Pathogenic Bacteria

Lab. 2

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C-Biochemical Identification:

Biochemical tests that investigate the enzymatic activities of cells are powerful tests in the identification of bacteria, because colony and cellular morphology may permit preliminary identification.

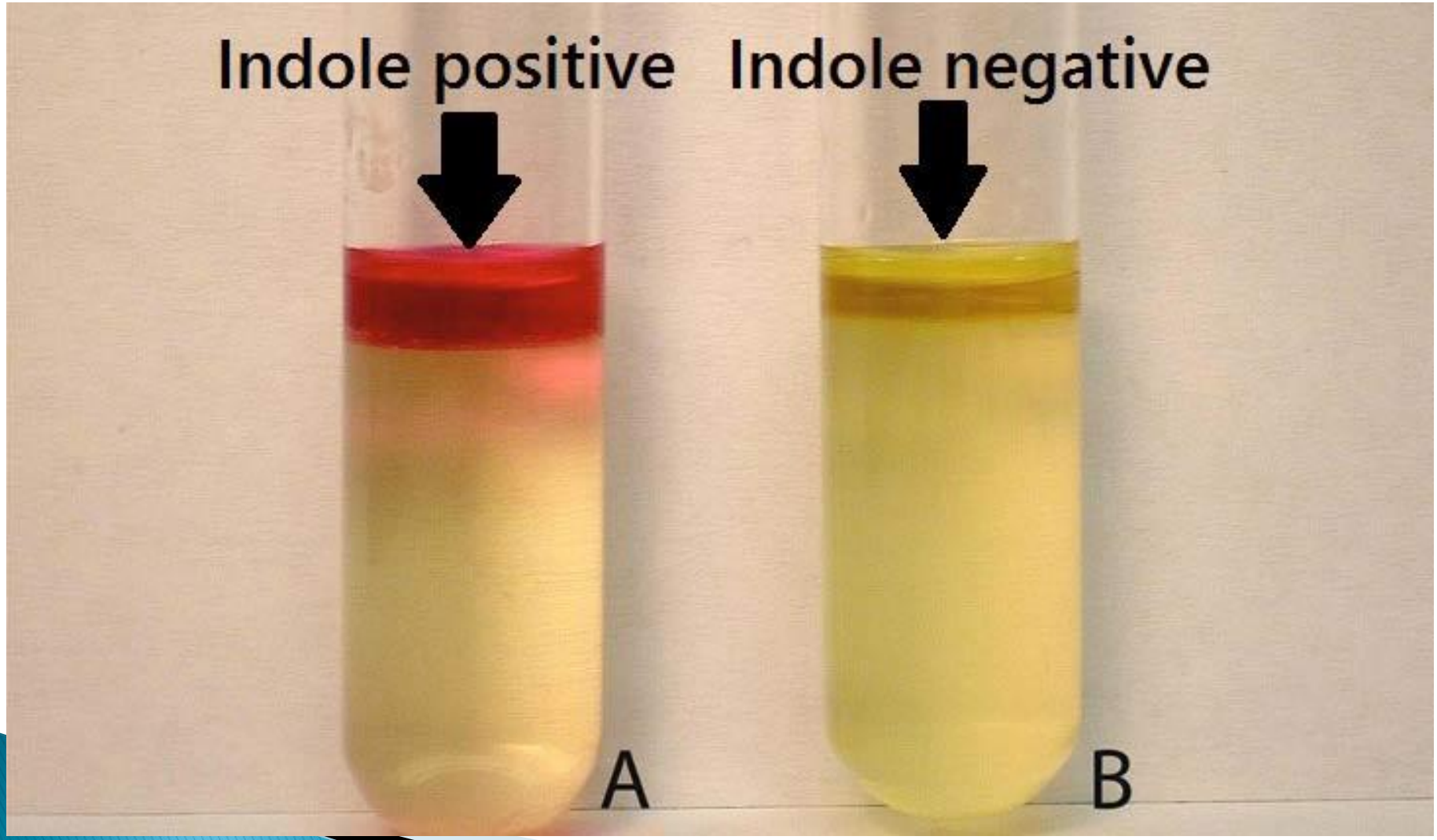
Indole, Methyl Red, Voges-Prosakaur, Citrate (IMViC) Tests:

The following four tests allows for the differentiation of the various members of Enterobacteriaceae.

- **Indole Test**

  Principle: It tests for the bacteria species' ability to produce indole. Bacteria use an enzyme, tryptophanase to break down the amino acid, tryptophan, which leads to the formation of pyruvic acid, indole and ammonia. The presence of indole is detected by addition of Kovac's reagent. pink color in the top layer indicates the presence of indole (indole is positive). The absence of color means that indole was not produced, indole is negative.

