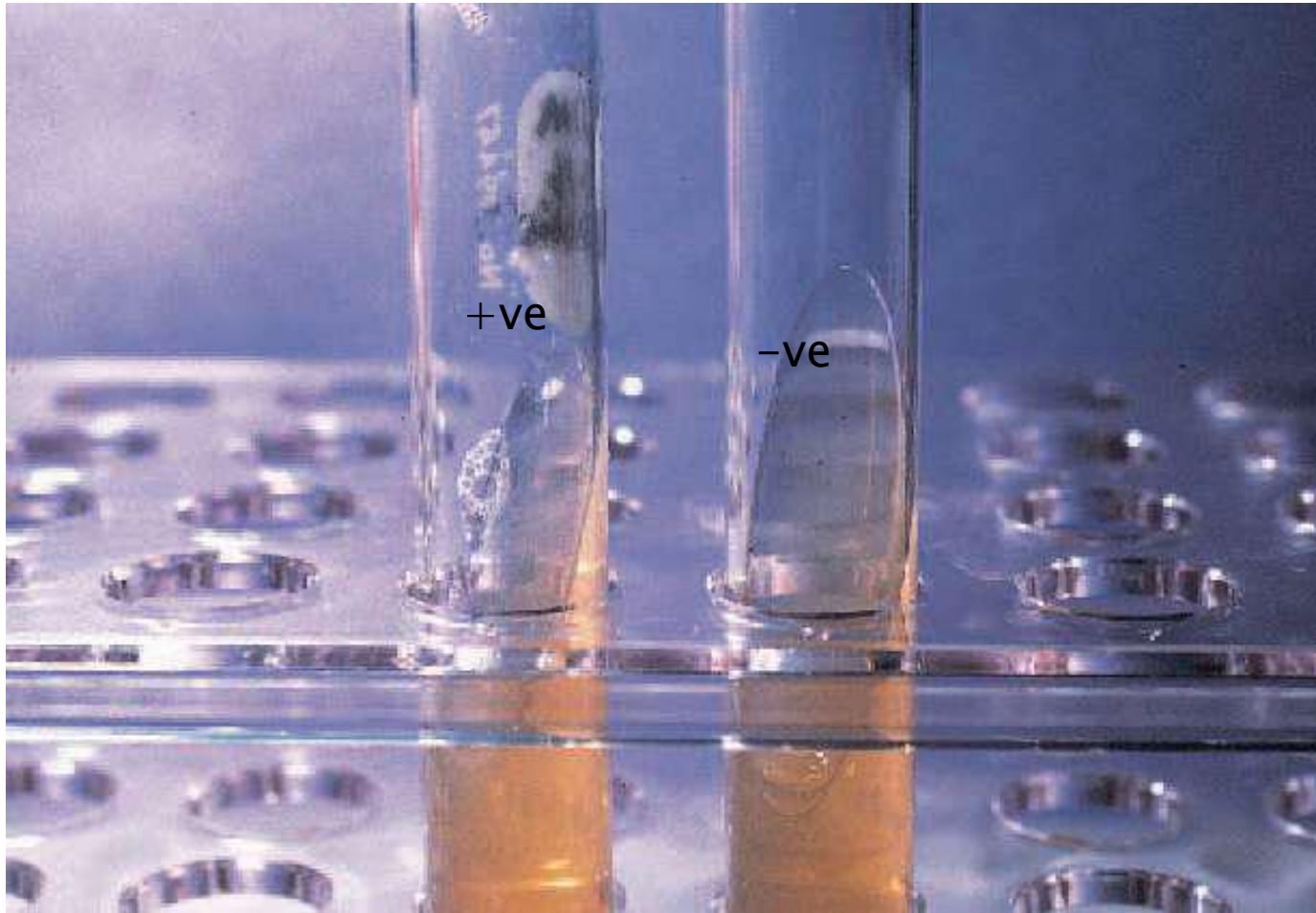
- ❖ Staph.aureus
- ❖ Bordetella avium
- ❖ E.coli

❖ Negative result in case of;

