

In the name of God

Gram-positive bacteria

Staphylococci

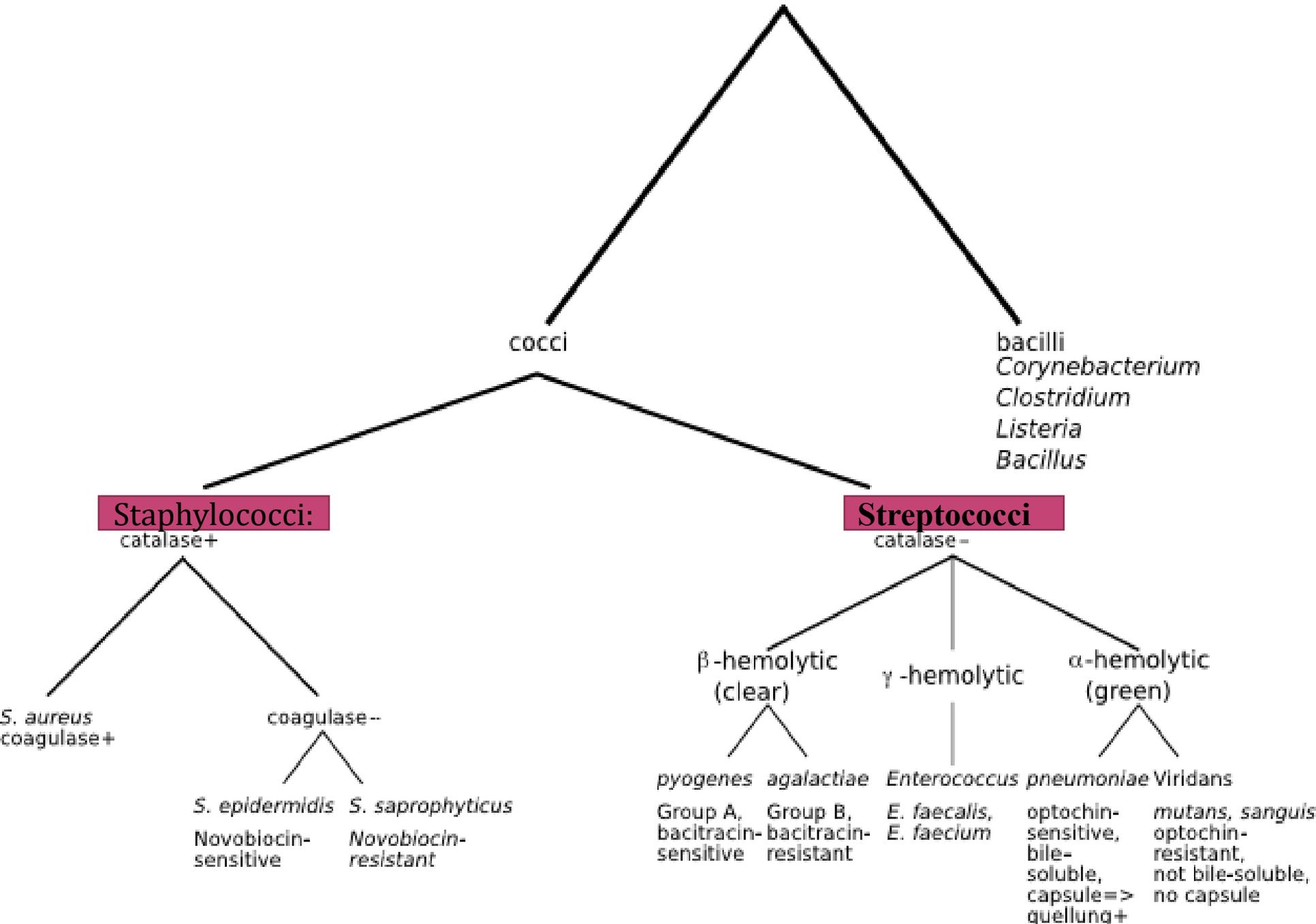
Assist.prof.Dr. Rasha jasim ALwarid

Staphylococci



Gram-positive bacteria are bacteria that give a positive result in the Gram stain test. Gram-positive bacteria take up the crystal violet stain used in the test, and then appear to be purple-coloured when seen through a microscope. This is because the thick peptidoglycan layer in the bacterial cell wall retains the stain after it is washed away from the rest of the sample, in the decolorization stage of the test.

