

وَقُلْ رَبِّ زِدْنِي عِلْمًا



RESPIRATORY SYSTEM

HAYAT BATCH



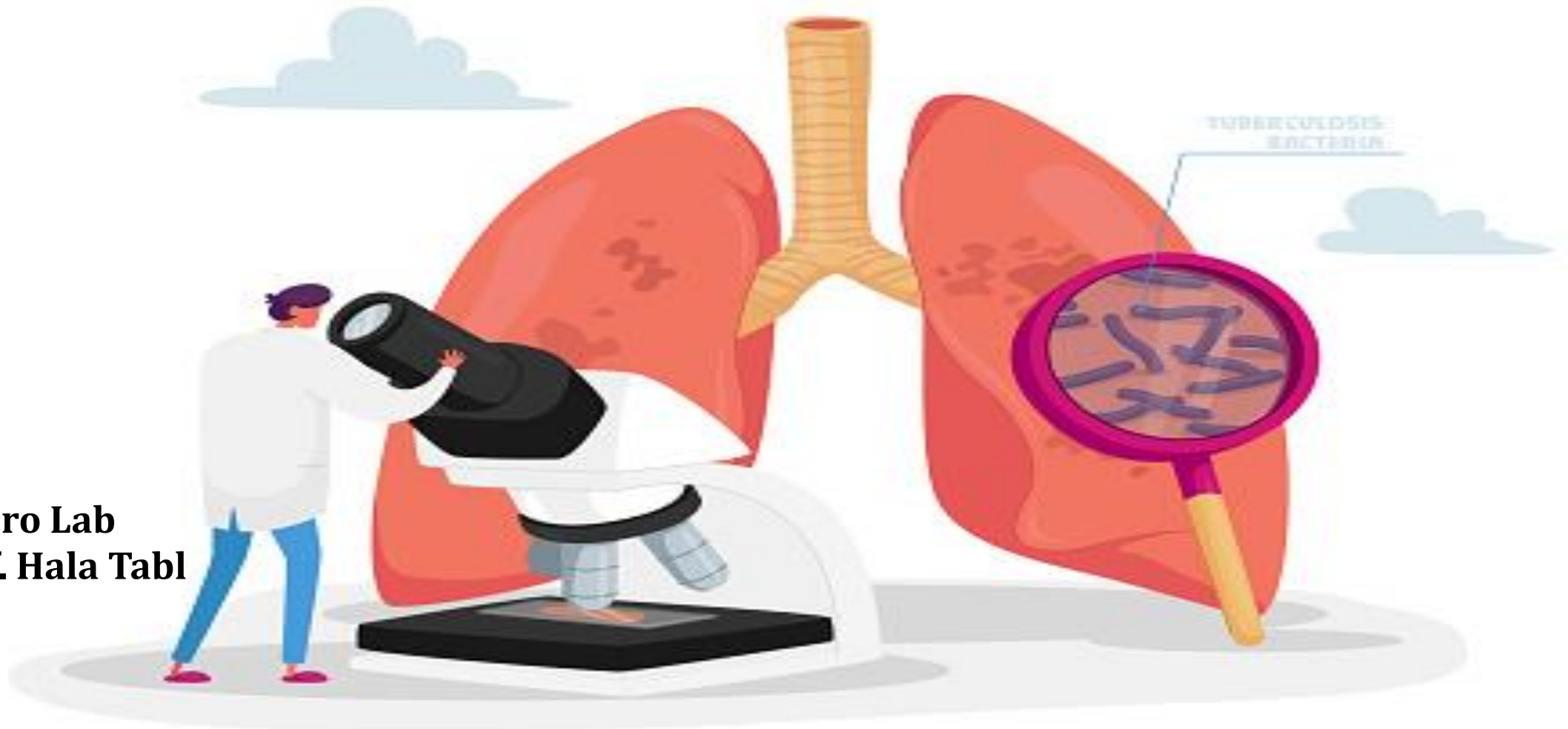
SUBJECT : Micro Lab

LEC NO. : _____

DONE BY : Lujain alzaareer

Laboratory Diagnosis of Respiratory Tract Infections

Micro Lab
By Prof. Hala Tabl



بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ

Sputum (phlegm)

((البلغم))

used in lower respiratory tract infection

The most commonly isolated organisms

Streptococcus pneumonia

Haemophilus influenza

Staphylococcus aureus

Klebsiella pneumonia

Pseudomonas aeruginosa



A) Collection of sputum sample

➤ Sputum either:

Ⓐ Expectorate, patient cough deeply and spit any sputum.

