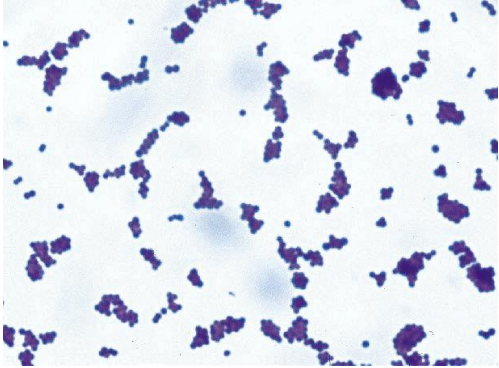


## Lab diagnosis---*Staphylococcus*

Sample: Nasal swab, skin swab, urine sample

### 1. Gram stain and KOH test



grapelike clusters of gram-positive cocci

### 2. Culture on Blood agar

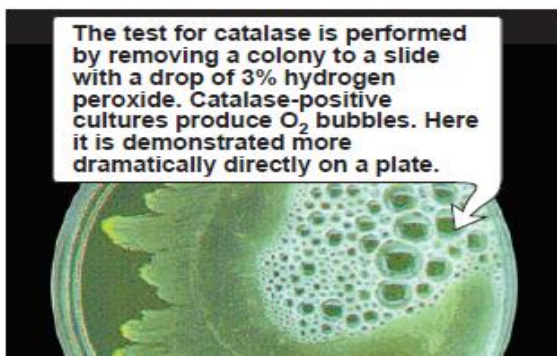


*Staphylococcus epidermidis* on **Blood Agar**. Notice the **white**, opaque, **nonhemolytic**, smooth colonies



*S aureus* on **Blood Agar**.  
-β large, smooth, **hemolytic** colonies. The lysis of the RBCs is due to alpha toxin

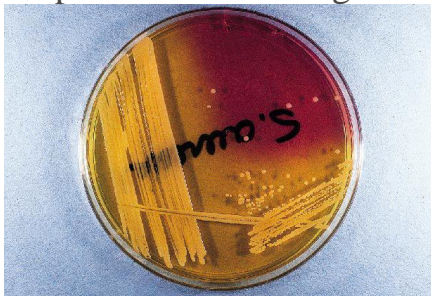
### 3- Catalase test



#### 4- Culturing on Mannitol salt agar

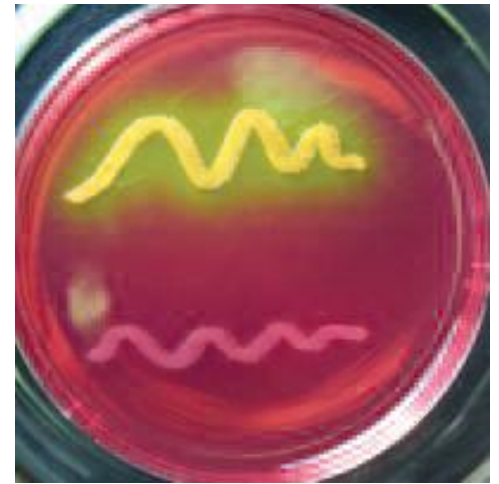
**Mannitol salt agar** is a commonly used growth medium in microbiology. It encourages the growth of a group of certain bacteria while inhibiting the growth of others. It contains a high concentration (~7.5%-10%) of salt (NaCl), making it selective for gram positive bacteria *Staphylococci* (and *Micrococcaceae*) since this level of NaCl is inhibitory to most other bacteria.

It is also a **differential medium for mannitol fermentors**, containing mannitol and the indicator phenol red. *Staphylococcus aureus* produce yellow colonies with yellow zones, whereas other *Staphylococci* produce small pink or red colonies with no colour change to the medium. If an organism can ferment mannitol, an acidic byproduct is formed that will cause the phenol red in the agar to turn yellow.



##### ***Staphylococcus aureus* on Mannitol Salt Agar.**

Notice that the medium has turned yellow around the growing bacteria since the bacteria are able to ferment the mannitol and produce an acid pH.

