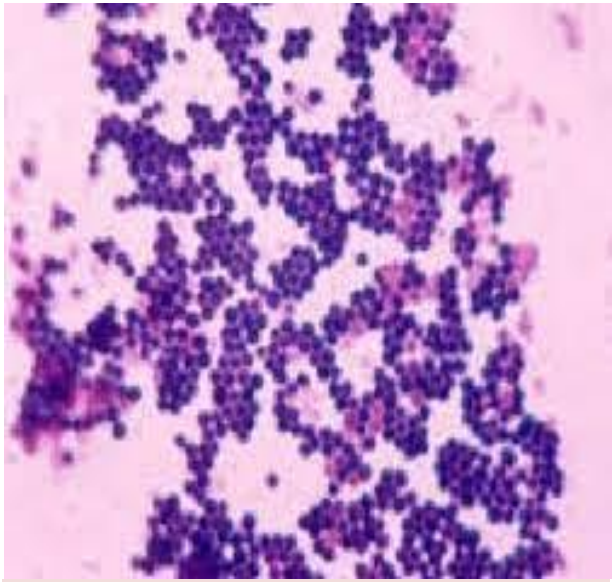
Lab – 8

Genus Staphylococci

Staphylococci

- They are **gram-positive cocci** arranged in irregular **grape-like clusters.**
- are often found in the human nasal cavity (and on other mucous membranes) as well as on the skin.
- Non motile
- The staphylococci are **catalase positive.**
- Tolerate relatively **high concentrations of sodium chloride** (7.5-10%). This ability is often employed in preparing media selective for staphylococci.



Staphylococci arranged in irregular **grape-like clusters.**

Three species of staphylococci commonly associated with clinical infections:

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

Human Infections caused by Staphylococcus

❖ **S. aureus** cause:

- **Skin infections** like impetigo (rashes ,red sores), folliculitis(infection in hair follicles), wound infection.
- **Systemic infections** like bacterimia (viable bacterial cells in blood), pneumonia, meningitis, deep-seated abscess.
- **Toxin mediated infections** like food poisoning, toxic shock syndrome.