يضع المريض يده ويحاو ان يطالع البلغم

Ⓑ Induced sputum, produced by using nebulized hypertonic saline.

② يحر ب عي مجتير ③ حاج على الظهر ④ نستخدم
very thick ← sputum ال في حالة ال
نزل بيفتار ونصح
Saline حاد
Fluid بنسجه
* اذا كان عنده صعوبات بالعضلات ومنه قادر يطلع السعال
* في حالة ال sputum ال

➤ Collected into a clean, dry, wide-necked, leak-proof container.

➤ It is important that true sputum (not saliva).

➤ Sputum is best collected in the morning soon after the patient wakes.

➤ When pulmonary tuberculosis is suspected, up to three specimens may need to be examined to detect AFB.

لو نؤخذ ثلاث عينات خلال ثلاث أيام مختلفه

➤ The sputum should be delivered to the laboratory without delay because organisms such as

S. pneumoniae and H. influenzae do not survive well in specimens.

↓
"autolysis"



Sputum Sample Container

1

CLEAR YOUR MOUTH



Spit into water



2

BREATH IN AND OUT 3 TIMES



3

GIVE A SPUTUM SAMPLE





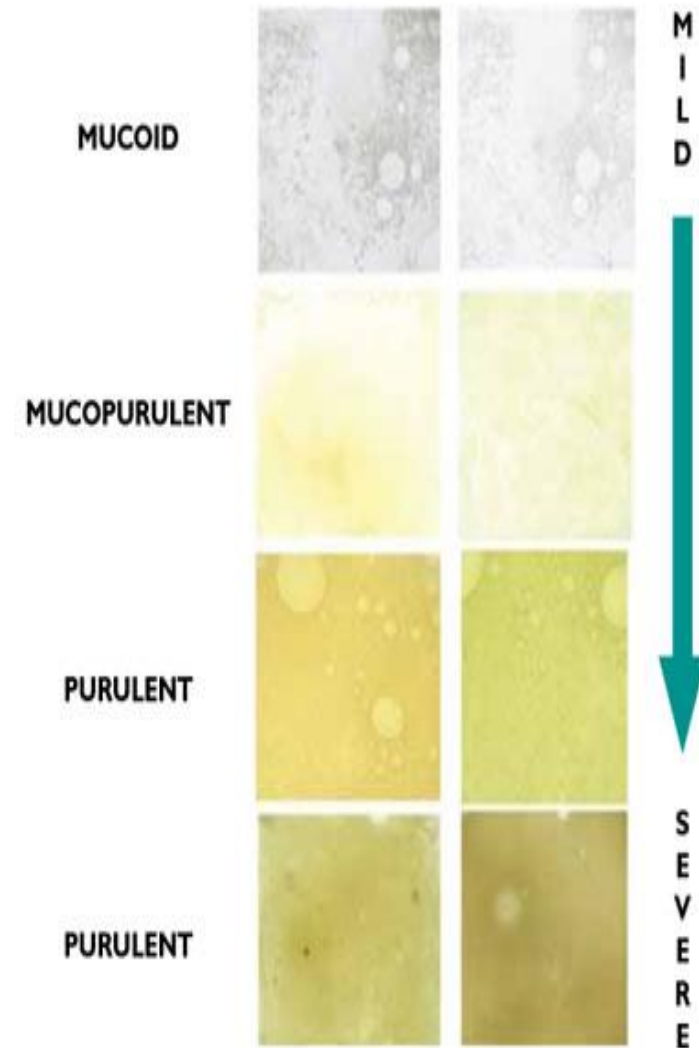
- **From lung.**
- **Thick & sticky.**
- Contains **many neutrophils** and **few epithelial cells.**
- **Accepted.**

- **From mouth.**
- **Thin & watery.**
- Contains **many epithelial** cells and **few neutrophils.**
- **Rejected.**

إذا كان في العينة Epithelial ما بعد عليها & نه فاي في sputum

B) Macroscopic examination of the sputum

- **Normal sputum:** Thin and clear colorless mucus.
- **Mucopurulent:** Yellow / green with pus and mucus.
- **Purulent:** Yellow / green, opaque, mostly pus (large numbers of white blood cells).
- **Bloody sputum (hemoptysis):**
 - ✓ Blood-streaked or Massive blood: could be due to lung cancer, tuberculosis, lung abscess.
 - ✓ Currant jelly sputum: *K. pneumonia*.
- **Rusty sputum** (due to decomposed Hemoglobin): it is typical for *S. pneumonia*.
- **Green / blue sputum** (due to exopigment): *P. aeruginosa*.



