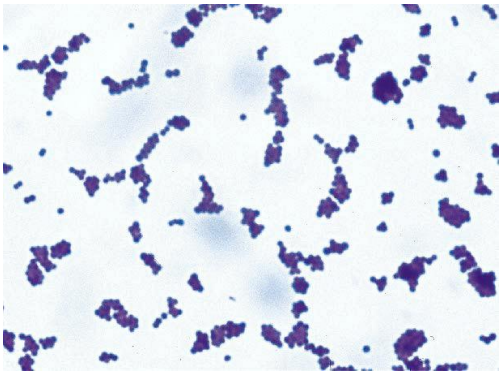
Lab diagnosis---*Staphylococcus*

Sample: Nasal swab, skin swab, urine sample

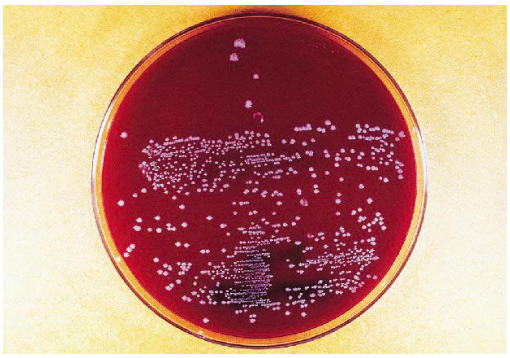
1. **Gram stain**



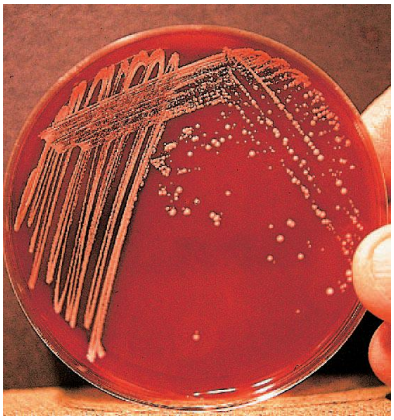
grapelike clusters of gram-positive cocci

2. **Culture:**

A. Blood agar



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



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

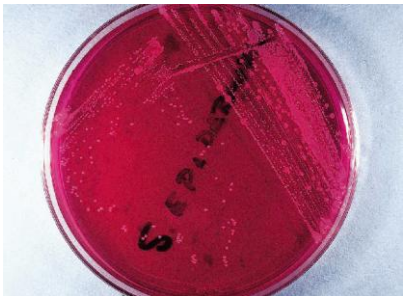
B. Mannitol salt agar

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.

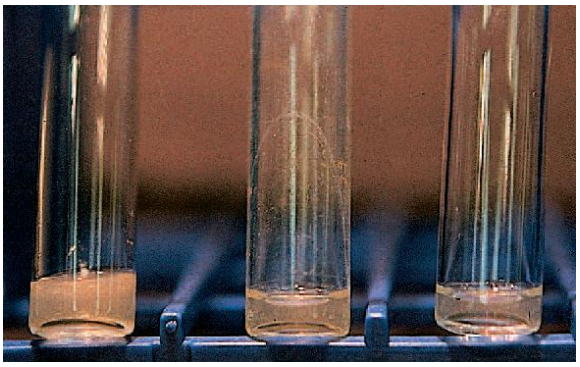
It is also **a differential medium for mannitol fermentors**, containing mannitol and the indicator phenol red. *Staphylococcus aureus* produce yellow colonies with yellow zones, whereas other *Staphylococci* produce small pink or red colonies with no colour change to the medium. If an organism can ferment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



***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.

3. Coagulase Test (slide and 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 Slide coagulase test:

Bound coagulase is also known as clumping factor. It cross-links α and β chain of fibrinogen in plasma to form fibrin clot that gets deposited on the cell wall of the cocci. As a result, individual coccus sticks to each other and clumping can be observed.

Procedure for Slide Coagulase test:

A. Emulsify few colonies of Staphylococci from culture in a drop of normal saline on two ends of clean glass slide.

B. Label one as “test” and the other as “control”. The control suspension serves to rule out false positives due to autoagglutination.

C. Mix a drop of rabbit or human plasma with the test suspension.

D. Observe agglutination or clumping of cocci. Agglutination within 5-10 seconds is considered as positive. Some strains of *S. aureus* may not produce bound coagulase, and such strains must be identified by tube coagulase test.

Slide Coagulase Test

Principle of Tube coagulase test:

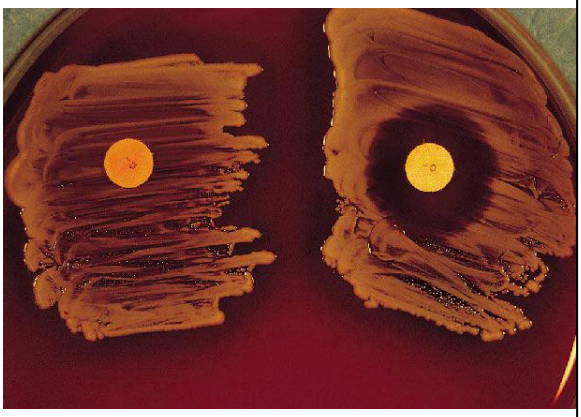
The free coagulase secreted by *S. aureus*reacts with coagulase reacting factor (CRF) present in plasma to form a complex, thrombin. This converts fibrinogen to fibrin resulting in clotting of plasma.

