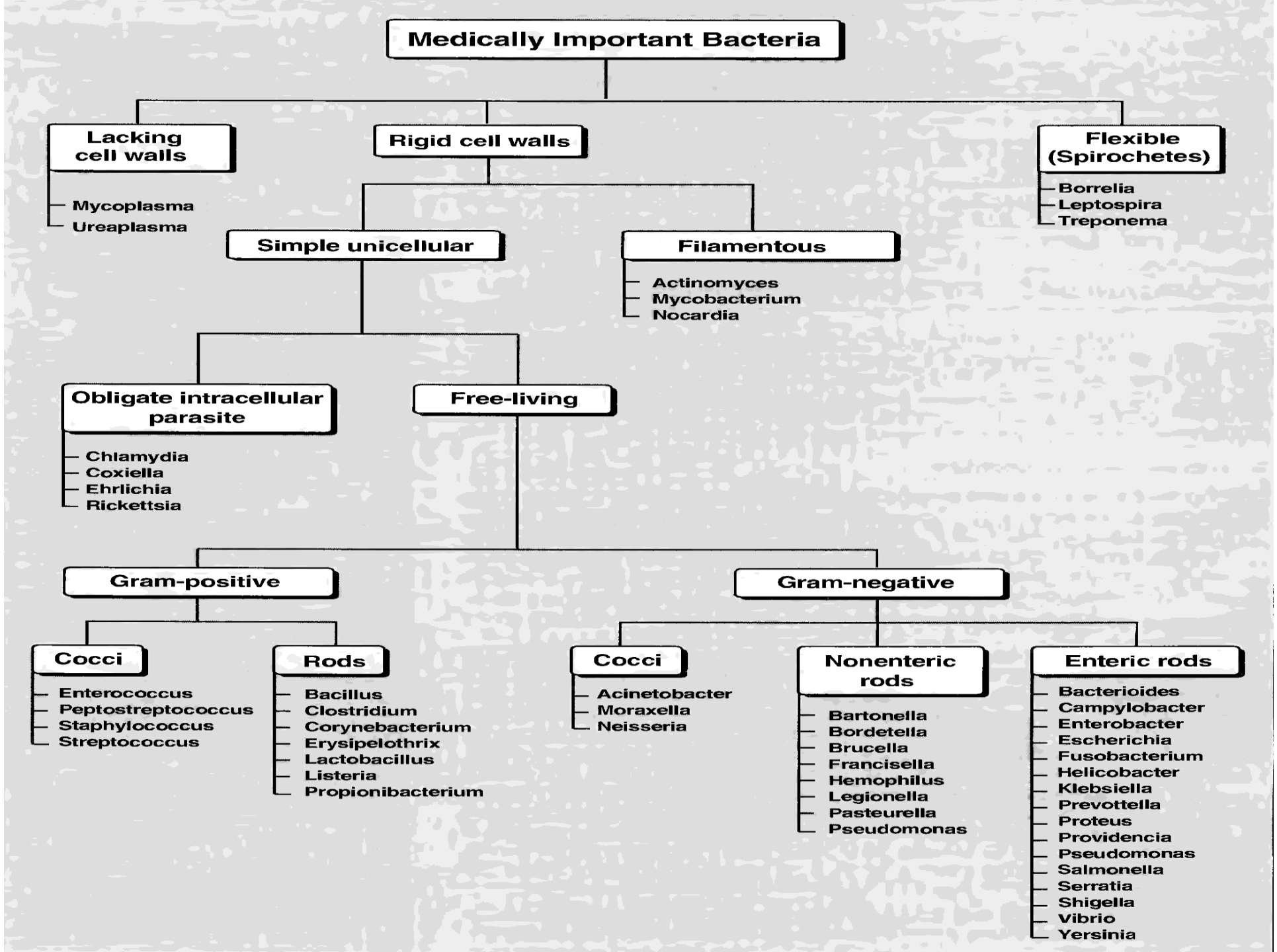
Practical No.9

STAPHYLOCOCCI

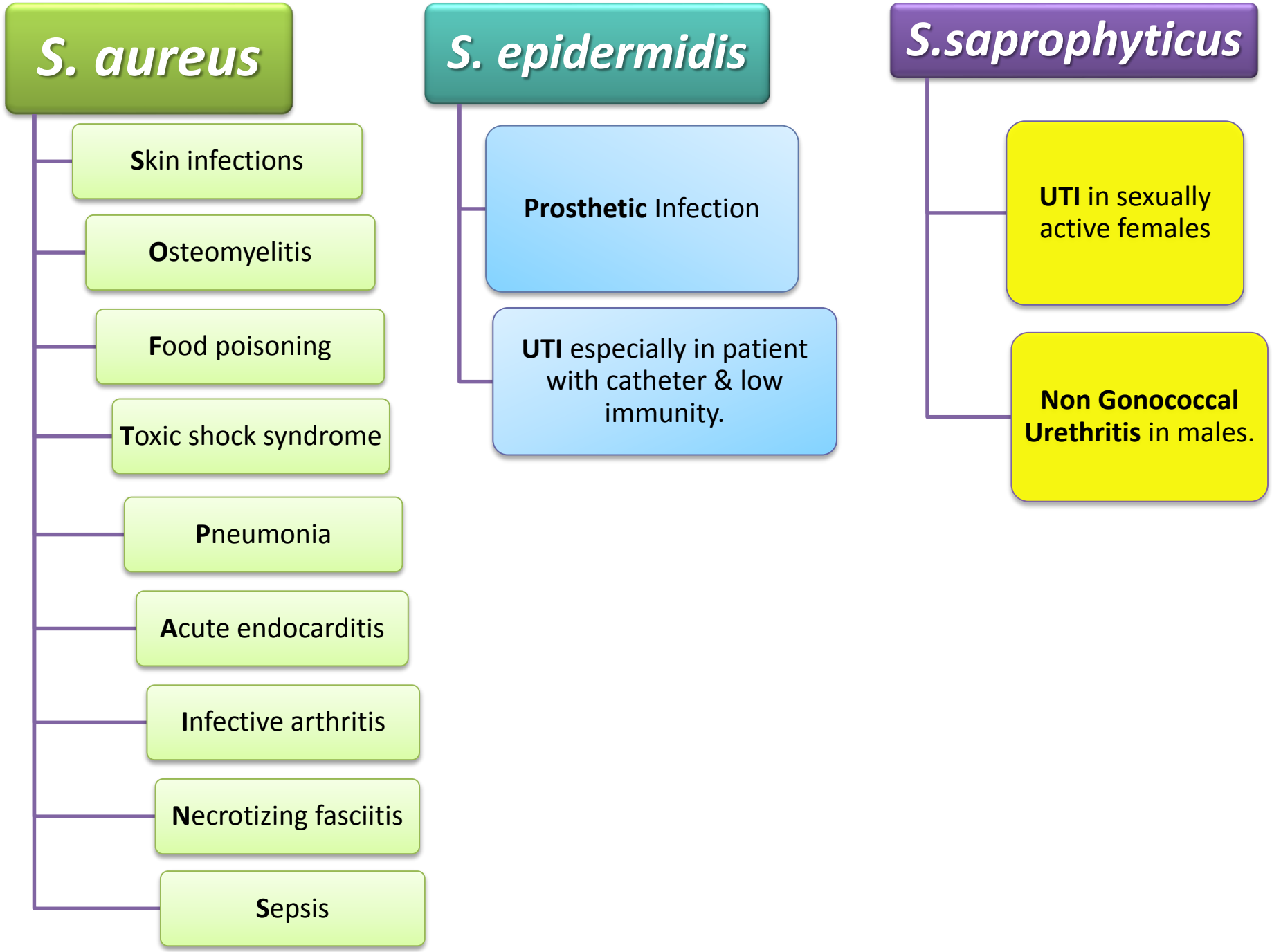
Department of Microbiology
College of Medicine