- ▶ Strept.zooepidemicus
- ▶ Haemophillus paragallinarum
- ▶



Tryptic soy agar slants



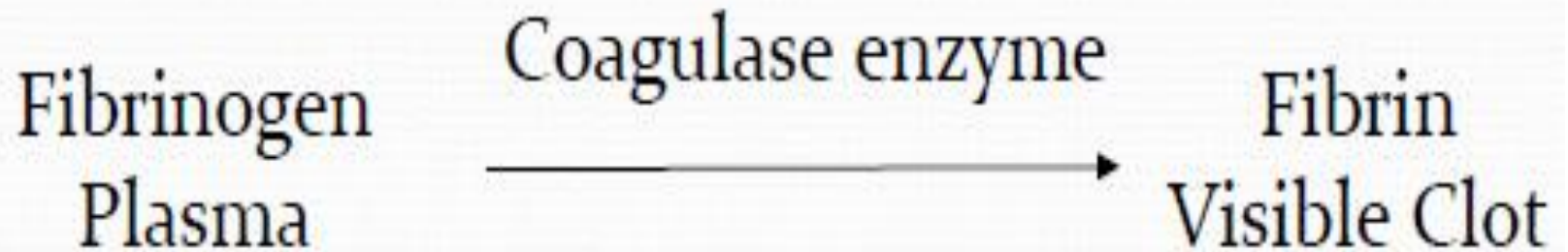
Coagulase Test

▶ Principle:

Fibrinogen
Plasma

Coagulase enzyme

Fibrin
Visible Clot



The diagram illustrates the principle of the Coagulase Test. It shows a horizontal flow from left to right. On the left, the text 'Fibrinogen Plasma' is stacked vertically. In the center, the text 'Coagulase enzyme' is positioned above a horizontal arrow pointing to the right. On the right, the text 'Fibrin Visible Clot' is stacked vertically. This represents the enzymatic conversion of fibrinogen in plasma into fibrin, which forms a visible clot.

PURPOSE

- ▶ To determine the ability of the organism to produce coagulase which clots plasma.
- ▶ To distinguish the **pathogenic** coagulase **positive** staphylococcus(staph.aureus) from the **nonpathogenic**(staph.epidermidis) coagulase **negative** staphylococcus.

Two forms of coagulase

- **bound coagulase** (clumping factor) – detected coagulase test in the slide.
- **free coagulase** – detected in the coagulase tube test.