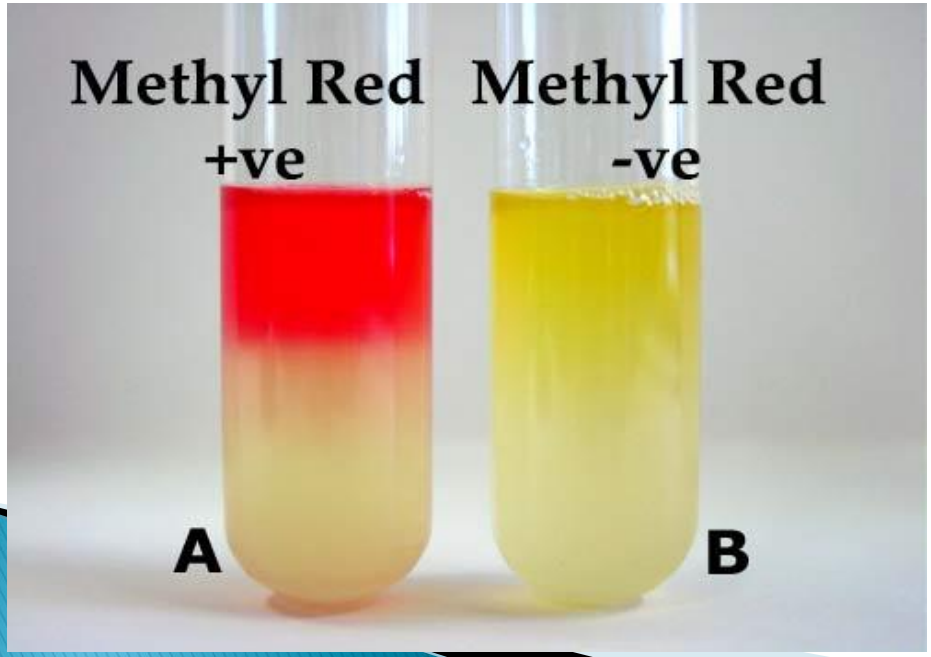
Indole Test



Methyl Red/Voges Proskauer (MR/VP) Test

Methyl Red Test:

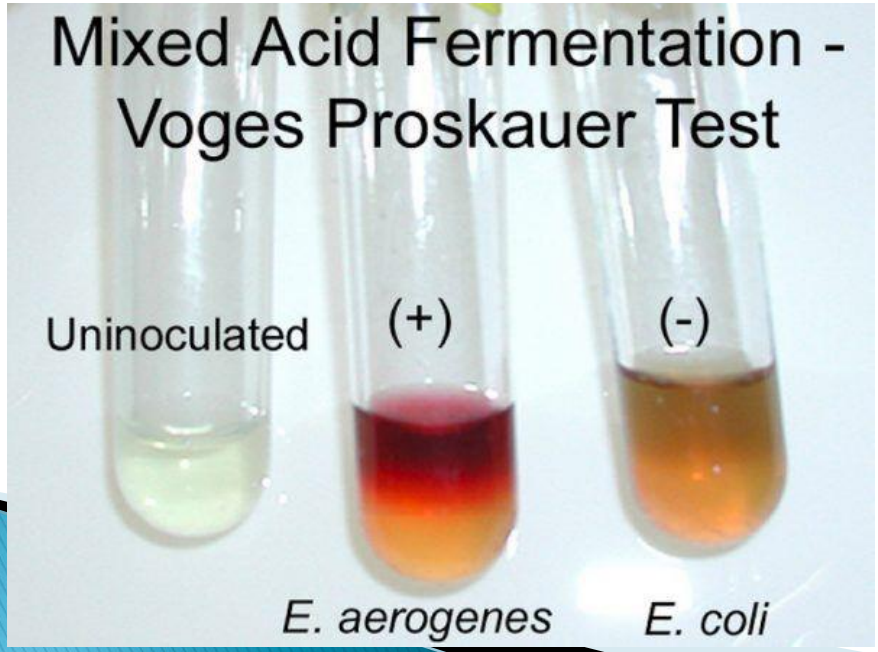
Principle: All enteric bacteria can utilize sugar for their energy demands. Mixed acid fermenters such as *E. coli* ferment glucose to produce large amounts of acids as end products. The large amount of acid drops the pH below 5.0. Methyl red (MR) indicator is using, at pH less than 5.0 MR indicator is red in color. Whereas the pH is above 6.0, the color will be yellow/orange



Voges Proskauer Test:

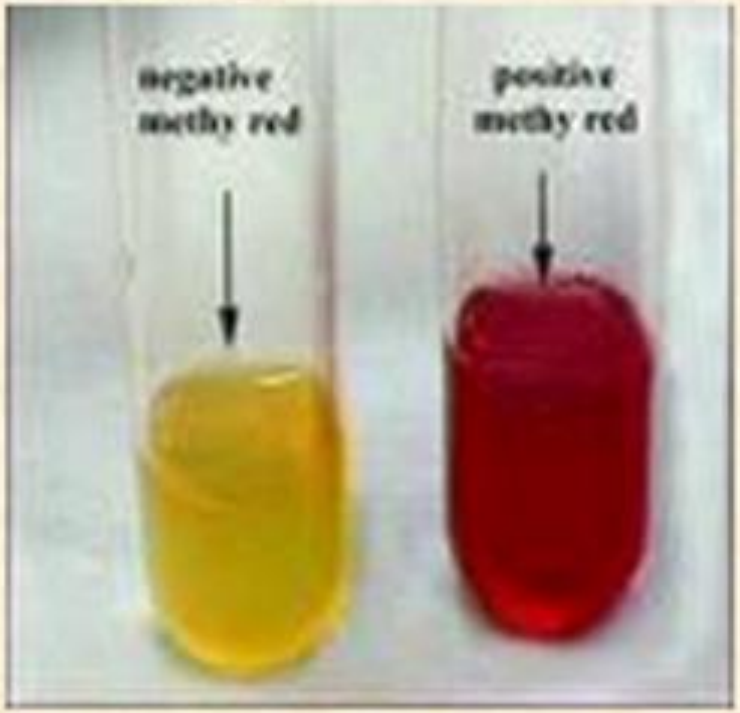
Principle: Some bacteria, rather than producing abundant acid in the fermentation of glucose, produce other products such as Acetoin (acetylmethylcarbinol).

A test for Acetoin is performed. If VP reagent is added, a pink color developing after few minutes indicates the presence of Acetoin and the test is positive.