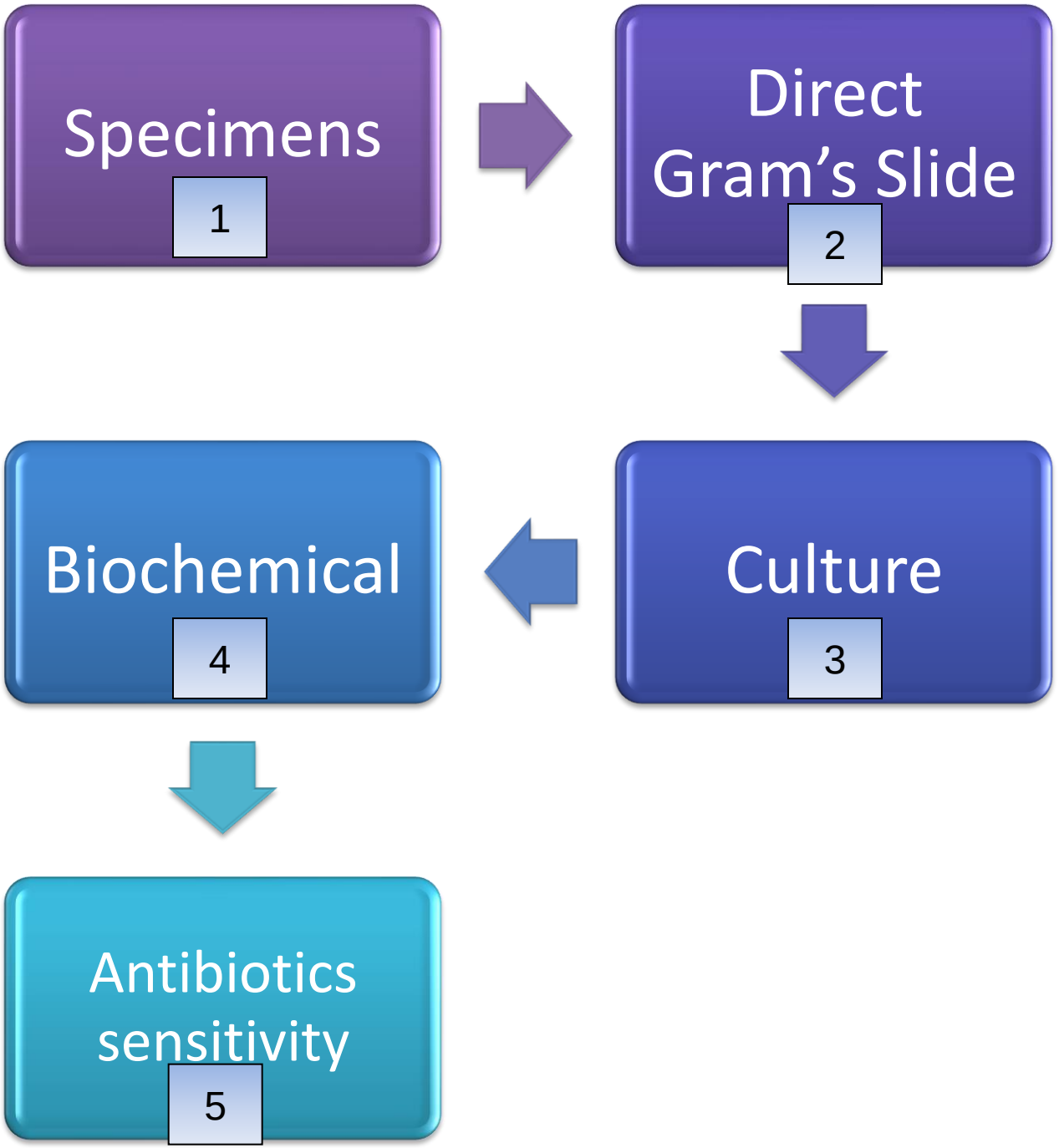
❖ **S. epidermidis** cause :

- Opportunistic infections like intravenous catheter infections, CSF shunt infections. **Prosthetic** Infection

❖ **S. saprophyticus** cause:

- UTIs.

Laboratory Diagnostic steps

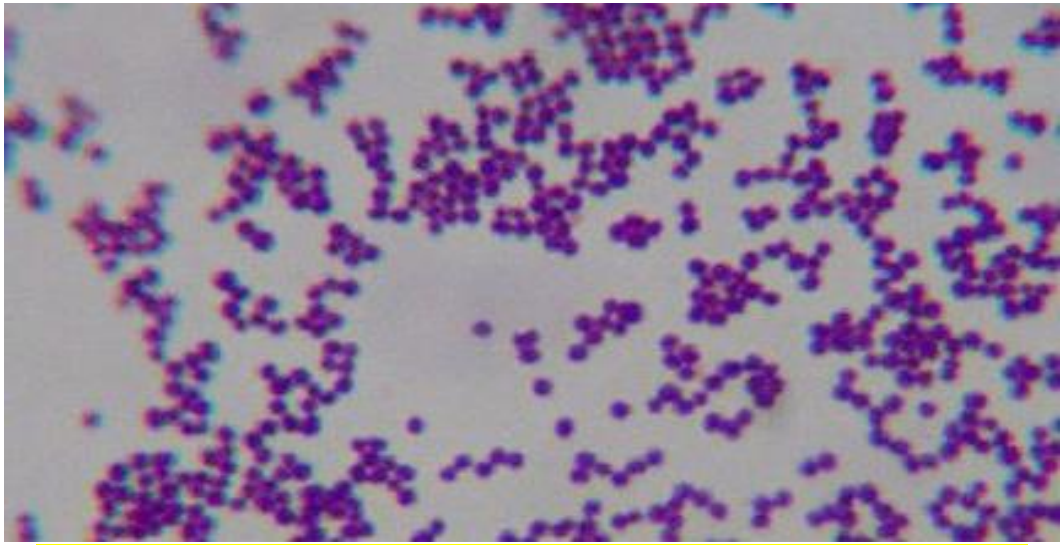


1. Specimens

Various specimens collected in staphylococcal infections	
Specimen	Condition
Pus	Supurrative lesions
Sputum	Respiratory infections
Blood	Bacterimia
Feces vomitus	Food poisoning
Urine	UTIs