Gram-Positive



cocci

bacilli
Corynebacterium
Clostridium
Listeria
Bacillus

Staphylococci:

Streptococci

catalase+

catalase-

S. aureus
coagulase+

coagulase-

S. epidermidis
Novobiocin-sensitive

S. saprophyticus
Novobiocin-resistant

β-hemolytic
(clear)

γ-hemolytic

α-hemolytic
(green)

pyogenes
Group A,
bacitracin-sensitive

agalactiae
Group B,
bacitracin-resistant

Enterococcus
E. faecalis,
E. faecium

pneumoniae
optochin-sensitive,
bile-soluble,
capsule=>quellung+

Viridans
mutans, sanguis
optochin-resistant,
not bile-soluble,
no capsule

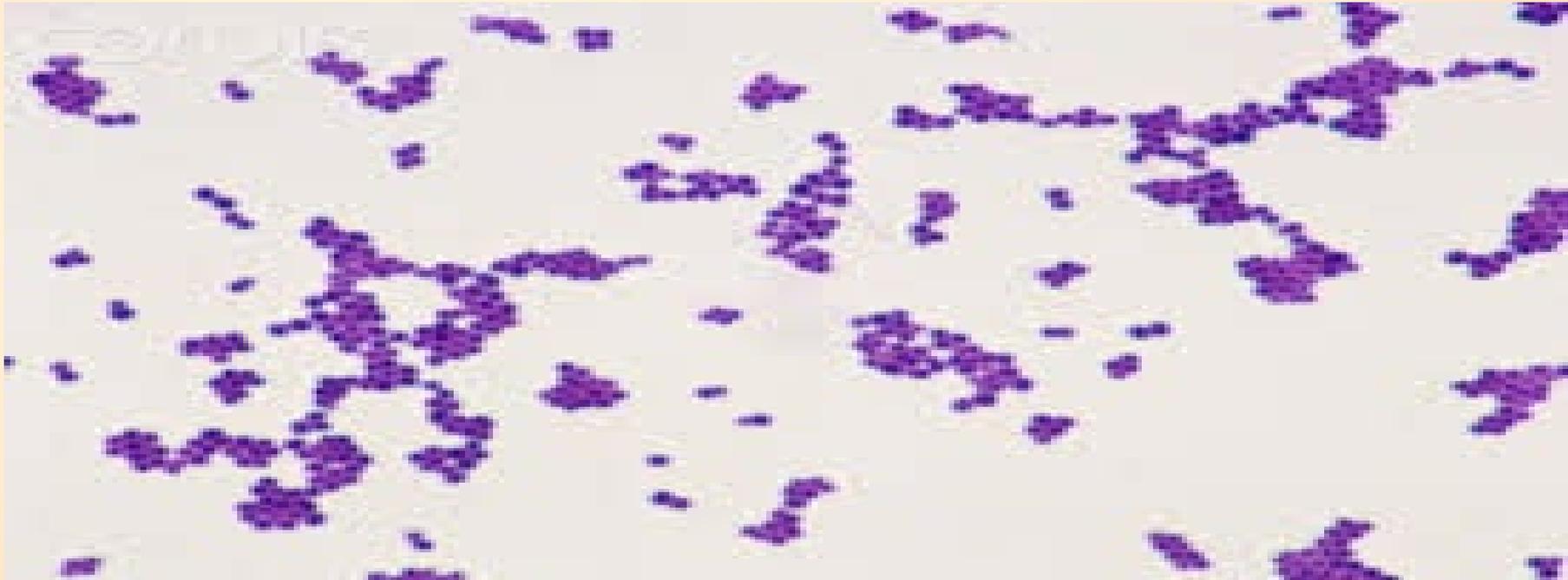
- Staphylococci and streptococci constitute the main groups of medically important gram-positive cocci.
- The most virulent of the genus, *Staphylococcus aureus*, is one of the most common causes of
- Bacterial infections,
- Food poisoning
- Toxic shock syndrome.

- Staphylococcus epidermidis is an important cause of prosthetic implant infections.
- Staphylococcus saprophyticus causes urinary tract infections, especially cystitis in women