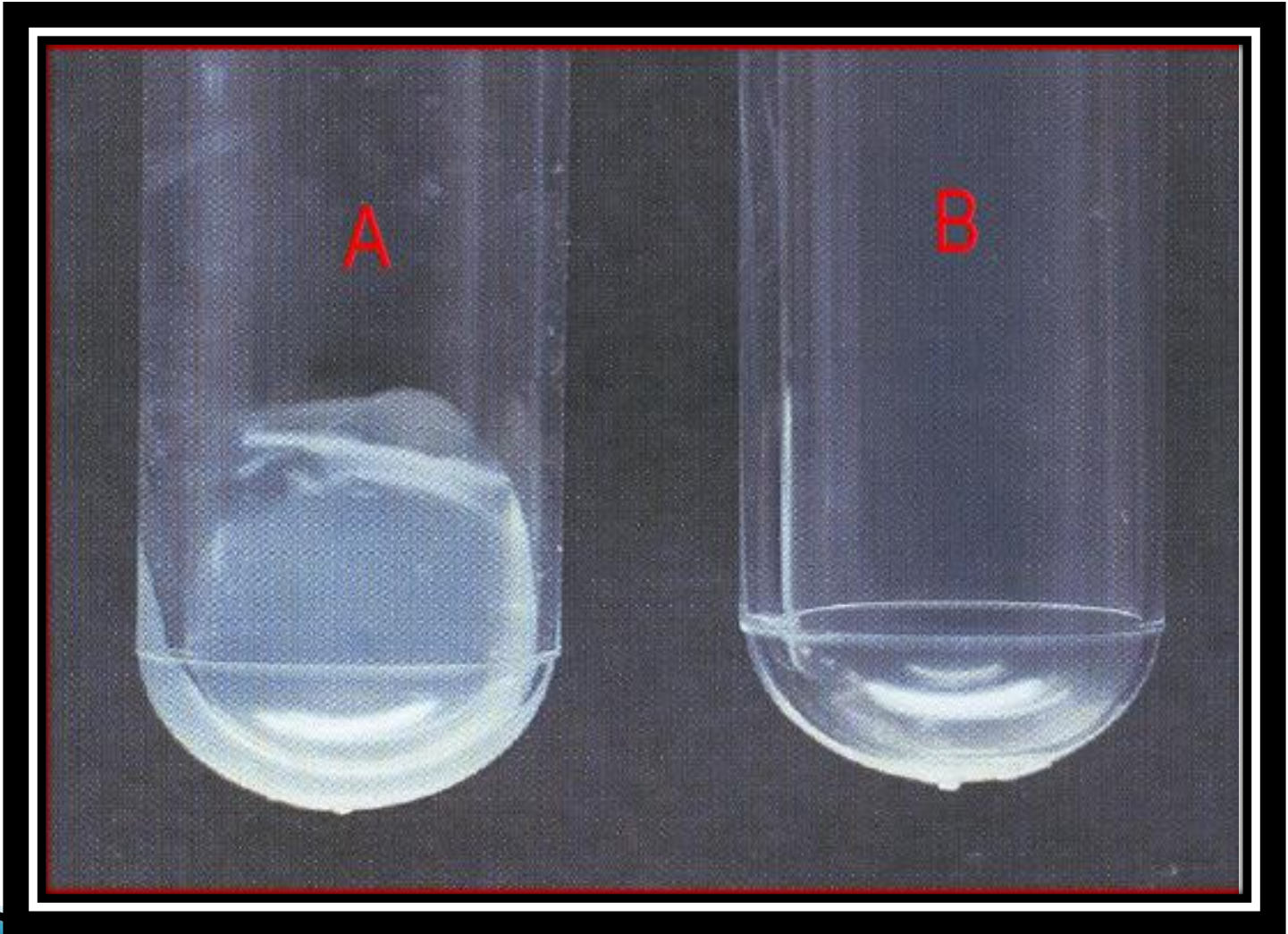
❖ Slide Coagulase test

- ▶ Positive – white fibrin clots in plasma
- ▶ Negative – smooth suspension

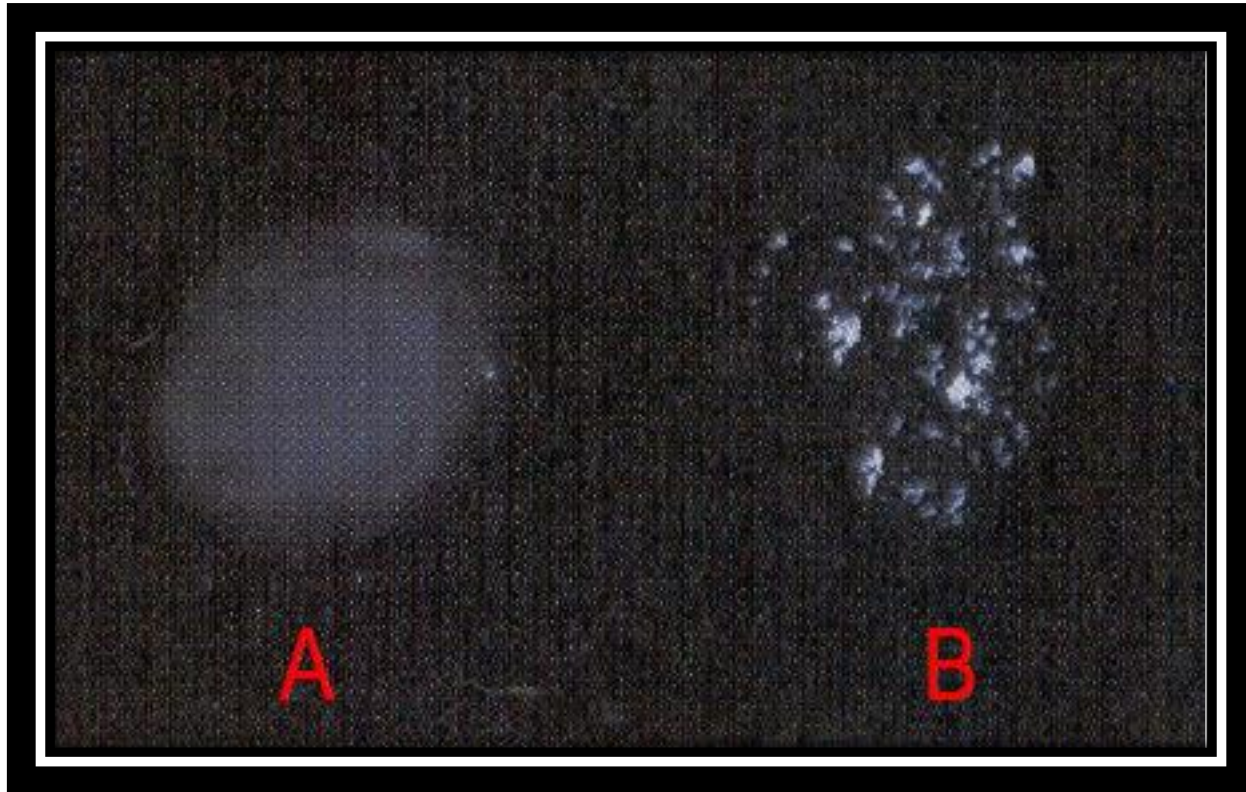
❖ Tube Coagulase test

- ▶ Positive – formation of fibrin clot
 - ▶ Negative – no clot is formed
- 

Tube coagulase test

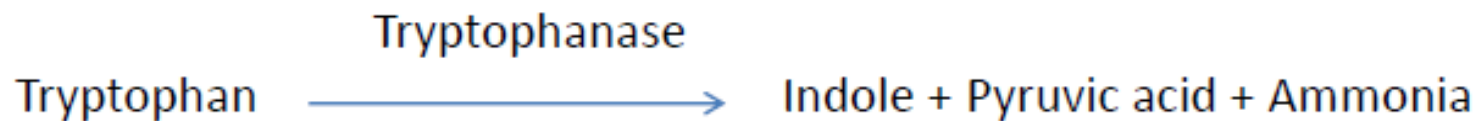


Slide coagulase test



Indole test

- ▶ **Aim:** To determine the ability of microbe to **degrade the amino acid tryptophan**
- ▶ **Principle:**



- **Positive** – red ring at the interface of reagent of broth at top of tube.
 - **E.coli**
 - **S.pullorum gallinarum**
- **Negative** – no color development
 - **Riemerella anatipestifer**

+ve



Oxidase test

- ▶ **Aim:** To determine the ability of microbes to produce Oxidase enzyme play role during aerobic respiration.