Medically Important Bacteria
Lacking cell walls
Mycoplasma
Ureaplasma
Rigid cell walls
Flexible (Spirochetes)
Borrelia
Leptospira
Treponema
Simple unicellular
Filamentous
Actinomyces
Mycobacterium
Nocardia
Obligate intracellular parasite
Chlamydia
Coxiella
Ehrlichia
Rickettsia
Free-living
Gram-positive
Gram-negative
Cocci
Enterococcus
Peptostreptococcus
Staphylococcus
Streptococcus
Rods
Bacillus
Clostridium
Corynebacterium
Erysipelothrix
Lactobacillus
Listeria
Propionibacterium
Cocci
Acinetobacter
Moraxella
Neisseria
Nonenteric rods
Bartonella
Bordetella
Brucella
Francisella
Hemophilus
Legionella
Pasteurella
Pseudomonas
Enteric rods
Bacterioides
Campylobacter
Enterobacter
Escherichia
Fusobacterium
Helicobacter
Klebsiella
Prevottella
Proteus
Providencia
Pseudomonas
Salmonella
Serratia
Shigella
Vibrio
Yersinia

Staphylococci are often found in the human nasal cavity (and on other mucous membranes) as well as on the skin. They are gram-positive cocci 0.5-1.0 μm in diameter and occur singly, in pairs, in short chains, and most commonly, in irregular grape-like clusters. The staphylococci are strongly catalase positive, reduce nitrates to nitrites, and generally tolerate relatively high concentrations of sodium chloride (7.5-10%). This ability is often employed in preparing media selective for staphylococci.

Five species of staphylococci commonly associated with clinical infections:

- ***Staphylococcus aureus***
- ***S. Epidermidis***
- ***S. Haemolyticus***
- ***S. hominis***
- ***S.saprophyticus***


S. aureus
Skin infections
Osteomyelitis
Food poisoning
Toxic shock syndrome
Pneumonia
Acute endocarditis
Infective arthritis
Necrotizing fasciitis
Sepsis
S. epidermidis
Prosthetic Infection
UTI especially in patient with catheter & low immunity.
S.saprophyticus
UTI in sexually active females
Non Gonococcal Urethritis in males.

Coagulase-positive Staphylococci (*Staphylococcus aureus*):

Since most ***S. aureus*** strains produce the enzyme coagulase they are often referred to as coagulase-positive staphylococci.

Staphylococcus aureus is the most pathogenic species and is implicated in a variety of infections. Approximately 30% of adults and most children are healthy periodic nasopharyngeal carriers of ***S. aureus.*** Around 15% of healthy adults are persistent nasopharyngeal carriers.

S. aureus causes**;** Pus-filled inflammatory lesions known as abscesses. Depending on the location and extent of tissue involvement, the abscess may be called: pustule**,** furuncle or boil, carbuncle , Impetigo, a superficial blister-like infection of the skin usually occurring on the face and limbs and seen mostly in young children.

-Cellulites.

-Accidental wound and postoperative wound infections

-Systemic infections include septicemia, septic arthritis, endocarditis, meningitis, and osteomyelitis, as well as abscesses in the lungs, spleen, liver, and kidneys. Pneumonia caused by ***S. aureus*** is considered as a secondary respiratory complication of viral infections such as measles and influenza. Finally, *S. aureus* is frequently introduced into food by way of abscesses or the nasal cavity of food handlers. If it is allowed to grow and produces an enterotoxin, it can cause staphylococcal food poisoning. It is also the causative agent of scalded skin syndrome and toxic shock syndrome.

Coagulase-Negative Staphylococci

Clinically common species of staphylococci other than ***S. aureus*** are often referred to as coagulase-negative staphylococci.

These staphylococci are normal flora of the skin and, as such, frequently act as opportunistic pathogens, especially in the compromised host. *S. saprophyticus* is a relatively common cause of urinary tract infections, especially in healthy young women, but is seldom isolated from other sources. The great majority of infections caused by other coagulase-negative staphylococci, including *S. epidermidis, S. haemolyticus,*

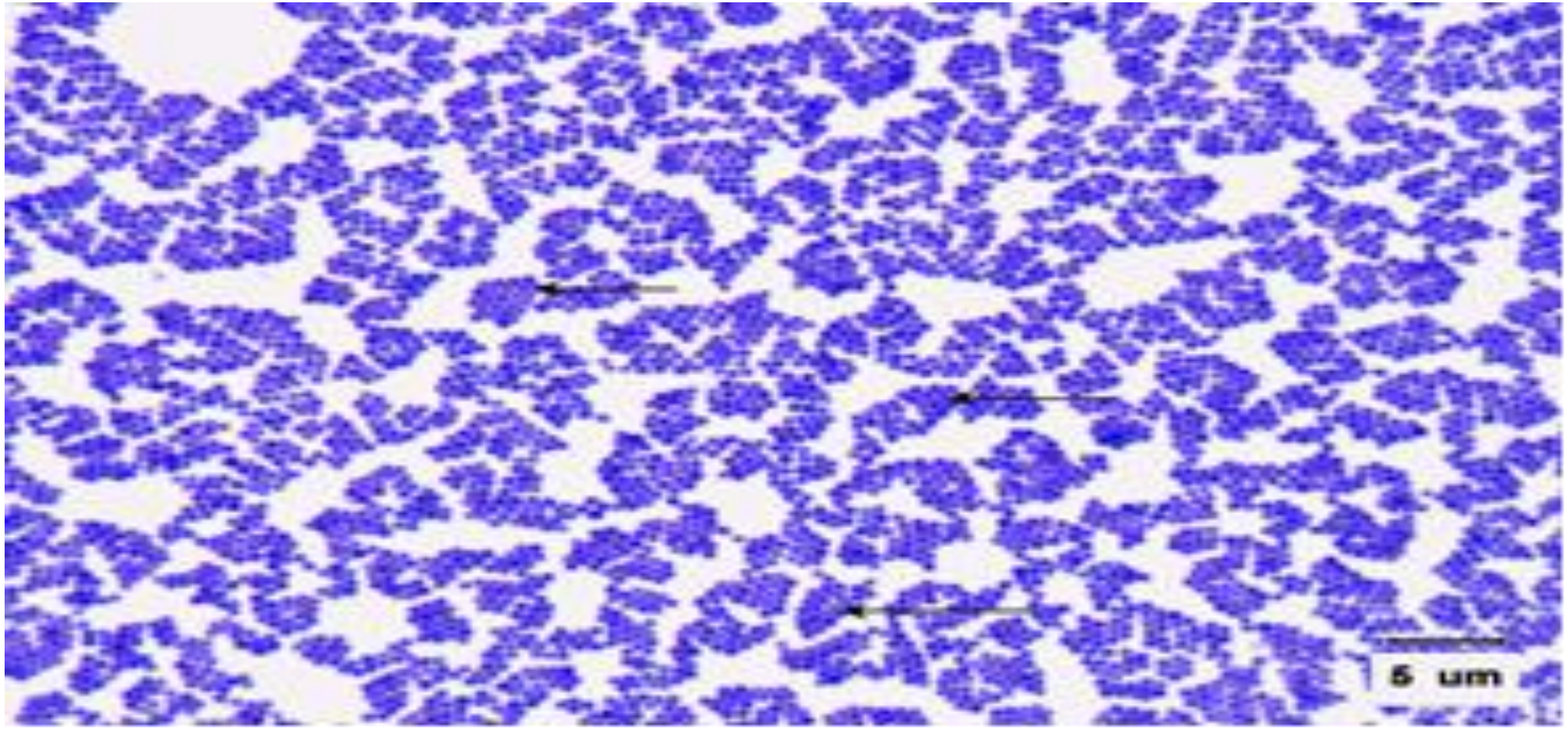
and *S. hominis,* are associated with intravascular devices (prosthetic heart valves and intra-arterial or intravenous lines) and shunts. Also quite common are infections of prosthetic joints, wound infections, osteomyelitis associated with foreign bodies, and endocarditis. Although certain reactions may vary from strain to strain, a series of biochemical tests will usually differentiate the most common clinically encountered species of staphylococci.

Today we will use a number of tests to isolate and identify ***S. aureus, S. epidermidis,*** and ***S. saprophyticus.***

Isolation and identification of Staphylococci

Specimen: blood, urine, stool, CSF, pus.