IMViC test: MR/VP test

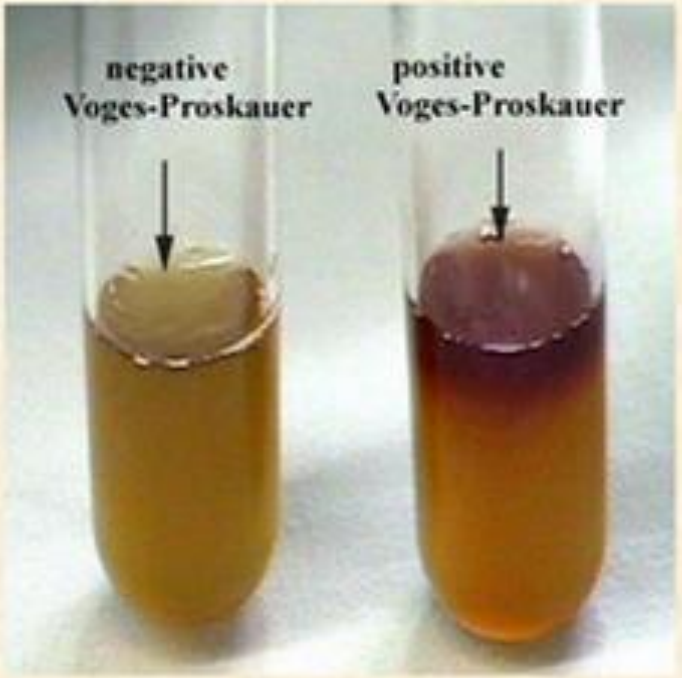
❖ Results



Methyl Red test

✓Red: Positive MR (E. coli)

✓Yellow or orange: Negative MR (Klebsiella)



Voges-Proskauer test

✓Pink: Positive VP (Klebsiella)

✓No pink: Negative VP (E. coli)

Citrate Test

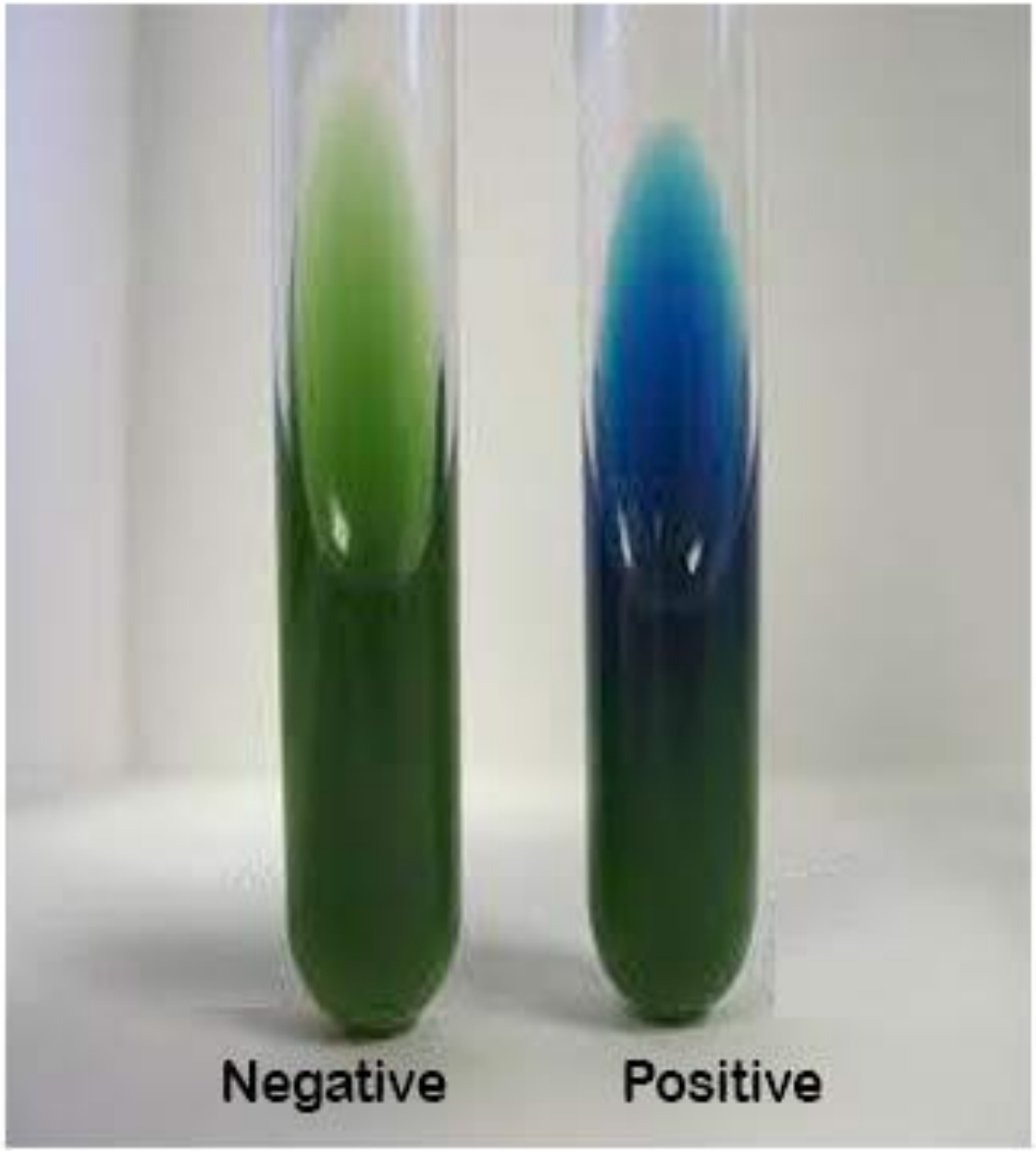
Principle: Some bacteria can metabolize citrate as a sole of carbon source.