Fibrinogen —Coagulase / CRF→ Fibrin (clot)

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^{o}C and observe up to four hours.

4. **Novobiocin Susceptibility Test.**



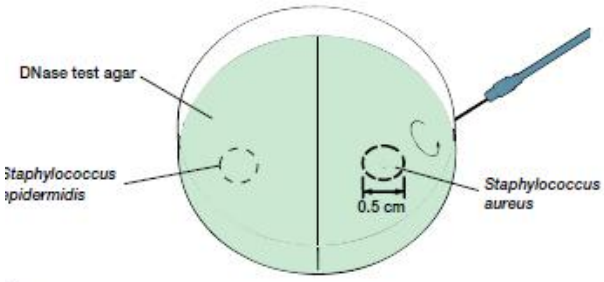
(Left on plate) Novobiocin resistance evidenced by lack of zone of inhibition (or a zone less than 17 mm) surrounding a novobiocin disk. Resistance is typical of *Staphylococcus saprophyticus.* (Right on plate) Novobiocin susceptibility evidenced by a zone of inhibition greater than 16 mm surrounding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

5. DNase test

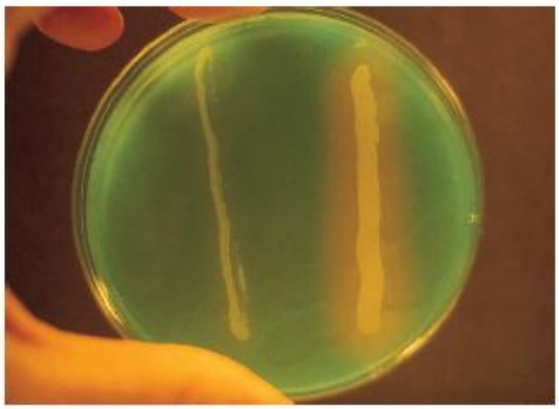
Pathogenic strains of staphylococci produce a nuclease enzyme called **DNase.** DNase degrades host DNA and increases the pathogenicity of staphylococci that possess it. To demonstrate the presence of DNase, agar containing dissolved DNA is spot inoculated with staphylococci. A zone of clearing around the colony indicates a positive DNase test.

DNase agar medium is **pale green in color** because of DNA-methyl green (indicator) complex (Note: Methyl green is a cation which binds to the negatively-charged DNA). It also contains nutrients for the bacteria.

If the organism that grows in the medium produces Deoxyribonuclease, it breaks down DNA into smaller fragments. When the DNA is broken down, it no longer binds to the methyl green, and **green color fades and the colony is surrounded by a colorless zone**

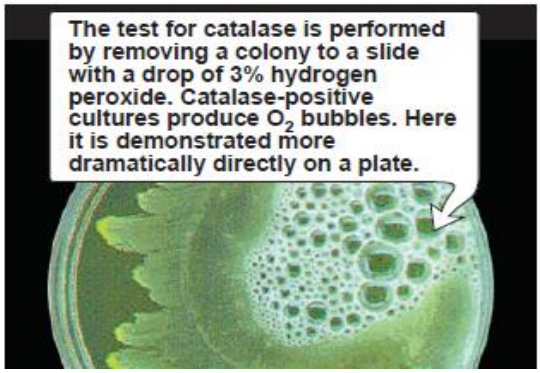


(a)



(b)

6. Catalase test



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_2O_2
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

d. Gelatinase activity. Test for gelatin hydrolysis

e. catalase test.

Lab report

Name: ——————————————————————

Staphylococcus
Q1: Complete the following table.

Biochemical Results

Coagulase test (+ or –) _____ _____

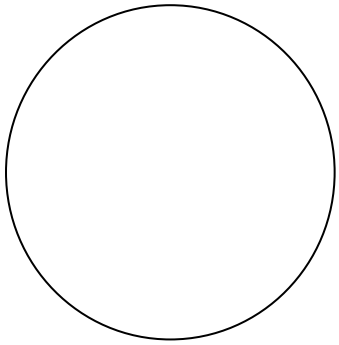
DNase test (+ or –) _____ _____

Blood agar ---------- ----------

Mannitol Salt agar ---------- ----------

Antibiotic Susceptibility ---------- ----------

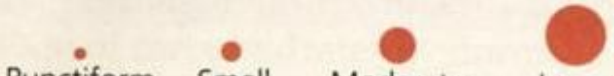
Q2: Make a drawing of your DNase test agar plate observations.



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