1- Gram stain:



Note staphylococcus arrangement (cocci in irregular, often grape-like clusters).

2-Catalase test:

Although microscopic examinations of stained smears presumptively permit distinction between Staphylococci and streptococci, a definitive classification can be made on the basis of the presence or absence of the enzyme catalase.

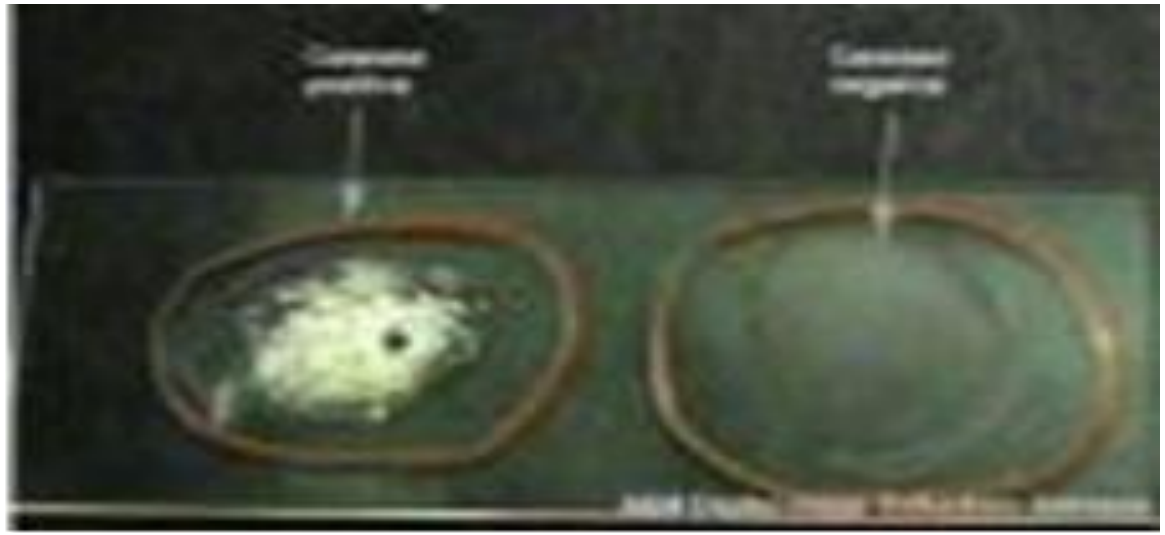
Staphylococci contain this enzyme, streptococci do not.

Procedure:

1. Place a drop of 3% hydrogen peroxide on a clean microscope slide.
2. Place a heavy loopful of cells from isolated colonies into the liquid (pick four to five colonies). Immediate generation of gas bubbles constitutes a positive test.
3. Avoid the inclusion of blood cells from blood agar plates as RBCs contain catalase. Lack of bubbles is a negative test.

Catalase

$2H_2O_2$ ----------> $2H_2O + O_2$ (bubbles)



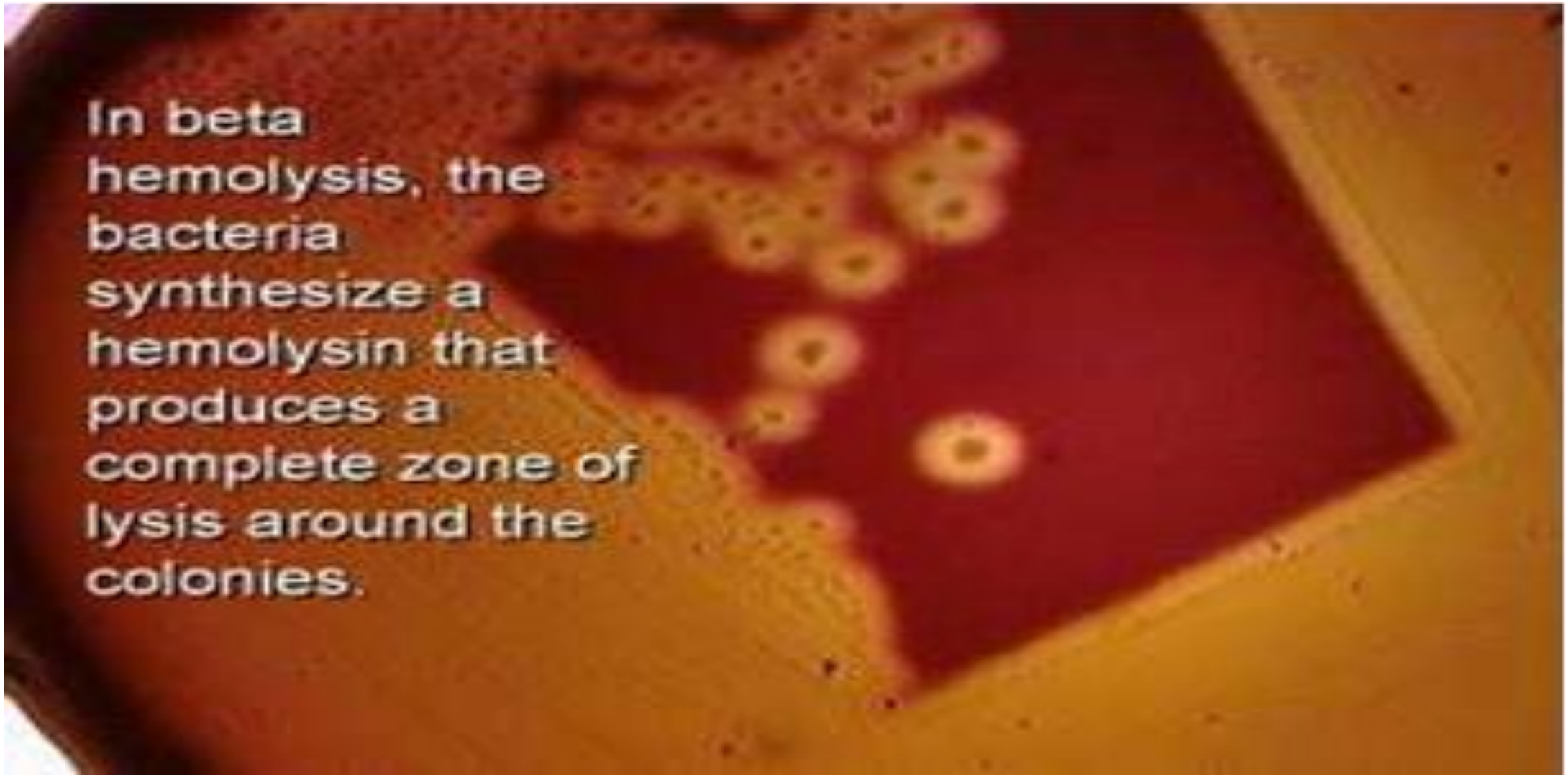
Catalase test

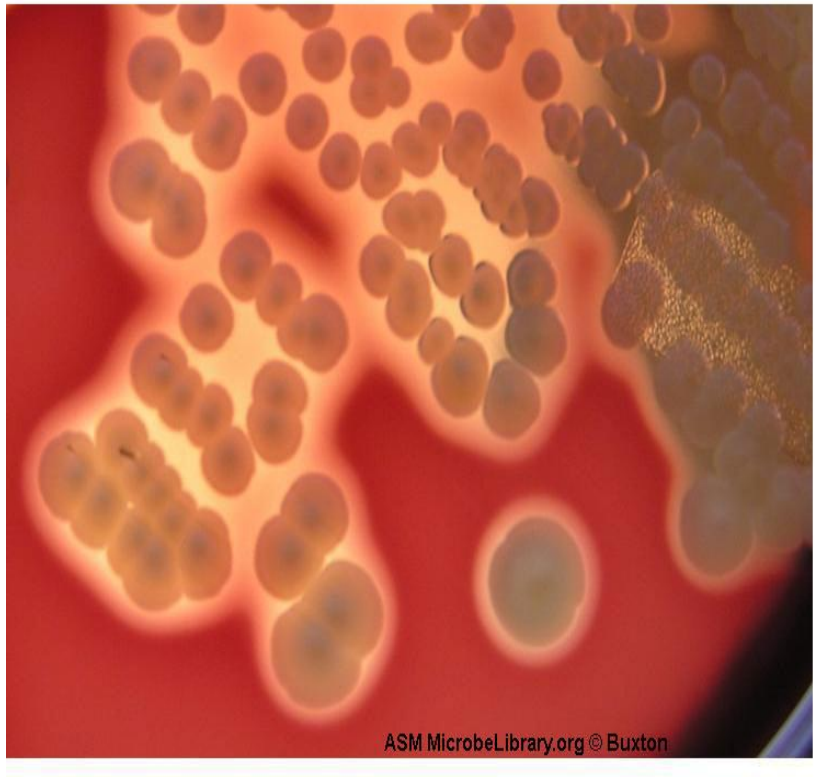
3- Hemolysis on blood agar:

