Microbiology Lab Edited Slides
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Respiratory system

Microbiology laboratory section

In this lecture, we will be taking the most common microorganisms (bacteria and fungi) that may affect the respiratory system.

THROAT SWAB

The swab is cultured on a semi-solid media:

1)Enrichment media like sheep blood agar.
2) Selective media. Allows for a certain one type of bacteria to grow while the others not.
3) Differential media: some chemical reactions that allow for 2 types of bacteria to grow but each with a unique color.

Gram Positive Coccus

A throat swab culture is a test commonly used to diagnose bacterial infections in the throat.

These are the common gram positive bacteria that may cause throat infection. We will see how we can differentiate between the species.

Staphylococcus Spp.

Streptococcus Spp.

Staphylococcus

- Arranged in grape like clusters.
- Includes at least 40 species. The most common species associated with clinical infection are Staph aureus, Staph epidermidis, Staph hemolyticus, Staph hominins, and Staph albus.

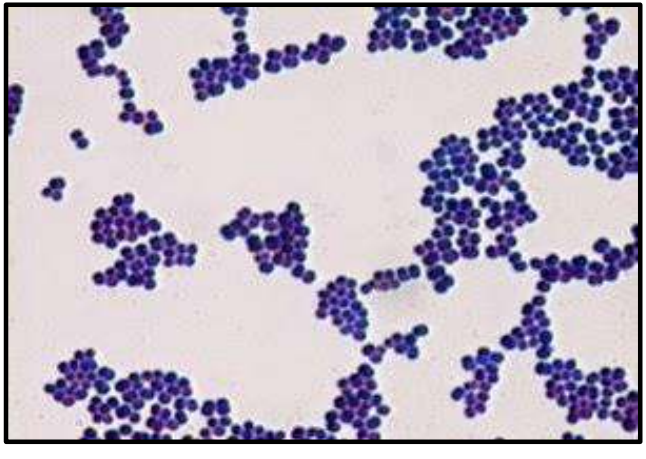
Streptococcus

- Arranged in chains or diplococci.

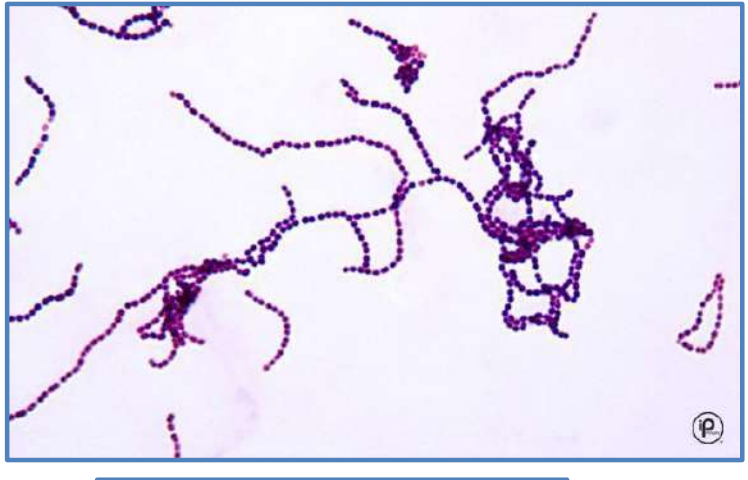
GRAM STAIN

Both are gram positive, stain blue/violet
Cocci shape, in staph it forms clusters, in strep it forms pinpoint chains.

Staphylococcus



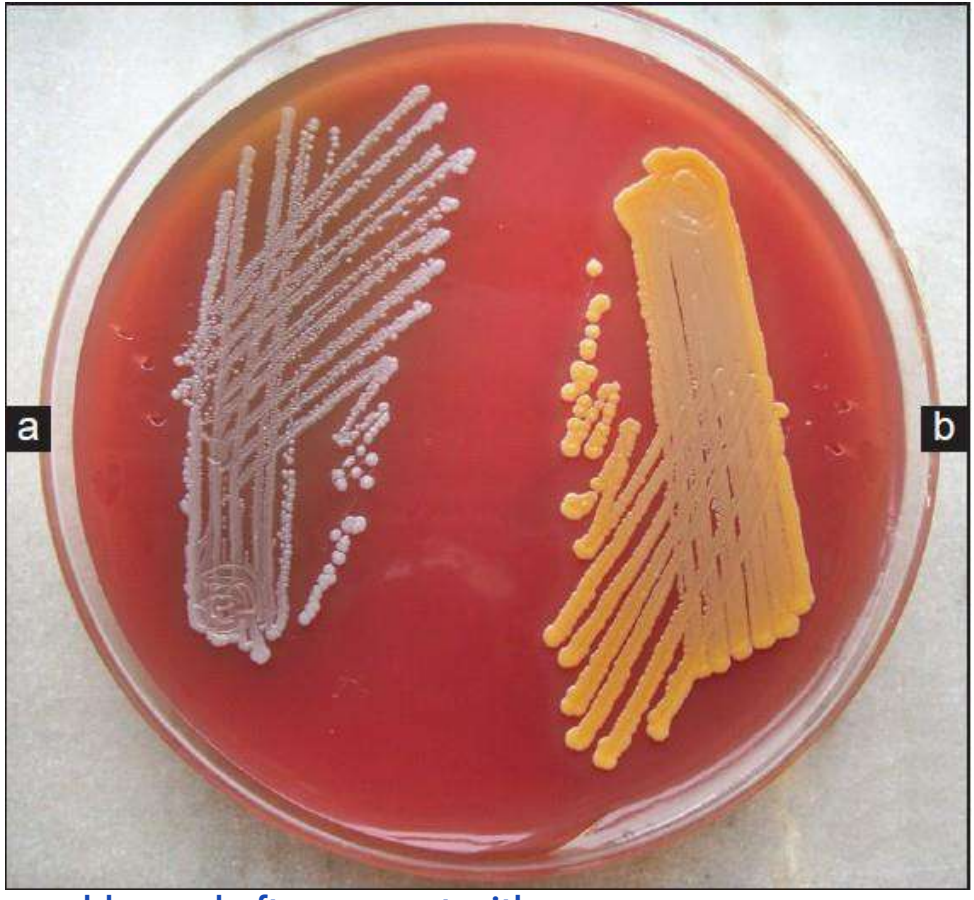
Streptococcus



Other staph species such as staph albus or epidermidis appear as white colonies on blood agar.

A- staphylococcus .albus

B- staphylococcus.Aureus



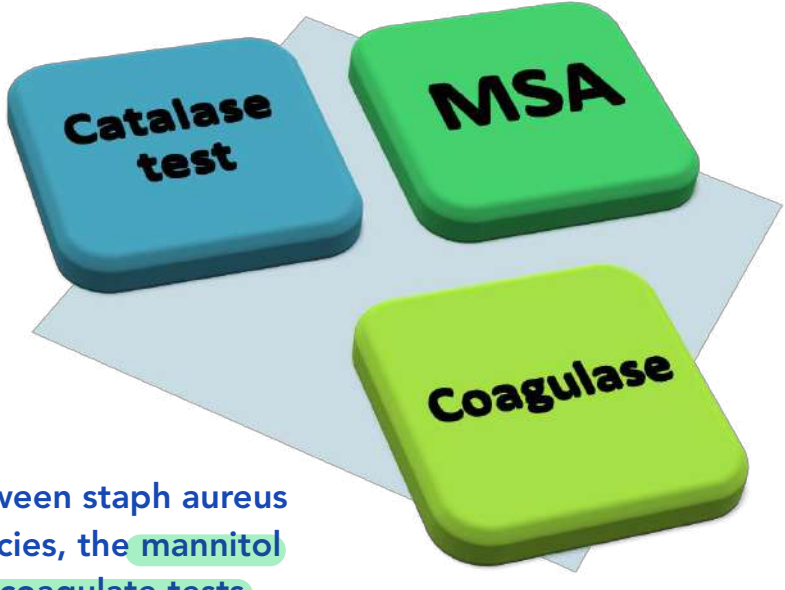
Blood agar

Staph aureus colonies appear yellow-golden and often present with hemolysis when grown on blood agar plates.

The golden appearance is the etymological root (origin) of the bacteria's name "aureus," as the word means golden in Latin.