The citrate is broken down to pyruvate and CO_2. The carbon dioxide combines with sodium in the medium and water to form sodium carbonate, an alkaline product.

The rise in pH is detected by the color change in bromothymol blue indicator present in the medium from green to deep blue.

Citrate $\rightarrow$Pyruvate $\rightarrow CO_2 + Na + H_2O \rightarrow Na_2CO_3 \rightarrow \uparrow$ pH

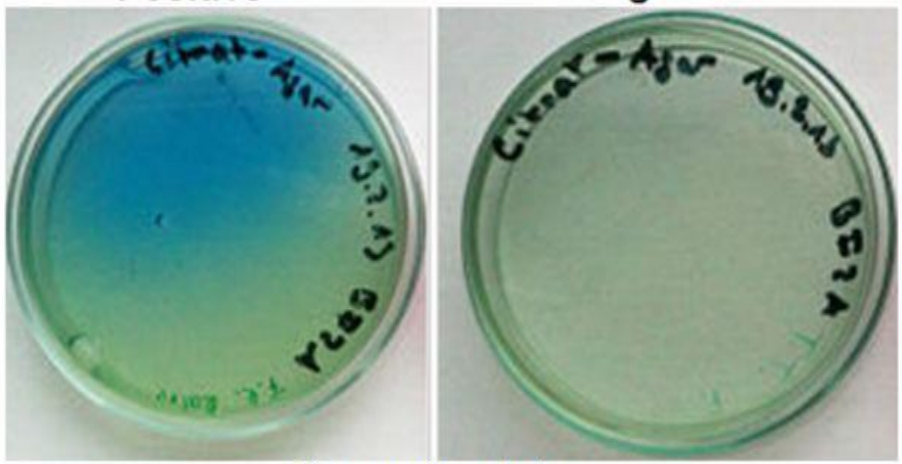
Citrate Utilization Test



Citrate Utilization Test

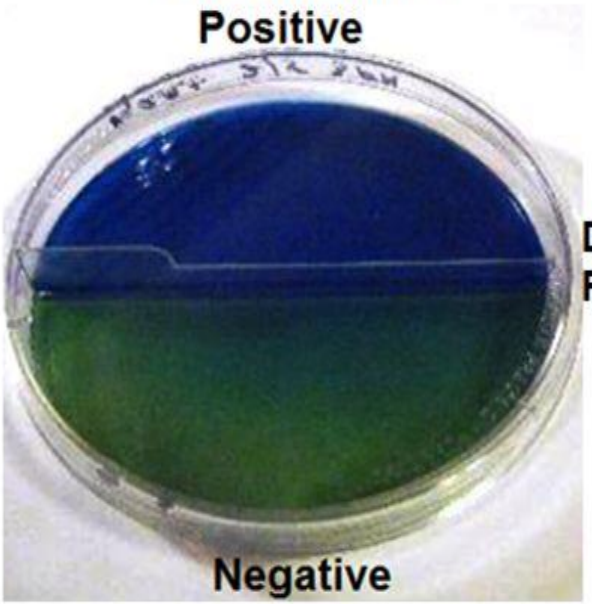
Positive

Negative



Separate plates

Positive

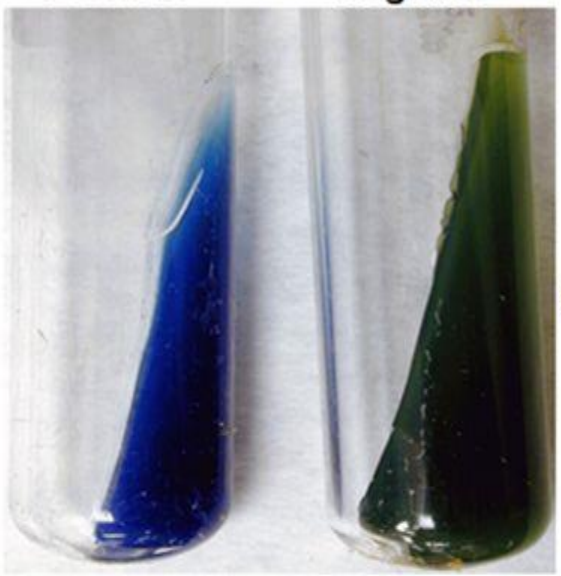


Negative

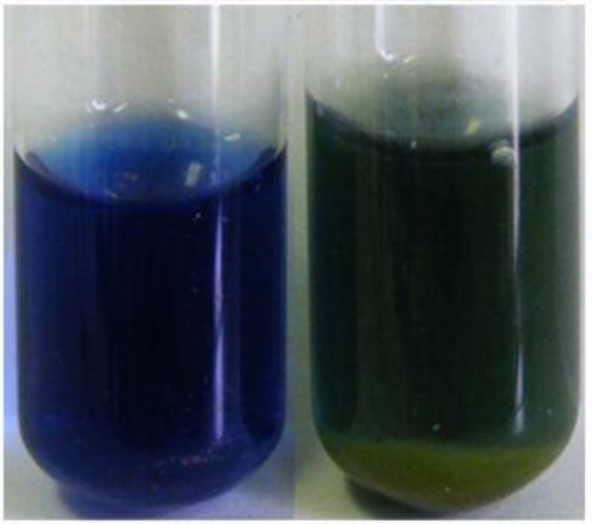
Divided Plate

Positive

Negative



Slant



Butt

Urease Test

Principle: Urease is enzymes that hydrolyze urea to CO_2 and NH_3. It's useful in identifying *Proteus, Klebsiella* and *Enterobacter*. The Christensen's urea agar used for the test contains urea and the indicator phenol red.