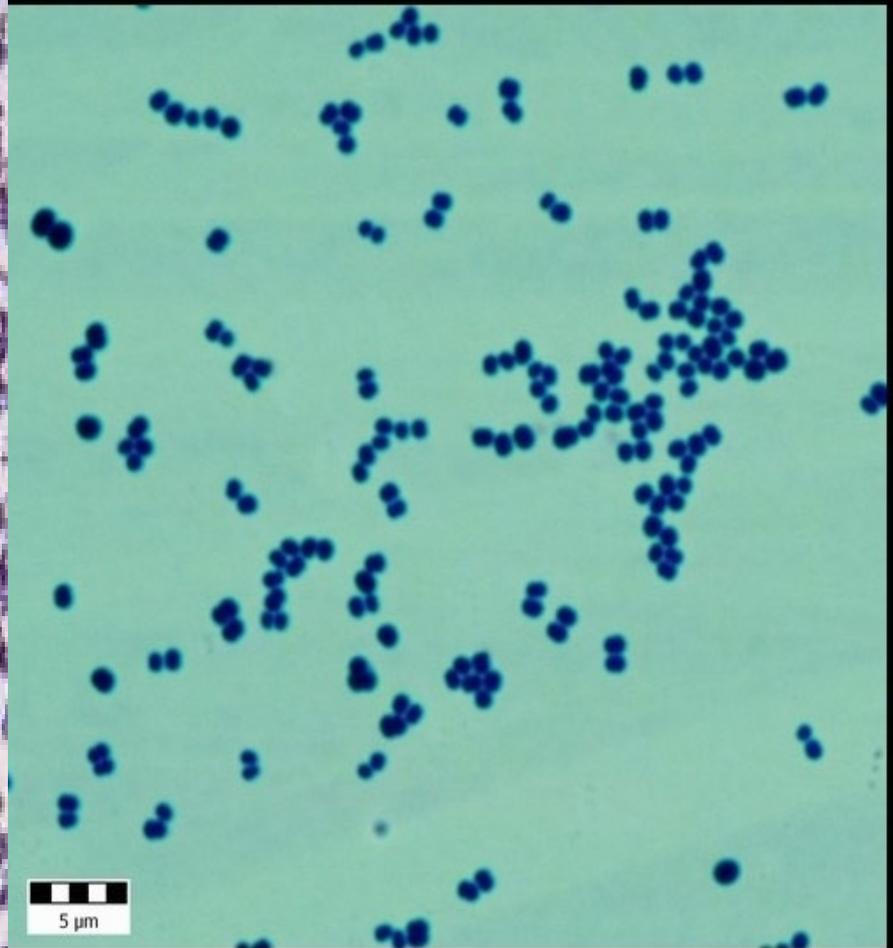
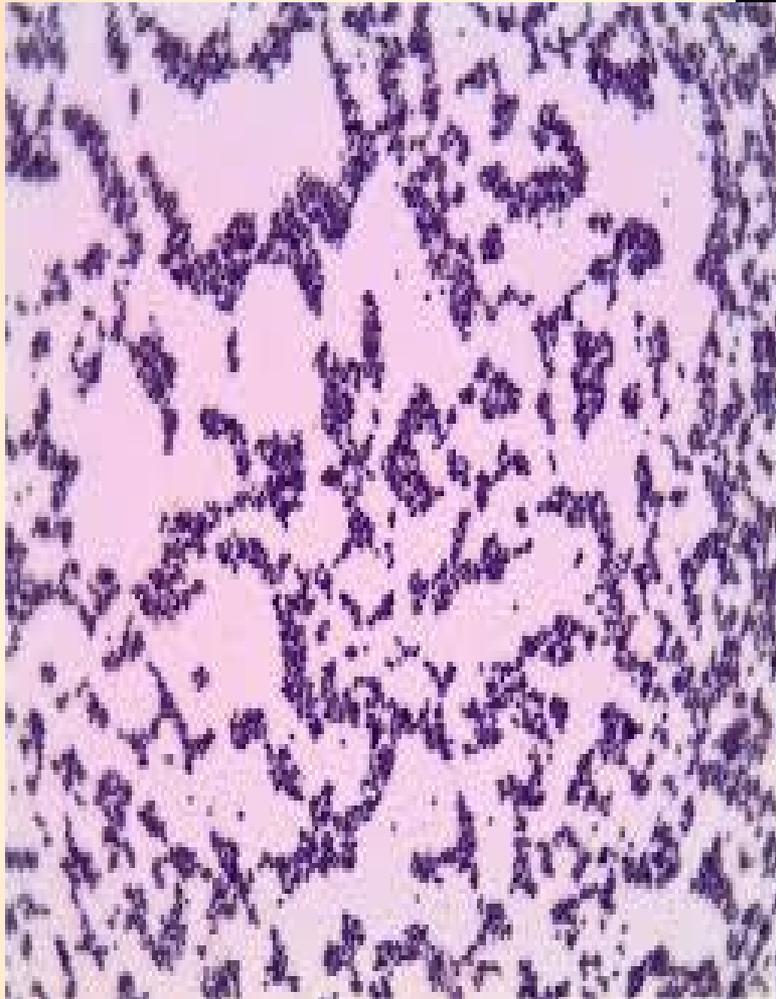
GENERAL FEATURES

- *Non motile**
- *Non-spore-forming**
- *None capsulated**
- *Catalase-producing**
- *Oxidase: negative**
- *Glucose fermenters**
- *Primarily aerobic, some facultative anaerobic**

- Staphylococci generally stain darkly gram positive. They are round rather than oval and tend to occur in bunches like grapes.