Test for differentiation of Staphylococcus species

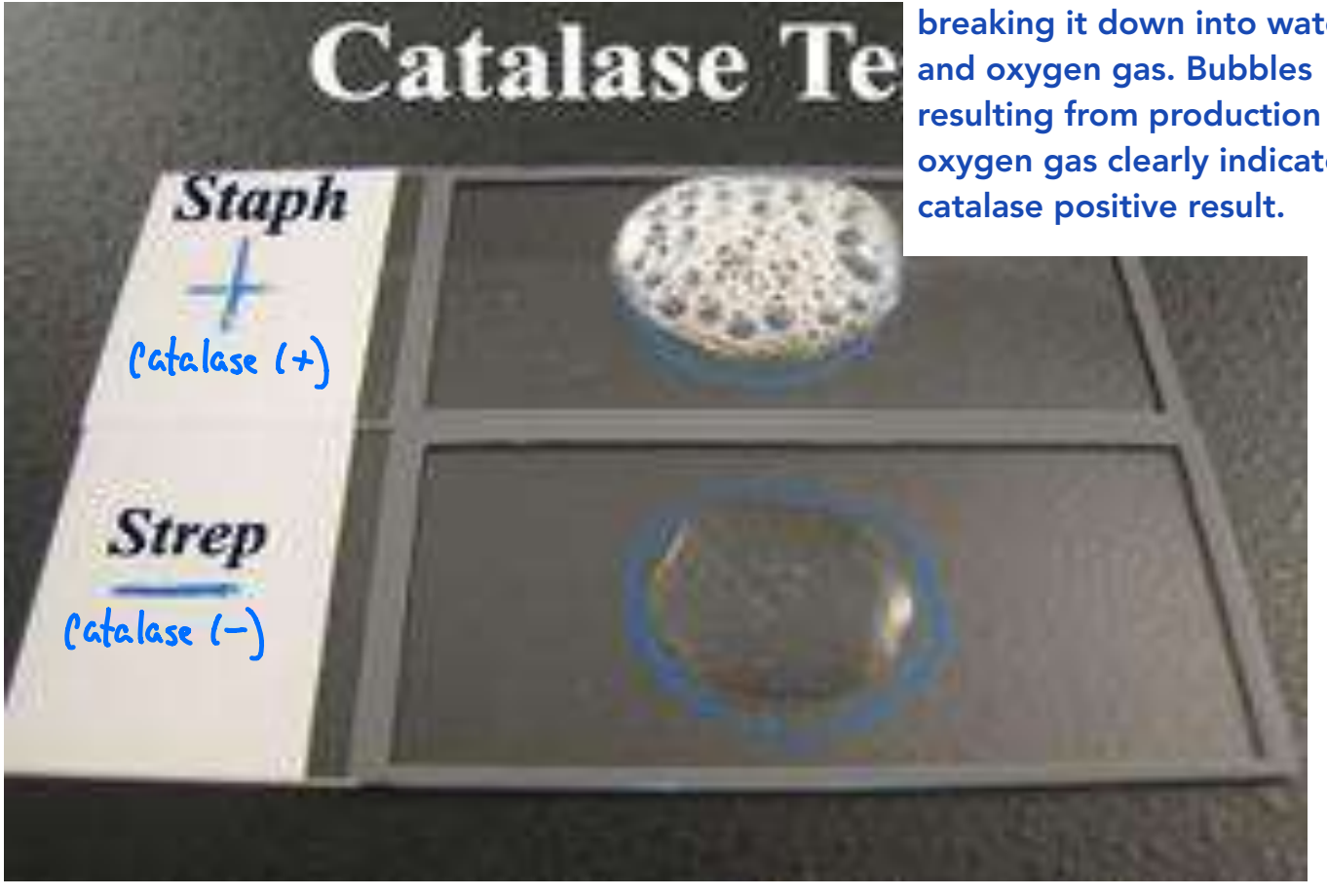
The major test reaction that should be used to differentiate between staphylococcus and streptococcus species is the catalase test.



To differentiate between staph aureus and other staph species, the mannitol salt agar (MSA) and coagulate tests should be used.

Catalase test

The catalase test is used to identify organisms that produce the enzyme catalase. This enzyme detoxifies hydrogen peroxide by breaking it down into water and oxygen gas. Bubbles resulting from production of oxygen gas clearly indicate a catalase positive result.



MSA
Mannitol salt agar media

The MSA is a selective and differential medium. Its high concentration of salt (7.5%) selects for members of the genus Staphylococcus since they can tolerate high saline levels. Organisms from other genera may grow but they grow weakly.

Ferments Mannitol

S.aureus

Does not ferment Mannitol

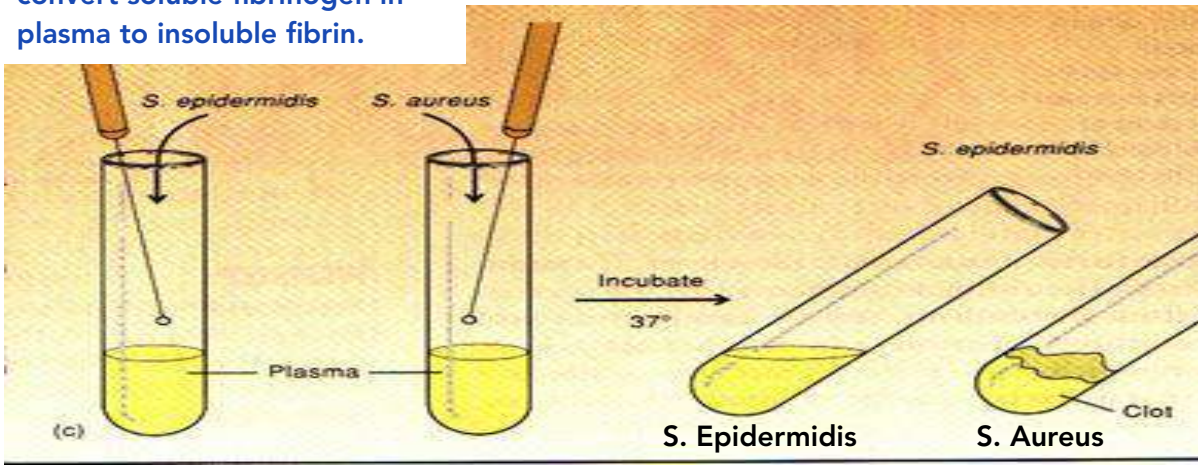
S.albus

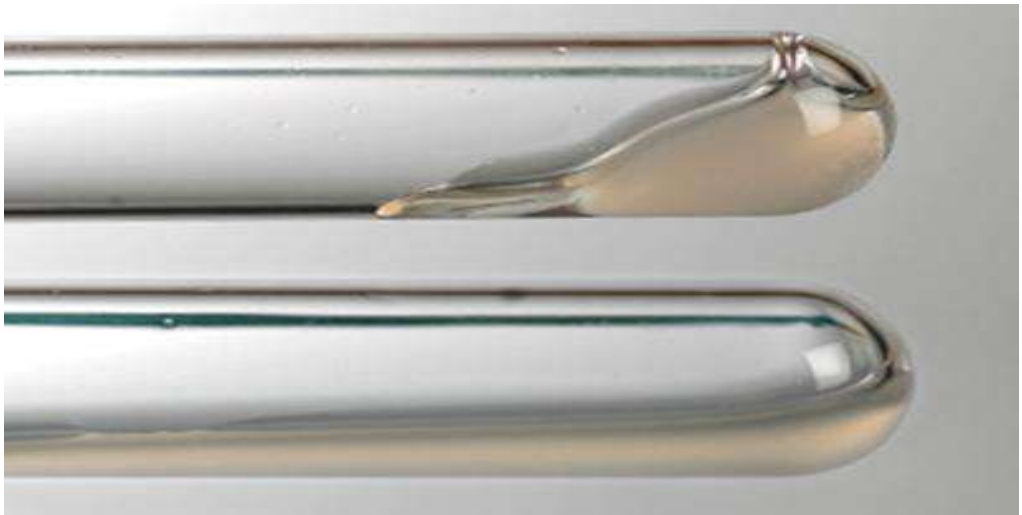
MSA also contains the sugar mannitol and the pH indicator phenol red. If an organism can ferment mannitol, an acidic byproduct is formed that will cause the phenol red in the agar to turn yellow. Staph aureus can ferment mannitol so its media will turn yellow while other Staph species will not ferment mannitol and it will remain red in color.

Coagulase test

The coagulase test is used to differentiate staph aureus (+) from other staph species (-). Coagulase is an enzyme produced by staph aureus to convert soluble fibrinogen in plasma to insoluble fibrin.