In the presence of urease, urea split ammonia and CO_2 are produced. Ammonia combines with water to produce ammonium hydroxide, a strong base which raises the pH of the medium. Turn color of medium from yellow to pink indicates positive test.

Urease test

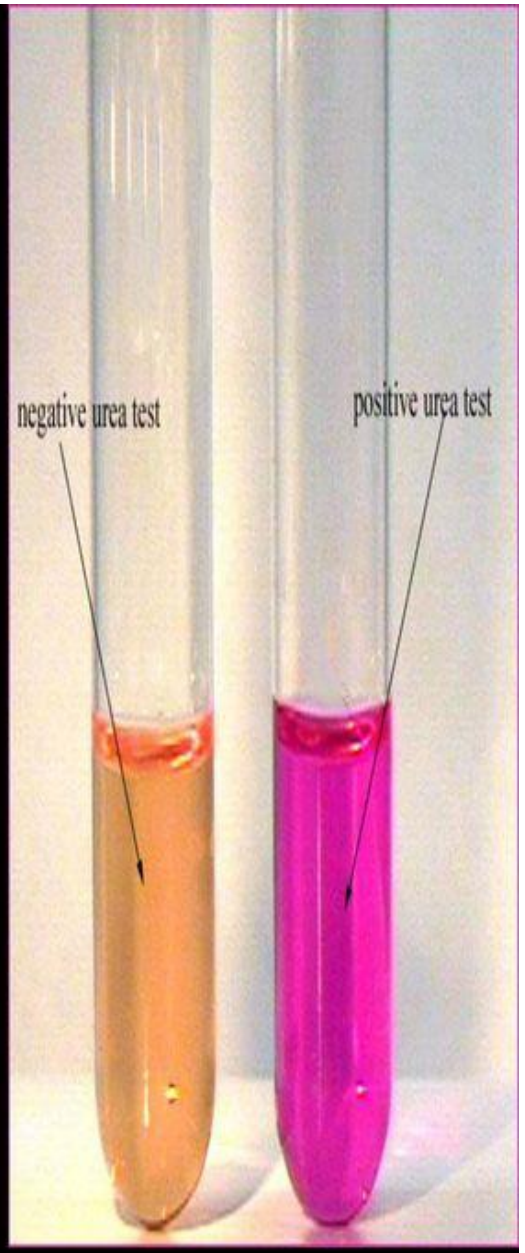
Principle

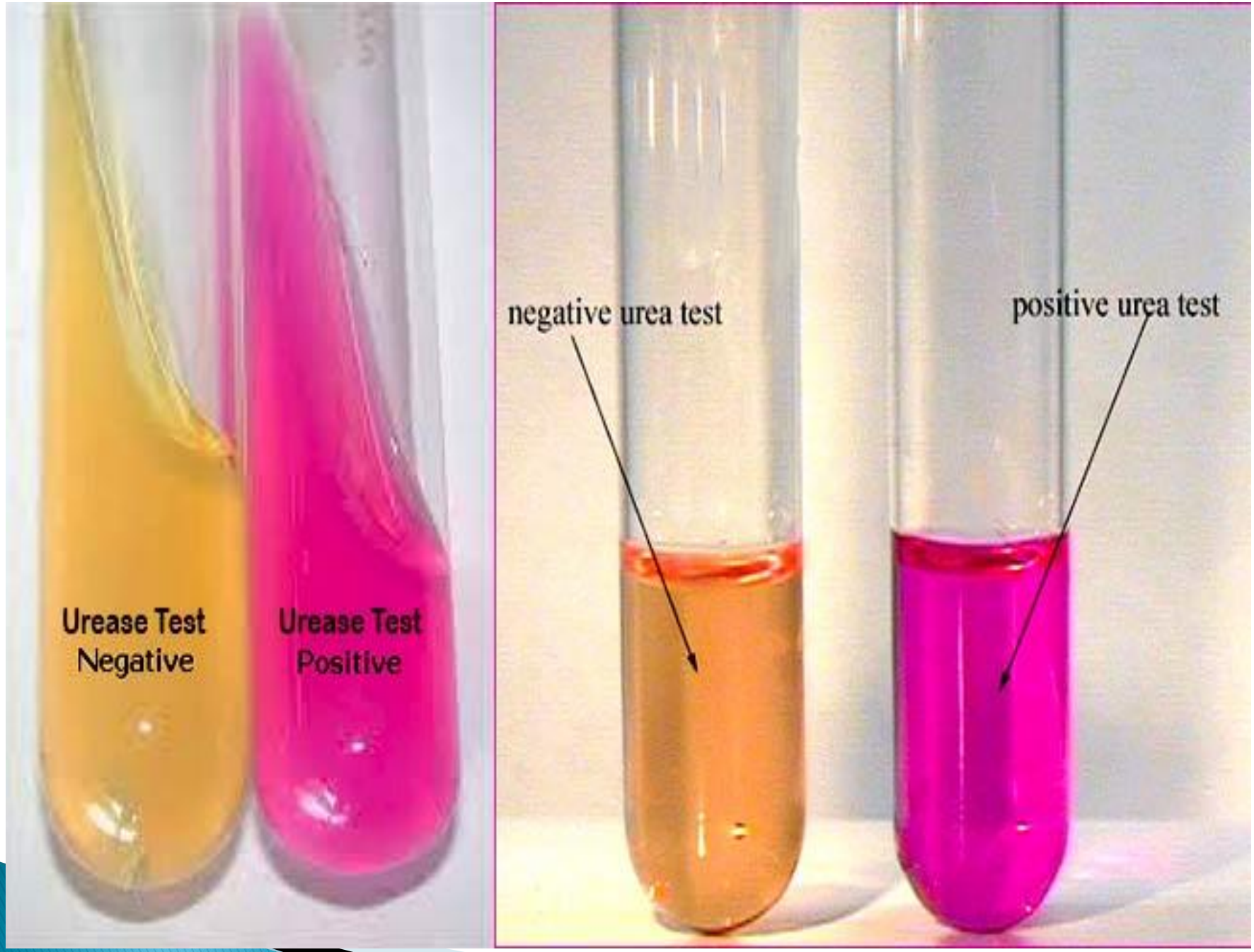
To determine the ability of the organism to split urea forming 2 molecules of ammonia by the action of the enzyme urease with resulting alkalinity. **PH indicator is phenol red which become red in alkalinity**