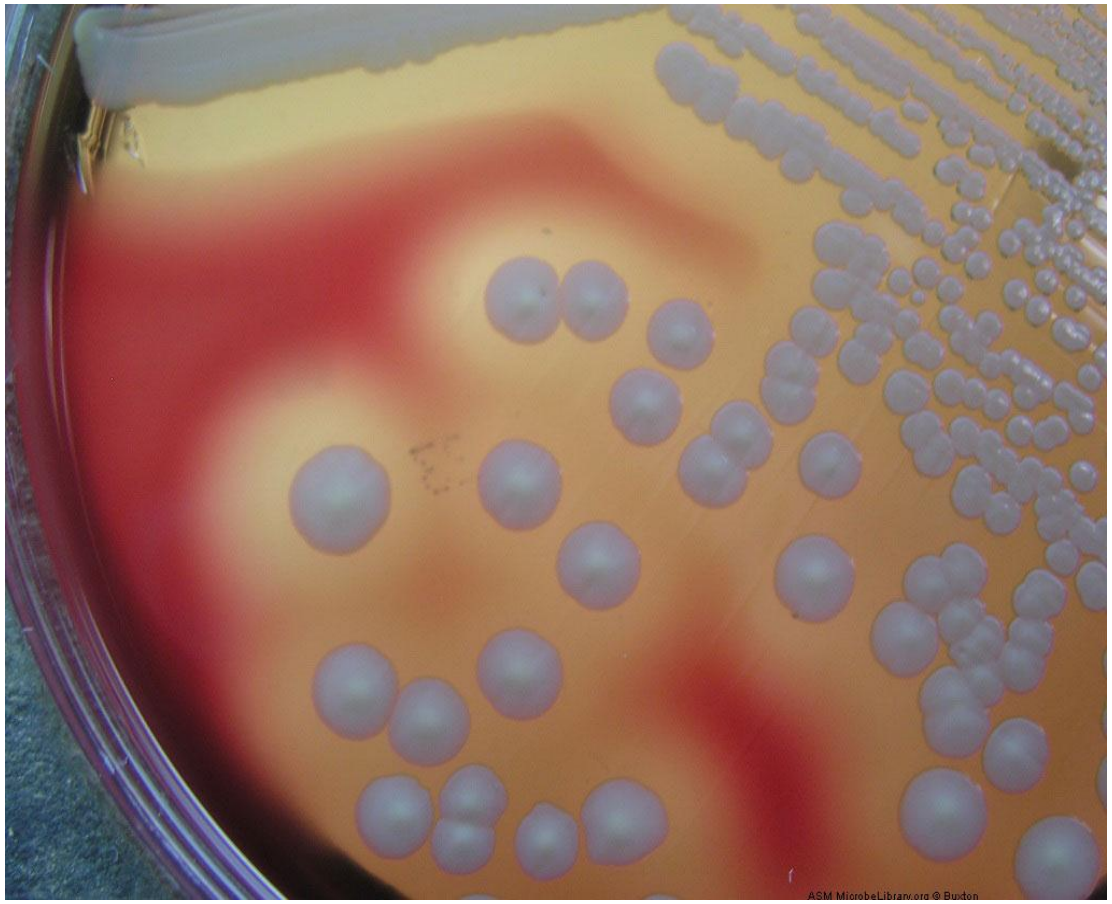
Some bacteria produce hemolysins, exotoxins that cause red blood cells (RBC's) to burst open (hemolyse). When these bacteria are cultured on blood agar, this hemolysis is visible as an area of clearing around the colony (zone of hemolysis). If the organism produces enzymes that completely lyse the RBC's, this is termed beta hemolysis (β-hemolysis).

Partial destruction of the RBC's produces a greenish color to the zone of hemolysis and is termed alpha hemolysis (α-hemolysis). Organisms lacking hemolysins cause no change in the color or opacity of the media and are termed gamma hemolytic or none hemolytic.

Test	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Hemolysis	Usually beta	Usually none	Usually none







Staphylococcus aureus **cause haemolysis on blood agar because of haemolysin enzyme, a zone of β – haemolysis will appear around the colony**