A suspension of the organism is suspended and incubated in plasma at 37C in a tube. Clot formation within four hours indicates a positive test, or the presence of staph aureus. Negative tubes should be left at room temperature overnight and re-examined the next day. This step is essential for some strains of staph aureus, including MRSA strains, as they produce a delayed clot which is rapidly lysed at 37C by staphylokinase

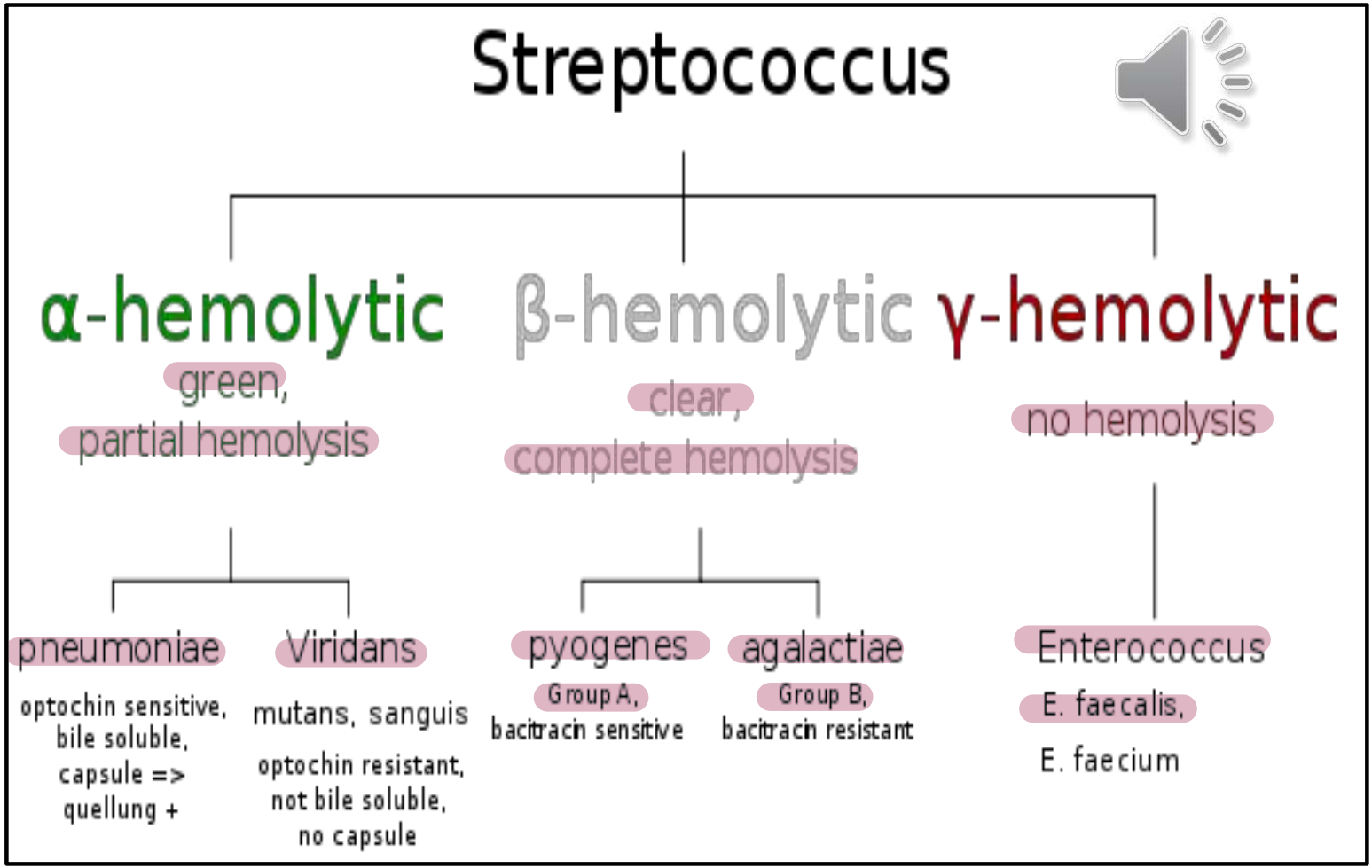




S.aureus

S.albus

Streptococci are gram positive aerobic organisms that cause many disorders including pharyngitis, pneumonia, skin infections, sepsis, and endocarditis. Three different types of strep are initially differentiated by their appearance when they are grown on sheep blood agar.



Hemolysis on sheep blood agar

Blood Agar:

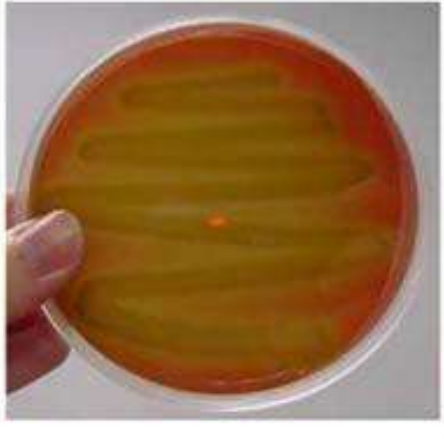
Shows three types of hemolysis

α Hemolysis

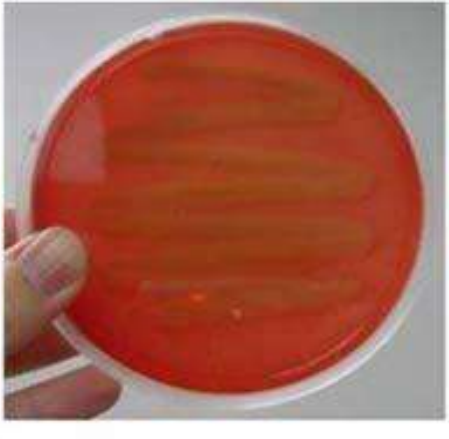
β Hemolysis

γ Hemolysis

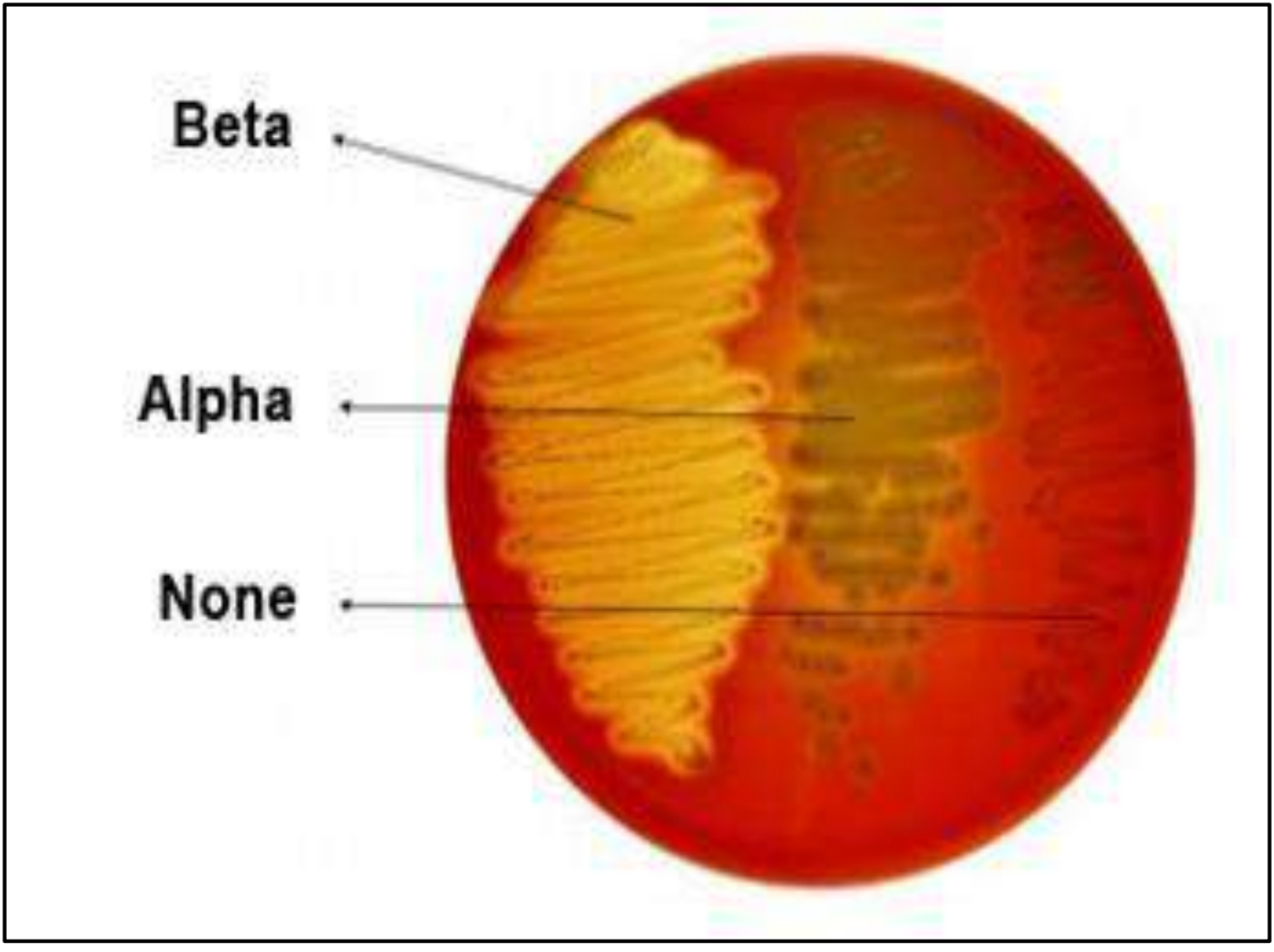
Beta Hemolysis



Alpha Hemolysis



Gamma Hemolysis


Beta
Alpha
None

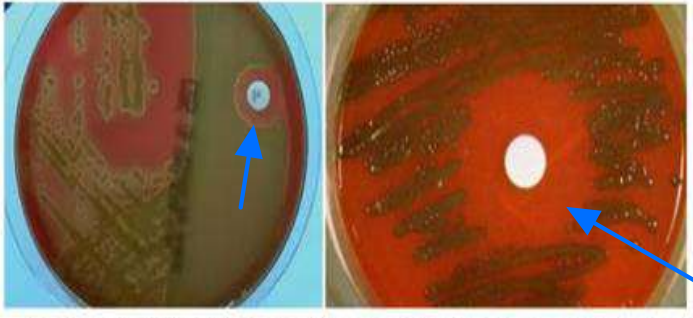
Differentiation between α-hemolytic streptococci

