* Microbiology * Differentiation * Lab #4

→ Objectives:

- How to collect the throat swap?
- How to differentiate between different MO?

---

1. How to collect the throat swap?:

→ 3 Tools that are needed: - tongue depressor "spatula"
- sterile swap "to carry the swap"
- transport media

→ procedures:

1. Permission must be taken from the patients to take the swap
2. Explain to the patient what you are going to do, and recommend him to be helpful with you
3. Tongue depressor/ spatula is used to simplify the access to the pharynx
   → Note:
   in this step ask te patient to say "ah" helping you to access much more easily, this important in decreasing the chance for the required sample to be contaminated with other MO in nearby places
4. Internalize the swap stick into the pharynx and apply a swap on the lateral wall of the pharynx : Tonsiller area
5. Eliminate the swap rapidly and in straight way (without touching the tongue, mucosa of cheeks, or the saliva) so as not to be contaminated
6. Inoculate on the transport media, close it firmly
7. Write details about the corresponding patient (name/ date/ # of sample), then send it to the lab.

---

2. How to differentiate between different MO?:

A. (strepto vs. staph.):

→ both of streptococci and staphylococci are: gram positive
→ strepto. arranged in strepts, strains
→ staph. Arranged in clusters
→ to differentiate between them we apply the catalse test: We put H2O2 on the colonies: if there is bubbles then it's catalase positive → staph. Not strept

B. (Staphylococcus aureus vs. coagulase negative staphylococci):

→ the differentiayion through the coagulase test

→ most types of staphylococcus aureus are carachterized by having 2 types of coagulase:

1. Free coagulase : can be detected by test tube (plasma serum)
2. Bound coagulase: - can be detected using the slides
   - called the clumping factor:
   - when we put the bacteria in the plasma, it will crosslink with alpha and beta chains of the fibrinogen that exist in the plasma converting it to fibrin therefore forming a clot "clumps"

→ if a clot results from the test the it's aureus : coagulase positive

C. (myogenic vs. commensal streptococci):

→ differentiation between TYPES of streptococci "myogenic, commensal" is through the hemolytic activity:

1. Put the bacteria on BLOOD agar (blood= RBCs)
2. Observe the hymolytic activity : 3 types of responses
   - beta hemolysis:
     * clear zone around the colony as a result of the bacteria hemlysae that lysis the RBCs around the colony
     * clear zone = myogenic strepto. → pathogenic
   - alpha hemolysis:
     * greenish discoloration around the colony as a result of action of H2O2 "existed in the bacteria" on the hemoglobin by hydroxidizing it, converting it to green methmoglobin
     * alpha bacteria include: 1. Viridian streptococci
       2. Streptococcus pnemonea
   - no hymolysis :
     * neither alpha nor beta
     * include : 1. Streptococcus mutants "causative of carries"
       2. Sulvarious group|

→ Details of Beta hemolysis:

** Note: in beta hemolysis , we can't stop testing by only determining the pathogenicity of bacteria " myogenic " so it's streptococcus , noooo, we must go through further test", so we mustn't stop at hemolysis test.

→Lancefield grouping test:

- it's a serological test that depends on the different versions of the cell wall polysaccharides, because each subtype of streptococcus has a specific version of polysaccharides, and according to that, these subtypes are classified as groups:
    1. Beta hemolytic Group A
    2. Beta hemolytic Group B
    3. Beta hemolytic Group C
    4. Beta hemolytic Group D …. Etc.