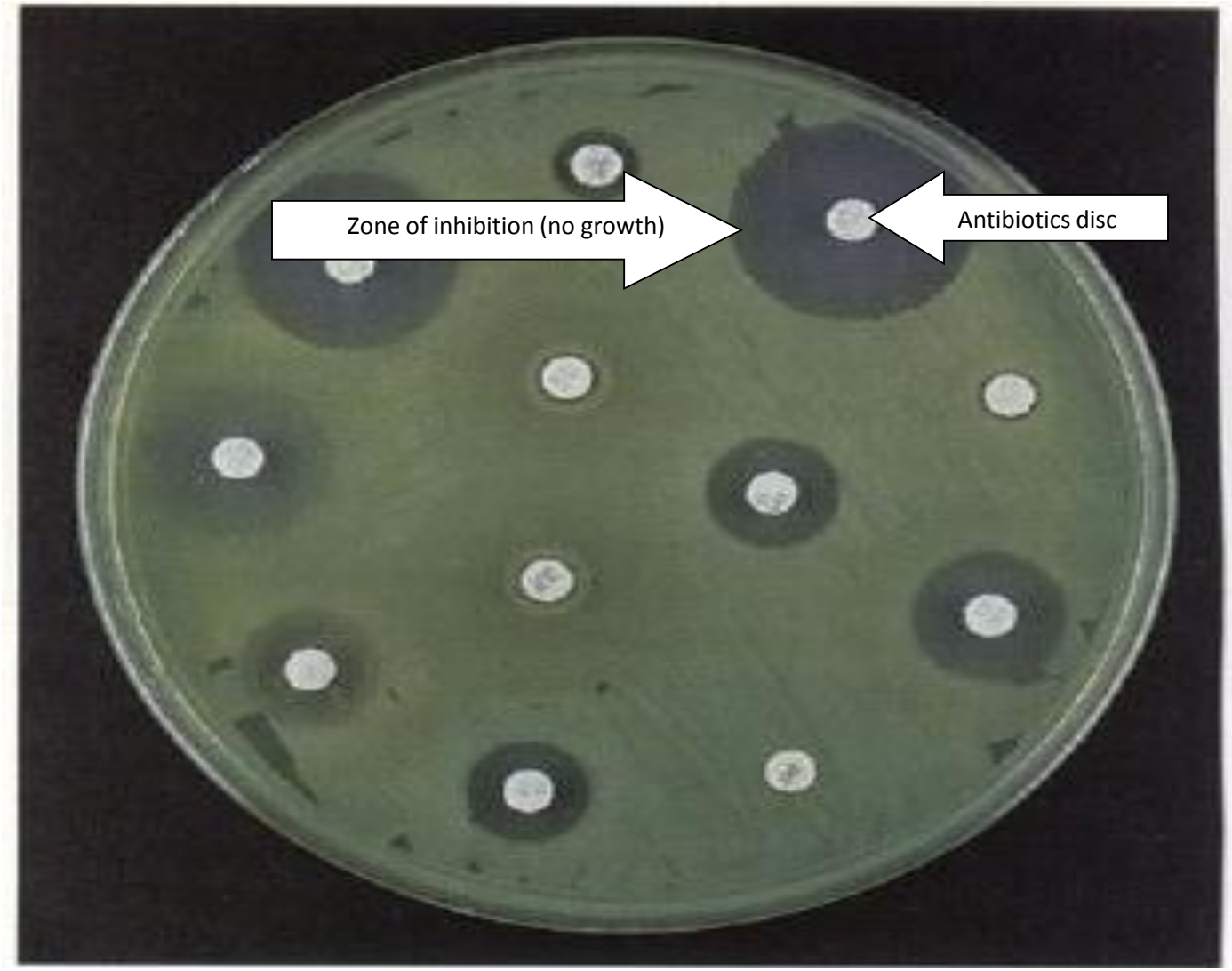

s.aureus
s.epidermidis

4- Agar plates with a novobiocin (NB) disc

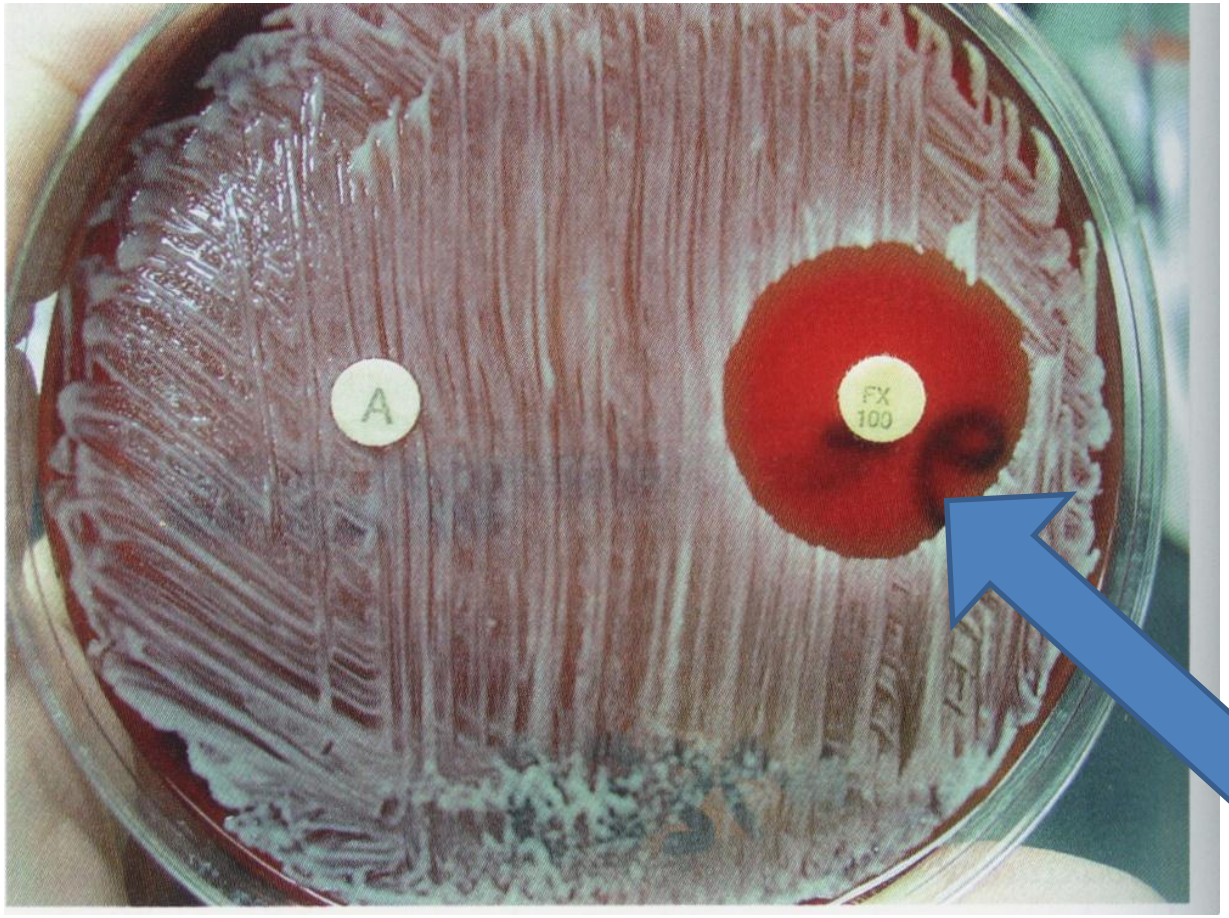
Novobiocin is an antibiotic to which Staphylococcus sp. are either resistant or sensitive. The appearance of a zone of inhibition > 16 mm indicates sensitivity.

Filter paper discs impregnated with the appropriate chemical are placed on an agar surface. The chemical diffuses through the agar. Organisms that are susceptible to the chemical will not grow on the agar containing the chemical. The size of the zone of growth inhibition determines the organisms' susceptibility to the chemical.

Test	***S. aureus***	***S. epidermidis***	***S. saprophyticus***
Novobiocin test	Sensitive	Sensitive	Resistant (at a concentration of 5 mg),



Antibiotic Sensitivity test



Antibiotic Sensitivity test

Zone of inhibition

5- Production of staphyloxanthin (pigmentation)

Staphyloxanthin is an orange pigment produced by Staphylococcus aureus that contributes to its virulence. It is the main pigment of this pathogen. Milk agar provides a white background to visually observe the colonies of *Staphylococcus aureus*

Procedure:

- Streaking colonies of staphylococci on milk agar plate

- incubating overnight

- observing the golden yellow pigment of ***Staphylococcus aureus***

Test	***S. aureus***	***S. epidermidis***	***S. saprophyticus***
Pigment	Often creamy gold	Usually white	Usually white

6- Mannitol fermentation on Mannitol Salt agar (MSA)

Staphylococci are able to tolerate the high salt concentration found in Mannitol Salt agar and thus grow readily. If mannitol is fermented, the acid produced turns the phenol red pH indicator from red (alkaline) to yellow (acid).

Procedure

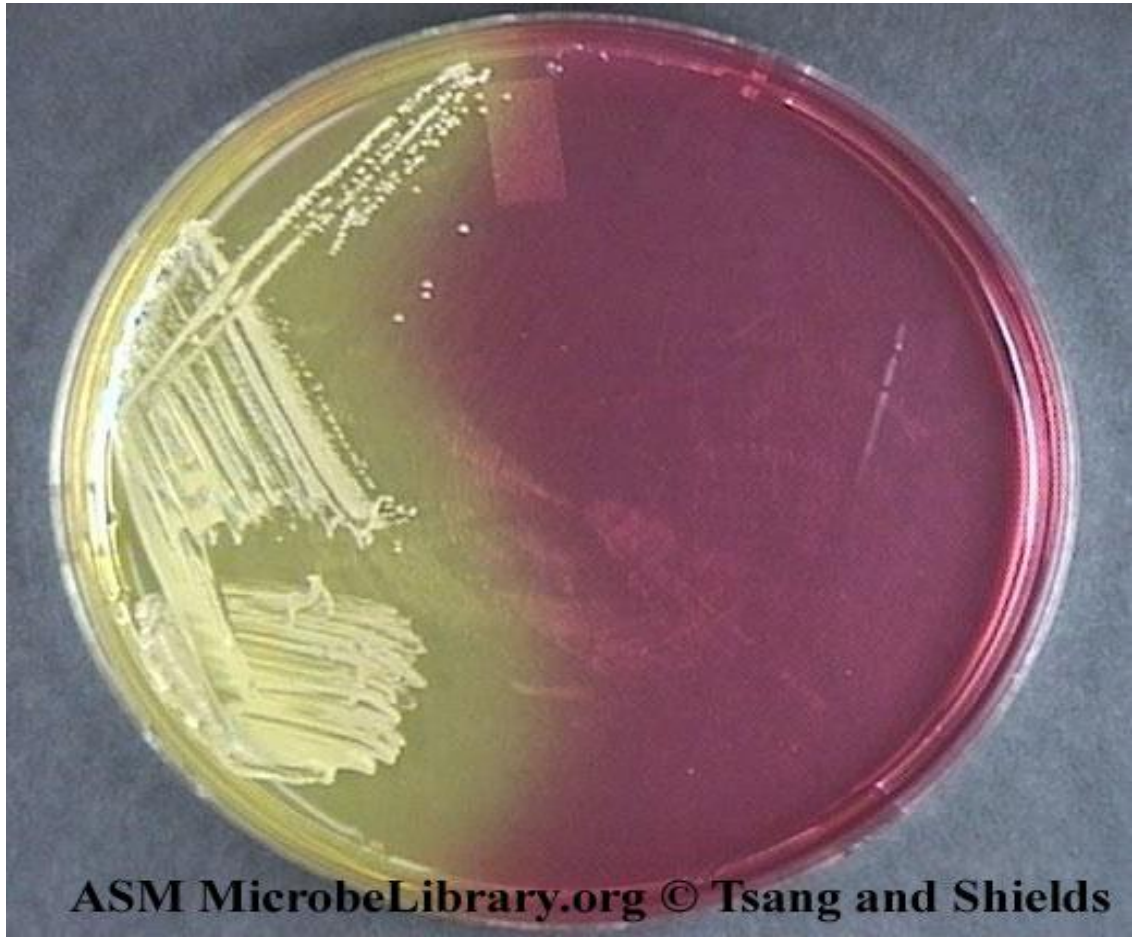
Mannitol-Salt Agar Tests.