	Hemolysis	Optochin sensitivity
S. pneumoniae	α	Sensitive (≥ 14 mm)
Viridans strep	α	Resistant (≤13 mm)

Streptococcus viridians can be differentiated from S. pneumoniae using an optochin test.
Viridans streptococci are optochin resistant; they also lack either the polysaccharide-based capsule typical of S. pneumoniae or the Lancefield antigens of the pyogenic members of the genus.

Optochin test

Streptococcus pneumoniae



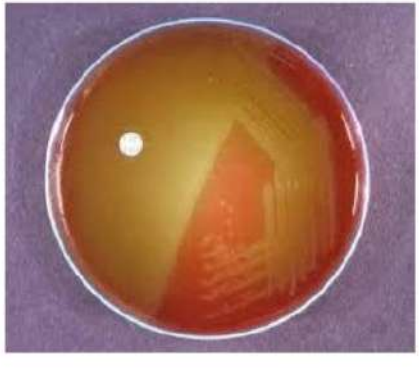
Streptococcus pneumoniae strain on blood agar showing alpha hemolysis (green zone surrounding colonies). Note the **zone of inhibition** around a filter paper disc impregnated with optochin. (**sensitive** to optochin)

The zone is >=14 mm

A zone of inhibition appears on a strep pneumoniae culture as it is sensitive to optochin.

Optochin test

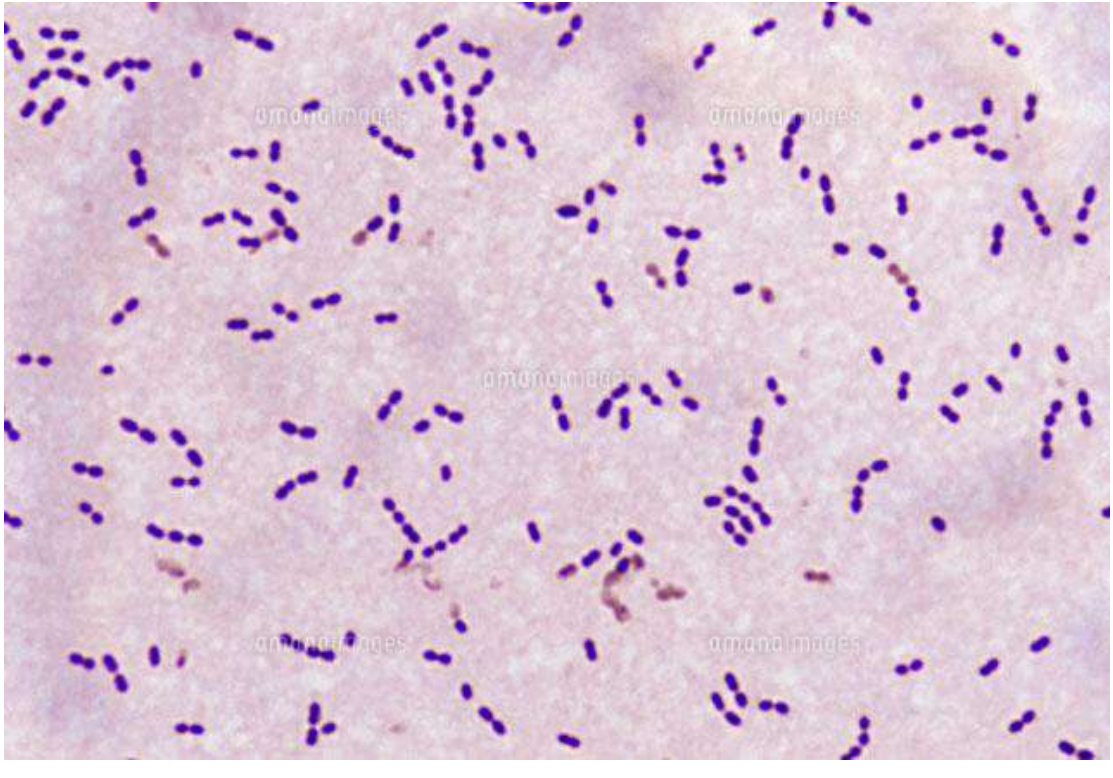
Streptococcus viridans



Streptococcus viridans strain on blood agar showing alpha hemolysis (green zone surrounding colonies). **No zone of growth inhibition (Resistant)** around a filter paper disc impregnated with optochin.

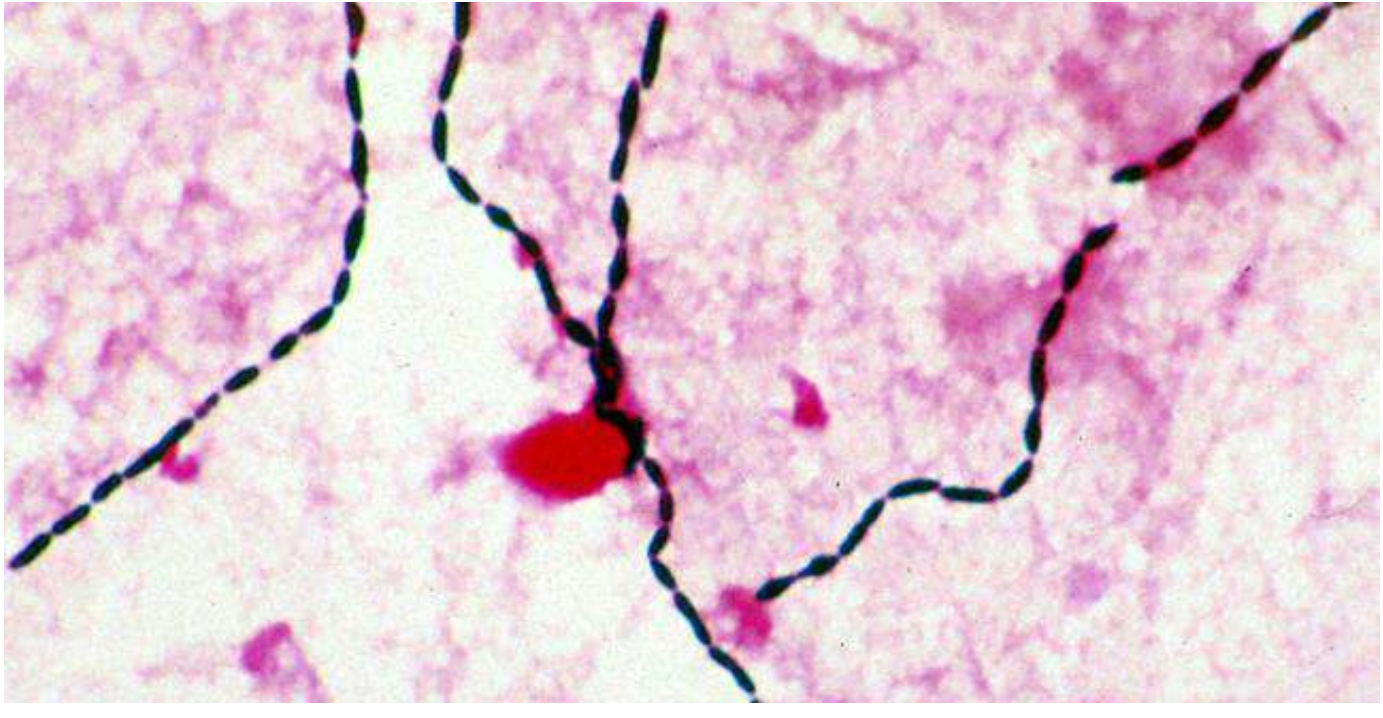
Streptococcus pneumoniae

Strep pneumoniae are gram positive, lancet shaped elongated cocci with a slightly pointed outer curvature. Usually they are seen in pairs (diploccoi) but they can be singular or in short chains.