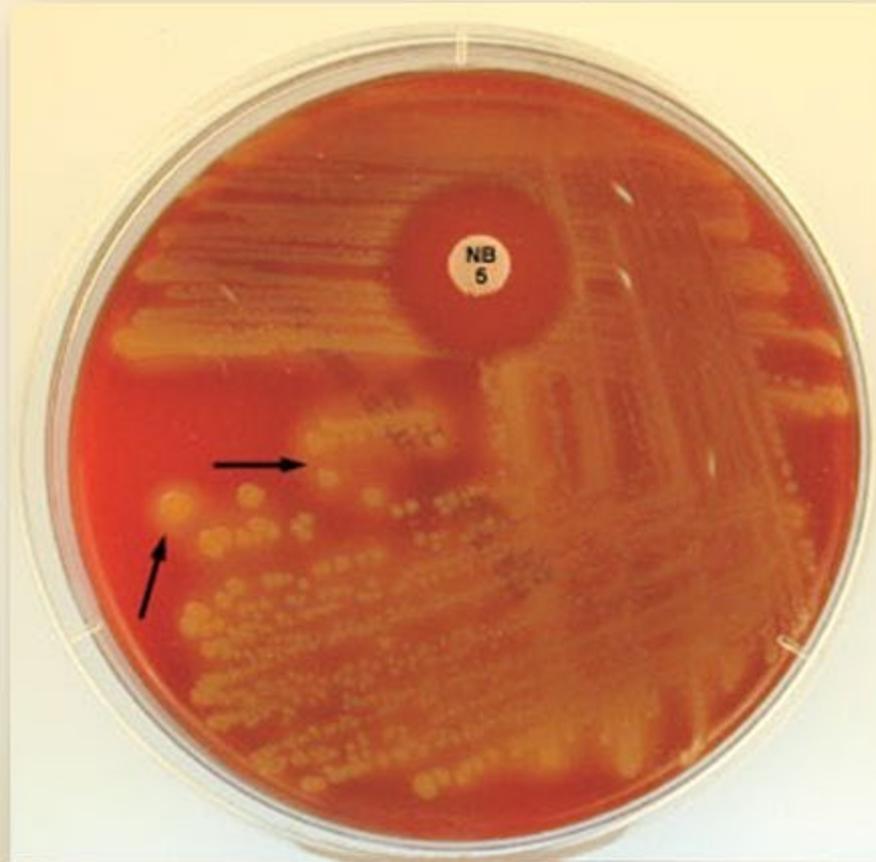
- Growth of staphylococci requires supplementation with various amino acids and other growth factors, they are routinely cultured on enriched media containing nutrient broth and/or blood
- Staphylococci are hardy, being resistant to heat and drying, and thus can persist for long periods on fomites, which can then serve as sources of infection



Staphylococcus epidermidis

Staphylococcus aureus Growing on Blood Agar

Blood Agar



Note beta hemolysis (complete lysis of the red blood cells around the colonies; see arrows) on the blood agar and the organism is sensitive to the antibiotic novobiocin.

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Hem-N.

Staphylococcus aureus

Cultural Characteristics:

- i) **On nutrient agar-** The colonies are large, circular, convex, smooth, shiny, opaque and easily emulsifiable. Most strains produce **golden yellow** pigments.



Catalase test

Catalase is an enzyme, which is produced by microorganisms that live in oxygenated environments to neutralize toxic forms of oxygen metabolites; H_2O_2 .

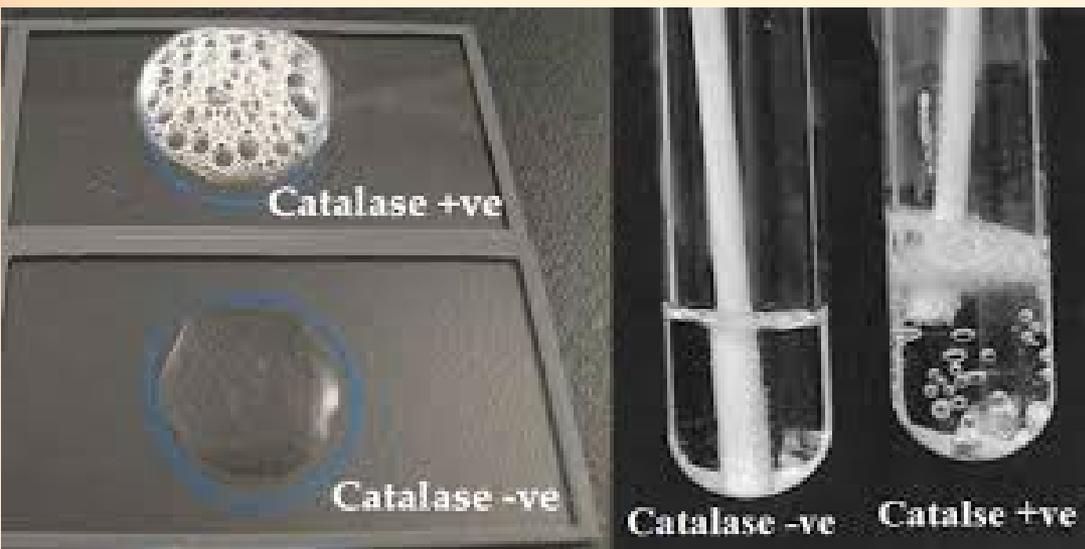
Catalase mediates the breakdown of hydrogen peroxide H_2O_2 into oxygen and water.

Procedure of catalase test (Slide Test)

1. Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick
2. Place a drop of 3% H₂O₂ on to the slide and mix.
3. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling.
4. A negative result is no bubbles or only a few scattered bubbles.
5. Dispose of your slide in the biohazard glass disposal container.

Tube Catalase Test-Procedure and Results

1. Add 4 to 5 drops of 3% H₂O₂ (Hydrogen peroxide) to in a test tube
2. Using a wooden applicator stick, collect a small amount of organism from a well-isolated 18- to 24-hour colony and place into the test tube
3. Place the tube against a dark background and observe for immediate bubble formation (O₂ + water = bubbles) at the end of the wooden applicator stick.