C) Microscopic examination of sputum: وَهَبْ لَنَا مِنْ لَدُنْكَ رَحْمَةً إِنَّكَ أَنْتَ الْوَهَّابُ ♥

1) Gram stained smear: routine

➤ Gram stained smears of sputum must be reported with caution. Cocci, diplococci, streptococci, and rods may be seen in normal sputum because these organisms form part of the normal microbial flora of the upper respiratory tract.

➤ Noting the predominant organism.

pathogenic organism: predominant in culture
له يعني يكون موجود بكثرة في العينة

➤ *Note:* When pus cells are present but no bacteria are seen in a Gram stained smear, this may indicate the presence of microorganisms such as *M. tuberculosis*, *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, *Legionella pneumophila* or viruses.

2) Ziehl-Neelsen smear: when M. tuberculosis infection is suspected.

سهم جذا
عليه سؤال

Decontamination & concentration (Petroff's method):

1. Liquefies the specimen, so release the tubercle bacilli.

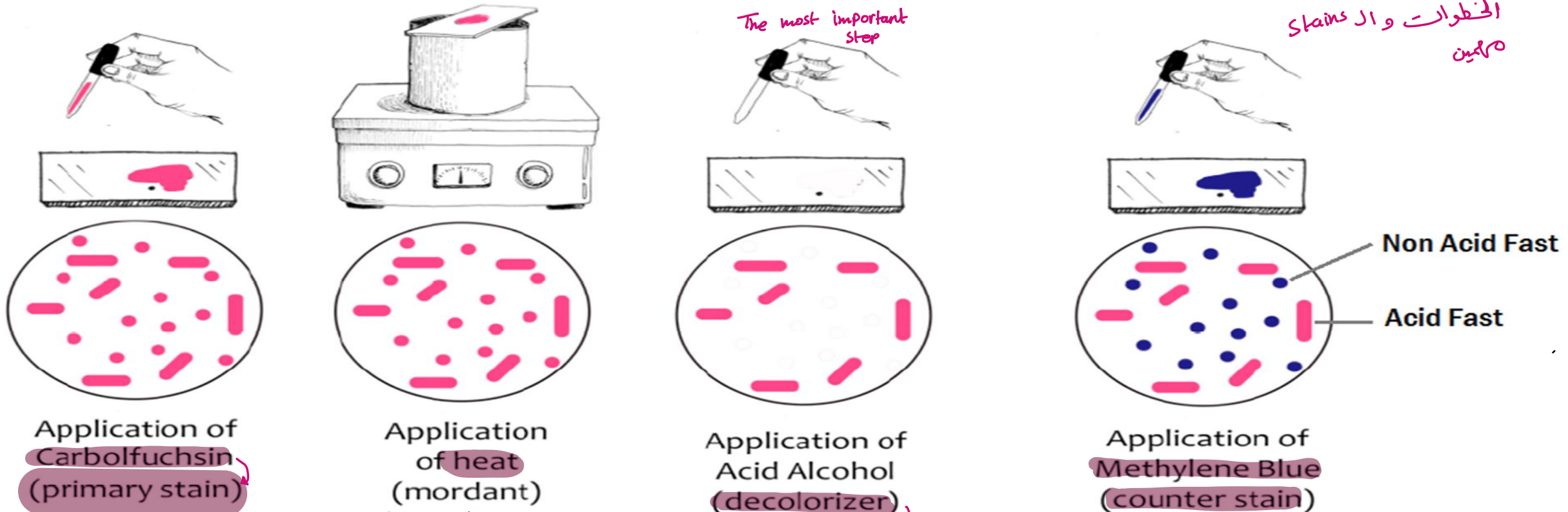
killing any bacteria and flora التي اكل

بترض م فن
alkaline
acidic
compound

2. Destroys bacteria other than T. B bacilli.

(concentration) Centrifuge و نعمل liquefies العينة حتى نكشف العينة للمخبرية وبعد يها نطها في

4% NaOH + specimen → incubate (30min, 37°C) → centrifuge (30min) → neutralize deposit by 8% HCL



The most important step

الخطوات و ال Stains
صالحين

نحط الريبعة وعليها organism و نحط عليها
primary stain

نسخن العينة بي ما
توصل للغليان تقريبا 60 درجة
حيث كل organism اخذت صبغة حمرا

أشبه الصبغة عن السريعة ذلك حتى يبروز عنه
الصبغة ال (TB) تحفظ بالصبغة وما يبروز عنها
فان هيكون بنميتها acid fast bacilli

الصبغة يكون لونها ازرق وكل السريعة لونها ازرق ال TB يكون لونها
احمر

3) Potassium hydroxide (KOH) or Lactophenol cotton blue preparation:

When Aspergillus infection is suspected.