- also in order to be sure that, it's a group A beta hemolysis, we do a test called passerine sensitivity test:
    → The concept of this test same as that of the antibiotic sensitivity test
    → put it on the media and next day we observe;
        - if there is a clear zone, then it's sensitive: meaning that it's group A beta hemolysis

---

→ Details of alpha hemolysis:

- as we said, alph include: - viridian
  - pnemonea

- How can I identify the streptococcus *pnemonea*: through 3 ways:

1. It's diplococci, then

2. Optician dic:
    - that contains chemicals that are toxic for some organisms "eg:pnemonea" and harmless for other MO, so when clear zone appear, then it's a streptococcus pnemonea

3. Bile solubility test:

- the bile salts that are found in the media, activates the enzyme found in streptococcus pnemonea called → Amidase
- once this enzyme gets activated, it will activate the autolysis process of the streptococcus pnemonea

→ By these 3 test, We can confirm that, this is alpha bacteria particularly → pnemonea

---

→ Bile esculin test:

- used to differentiate between the enterococci and the usual streptococci
- enterococci: → gram positive
  → cocci characterized by short chains less than 8 colonies

- we use the esculin test, because the esculin material can be hydrolyzed by the ENTERCOCCI converting it's color from green to black or dark brown
- then as the color of esculin media is converted from green to black, then the bacteria is enterococcus garm + bacteria

---

→ Dephtheroids:

- example : corynebacteria
- usuall, it's resemble the Chinese letters under the microscope
- it's gram +
- RODS either: - straight
  - or lightly curved

Practical part

→ Notes:

- swap is disposable
- in case if the transport media isn't available, we bring a tube filling it with saline and then put the swap in it then close it,,, this way protect the bacteria from getting dry
- the sample is cultured on blood agar and on cled

➔ **Blood agar**

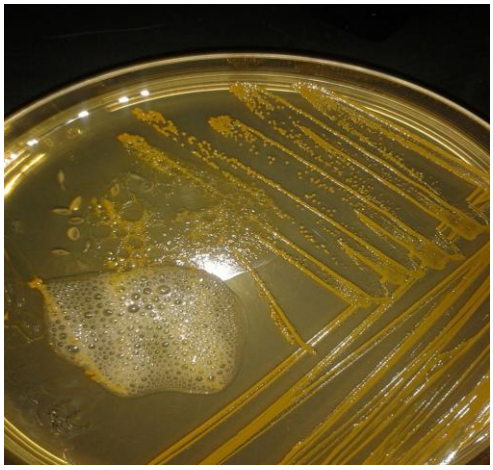
➔ **Appear on it most tyupes of bacteria because It's enriched "supplied by extra media"**

➔ **We expect after culturing that we will get:**

- **MIXED media, because the area from which we to the swap "pharynx" is contaminated with many types of bacteria other than the pathogenic one**
- **areas of hemolysis**

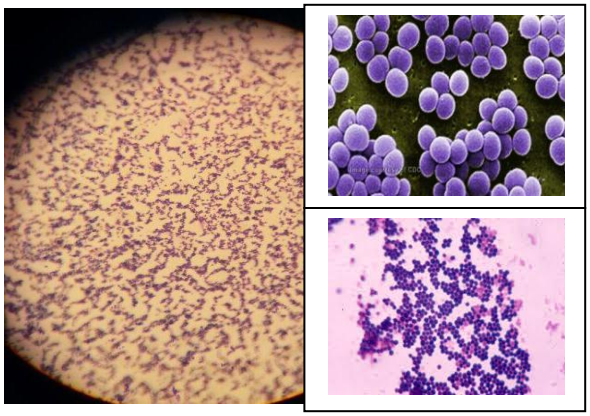
➔ **We must separate the bacteria in the mixed media by picking colonies of different sizes doing the:**

1. **Catalase test : to differentiate between staph(+) and strept(-)**
2. **gram stain: help us very much in identification of staph, strept, pne,onea, diphteroids, … etc**

