Ministry of Higher Education
& Scientific Research
University of Sawah
Dep. Of laboratories

Microbiological methods for identification of microorganisms

Staining

Grade: 4

Lecture : 6

By
Prof. Dr. Naer Alkaabi

There are many types of stains, each with specific applications.

- **Simple stains** are directed toward colouring the forms or present shapes
- **Differential stains** are directed toward colouring specific components of the elements present,
- **Diagnostic antibody or DNA probe–mediated stains** are directed specifically at identification of an organism.

Staining techniques

Structural details of bacteria cannot be seen under light microscope due to lack of contrast. Hence, it is necessary to use staining methods to produce color contrast and thereby increase the visibility. Before staining, the fixation of the smear to the slide is done

Fixation is the process by which

- **the internal and external structures** of cells are preserved and fixed in position. It also

-**Inactivates the enzymes** that might disrupt cell morphology.

-**Toughens (hardens)** cell structure so that they do not change during staining. It kills and fixes the cells on to the slide.

There are two types of fixation as follows:

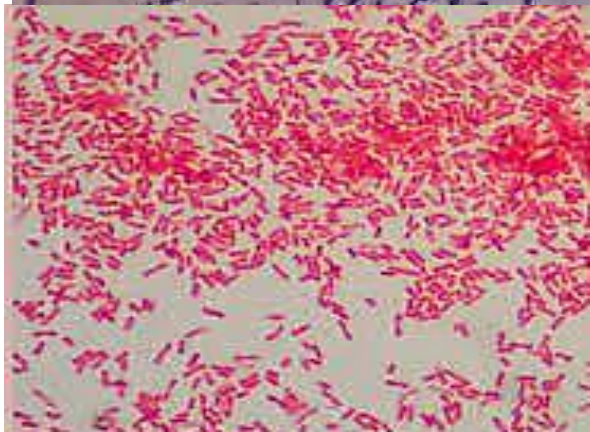
1. **Heat fixation**: **It is usually done for bacterial smears by gently flame heating an air-dried film of bacteria. This adequately preserves overall morphology but not structures within the cells.**

2. **Chemical fixation**: **It can be done using ethanol, acetic acid, mercuric chloride, formaldehyde, methanol and glutaraldehyde. They are used to protect the fine internal structure of the cells. This is useful for examination of blood smears. The fixed smear is stained by appropriate staining technique.**

Common staining techniques used on microbiology

Simple stain

Basic dyes, such as methylene blue or basic fuchsin are used as simple stains. They provide the colour contrast, **but** impart the same colour to all the bacteria in a smear.



Common staining techniques used on microbiology

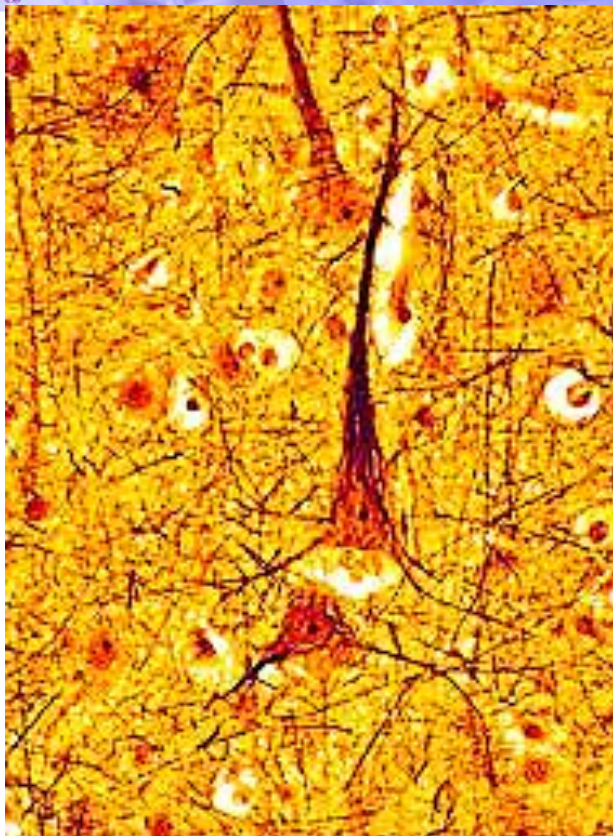
Negative staining

A drop of bacterial suspension is mixed with dyes, such as India ink or nigrosin. The background gets stained black whereas unstained bacterial/yeast capsule stand out in contrast. It is very useful in the demonstration of bacterial/yeast capsules which do not take up simple stains.