Streptococcus viridans

Strep viridans are gram positive (often elongated) cocci that form short to long chains.



Differentiation between β-hemolytic streptococci

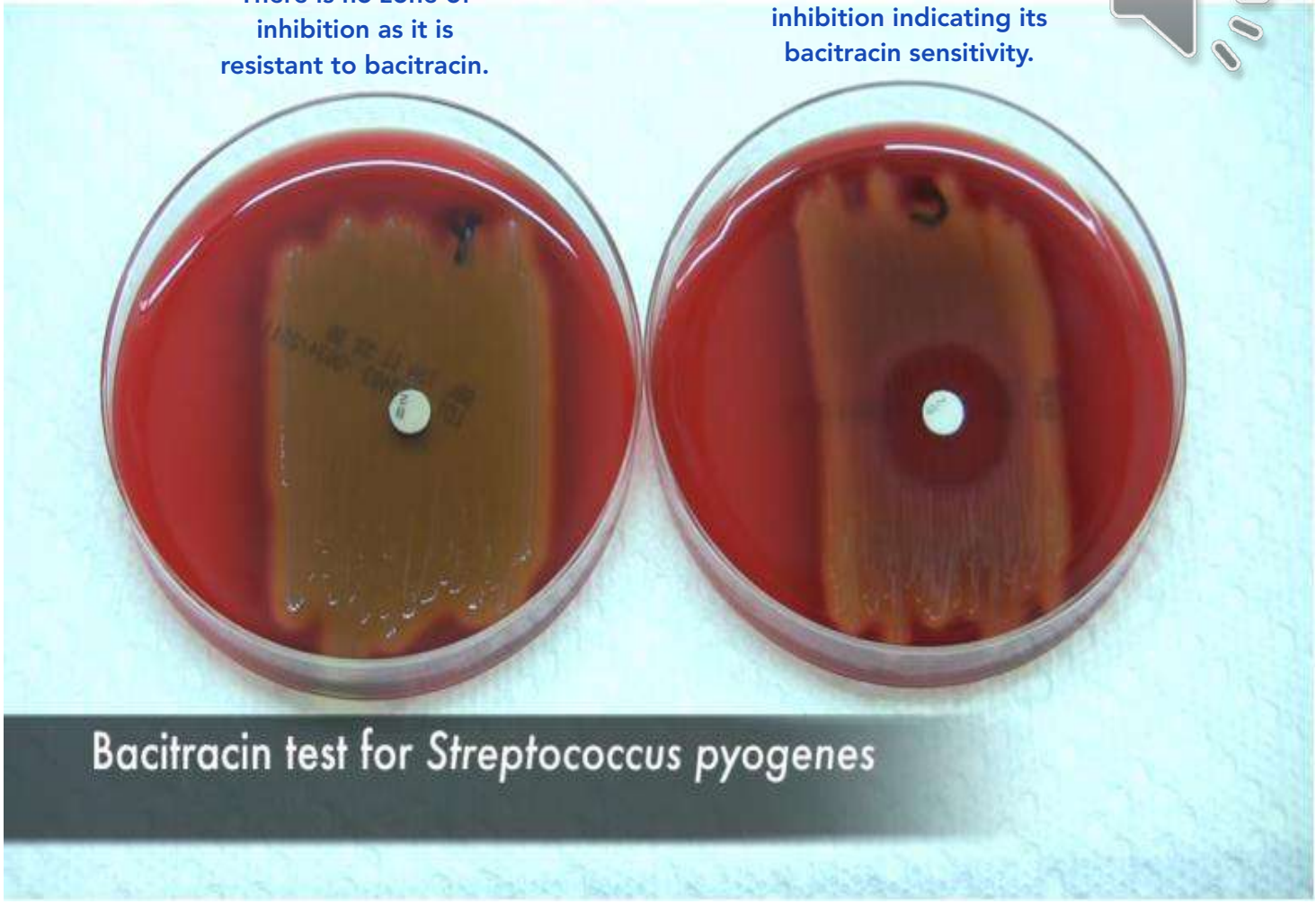
	Hemolysis	Bacitracin sensitivity
S. pyogenes	β	Susceptible
S. agalactiae	β	Resistant

The bacitracin sensitive test is used to distinguish group A S. pyogenes from other streptococci such as group B S. agalactiae.

When grown on blood agar, S. pyogenes is sensitive to bacitracin and will exhibit a zone of inhibition. While S agalactiae will not be affected and will not have a zone of inhibition.

Strep agalactiae
There is no zone of inhibition as it is resistant to bacitracin.

Strep pyogenes
We can see the zone inhibition indicating its bacitracin sensitivity.



Gamma hemolysis

(Non-hemolytic)

streptococcus

Enterococcus Group D

- E.feacalis

Other than Enterococcus group D

Bile-Esculin

- The bile-esculin test is used to differentiate between enterococcus group D and non-enterococcus species.
- Enterococcus group D species give a positive test.
- The bile-esculent test is based on the hydrolysis of esculin into glucose and esculetin by microorganisms that can produce esculinase.
- Esculetin then can interact with an iron salt, ferric citrate, in the medium to form a phenolic iron complex, which produces a dark brown or black color.

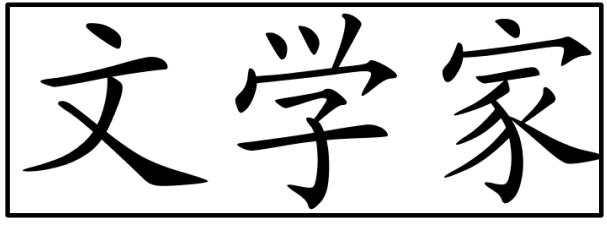
Diphtheroids

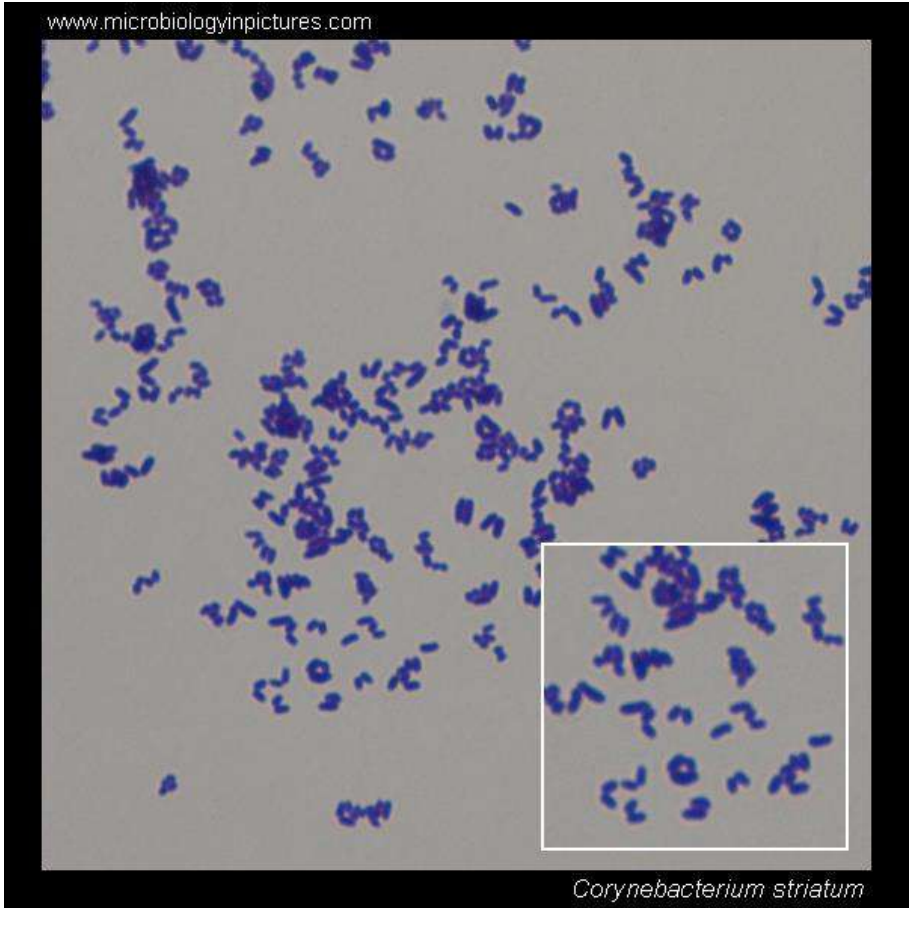
Diptheroids are aerobic non-sporulating pleomorphic gram postive bacilli which are more uniformly stained than Corynebacterium diptheria.