2. Gram stain:

Sample can be stained by gram stain and bacteria has the features in picture below



<u>Microscopic characteristics</u>

Gram's positive cocci, Grape-like cluster

3. Culture

- **Aerobic or facultative anaerobic**
- **Grow on (nutrient agar & blood agar) & can tolerate 7.5 – 10 % NaCl.**
- **The colonies are round, smooth, raised, & glistening, producing pigments**

- ***Staphylococcus epidermides* : gray to white**
- ***Staphylococcus aureus :* gray to golden – yellow**

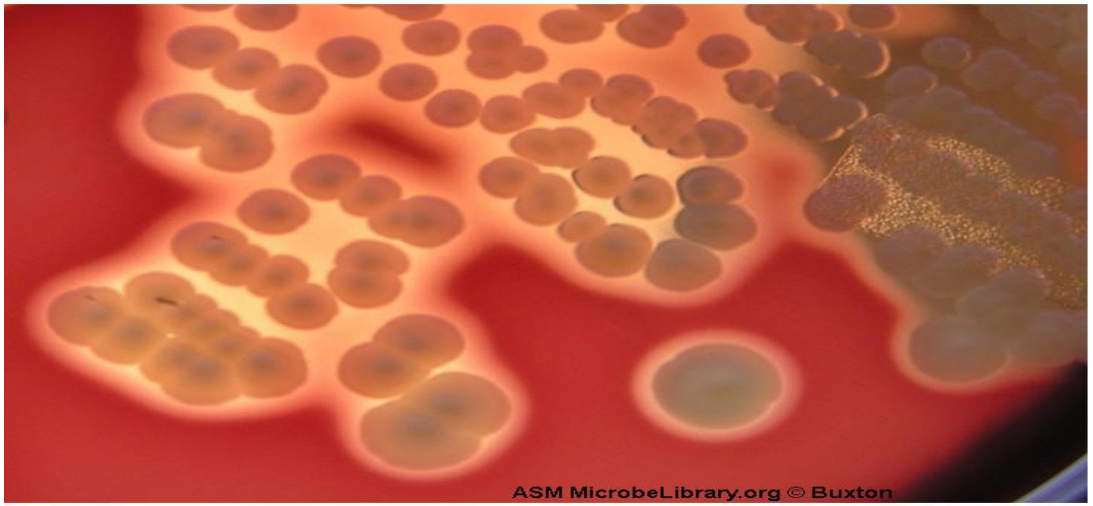
Only *Staphylococcus aureus* produce β-haemolysis on blood agar & ferment mannitol

Colonies on biood agar

a/ on blood agar (to detect type of hemolysis)

Some bacteria produce **hemolysins,** that cause red blood cells (RBC's) to burst open (hemolysed). On blood agar hemolysis is visible as an area of clearing around the colony (zone of hemolysis).

- If the organism **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**.