Coagulase test

Coagulase is a protein enzyme produced by several microorganisms that enables the conversion of fibrinogen to fibrin. In the laboratory, it is used to distinguish between different types of Staphylococcus isolates.

Coagulase test

The coagulase test has traditionally been used to differentiate *Staphylococcus aureus* from coagulase-negative staphylococci. *S.aureus* produces two forms of coagulase (i.e., bound coagulase and free coagulase). Bound coagulase, otherwise known as "clumping factor", can be detected by carrying out a slide coagulase test, and free coagulase can be detected using a tube coagulase test.

Slide test

A slide coagulase test is run with a negative control to rule out autoagglutination. Two drops of saline are put onto the slide labeled with sample number, Test (T) and control (C). The two saline drops are emulsified with the test organism using a wire loop, straight wire, or wooden stick. A drop of plasma (rabbit plasma anticoagulated with EDTA is recommended)[5] is placed on the inoculated saline drop corresponding to test, and mixed well, then the slide is rocked gently for about 10 seconds.

If 'positive', macroscopic clumping would be observed in the plasma within 10 seconds, with no clumping in the saline drop.

If 'negative', no clumping will be observed.

Tube test

A fibrin clot formed in a test tube by the coagulase reaction
The tube test uses rabbit plasma that has been inoculated with a staphylococcal colony (i.e., Gram-positive cocci which are catalase positive). The tube is then incubated at 37 °C for 1.5 hours. If negative, then incubation is continued up to 18 hours.

If 'positive' (e.g., the suspect colony is *S. aureus*), the plasma will coagulate,[6] resulting in a clot (sometimes the clot is so pronounced, the liquid will completely solidify).

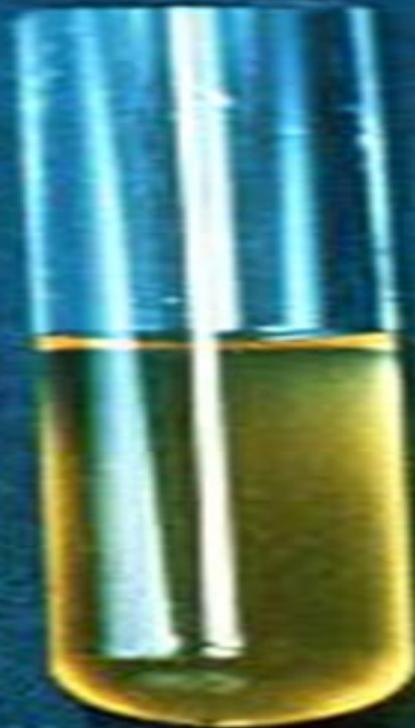
If 'negative', the plasma remains a liquid.



COAGULASE TEST



POSITIVE



NEGATIVE

Mannitol salt agar or MSA is a commonly used selective and differential growth medium in microbiology.

- It encourages the growth of a group of certain bacteria while inhibiting the growth of others.
- This medium is important in medical laboratories by distinguishing pathogenic microbes in a short period of time.
- It contains a high concentration (about 7.5%-10%) of salt (NaCl), making it selective for Gram-positive bacteria (*Staphylococcus*)
- [It is also a differential medium for mannitol-fermenting staphylococci, containing carbohydrate mannitol and the indicator phenol red, a pH indicator for detecting acid produced by mannitol-fermenting staphylococci.
- *Staphylococcus aureus* produces yellow colonies with yellow zones, whereas other coagulase-negative staphylococci produce small pink or red colonies with no colour change to the medium

Staphylococcus epidermidis



Staphylococcus aureus

Mannitol Salt Agar

Mannitol Salt agar



Staphylococcus aureus



Staphylococcus epidermidis



Micrococcus luteus

MSA (Mannitol Salt Agar)

Treatment

Serious *S. aureus* infections require

aggressive treatment,

- including incision
- drainage of localized lesions, as well as systemic antibiotics.
- Antibiotics commonly prescribed to treat staph infections include certain **cephalosporins, nafcillin** or related antibiotics, **sulfa drugs or vancomycin**.

- **Prevention**

There is no effective vaccine against *S. aureus*. Infection control procedures, such as barrier precautions and disinfection of hands and fomites, are important in the control of nosocomial *S. aureus* epidemics

COAGULASE-NEGATIVE STAPHYLOCOCCI

- **A. Staphylococcus epidermidis**
- is present in large numbers as part of the normal flora of the skin .
- Despite its low virulence, it is a common cause of infection of implants such as heart valves and catheters.
- Acquired drug resistance by *S. epidermidis* is even more frequent than by *S. aureus*.
- Vancomycin sensitivity remains the rule,
- *S. epidermidis* produces an extracellular polysaccharide material called polysaccharide intercellular adhesin (sometimes called “slime”), that facilitates adherence to bioprosthetic material surfaces, such as intravenous catheters, and acts as a barrier to antimicrobial agents.

Staphylococcus aureus

Staphylococcus epidermidis

Tryptic soy agar



B. Staphylococcus saprophyticus

This organism is a frequent cause of cystitis in women,

It tends to be sensitive to most antibiotics, even penicillin G.

