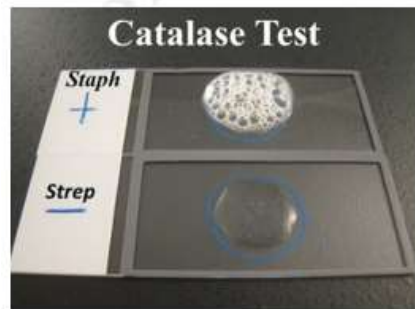


Single enzyme tests

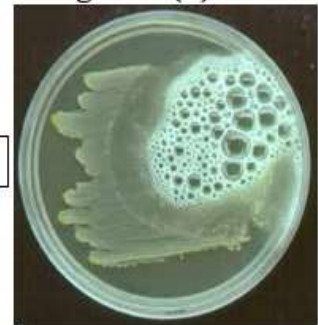
By Prof. Dr. Habeeb S. N.

1- Catalase test



Catalase test on slide

Gas bubbles



Catalase test on plate

2- Oxidase test: The reagent is 1% Kovács oxidase

Oxidase test:

The oxidase test is used to determine if a bacterium produces certain cytochrome c oxidases. The reagent turns dark blue when oxidized (oxidase positive). The reagent is colorless when reduced (oxidase-negative)

Proteus spp. Oxidase negative



3- Indole test: It is a biochemical test which differentiates the coliform from other members of *Enterobacteriaceae* by detecting their ability to produce the enzyme tryptophanase. This enzyme hydrolyses the amino acid tryptophan into indole, pyruvic acid and ammonia. It is the intracellular enzyme (endoenzyme).

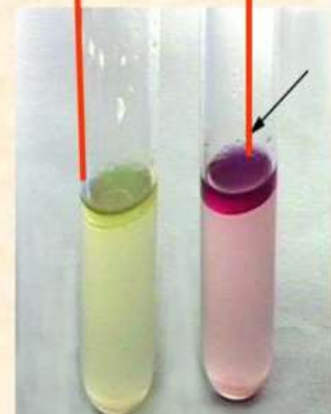
Result:

- A bright pink color in the top layer indicates the presence of indole
- The absence of color means that indole was not produced i.e. indole is negative

Special Features:

- Used in the differentiation of genera and species. e.g. *E. coli* (+) from *Klebsiella* (-).

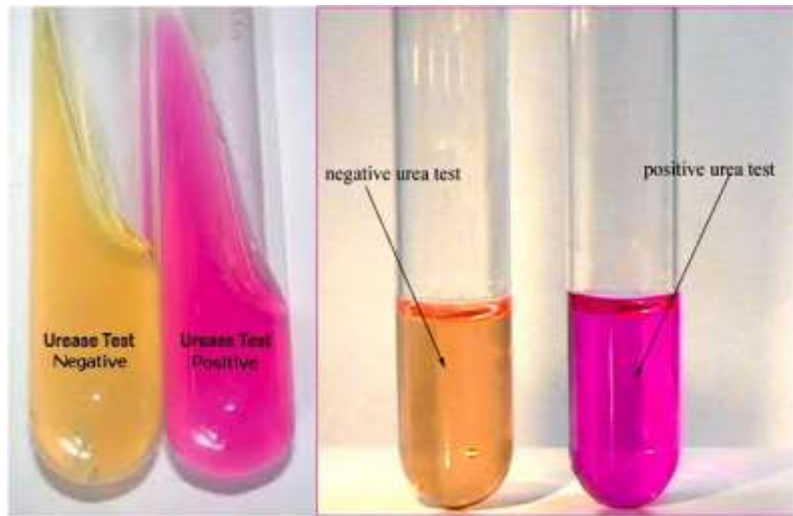
Negative test e.g. *Klebsiella* Positive test e.g. *E. coli*



4- Urease test:

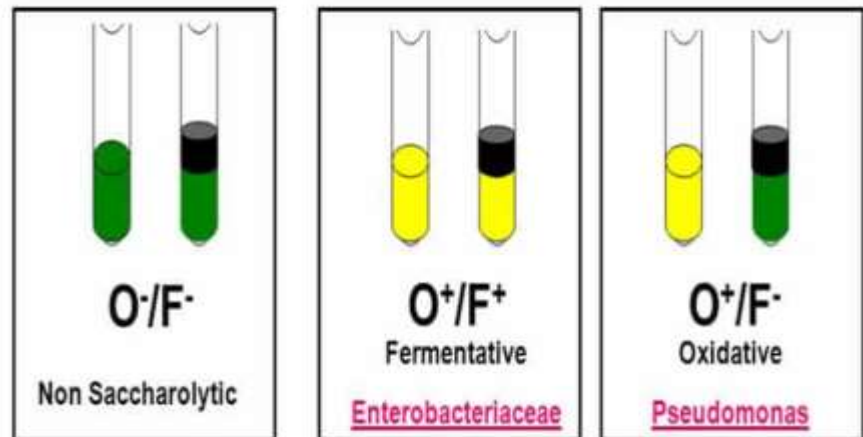
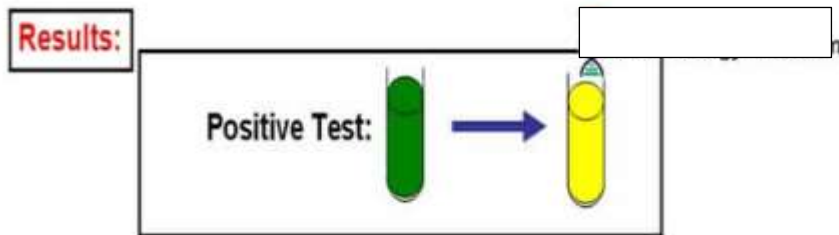
Hydrolysis of **urea** produces **ammonia** and **CO₂**. The formation of **ammonia**

alkalinizes the medium, and the pH shift is detected by the color change of **phenol red** from **light orange** at pH 6.8 to **magenta (pink)** at pH 8.1. Rapid urease-positive organisms turn the entire medium **pink** within 24 hours.



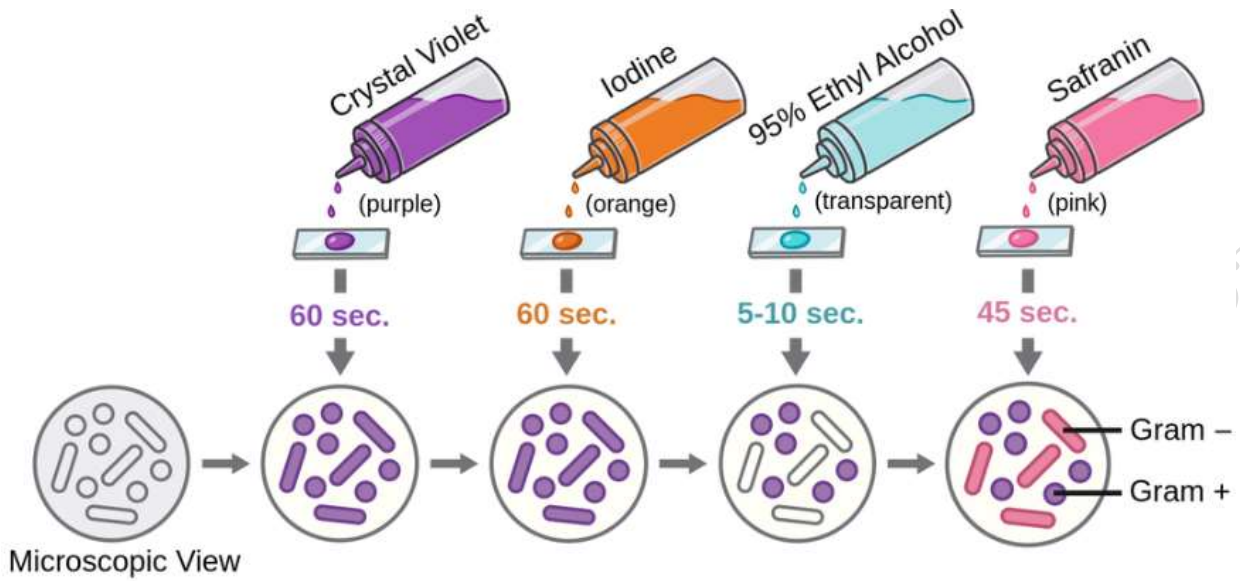
5- Oxidation and Fermentation Tests.