Beta hemolysis on blood agar

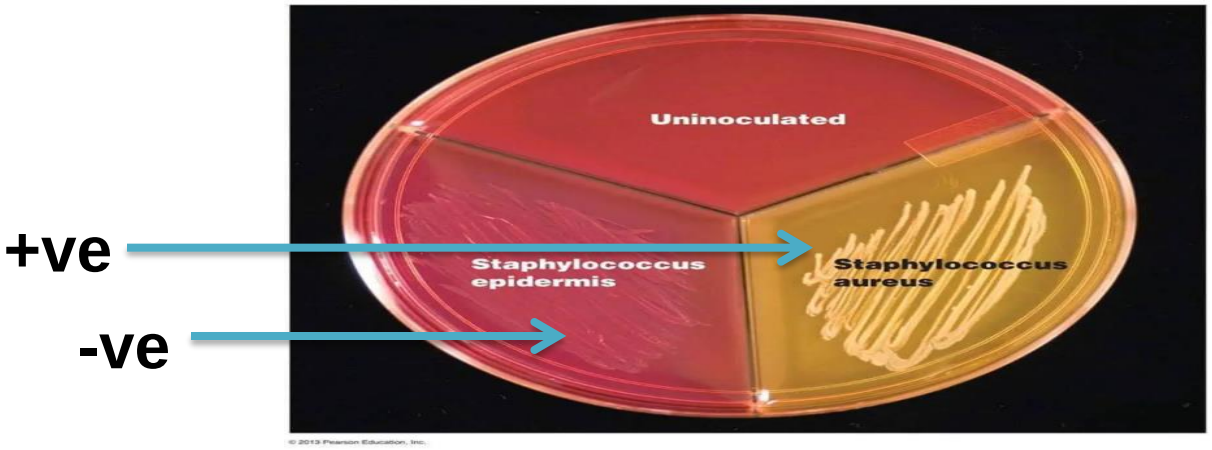
Staphylococcus aureus **cause β – haemolysis will appear around the colony on blood agar because of haemolysin enzyme**

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

b. on Mannitol Salt agar (MSA) to detect Mannitol fermentation

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).



Mannitol fermentation

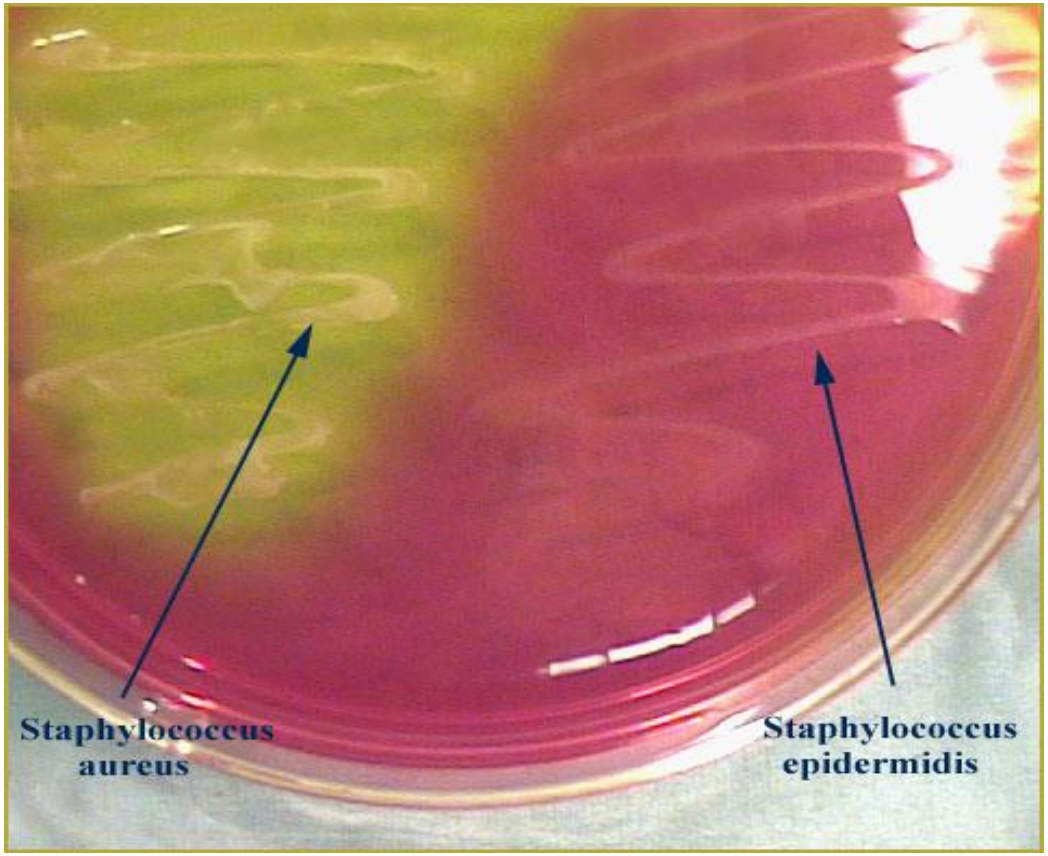
Positive = acid end products turn the phenol red pH indicator from red to yellow
negative = phenol red remains red