S. saprophyticus can be distinguished from *S. epidermidis* and most other coagulase-negative staphylococci by its natural resistance to novobiocin



Staphylococcus saprophyticus Growing on Blood Agar



Note there is no hemolysis (gamma reaction) on the blood agar and the organism is resistant to the antibiotic novobiocin.

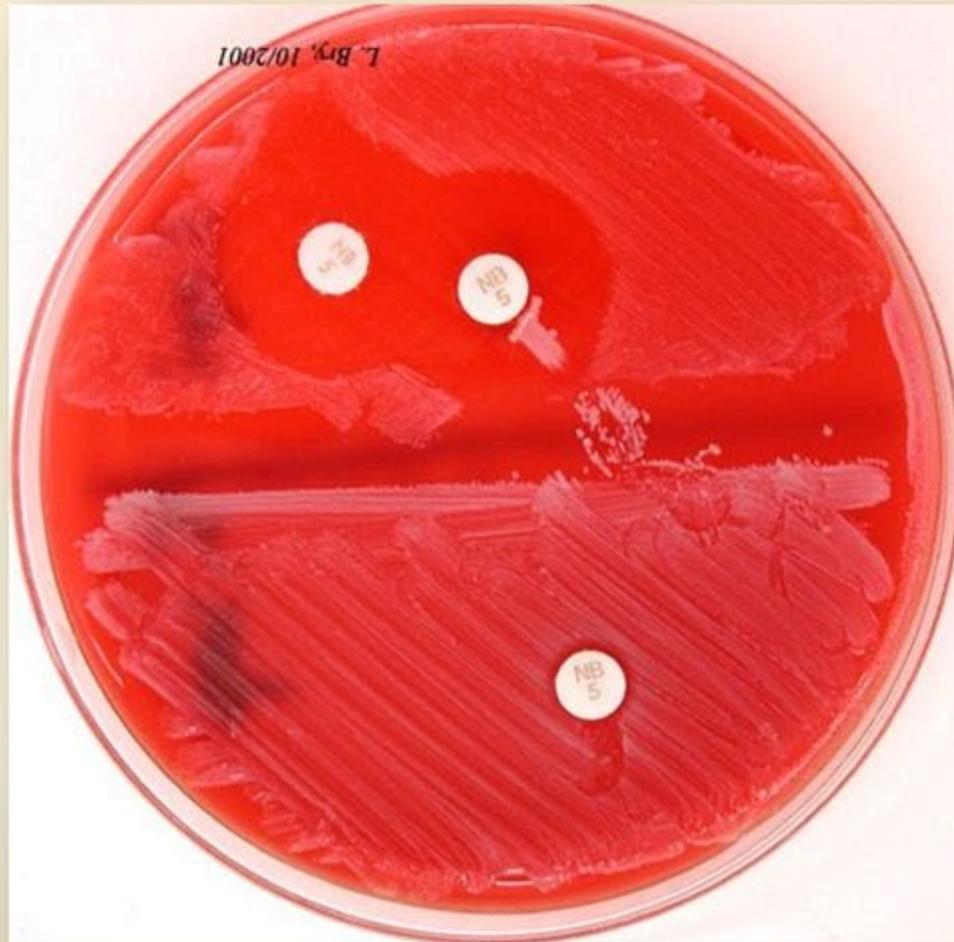
- ***S. Saprophyticus***

- white-yellow colony

- no haemolysis of red blood cells

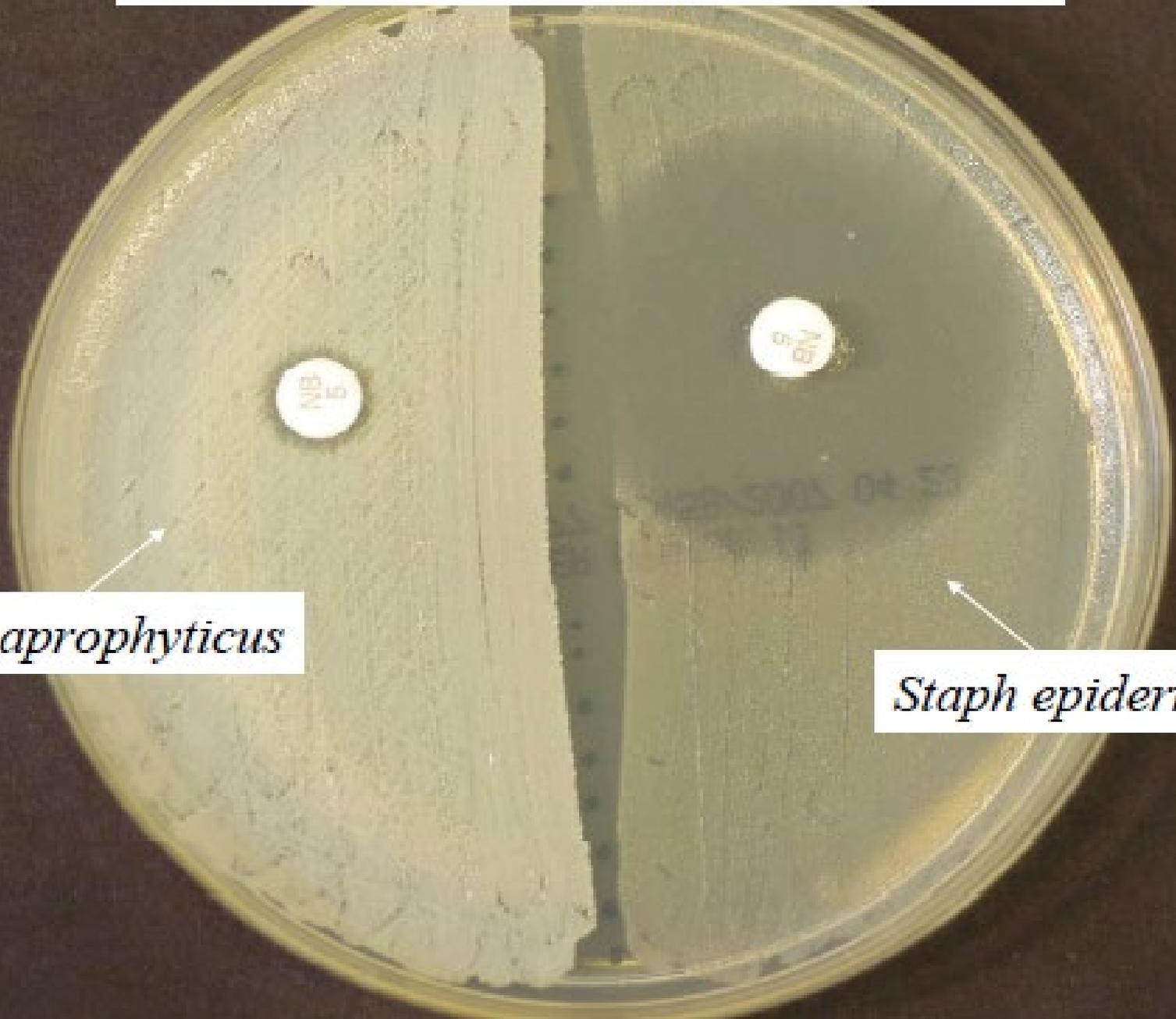


Novobiocin test



A novobiocin disk will be placed on the plate, Novobiocin is an antibiotic that many *Staphylococcus* strains are sensitive to with the exception of one, *Staph. saprophyticus* that is resistant to novobiocin antibiotic.