Gram Positive Cocco-bacilli

They lack metachromatic granules and are arranged as in what is known as the 'Chinese letter' appearance. They are usually commensals in the skin and mucous membranes.

Arrangement as Chinese letter





Candida Species

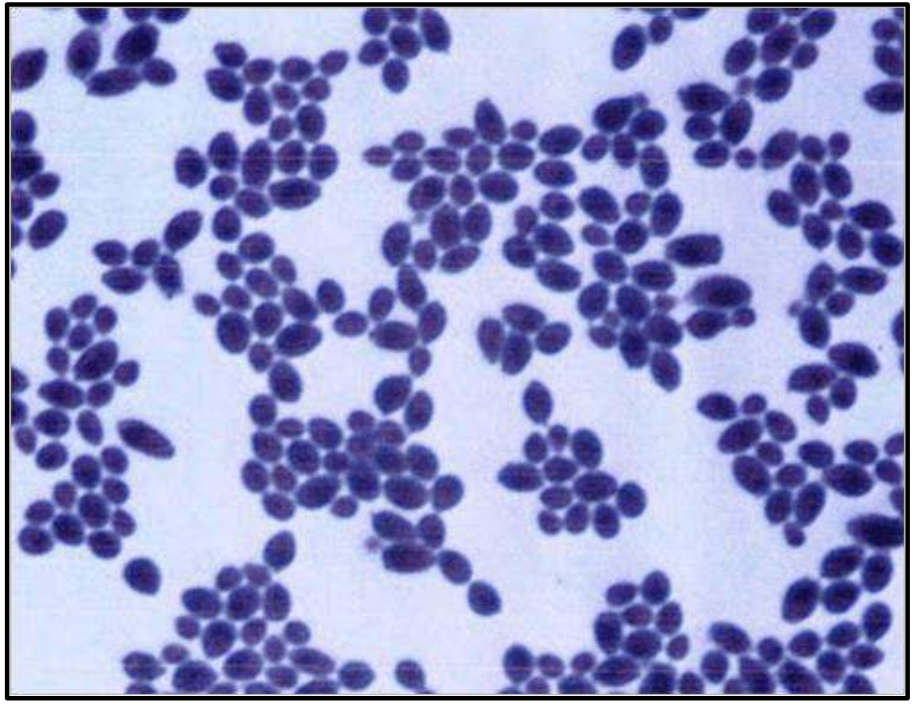
Candidiasis is a fungal infection caused by a yeast known as Candida. Some species of Candida can cause infections in humans, most commonly C. albicans.

Candida normally lives on the skin and inside the body in places such as the mouth, throat, gut, and vagina, without causing any problems. In some circumstances, such as when taking a long course of Abx or a weakened immune system, the risk of Candida infection increases.

At least 30 Candida species have been implicated as causes of human infection. The most common species are those shown in the figure.

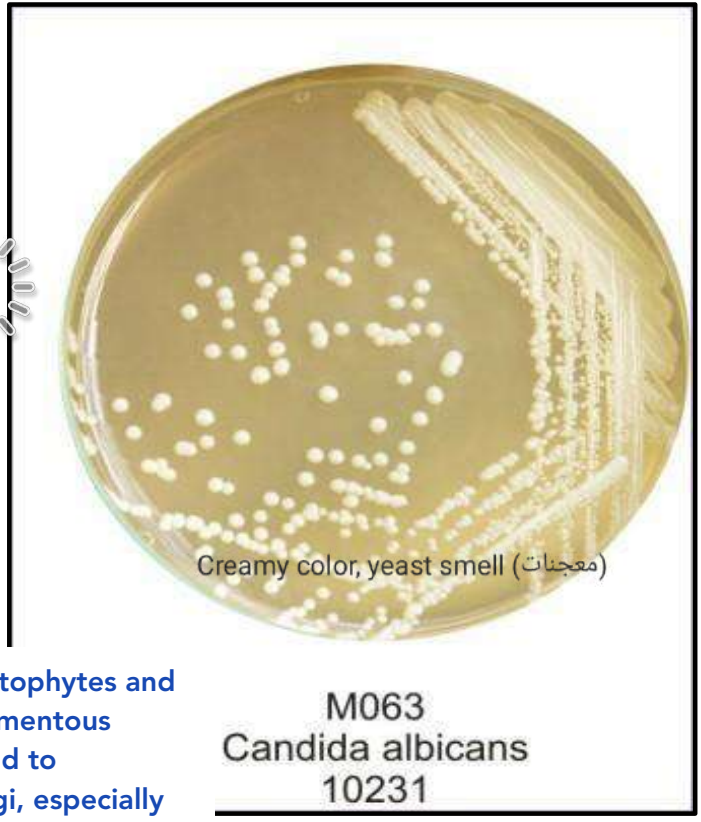
Candida Spp

- Larger than Bacteria
- Budding



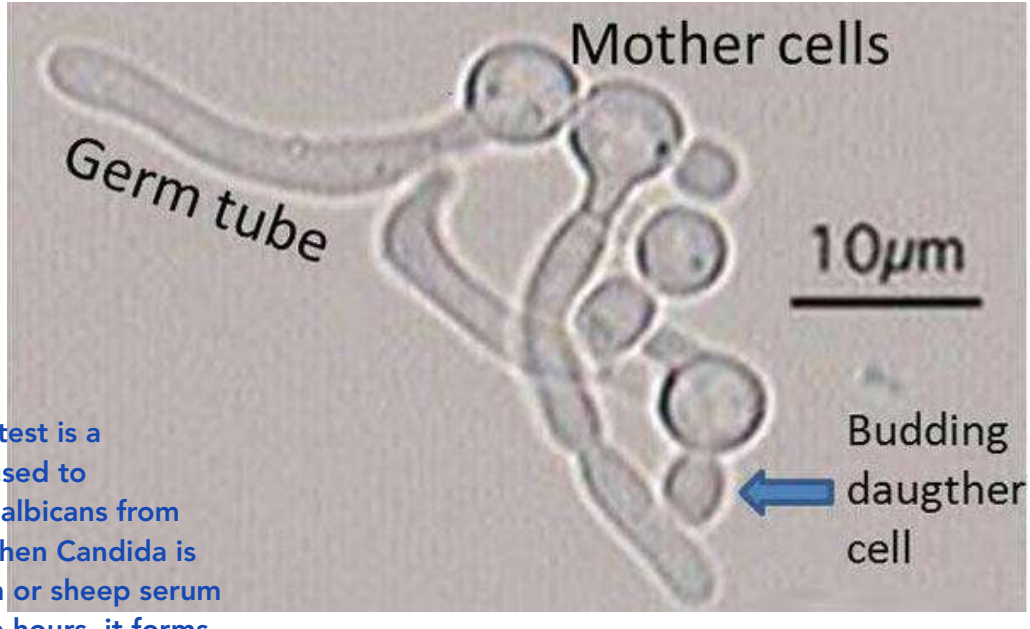
- Candida are unicellular fungi.
- They may be spherical, elliptical or cylindrical shaped.
- Their size varies greatly but they are generally larger than bacteria.
- They typically grow asexually by budding.

sabouraud dextrose agar



This agar contains peptones. It is used to cultivate dermatophytes and other fungi (such as Candida) at 20C. It can also grow filamentous bacteria such as Nocardia. The pH of the media is adjusted to approximately 5.6 in order to enhance the growth of fungi, especially dermatophytes, and to slightly inhibit bacterial growth. Yeast will grow as creamy white colonies while molds will grow as filamentous colonies

To Differentiate between C.albican and other Species



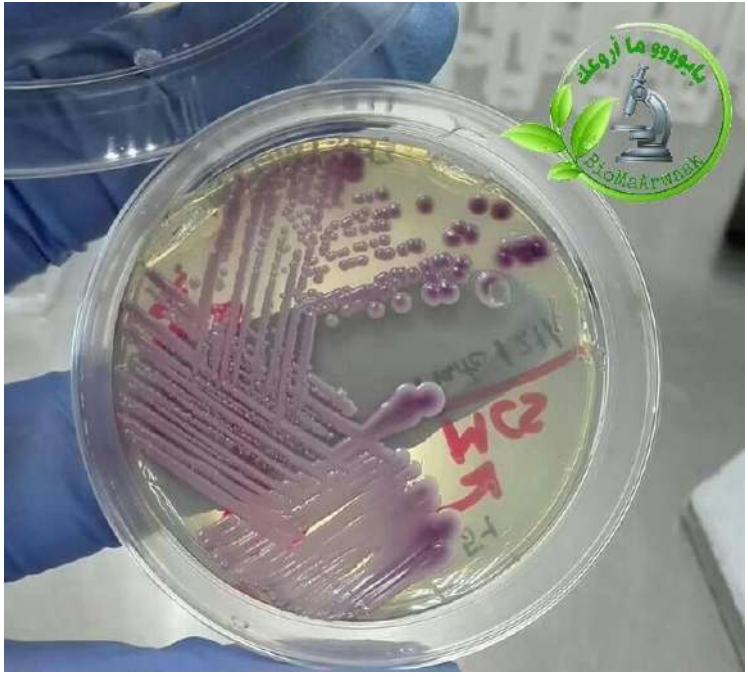
The germ tube test is a screening test used to differentiate C. albicans from other yeasts. When Candida is grown in human or sheep serum at 37C for three hours, it forms germ tubes. These germ tubes can be detected with wet KOH films as filamentous outgrowth extending from yeast cells. In this case, the sample is positive for C. Albicans.