Two separate tests are done with this plate. The plate contains 7.5% NaCl, mannitol, phenol red, and extracts of peptone and beef. If the bacteria in question grow on the plate they are osmotolerant and therefore NaCl growth positive. If surrounding the growth the media has turned from pink to yellow, the bacteria has fermented the mannitol and produced acid.

The acid lowers the pH of the media and the phenol red turns yellow. If yellow, the bacteria are positive for the ability to ferment mannitol.

Test	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Mannitol fermentation	**Positive**	**Negative**	**Usually positive**

Positive = acid end products turn the phenol red pH indicator from red to yellow
negative = phenol red remains red

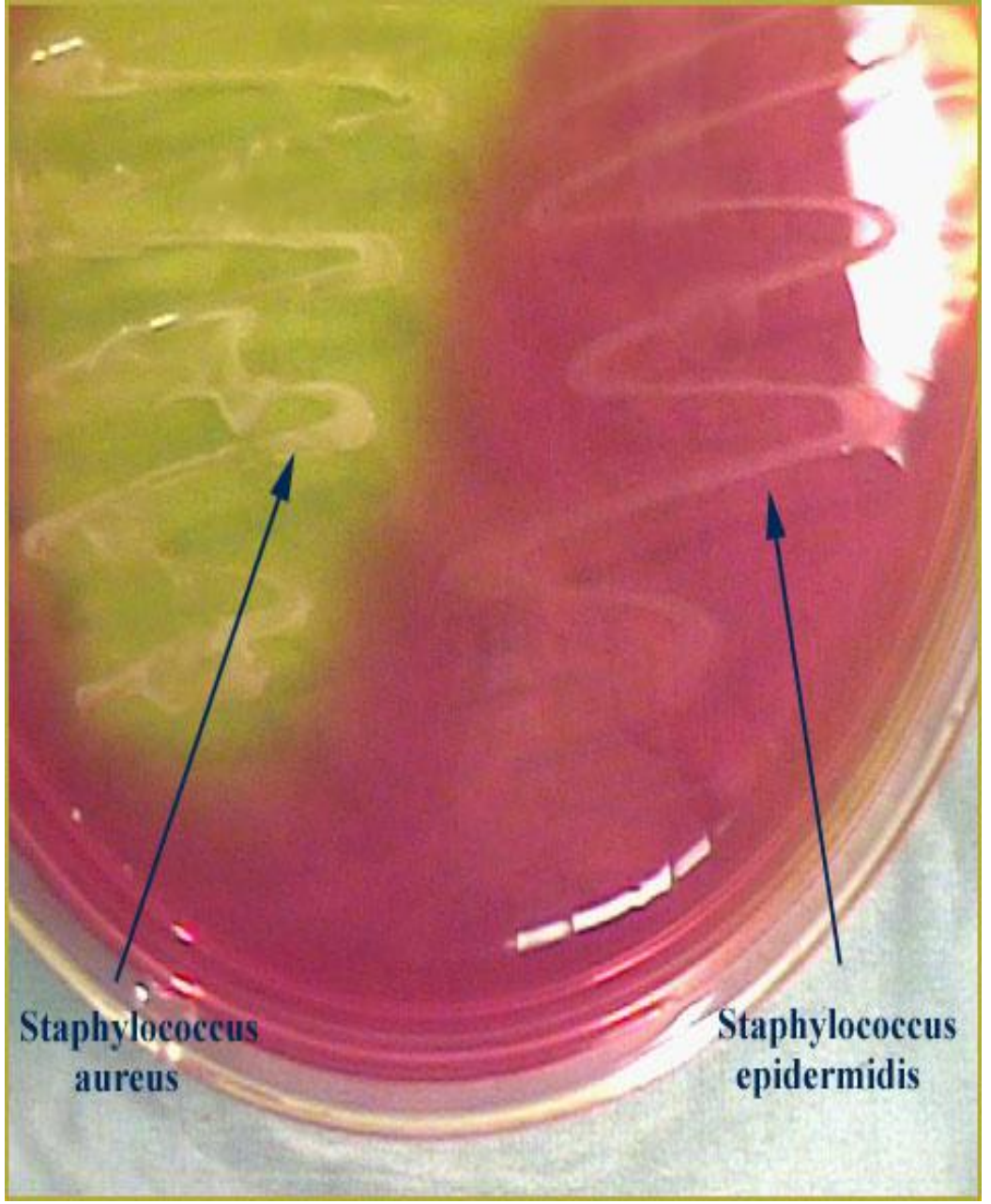


Staphylococcus aureus turning the indicator from red to yellow

MANNITOL SALT AGAR

NaCl 7.5% → selective

Mannitol → differential



Staphylococcus aureus can ferment mannitol, so the selective media used to isolate *Staphylococcus aureus* especially from nasal carriage is (**Mannitol Salt Agar**)

7- Production of deoxyribonuclease (DNase) on DNase agar

DNase agar contains 0.2% DNA. To detect DNase production, the plate is inoculated and incubated. After growth, the plate is flooded with 1N hydrochloric acid (HCl). DNase positive cultures show a distinct clear zone around the streaked area, where the DNA in the agar was broken down by the bacterial DNase. DNase negative cultures appear cloudy around the growth where the acid caused the DNA in the agar to precipitate out of solution.

Procedure

1- Inoculate by making a single streak line using inoculum from an agar slant or plate.

2- Incubate at 35 ± 2°C for 24-48 hours. Plates should be incubated in an inverted position.