MANNITOL SALT AGAR

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)**

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

3. Biochemical tests

A / **Coagulase test**

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

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

B/ **Catalase test**

To differentiate between Staph. and Streptococcus

Coagulase Test

PURPOSE

- To determine the ability of the organism to produce coagulase which clots plasma.
- To distinguish the pathogenic coagulase positive staphylococcus from the nonpathogenic coagulase negative staphylococcus.

Principle:

- Coagulase is an enzyme that converts soluble fibrinogen into insoluble fibrin.

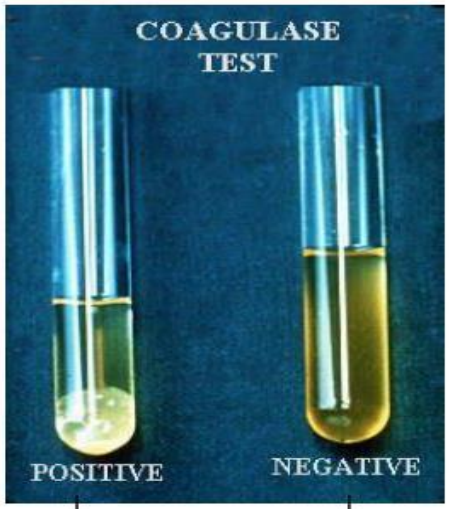
Two forms of coagulase:

1. Bound coagulase (clumping factor) –
   - Detected in the coagulase slide test
   - Can directly convert fibrinogen to insoluble fibrin and causes the staphylococci to clump together

2. Free coagulase
   - Detected in the coagulase tube test
   - Reacts with a globulin plasma factor (coagulase reacting factor-CRF) to form a thrombin like factor, staphylothrombin ---→ catalyzes the conversion of fibrinogen to insoluble fibrin

Coagulase test

- **The tube coagulase test (Free):**
- Procedure:
  - Mix 0.1 ml of culture + 0.5 ml of plasma
  - Incubate at 37C for 4 h
  - Observing the tube for clot formation
  - Any degree of clotting constitutes a positive test
- Advantage
  - More accurate
- Disadvantage
  - Time consumed



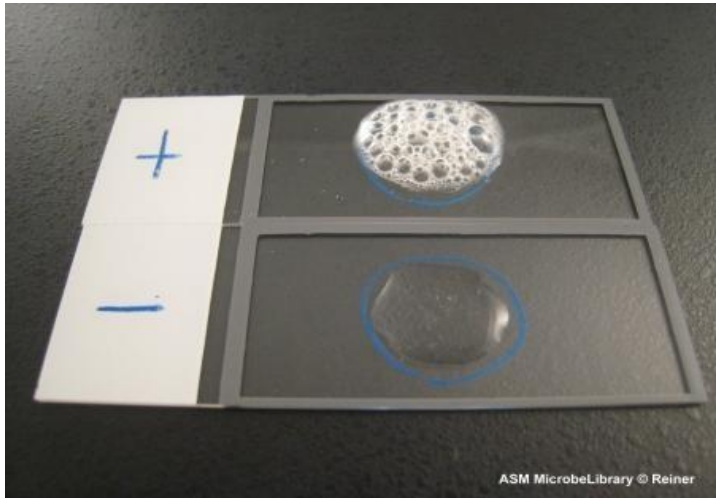
S. aureus *S. epidermidis*

- **The slide coagulase test**
- Procedure:
  - Used to detect bound coagulase or clumping factor
  - Add one drop heavy bacterial suspension and one drop of plasma on slide
  - Mixing well and observing for clumping within 10 seconds
- Advantage
  - Rapid diagnosis
- Disadvantage
  - Less accurate