Germ tube

[Serum + candida]

Chrom agar is a novel differential culture medium that facilitates the isolation and identification of some clinically important yeast species.

Chrom agar



C.glabrata : violet (dark pink) glistering

C.albicans : Green

Chrom agar

C. Krusei isolates forms highly characteristic rough, spreading colonies with pale pink centers and white edges.

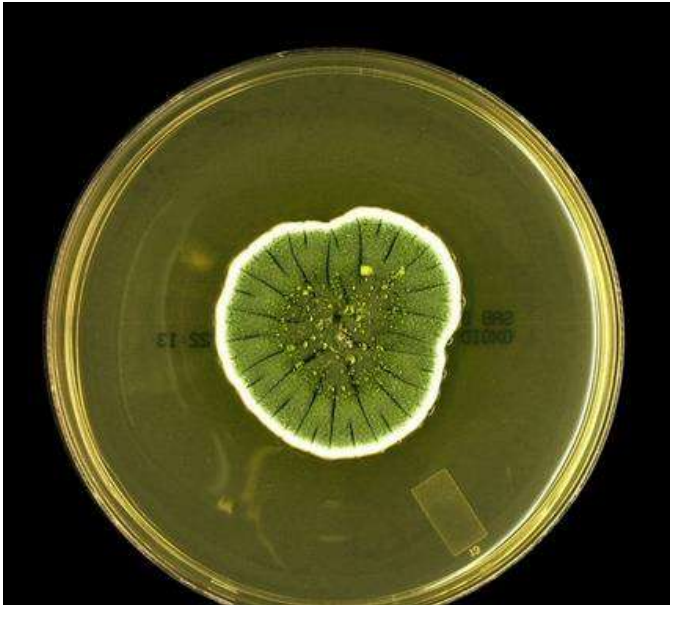
C.Krusei : rough dry pale pink

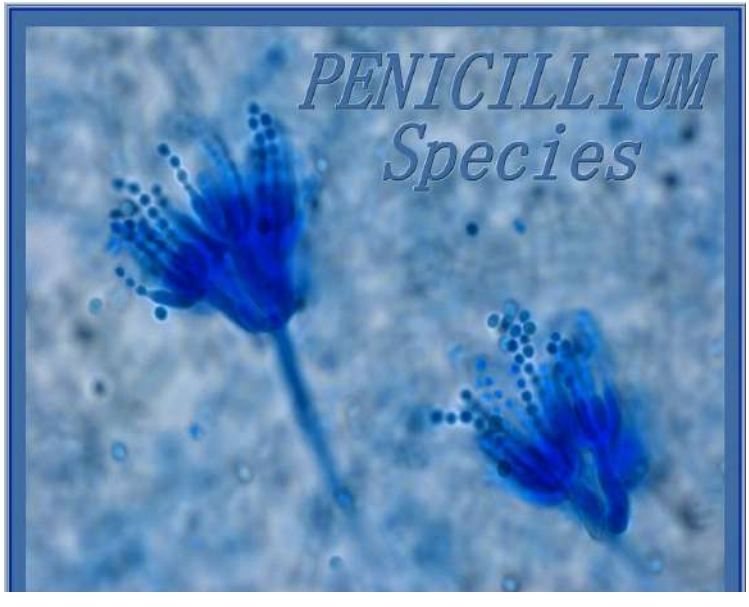
C.Tropicalis Blue/gray

Aspergillus Niger

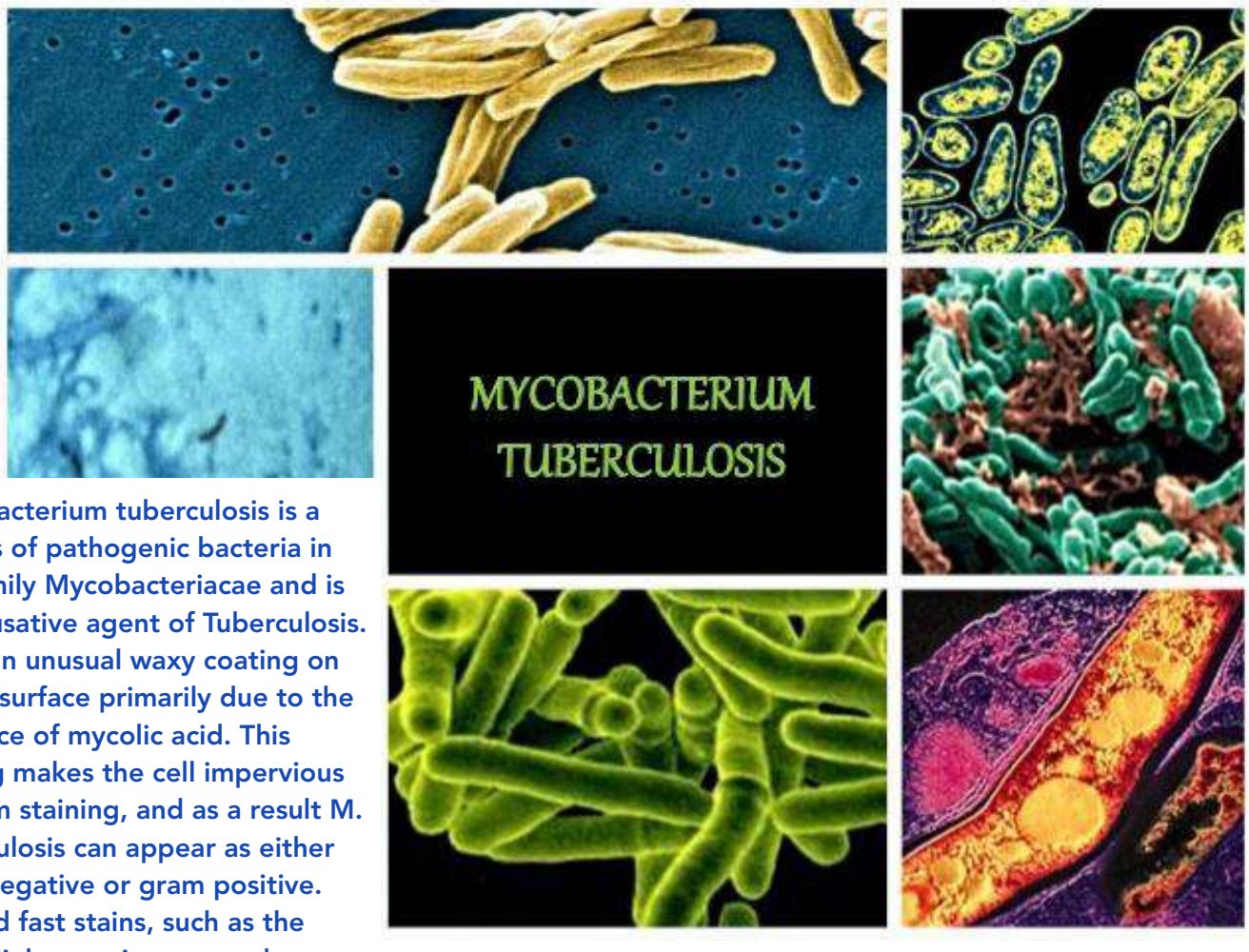
Aspergillus niger is a fungus and one of the most common species of Aspergillus. It causes a disease called black mold on certain fruits and vegetables such as grapes, apricots, onions, and peanuts. It is a common contaminant of food. Aspergillus niger is one of the most common causes of otomycosis (fungal ear infection) which can cause pain or temporary hearing loss. In severe cases, it may damage the ear canal and tympanic membrane.

Penicillium Spp.





Penicillium is a genus of ascomycetous fungi that is of major importance in the natural environment, in food spoilage, and in food and drug production. Some members of the genus produce penicillin, a molecule that is used as an antibiotic, which kills or stops the growth of certain kinds of bacteria. Penicillium species are occasional causes of infection in humans and the resultant disease is known generally as penicilliosis. Penicilliums have been isolated from patients with keratitis, endophtalmitis, otomycosis, necrotizing esophagitis, pneumonia, endocarditis, peritonitis and urinary tract infections.