The medium used is called as O-F medium which contains tryptone and bromothymol blue (an indicator). One sugar "glucose" is added to the medium. An organism is inoculated to two tubes of each OF Medium. One tube is overlaid with mineral oil or melted paraffin producing an anaerobic condition. The other tube is left open to the air. Growth of microorganisms in this medium is either by **utilizing the tryptone** which



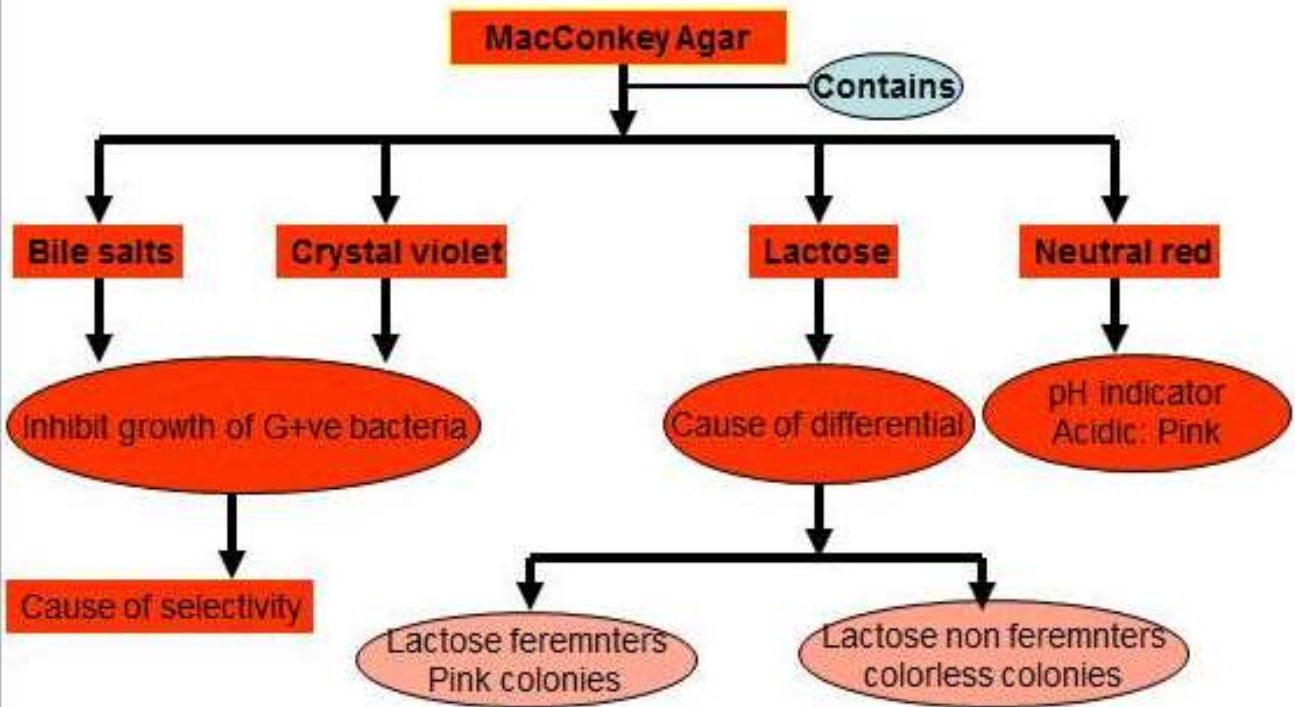
results in an alkaline reaction (dark blue color) or by utilizing glucose, which results in the production of acid (turning bromothymol blue to yellow). Oxidative utilization of the carbohydrate will result in acid production (yellow) in the open tube only. Fermentative utilization of the carbohydrate will result in acid production (yellow) in both the open and closed tubes. Acidic changes in the overlaid tubes are considered to be a result of true fermentation, while acidic development in the open tubes are due to oxidative utilization of the carbohydrate present. Asaccharolytic organisms will not produce acid in either tube.