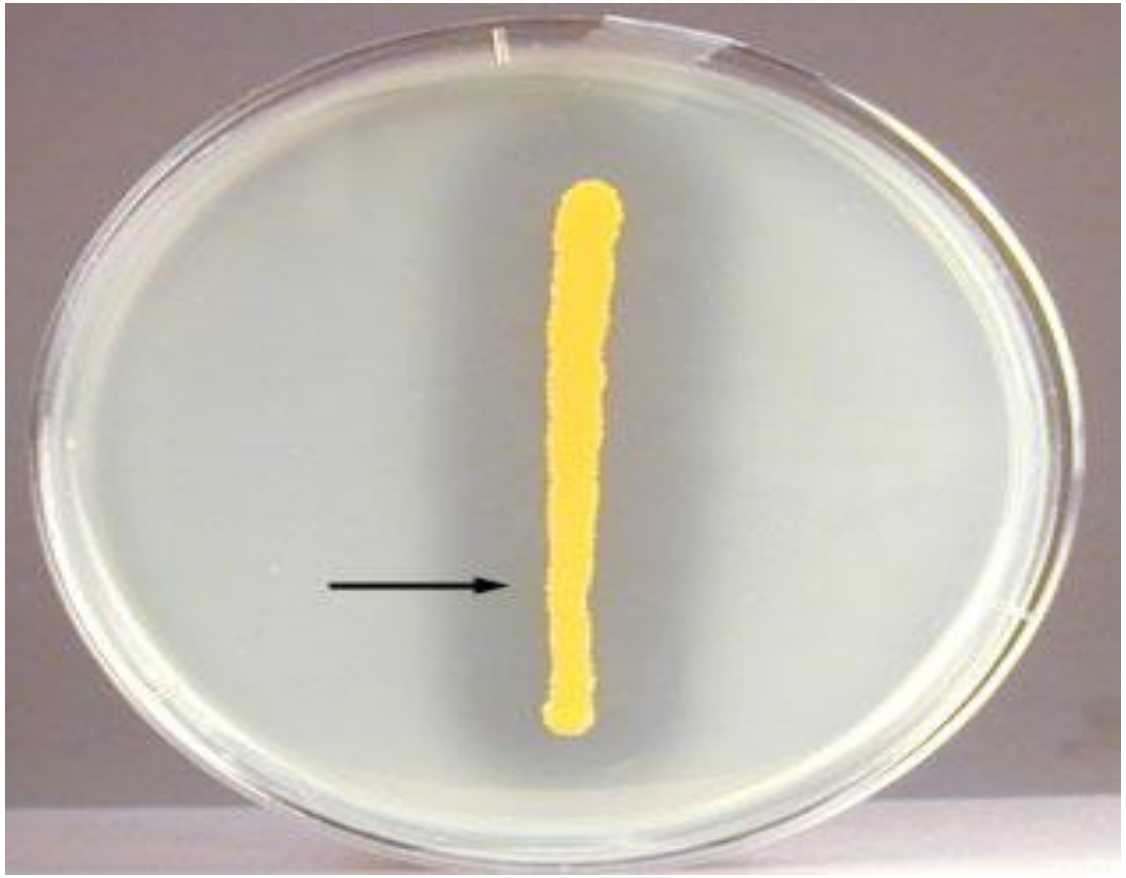
3-Following incubation, flood DNase Test Agar plates with 1N HCl reagent and observe for reaction.

Test	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
DNase production	Positive	Negative	Negative

Positive = clear zone around growth after adding 1N HCl (no DNA remaining in the agar)

negative = cloudy around growth after adding 1N HCl (DNA remains in the agar forming a precipitate)

A Positive DNase Test



Note there is breakdown of the DNA in the agar. There is a clear zone (arrow) around the bacterial growth where there is no longer any DNA left in the agar to precipitate out of solution after the HCl was added.

8- Production of coagulase

The staphylococcal enzyme coagulase will cause inoculated citrated rabbit plasma to gel or coagulate. The coagulase converts soluble fibrinogen in the plasma into insoluble fibrin. . In the laboratory, it is used to distinguish between different types of *Staphylococcus* isolates.

Procedure:

- Add 0.5 mL reconstituted lyophilized rabbit coagulase plasma with EDTA to a sterile 13 x 100mm tube

- Touch an isolated colony with an inoculating loop

- Place the loop, carrying some of the isolate, into the tube containing the rabbit plasma, and mix thoroughly

- Incubate the tube at 35º C for 6 hours

- Observe the tube for the presence of clotting.

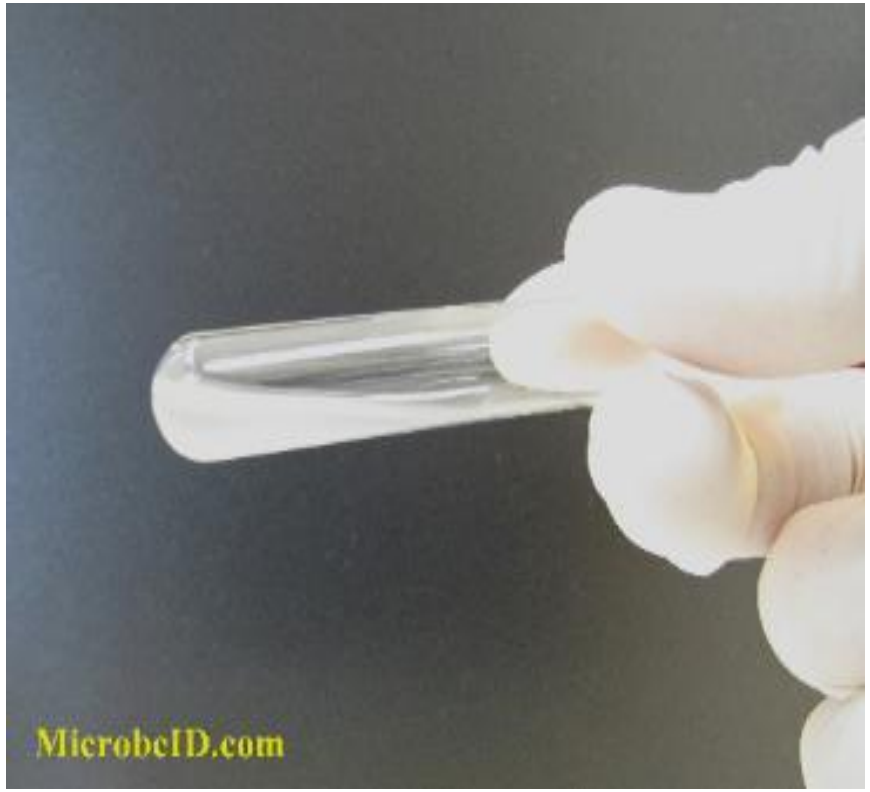
- If no clotting is observed, reincubate for 24 hours and observe again.

The reaction is positive if clotting is present. (The plasma will gel to a viscous form, where it will not flow down the tube when tilted at a 45º angle.)

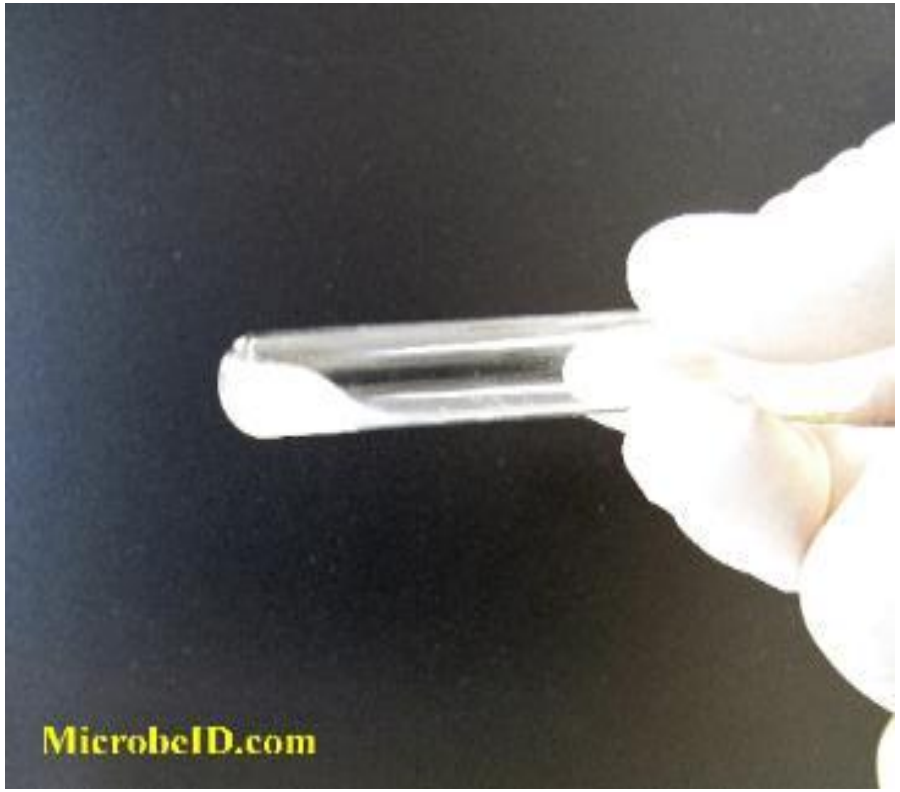
Test	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Coagulase production	Positive	Negative	Negative