□ **Positive** – blue/ dark purple/black color

Bordetella

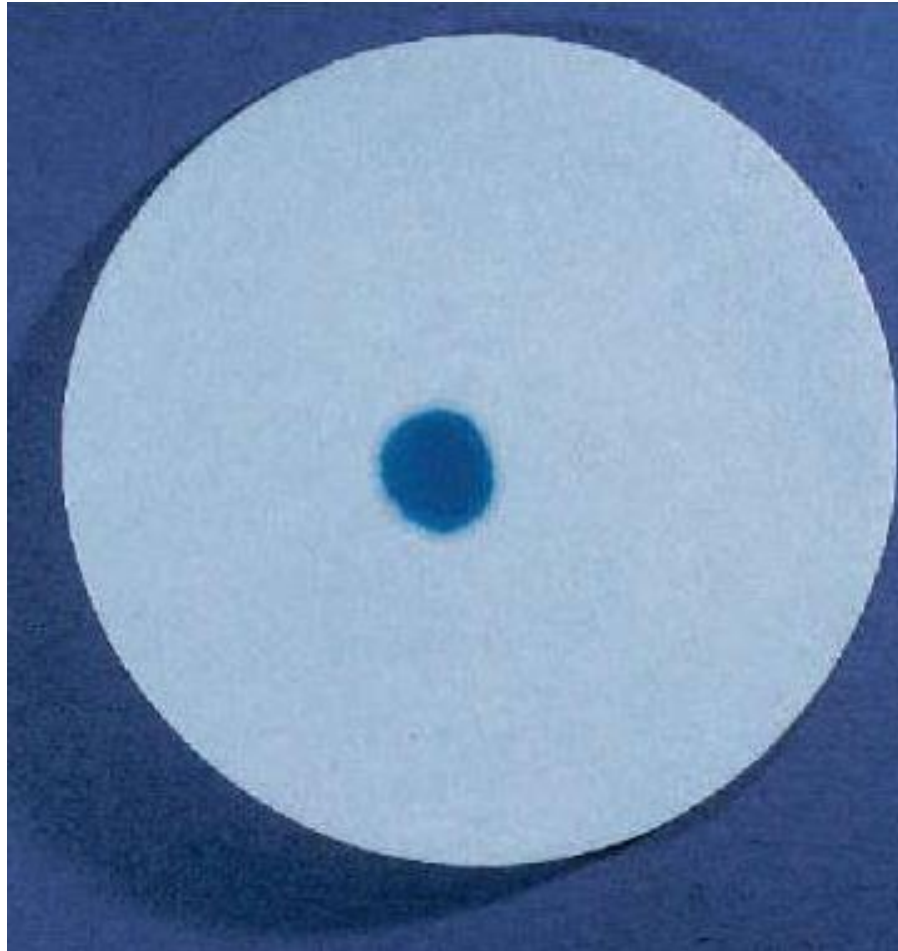
Haemophilus

Rimerella.

□ **Negative** – no color development

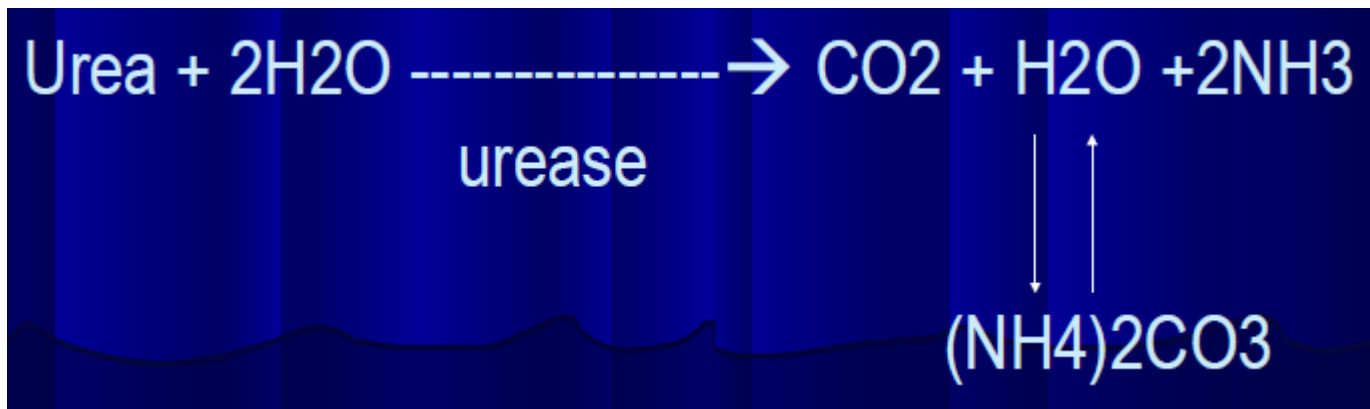
E.coli

purple to dark purple color



Urease test

- To determine the ability of an organism to produce the enzyme, urease, which hydrolyzes urea.
- **PRINCIPLE**



Positive – rapid urease activity; **red** throughout the medium

Proteus

☐ **Negative** – no urease activity; medium remains **yellow**

Salmonella

E.coli

Bordetella avium



Urease test

Stuart Urea broth



A. Uninoculated
B. Strong positive reaction-
Proteus spp.
C. Negative – Escherichia
coli

Nitrate Reduction Test

- ▶ Aim: To determine the ability of some microbes to reduce nitrate (NO_3) to nitrites (NO_2) in presence of reductases enzyme.

- Positive result give red color.
- ▶ Salmonella & E.coli
- Negative result no change
- ▶ Bordetella avium

Un inoculated