Common staining techniques used on microbiology

Impregnation methods

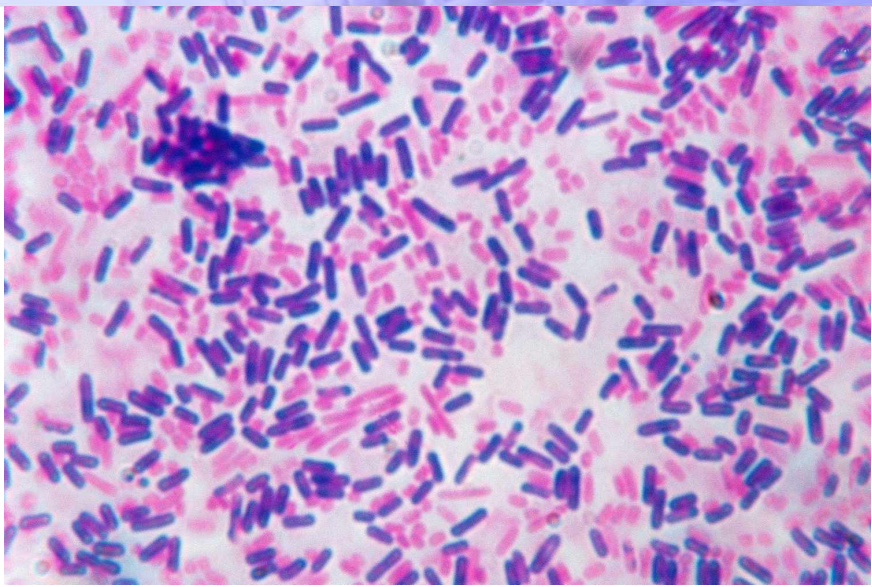
Bacterial cells and structures that are too thin to be seen under the light microscope, are thickened by impregnation of silver salts on their surface to make them visible, e.g. for demonstration of bacterial flagella and spirochetes.



Common staining techniques used on microbiology

Differential stain

two stains are used which impart different colors to different bacteria or bacterial structures, which help in differentiating bacteria.

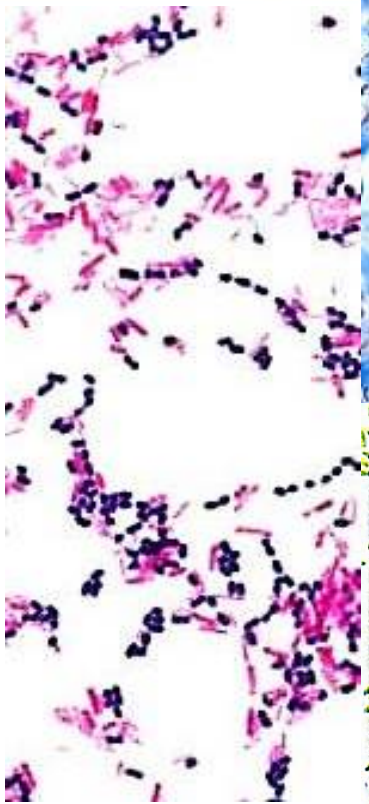


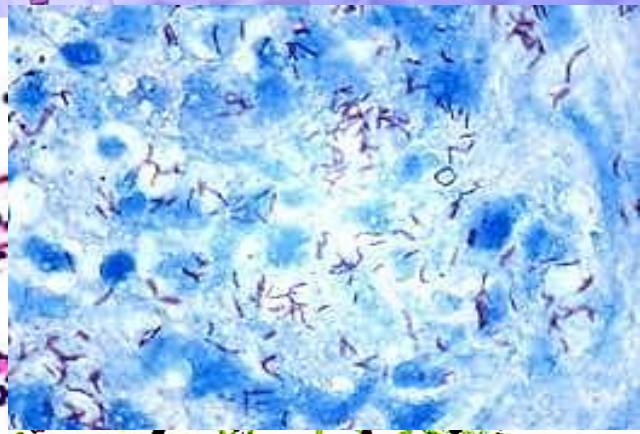
The most commonly employed differential stains are:

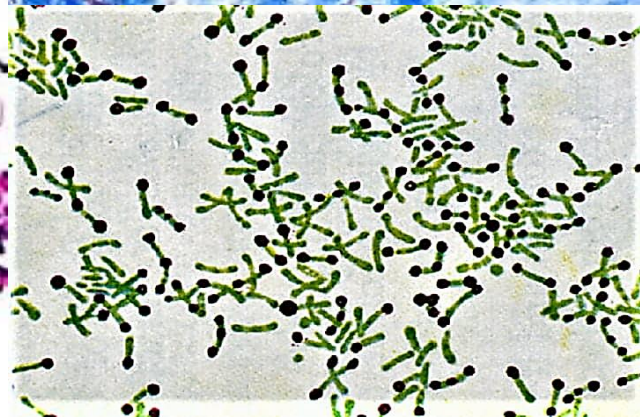
A- **Gram stain:** It differentiates bacteria into gram positive and gram negative groups (G+ or G- bacteria)

B- **Acid-fast stain:** It differentiates bacteria into acid fast and non-acid fast groups

C- **Chromatic granules** from other bacteria that do not have them.







Single Enzyme Tests (biochemical tests)

Several tests are commonly used to determine the presence of a single enzyme. These tests usually provide rapid results because they can be performed on organisms already grown in culture.

01 Catalase Test

02 Oxidase Test

03 Indole Test

04 Urease Test

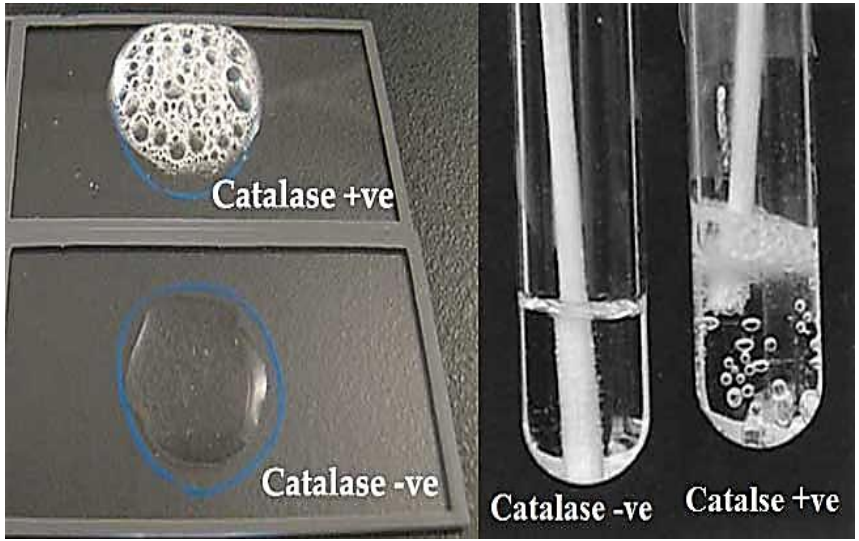
05 Oxidation and Fermentation

06 Amino Acid Degradation

Single Enzyme Tests (biochemical tests)

Catalase Test

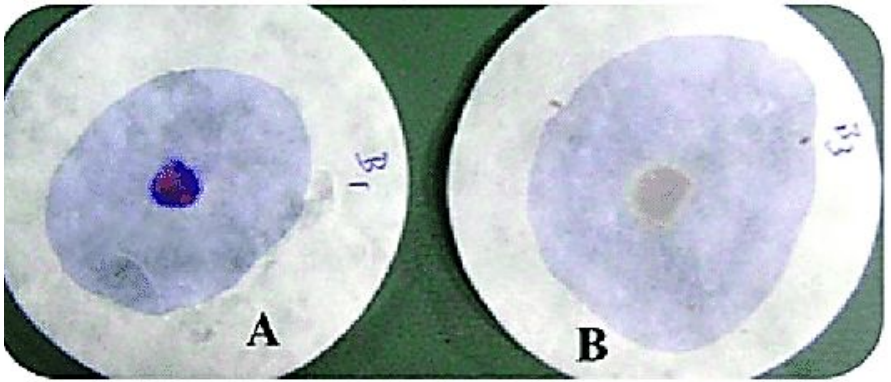
The enzyme catalase catalyzes the release of water and oxygen from hydrogen peroxide (H2O2 _ catalase H2O _O2); its presence is determined by direct analysis of a bacterial culture .The rapid production of bubbles when bacterial growth is mixed with a hydrogen peroxide solution is interpreted as a positive test.