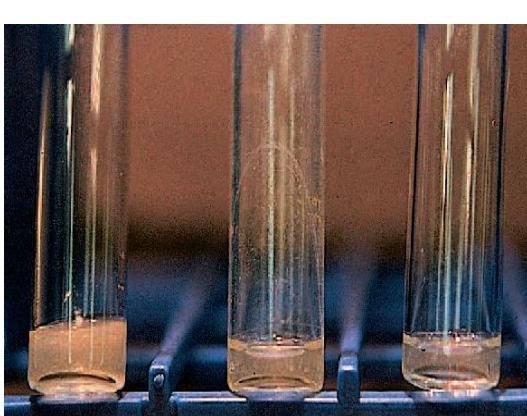


##### ***Staphylococcus epidermidis* on Mannitol Salt Agar.**

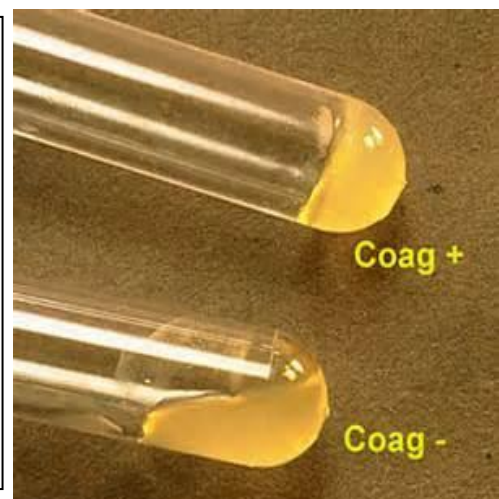
Notice the small white colonies that do not use the mannitol; that is, no color change is observed since no acid has been produced.



#### 5-Coagulase Test (tube coagulase test)

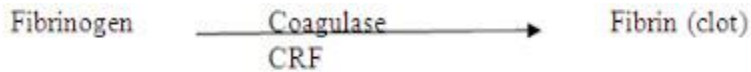


Coagulase producing strains of *S. aureus* form a clot (solid fibrin gel) when grown in plasma (tube on the left), whereas coagulase negative staphylococci (*S. saprophyticus* [middle tube] and *S. epidermidis* [tube on the right]) do not form a clot.



## Principle of Tube coagulase test:

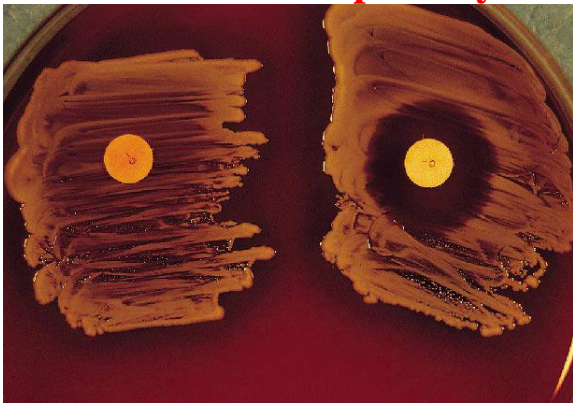
The free coagulase secreted by *S. aureus* reacts with coagulase reacting factor (CRF) present in plasma to form a complex, thrombin. This converts fibrinogen to fibrin resulting in clotting of plasma.



## Procedure for Tube coagulase test:

1. Take three test tubes and label them as “test”, “negative control” and “positive control”.
2. Fill each test tube with 1 ml of 1:6 dilution of rabbit or human plasma in normal saline.
3. Add 0.1 ml of overnight broth culture to the tube labeled test. Also add 0.1 ml of overnight broth culture of known *S. aureus* to the tube labeled positive control and 0.1 ml of sterile broth to the tube labeled negative control.
4. Incubate all the tubes at 37°C and observe up to four hours.

## 6-Novobiocin Susceptibility Test.

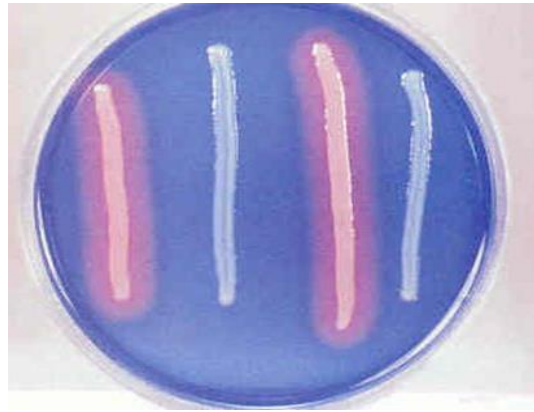
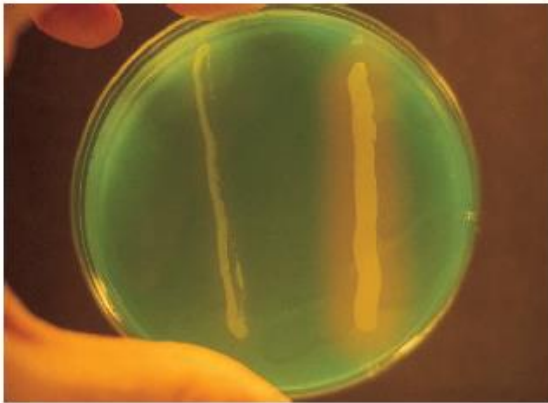