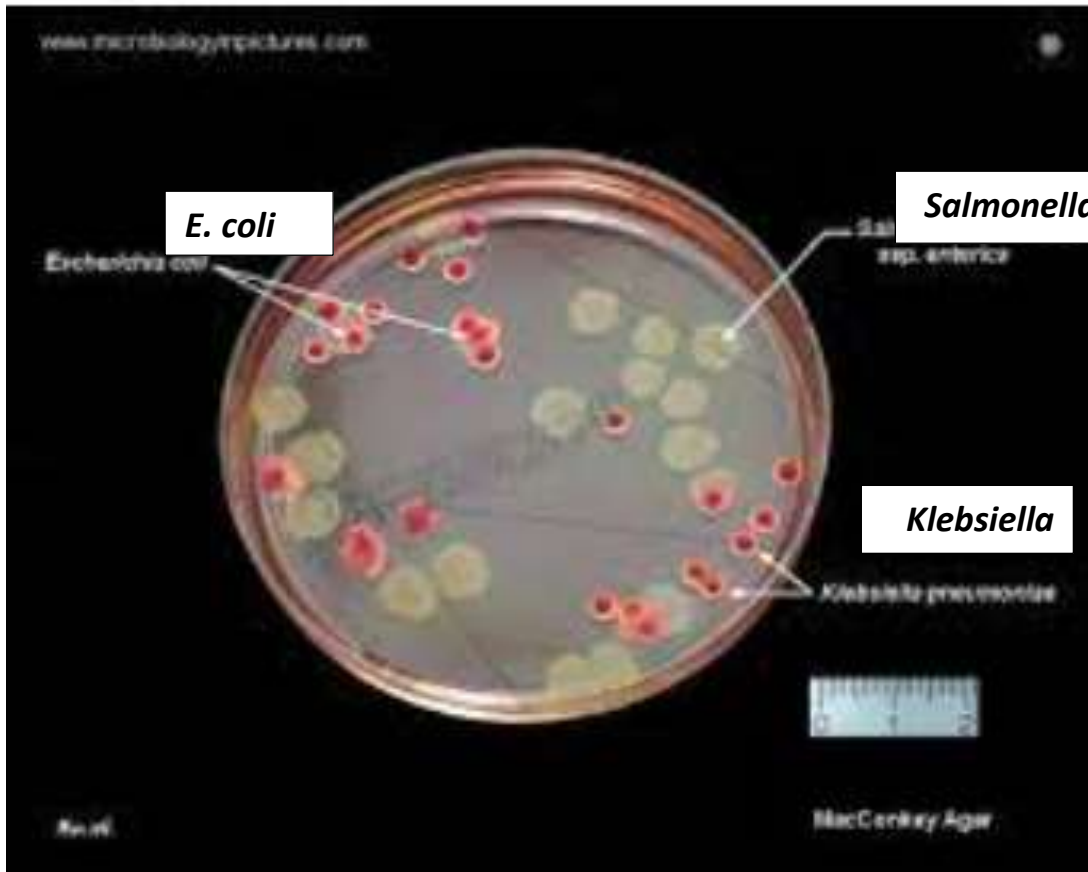
Steps of Gram staining method:



MacConkey agar:

➤ MacConkey agar is selective & differential medium for *Enterobacteriaceae*





MacConkey agar plate with salmonella, escherichia and klebsiella.

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Prof. Dr. Hussein