Single Enzyme Tests (biochemical tests)

Oxidase Test

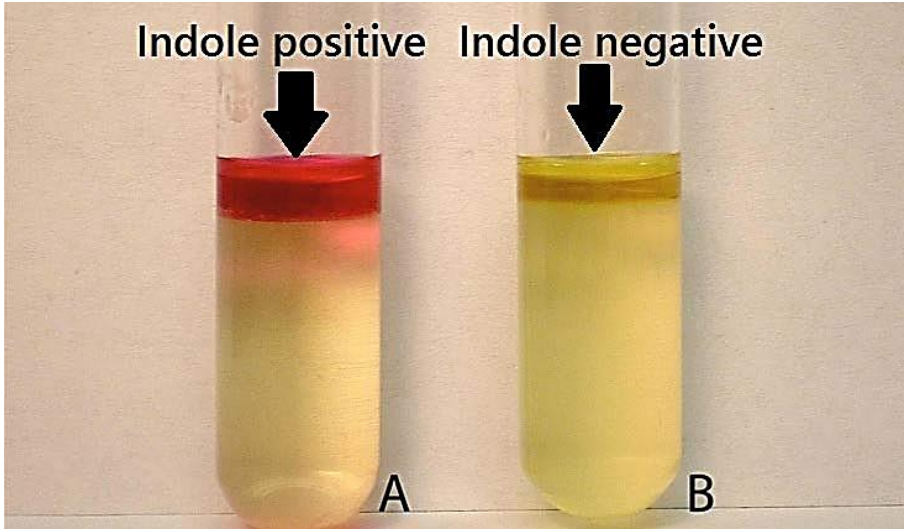
Testing for the presence of oxidase can be performed by flooding bacterial colonies on the agar surface with 1% tetramethylp-phenylenediamine dihydrochloride. Alternatively, a sample of the bacterial colony can be rubbed onto filter paper impregnated with the reagent. A positive reaction is indicated by the development of a purple colour.



Single Enzyme Tests (biochemical tests)

Indole Test

The enzyme tryptophanase are able to degrade the amino acid tryptophan into pyruvic acid,ammonia, and indole. Indole is detected by combining with an indicator Kovac's reagent), which results in a pink to red colour formation.



Single Enzyme Tests (biochemical tests)

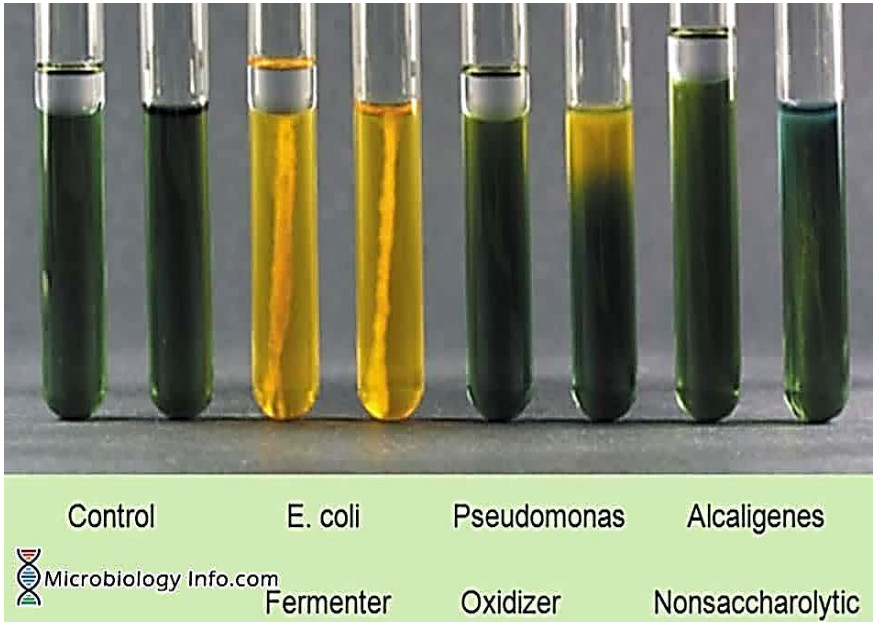
Urease Test

Urease hydrolyzes the substrate urea into ammonia, water, and carbon dioxide. The presence of the enzyme is determined by inoculating an organism to broth (Stuart's urea broth) or agar (Christensen's urea agar) containing urea as the primary carbon source followed by detecting the production of ammonia. Ammonia increases the pH of the medium so its presence is readily detected using a pH indicator. Change in medium pH is a common indicator of metabolic process and, because pH indicators change colour with increases (alkalinity) .The urease test helps identify Proteus spp., and other important bacteria such as Corynebacterium urealyticum and Helicobacter pylori