urease is an enzyme that hydrolyzes urea to carbon dioxide and ammonia.

$$(NH_2)_2CO + H_2O \rightarrow CO_2 + 2NH_3$$

Urease test media contain 2% urea and phenol red as a pH indicator. An increase in pH due to the production of ammonia results in a color change from yellow (pH 6.8) to bright pink (pH 8.2).




Urease Test
Negative
Urease Test
Positive
negative urea test
positive urea test

Coagulase Test

Principle: this test for the bacteria's ability to clot blood plasma using the enzyme coagulase. If the organism has coagulase it will clump plasma by converting fibrinogen into fibrin. Coagulase test used to classify staphylococci into 1- **Coagulase-positive staphylococci** (e.g. *S. aureus*) and 2- **Coagulase negative staphylococci** (*S. epidermidis, S. saprophyticus*).

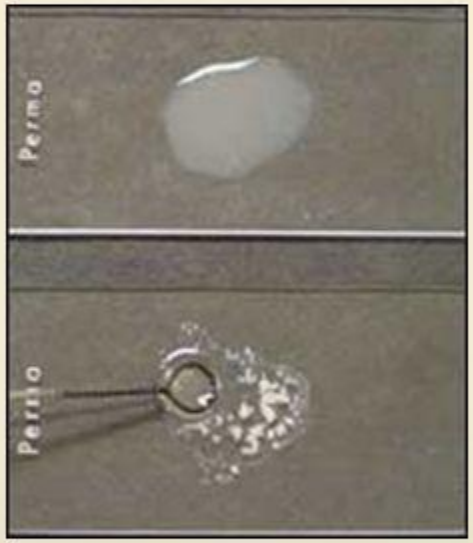
Coagulase

\+ → *Staphylococcus aureus*

− → CNS → *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus saprophyticus*