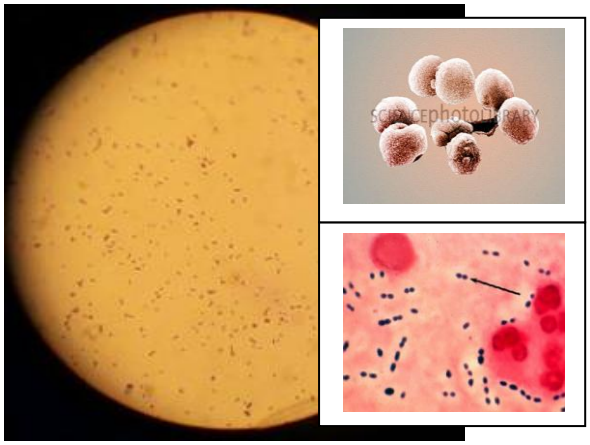


Catalase test:here we apply hydrogen peroxide on petri dish which contains the cultured microorganism so if the rxn is positive we can notice the air bubbles. This is staph.aureus and mostly all staph aureus are catalase +

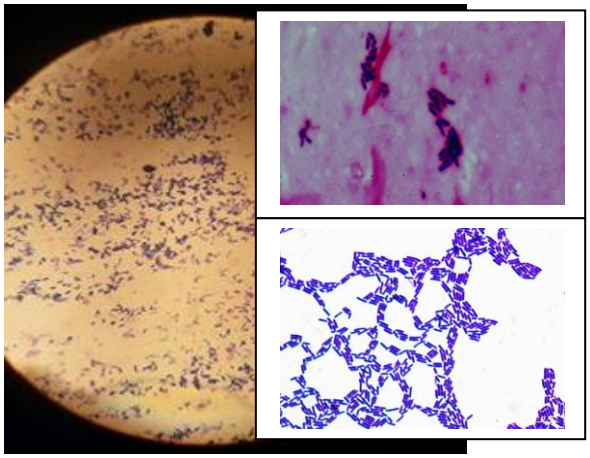
→Microscopal samples:



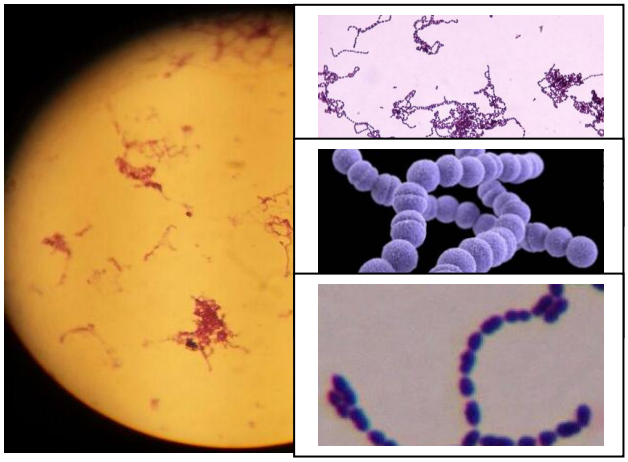
- **Staphcoccus aureus**
- **Gram +**
- **clusters**



- **Streptococcus pnemonea**
- **diplococcus**
- **Gram +**
- **lancet**



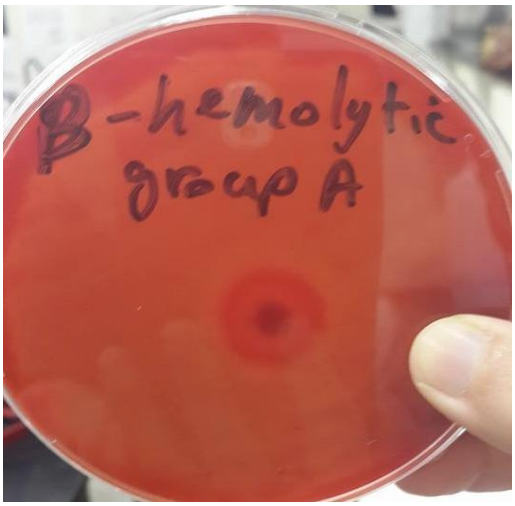
- **Diphteroids**
- **Gram +**
- **Chinese letters “rods”**



- **streptococcus**
- **Gram +**
- **chains**

➔ Beta hemolytic bacteria:

- after doing gram stain and differentiated staph from strept, then we pick one of these strept. Colonies and we do what's called ➔ sub-culture
- sub-culture: move the colony from media to another clean/new one for separation and purification
- candle jar: jar where the plates are put above each other, then we put a candle then we close it
- this candle will utilize some of the O2 found in the media releasing few Co2, providing a semi-anaerobic media for streptococci,,, after half to one min the candle will turn off because it utilized few O2 and release few Co2
- this semi-anaerobic media favoured by strept. Called ➔ micriaerobic condition
- then incubation, we will observe:



**** $ar6ain:**

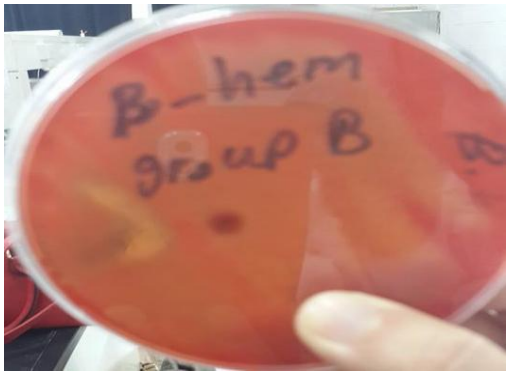
➔ beta hemolytic group A

➔ complete hemolytic activity "COMPLETE DISCOLORATION FOR BLOOD"

➔ with inhibition zone surrounding the optician ab discs and passitrasin disc at least 18mm ➔ type A

➔ the only bacteria sensitive to optician is pnemonea whether A or B

➔ if there is no inhibotio zone then it's viridan



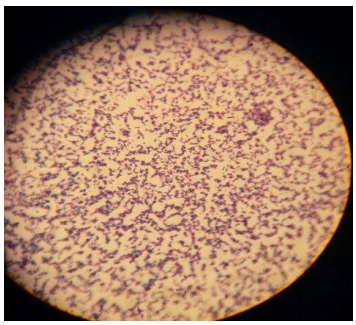
➔ **Beta hemolytic group B**
➔ **Complete discoloration of blood**
➔ **Optician test : + cuz it's pnemonea**
➔ **Passitacin test : - cuz it's B not A**

➔ **alpha hemolytic**
➔ **Partial discoloration of blood**
➔ **Redish → greenish/yallowish**

→ Staphylococcus:

- include many types : 1. Albus
  2. Areus
  3. Suprophyticus
- these types are isolated from different sites of infection, so it's important to know it's diagnosis

→ under the microscope:



- **gram+**
- **cluster shape**

➔ On agar:

- we isolate it on 3 types of culture media:

1.blood: - colonies of staph albus are large and white in color

→ Rule: if the colony was white on blood, then it will be white on cled and on mannitol, that's why it's called satph ALBUS "white" or epidermidis

→ on cled; there is no change in the color of the culture media

→ on mannitol is same result as cled : no change in color

2. Cled

3. Mannitol salt media: used to differentiate between the coagulase + and – bacteria

** To differentiate between albus and aureus:

1. In mannitol:

→ albus: no change in the color of media "pink→pink"

→aureus: change from pink to yallow

→ sthaph albus → coagulase -

→ staph aureus → coagulase +

2. On the cled agar: we take the sample from the blood agar "mixed" to cled "pure"

→ albus: no change in the media "original color of cled is blue"

→ aureus : change from blue to yallow

→ conclusion: - albus has a white color on blood/ mannitol/ cled

- aureus has a yallow color on blood/mannitol/cled

→ Staph. Aureus:

Changing the color of the media "blood" from red to yallow, then it's : staph aureus

→ + test: staph aureus

Changing the color of the media "cled" from blue to yallow, then it's : staph aureus

→coagulase test

Coagulase test:its a coagulase enzyme produced by several microorganisms that enables the conversion of fibrinogen to fibrin so we can notice the clumping on the glass slide if the rxn is positive and this results taken from staph.areus

→Esculin test:

Bile esculin agar test:its selective differential agar use to identify entrococcus resulting jn blackening of the medium if there is entrococcus

---

Done by: Dana Ayman