Single Enzyme Tests (biochemical tests)

Oxidation and Fermentation Tests

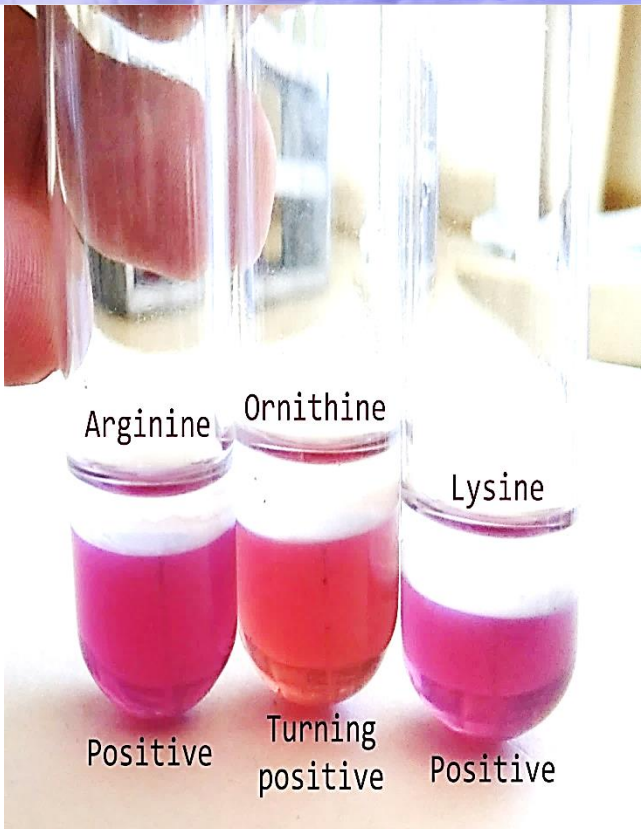
Bacteria utilize of carbohydrates (e.g., sugar or sugar derivatives) and protein substrates. Oxidation-fermentation determinations are usually accomplished using a special semisolid medium (oxidative fermentative [O-F] medium) that contains low concentrations of peptone and a single carbohydrate substrate such as glucose. The glucose fermentative or oxidative capacity is generally used to separate organisms into major groups (e.g., Enterobacteriaceae are fermentative; Pseudomonas spp. is oxidative).



Single Enzyme Tests (biochemical tests)

Amino Acid Degradation

The amino acid substrates most often tested include lysine, tyrosine, ornithine, arginine, and phenylalanine.(The indole test for tryptophan cleavage is presented.) Decarboxylases cleave the carboxyl group from amino acids so that amino acids are converted into amines; lysine is converted to cadaverine, and ornithine is converted to putrescine. Because amines increase medium pH, they are readily detected by colour changes in a pH indictor indicative of alkalinity. Decarboxylation is an anaerobic process that requires an acid environment for activation, the amino acid substrate of interest (i.e., lysine, ornithine, or arginine), and a pH indicator



non-traditional methods

*Complementary diagnostic methods are epi test and VITEC system.

There some non-traditional methods for identification of pathogens or their products include:

1- Molecular testing.

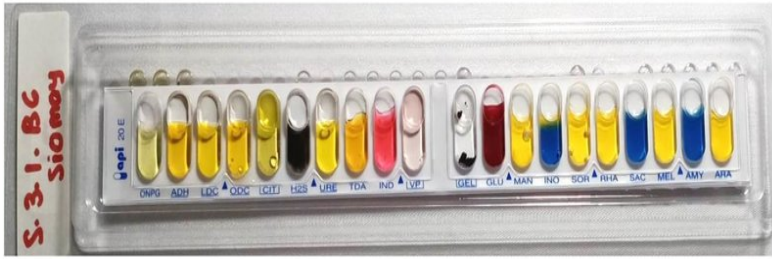
2- Saliva based testing.

3- Chromogeneic testing.

4- Rapid immune-chromatographic testing.

All these tests are expensive and not used as ordinary laboratory tests.

(a)



(b)

GOOD IDENTIFICATION	
Strip	API 20 E V5.0
Profile	1 4 4 4 5 5 2 5 7
Note	

Significant taxa	% ID	T	Tests against					
Escherichia coli 1	92.6	0.54	H2S	1%				

Next taxon	% ID	T	Tests against					
Citrobacter freundii	4.0	0.31	CIT	75%	IND	1%	SAC	99%

THANK YOU