Negative

Positive



Slide method

Tube method

Positive

Negative

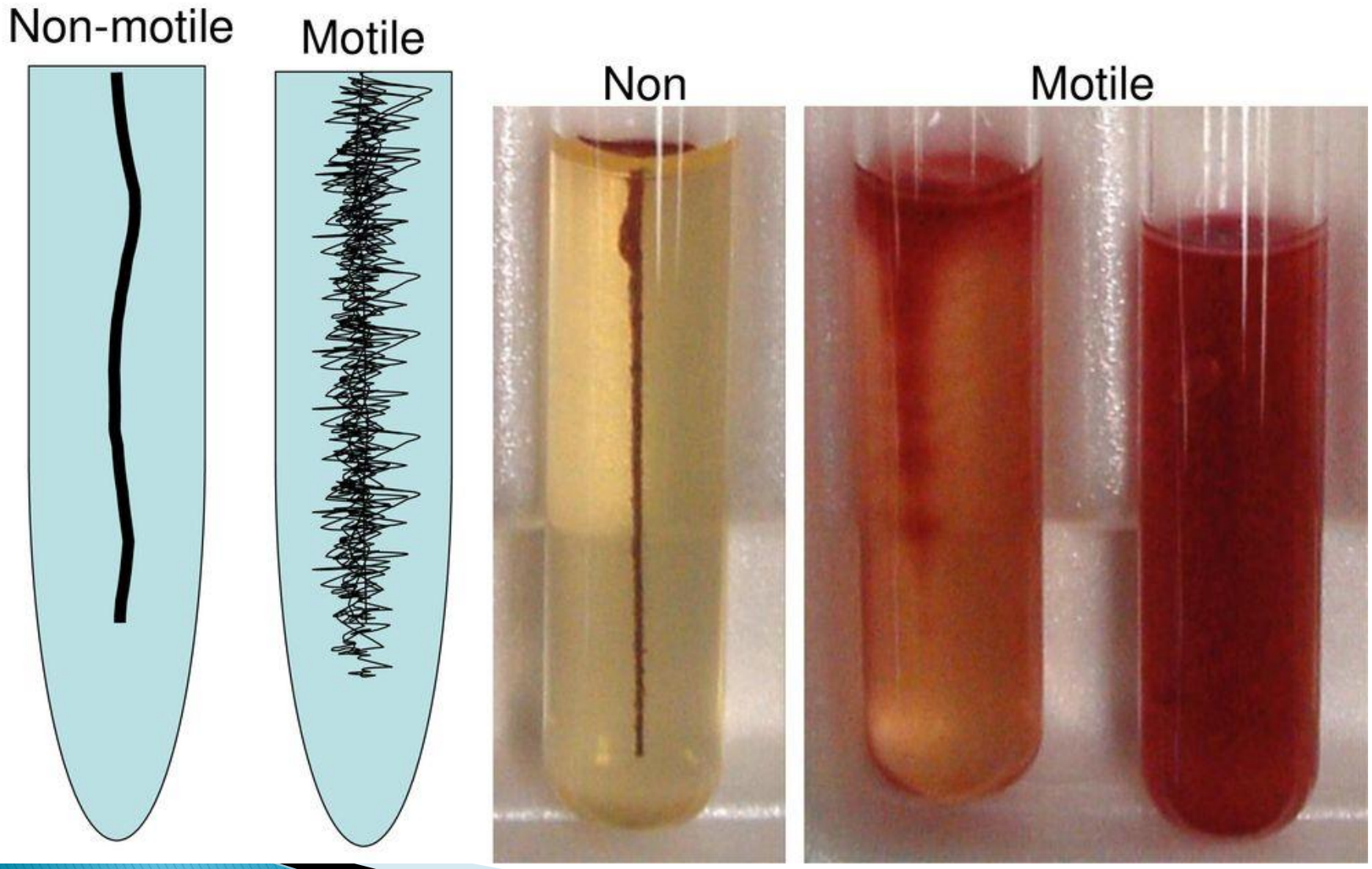
Coagulase test

Motility Test

Principle: This test is done to differentiate species of bacteria that are motile from non-motile species.

This test done by using motility media (semi solid media) and a color indicator (a colored indicator can be used to make the results easier to see).

Motility Test Media Results



Oxidase test

Principle: Oxidase test is done to determine the presence of cytochrome oxidase in bacteria.

During respiration electrons are transferred through a series of oxidation-reduction reaction to a terminal electron acceptor such as oxygen. The terminal link in the electron transport chain is cytochrome oxidase, which is given name because it mediates the transfer of electrons from reduced cytochrome *c* to molecular oxygen to form water.

The test is extremely beneficial in differentiating many groups of bacteria. All members of *Enterobacteriaceae* are oxidase negative, while other Gram-negative rods, such as *Pseudomonas*, are oxidase positive.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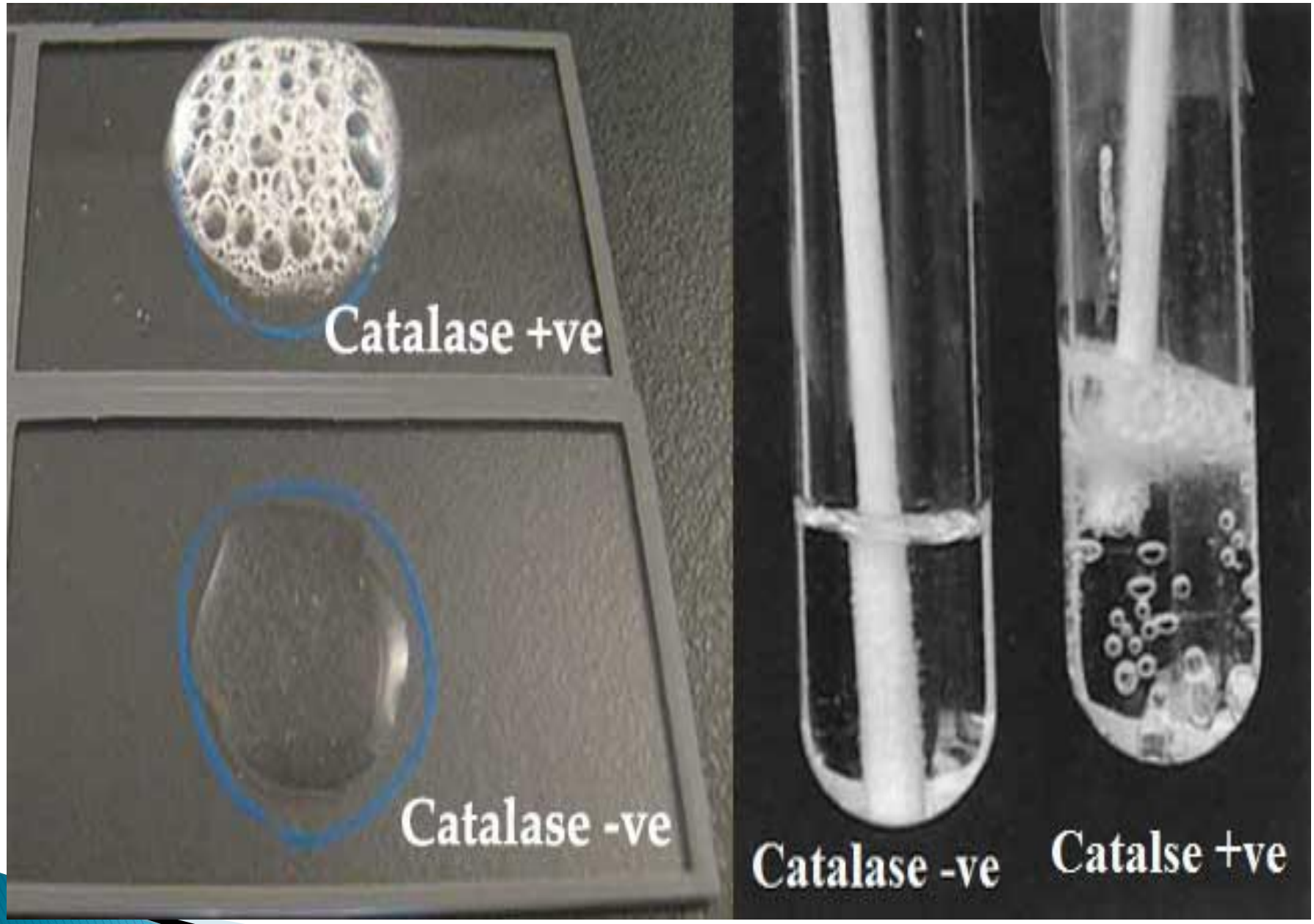
©
Staphylococcus epidermidis
OXI -
Pseudomonas aeruginosa
OXI +
Oxidase test
Hans N.