Catalase test

Procedure:

1. Place a drop of 3% hydrogen peroxide on a clean microscope slide.
2. Place a heavy loopfull 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.
4. Lack of bubbles is a negative test.



Catalase

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

C/Novobiocin Sensitivity test

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

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

D/ 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***

golden yellow pigment of *Staphylococcus aureus by* production of staphyloxanthin

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

E/ Production of deoxyribonuclease (DNase) on DNase agar

DNase agar contains 0.2% DNA. To detect DNase production.

•The plate is Inoculate by making a single streak line using inoculum from an agar slant or plate and then incubated.

•After growth, the plate is flooded with 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.

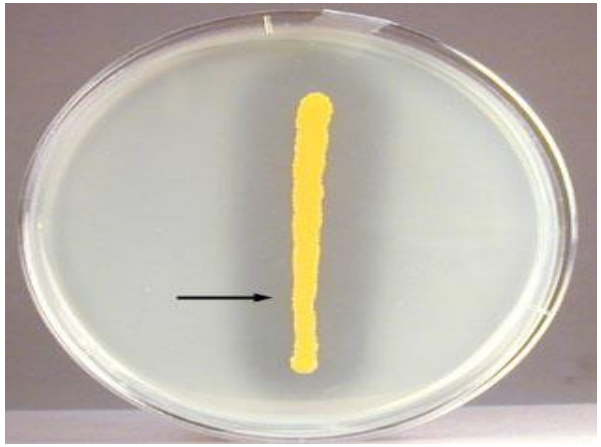
•**DNase negative cultures** appear cloudy around the growth where the acid caused the DNA in the agar to precipitate out of solution.

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

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

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

A Positive DNase Test

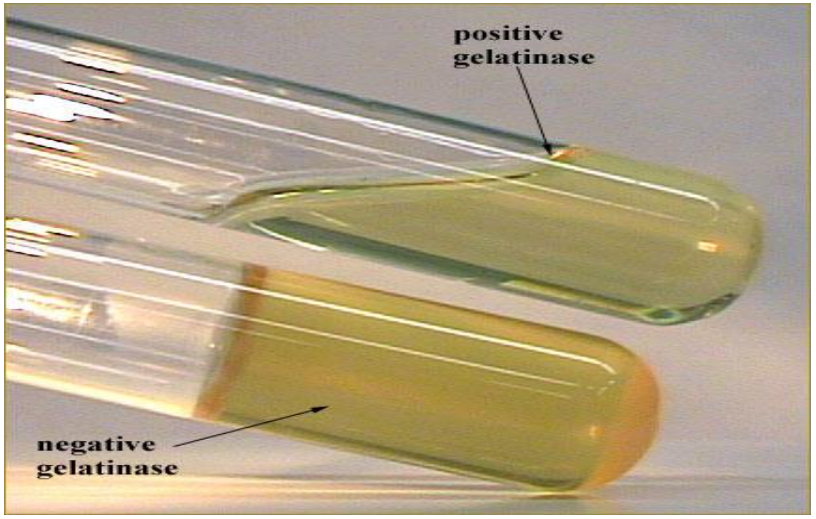


Note there is breakdown of the DNA in the agar. There is a clear zone (arrow) around the bacterial growth where there is no longer any DNA left in the agar to precipitate out of solution after the HCl was added.

F/ Gelatinase test

Nutrient gelatin is a differential medium that tests the ability of an organism to produce **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)	+	+	-

THANKS