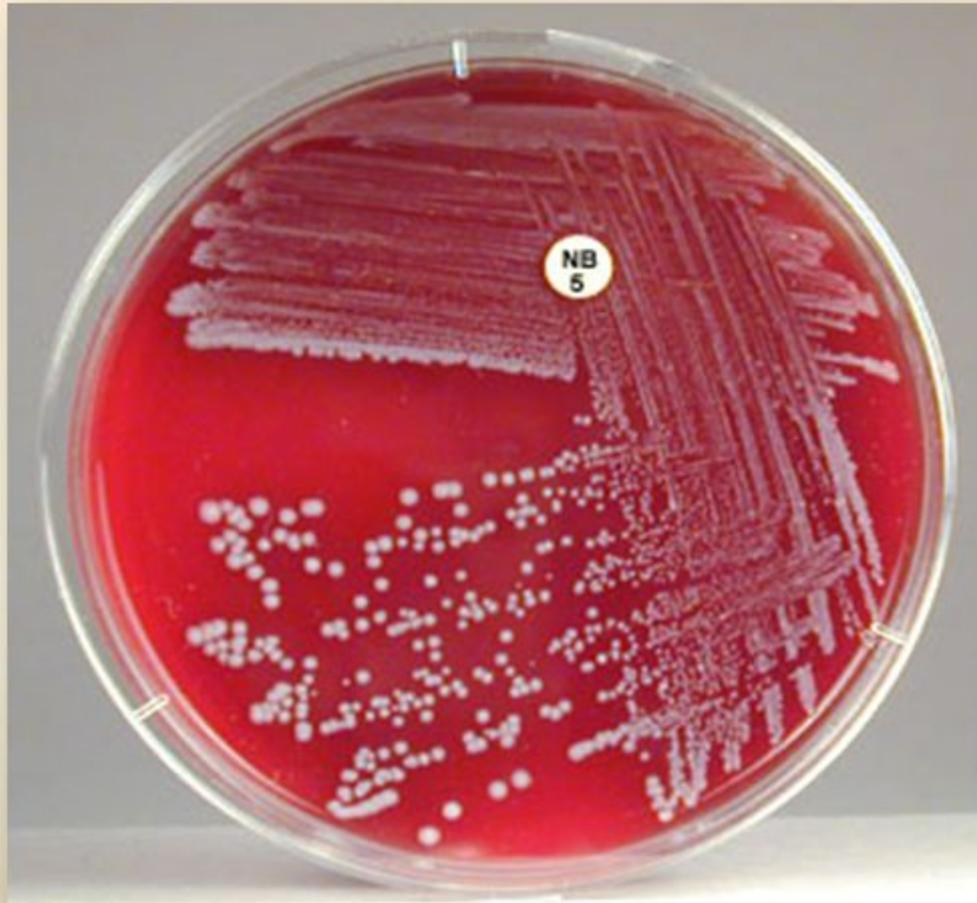
Novobiocin Susceptibility Test



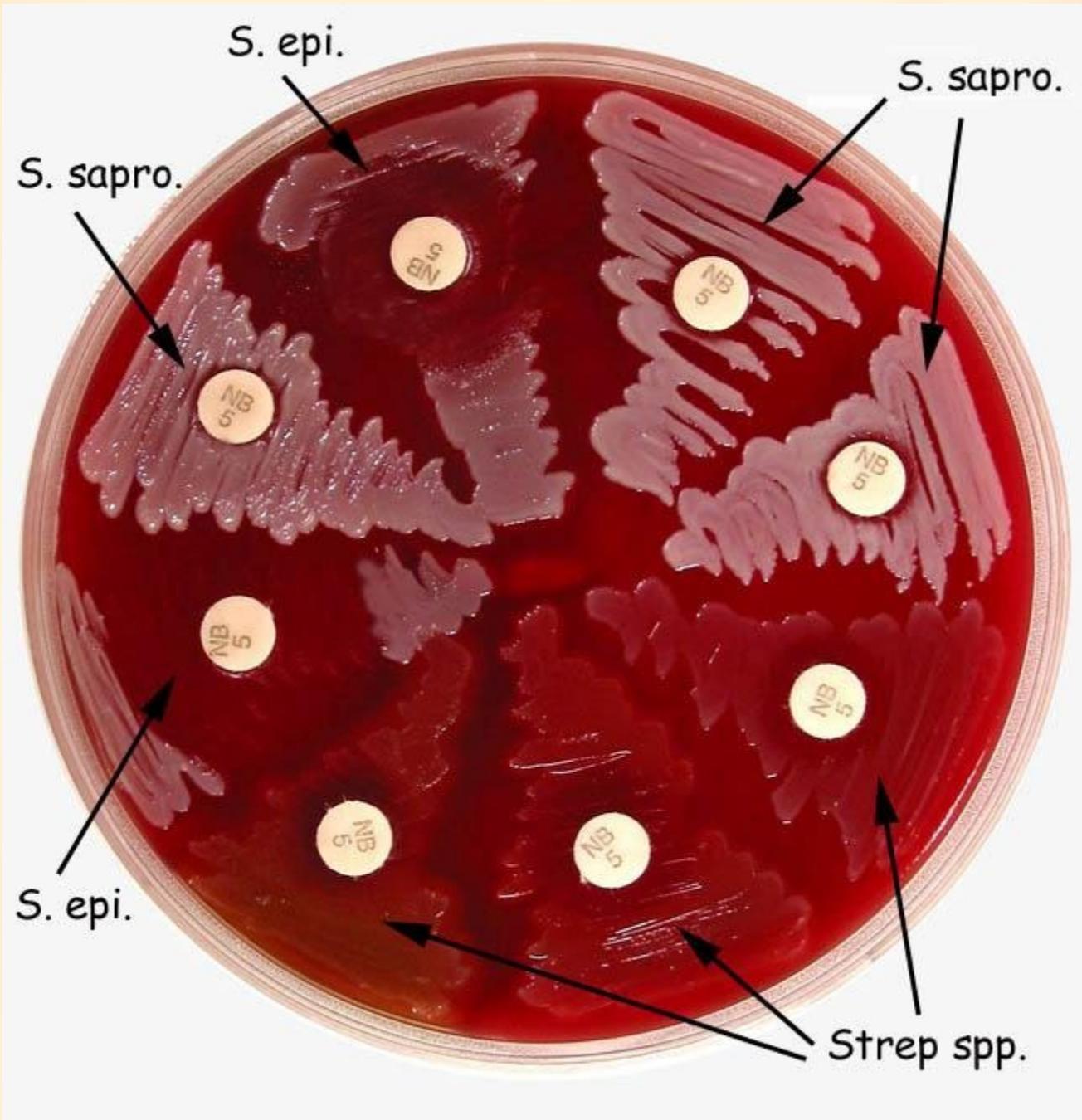
Staph saprophyticus

Staph epidermidis

Staphylococcus saprophyticus Growing on Blood Agar



Note there is no hemolysis (gamma reaction) on the blood agar and the organism is resistant to the antibiotic novobiocin.



S. epi.

S. sapro.

S. sapro.

NB 5

S. epi.

Strep spp.

Differences between Staphylococcal species

| Staphylococcal species | colony color | coagulase | Fermentation of mannitol | Beta haemolysis |
|-------------------------------|----------------------|------------------|---------------------------------|------------------------|
| S. aureus | golden yellow | + | + | + |
| S. epidermidis | white | - | - | - |
| S.saprophyticus | White -yellow | - | - | - |

THANKS