Catalase Test

- **Principle:** Catalase is an enzyme that split or catalyzes H_2O_2 to water and O_2.
- The function of catalase is to remove toxic hydrogen peroxide that forms during oxidation-reduction reaction. If the organism has catalase, bubble will occur from the production of O_2.
- The test is important in distinguishing streptococci (catalase-negative) from staphylococci which are catalase positive.


Catalase +ve
Catalase -ve
Catalase -ve
Catalse +ve

Normal flora

- is microorganisms such as bacteria that are frequently found in various body sites in normal, healthy individuals.
- The constituents and numbers of the flora vary in different areas and sometimes at different ages and physiologic states. These bacteria are known as commensal or “good” bacteria, because they perform some vital and useful functions in the human body.
- In the gut they aid digestion & produce essential vitamins (folic acid & vitamin K). Prevent other, pathogenic bacteria from colonization, by taking up space or competing for nutrients. Whereas can cause disease in certain circumstances such as if they get into the wrong site.

- Residents are strains that have an established niche at one of the many body sites, which they occupy indefinitely.
- Transients are acquired from the environment and establish themselves briefly but tend to be excluded by competition from residents or by the host's innate or immune defense mechanisms.

Bacteria are unicellular organisms, i.e. organisms that consist of one single cell.

They come in many shapes and sizes. Common shapes are rod-shaped (bacillus), sphere-shaped (coccus) and helix-shaped (spirilla). These shapes are caused by the growth of the cell wall of the bacterium.

The cell wall usually acts to protect the bacterium against invasion, by other organisms or by chemicals. However, sometimes, in particular environments, bacteria can exist without cell walls.

- **Microbiology** – study of microorganisms (simple forms of life visible only with a microscope) such as (**Viruses , bacteria, fungi, protozoa and some algae are all in this category)**
- **How do microorganisms cause disease?**
- Organisms cause disease by using nutrients needed by cells and tissues, damaging cells directly, or producing toxins.

What is Medical Microbiology?

the study of microorganisms (including bacteria, viruses, fungi and parasites) which are of medical importance and are capable of causing diseases in human beings.

Why is it Important?

Infection is one of the most important causes of mortality and morbidity in the population.

Approximately 30% of hospital patients are on antibiotics at any one time

1 in 10 patients acquires an infection whilst in hospital.

Microorganisms and Human Beings

Beneficial activities: Most microbes are of benefit to human beings, some are necessary(nitrogen, carbon cycles, etc)

Harmful activities: A portion of microbes cause diseases and are poisonous to human, and these are really that concern us in the study of medical microbiology, etc.

Steps in diagnostic isolation and identification of bacteria

Step 1. Samples of body fluids (e.g. blood, urine, cerebrospinal fluid) are streaked on culture plates and isolated colonies of bacteria (which are visible to the naked eye) appear after incubation for one to several days .

Each colony consists of millions of bacterial cells. Observation of these colonies for size, texture, color, and (if grown on blood agar) hemolysis reactions, is highly important as a first step in bacterial identification. Whether the organism requires oxygen for growth is another important differentiating characteristic.

Step 2. Colonies are Gram stained and individual bacterial cells observed under the microscope.

Step 3. The bacteria are speciated using these isolated colonies. This often requires an additional 24 hours of growth.

The identification of a bacterial species is based on many factors, including cell and colony morphology, chemical composition of cell walls, biochemical activities, and nutritional requirements. In order to begin identifying a bacterial species, you must start with a pure culture.

In addition to the Gram stain, microorganisms are also classified according to colony morphology and cell morphology. Bacterial colonies grow from a single cell and are composed of millions of cells. Each colony has a characteristic size, form or shape, edge, texture, degree of opacity, and color.

THE SPECIMEN

The primary connection between the clinical encounter and diagnostic laboratory is the specimen submitted for processing. If it is not appropriately chosen and/or collected, no degree of laboratory skill will rectify the error. Failure at the level of specimen collection is the most common reason for failure to establish an etiologic diagnosis.

Direct Samples: specimens are collected from normally sterile tissues (lung, liver) and body fluids (cerebrospinal fluid, blood) by using needle or surgical biopsy.

Indirect Samples: samples are specimens of inflammatory exudates (expectorated sputum, urine).

Specimen Collection and Transport

The sterile swab is the most commonly used tool for specimen collection; however, it provides the poorest conditions for survival and can only absorb a small volume of inflammatory exudate.

The best is a collection of 5 to 10 mL or more of the infected fluid or tissue. The volume is important because infecting organisms present in small numbers may not be detected in a small sample.

Specimens should be transported to the laboratory as soon after collection as possible, because some microorganisms survive only briefly outside the body.

CULTURAL CHARACTERISTICS

Cultural characteristics include the demonstration of properties such as unique nutritional requirements, pigment production, and the ability to grow in the presence of certain substances (sodium chloride, bile) or on certain media (MacConkey, nutrient agar). Demonstration of the ability to grow at a particular temperature or to cause hemolysis on blood agar plates is also used.

BIOCHEMICAL CHARACTERISTICS

The ability to attack various substrates or to produce particular metabolic products has broad application to the identification of bacteria.