Positive = plasma will gel or coagulate

negative = plasma will not gel



Coagulase negative



coagulase positive

Biochemical tests:

Coagulase test:

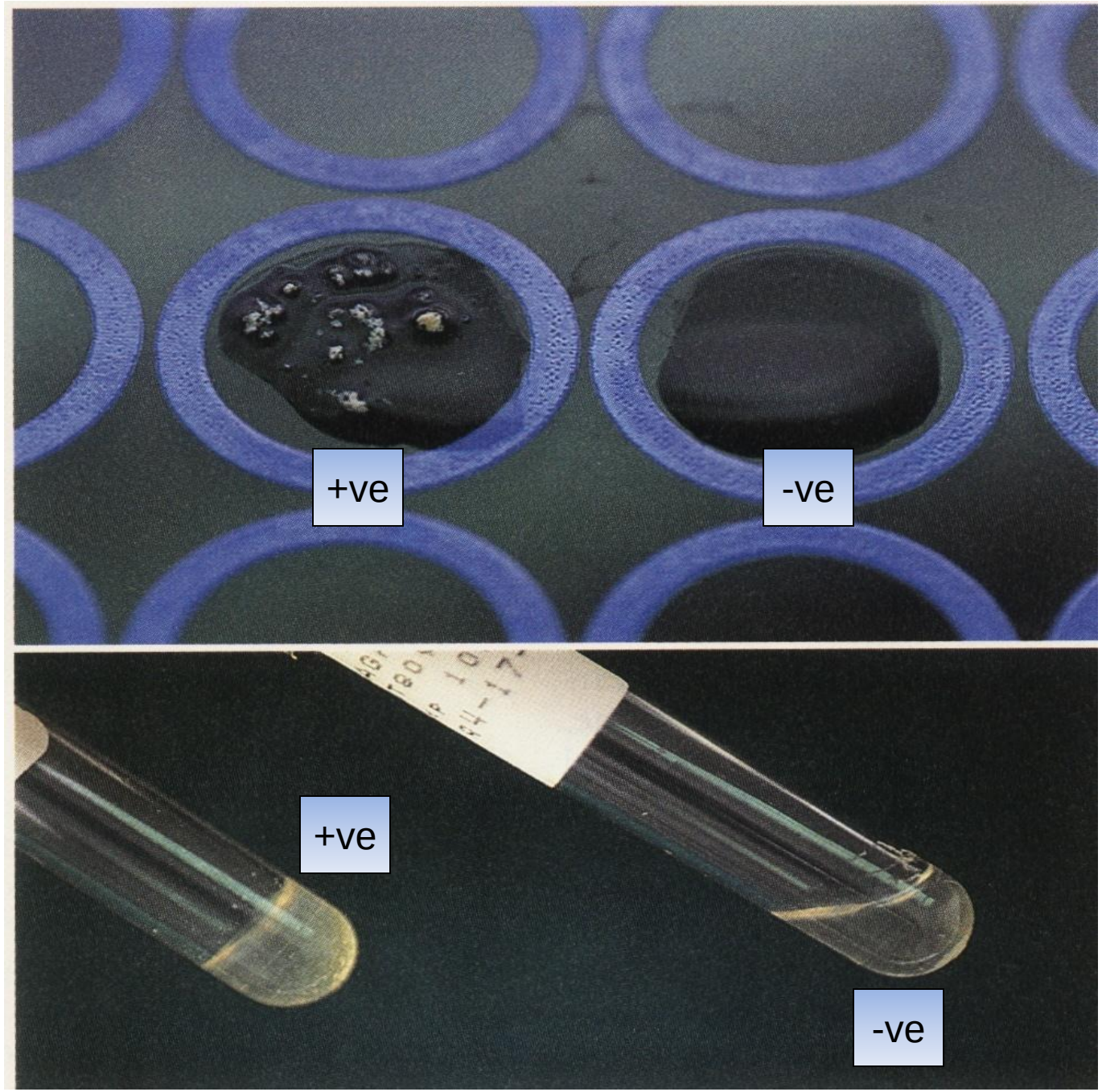
DETECT the ability of ***S. aureus*** to clot blood plasma (fibrinogen → fibrin).

- **Slide method**
- **Tube method**

Catalase test:

To differentiate between Staph. & Strepto.

Coagulase test



The Staphyloslide Latex Test for cell-bound coagulase (clumping factor) and/or Protein A

The Staphyloslide Latex Test is an agglutination test that detects cell-bound coagulase (clumping factor) and/or Protein A. This test uses blue latex particles coated with human fibrinogen and the human antibody IgG. Mixing of the latex reagent with colonies of the suspected

S. aureus having coagulase and/or Protein A bound to their surface causes agglutination of the latex particles.

Procedure:

1.Gently mix the Staph latex reagent bottle (make sure latex is resuspended and warmed to room temperature) and place 1 drop of the latex reagent (by holding dropper bottle vertically) into one circle of the reaction card.

2.Spread 1 colony (using either an applicator stick or an inoculating loop) onto the circle and then mix into the drop of latex reagent. Slowly blend the staphylococci into the reagent.

3.Spread the mixture over much of the circle. Discard the stick into biohazard waste or flame the loop.

4.Rotate the slide with circular motion for up to 60 sec. Aggregation of the black latex suspension with subsequent loss of black background represents a positive reaction for agglutination. For ***S. aureus***, this usually occurs within 15 sec. A negative reaction is reported as little or no agglutination within 60 sec.

Test	S. aureus	S. epidermidis	S. saprophyticus
Cell-bound coagulase (clumping factor) and/or Protein A	Positive	Negative	Negative

Positive = clumping of latex particles

negative = no clumping of latex particles

9- Gelatinase test

Nutrient gelatin is a differential medium that tests the ability of an organism to produce an exoenzyme, called gelatinase that hydrolyzes gelatin. The gelatinase test can be used to differentiate between *Staphylococcus aureus* and *Staphylococcus epidermidis.*

Test	S. aureus	S. epidermidis
Gelatinase	Positive	Negative

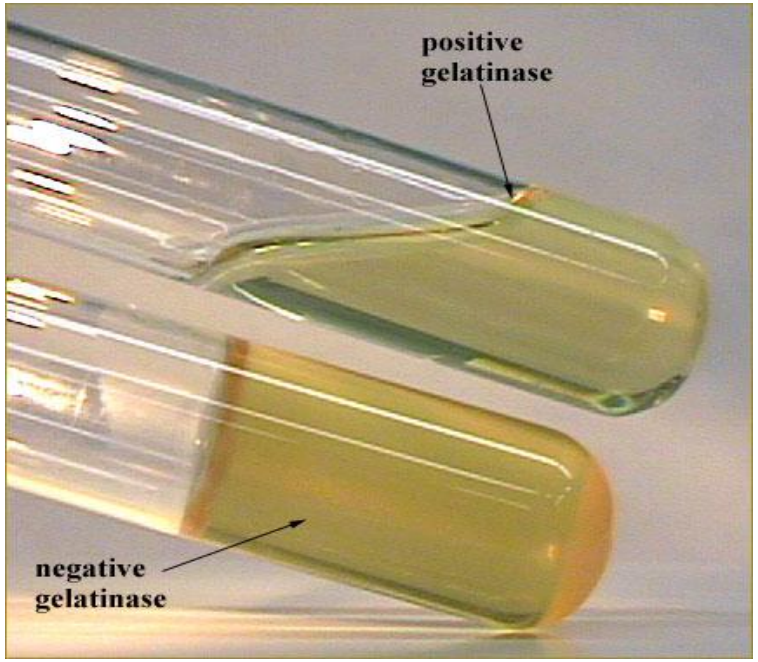


Table summarized the point of **differences** between 3 imp. *Staphylococci* species

Tests	*S. aureus*	*S. epidermidis*	*S.saprophyticus*
Catalase	+	+	+
Coagulase	+	-	-
β – haemolysis	+	-	-
Mannitol fermentation	+	-	-
Novobiocin sensitivity (0.5 µg)	+	+	-

Thank You