(Left on plate) Novobiocin resistance evidenced by lack of zone of inhibition (or a zone less than 17 mm) surrounding a novobiocin disk. Resistance is typical of *Staphylococcus saprophyticus*. (Right on plate) Novobiocin susceptibility evidenced by a zone of inhibition greater than 16 mm surrounding the novobiocin disk. Sensitivity is typical of *Staphylococcus epidermidis* and other coagulase-negative staphylococci, other than *S. saprophyticus*.

## 7- DNase test :

Pathogenic strains of staphylococci produce a nuclease enzyme called **DNase**. DNase degrades host DNA and increases the pathogenicity of staphylococci that possess it. To demonstrate the presence of DNase, agar containing dissolved DNA is spot inoculated with staphylococci. A zone of clearing around the colony indicates a positive DNase test. Three versions of the test are available, One formulation for DNase Agar consists of peptides, sodium chloride, and DNA as the substrate. After incubation, 1N HCl is added. Intact DNA will form a cloudy precipitate with the HCl, but not with nucleotides. Therefore, clearing around the growth is an indication of DNase activity (DNA hydrolysis). A modification of DNase test agar includes toluidineblue, which forms a blue complex with intact DNA, but appears pinkish when complexed with nucleotides. DNase activity is indicated by a pink

coloration around the growth. An alternate modification of DNase Test Agar contains methyl green dye. Bacterial colonies that secrete DNase produce clearing around the growth of DNase positive organisms.



(b)



Lab Work:

#### Materials required

1. Swabs for taking samples (nasal, skin), Normal saline (0.09% NaCl)
2. Syringe for taking blood plasma, heprinized tube
3. Preparation of blood agar, mannitol salt agar, DNase agar, Muller hinton agar
4. Gram stain (KOH), slides, Oil , HCL
5. 3% H<sub>2</sub>O<sub>2</sub>
6. Novobiocin antibiotic disc

#### First Period

##### 1. Taking sample

- Obtain a nose swab by rotating a moistened (0.85% saline) swab thoroughly around the perimeter of both nares (nostrils). Avoid touching the outside skin area.
- Obtain a skin culture by rolling a moistened swab up and down the arm. An alternative method is to swab underneath the fingernails of one hand.
- Obtain a urine sample (mid stream urine)

2. Inoculate Blood agar plate, Mannitol salt agar, Incubate for 24 hours at 37°C.

#### Second period

3. Choose one presumptive colony of *S. aureus* and *S. epidermidis* for further characterization. If you did not obtain one of each from your body, use a plate from one of the other students in the laboratory and inoculate the following:

a. Citrated rabbit plasma.

b. DNase test agar. Use a loop for inoculation. Incubate for 18 to 24 hours at 35°C. after incubation Flood the plate with 1 N HCl. **The acid precipitates unhydrolyzed DNA. DNA-ase-producing colonies are therefore surrounded by clear areas due to DNA hydrolysis**

c. Mueller-Hinton plate. Pencillin disc

e. catalase test.

### Lab report

Name: \_\_\_\_\_

Staphylococcus

Q1: Complete the following table.

#### Biochemical Results

Coagulase test (+ or -) \_\_\_\_\_

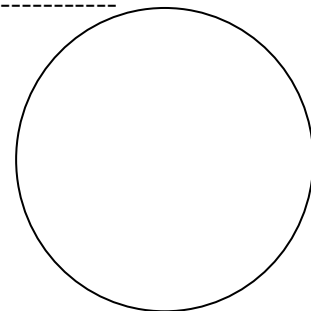
DNase test (+ or -) \_\_\_\_\_

Blood agar -----

Mannitol Salt agar -----

Antibiotic Susceptibility -----

Q2: Make a drawing of your DNase test agar plate observations.



Q3: Describe and draw the cellular morphology and arrangement of staphylococci after gram stain?

Q4: Describe and draw colony morphology of staphylococcus spp. ?

**Conclusion:**

**Shape**



**Margin**



**Elevation**



**Size**



**Texture**

Smooth or rough

**Appearance**

Glistening (shiny) or dull

**Pigmentation**

Nonpigmented (e.g., cream, tan, white)  
Pigmented (e.g., purple, red, yellow)

**Optical property**

Opaque, translucent, transparent