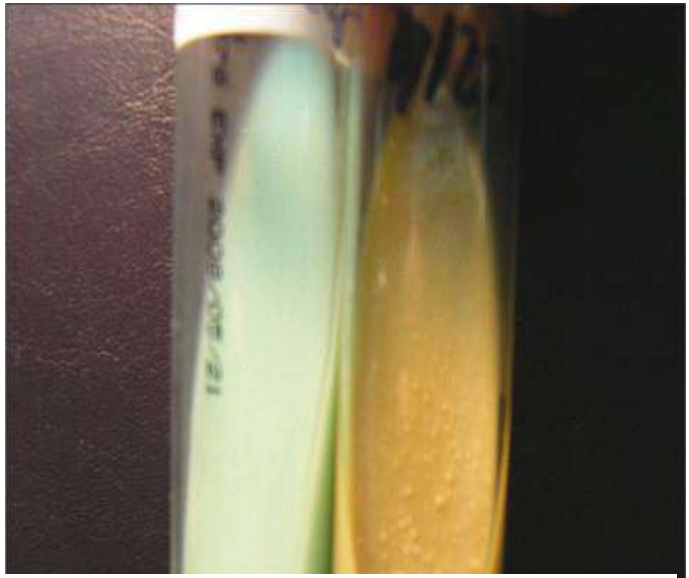


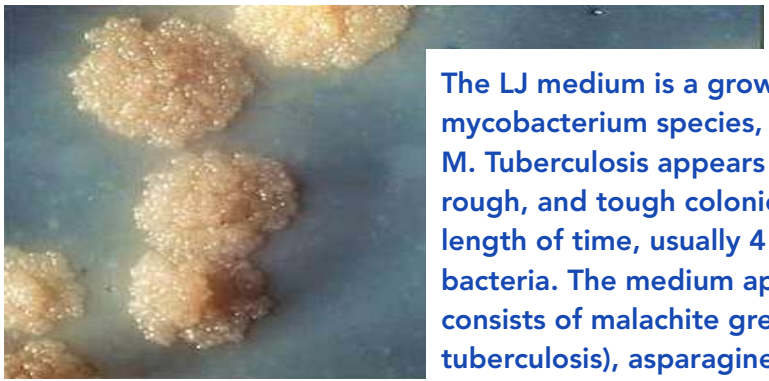
Mycobacterium tuberculosis is a species of pathogenic bacteria in the family Mycobacteriacae and is the causative agent of Tuberculosis. It has an unusual waxy coating on its cell surface primarily due to the presence of mycolic acid. This coating makes the cell impervious to gram staining, and as a result M. Tuberculosis can appear as either gram negative or gram positive. So, acid fast stains, such as the Ziehl-Nielsen stain, are used to identify it with a microscope.

Lowenstein –Jensen Medium

- Contain malachite green and egg albumin
- Media color : green
- Cell show :

Rough Tough Buff





The LJ medium is a growth medium specially used for the culture of mycobacterium species, especially M. Tuberculosis. When grown on LJ medium, M. Tuberculosis appears as brown granular colonies sometimes called buffs rough, and tough colonies. The medium must be incubated for a significant length of time, usually 4 weeks, due to the slow proliferation time of this bacteria. The medium appears green, opaque and opalescent. The medium consists of malachite green, glycerol (which enhances the growth of M. tuberculosis), asparagine, potato starch, coagulated eggs, mineral salt solution, potassium dihydrogen phosphate, and magnesium sulfate.

Incubation Period = 4 weeks

Glass

Put the media in covered tubes to avoid drying of media

It also helps to avoid contamination.

Because it needs a lot of time to grow so the chance of contamination is higher

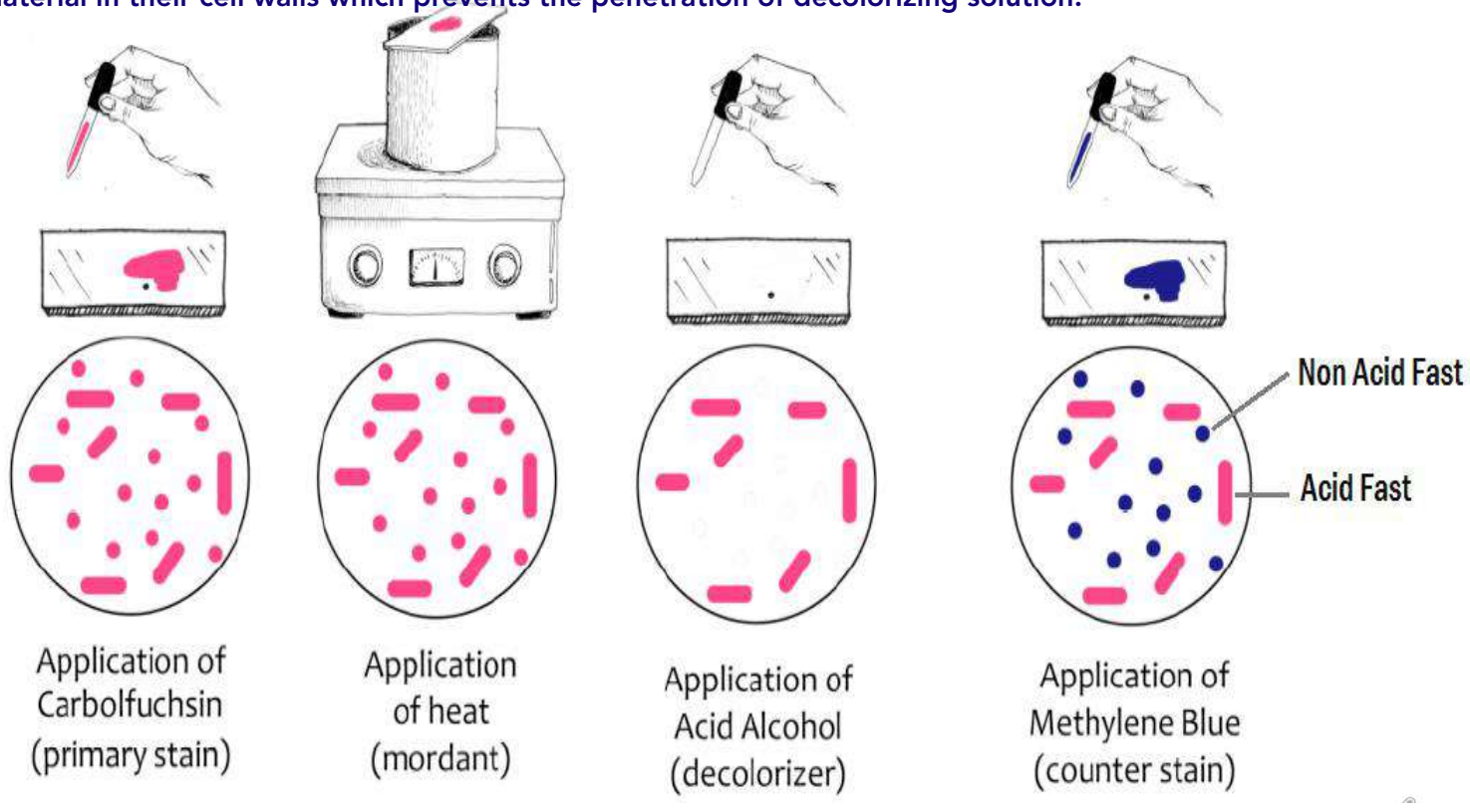
ziehl neelsen
Acid fast stain

- Mycobacterium Tuberculosis cell wall are waxed for that reason do heating while staining .

- Stain made of :

- **Carbol fuchsin** Primary stain
- **Hydrochloric acid alcohol (3% HCL)**
  Works as a de-colorizer
- **Methylen blue** Counter stain

When the smear is stained with carbol fuschin, it solubilizes the lipoid always material present in the mycobacterial cell wall. With the application of heat, carbon fuschin further penetrates through the lipoidal wall and enters into the cytoplasm. All cells now appear red. The smear is then decolorized with a decolorizing agent (3% HCl in 95% alcohol) but the acid fast cells are resistant due to the presence of large amounts of lipoidal material in their cell walls which prevents the penetration of decolorizing solution.



Non-acid fast organisms lack the lipoidal material in their cell wall and are therefore easily decolorized, leaving colorless cells. Then, the smear is stained with a counter stain, methylene blue. Only decolorized cells absorb the counter stain and take its color and appear blue while acid-fast retain the red color.

Here, the M, tuberculosis cells appear red/pink in color while other cells in the background appear blue.

T.B
Acid fast stain