ہا سوکت کرنا

4) Saline preparation:

When paragonimiasis is suspected.

5) Giemsa stained preparation:

When histoplasmosis or Pneumocystis infection is suspected.

D) Cultivation of sputum:

نؤخذ العينة وبعمل روتيني بزعمها (blood , cho chate , Macconkey) وبعرفها مرة aerobic و مرة anaerobic (إذا شكيت ب lung abscess) هيك صارو ستة عينات

A) Culture the specimen on Blood agar and chocolate agar and MacConkey agar.

Incubate the blood agar plate aerobically (and anaerobically, in lung abscess) and the chocolate agar plate in a carbon dioxide enriched atmosphere.

B) Culture on Lowenstein-Jensen (L-J) medium.

When M. tuberculosis infection is suspected.

C) Sabouraud's agar

When fungal infection is suspected.

E) Identification of the causative organism:

Based on colonial characters, biochemical tests and special tests according to the organism.

F) Antimicrobial susceptibility tests as required.

Throat swab

Upper Respiratory tract infection

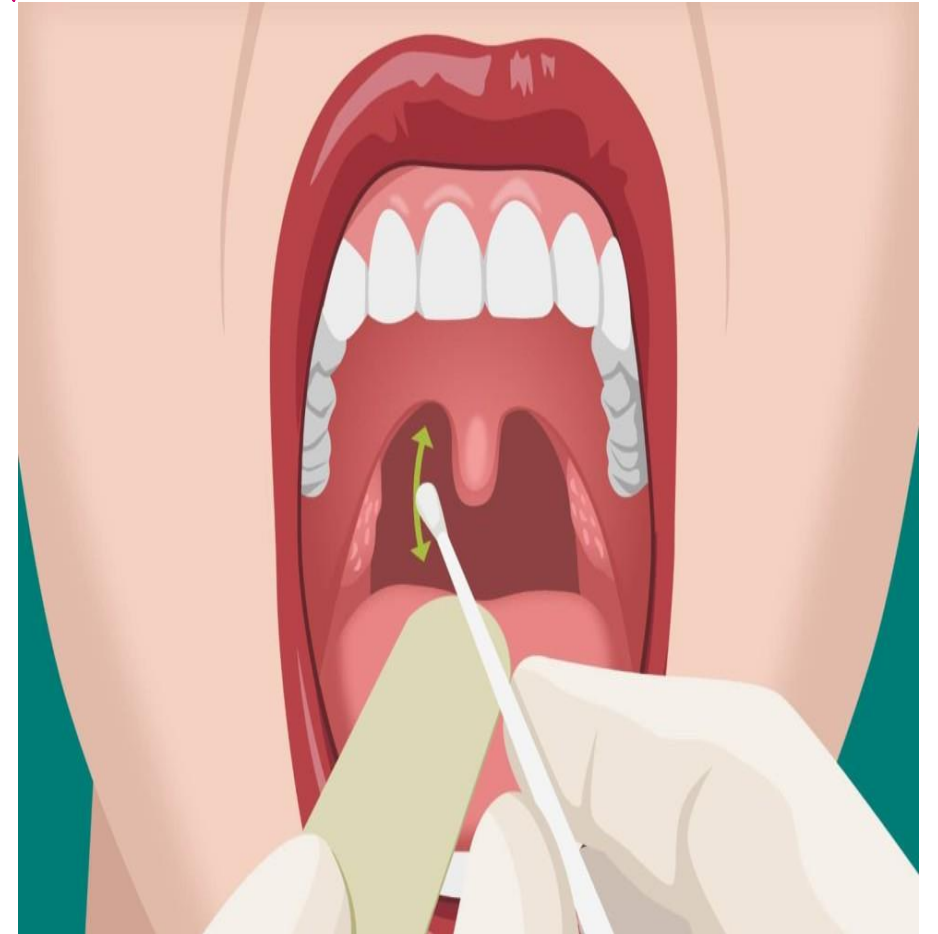
The most commonly isolated organisms

Streptococcus pyogenes

Corynebacterium diphtheria

Vincent's organisms

Candida albicans



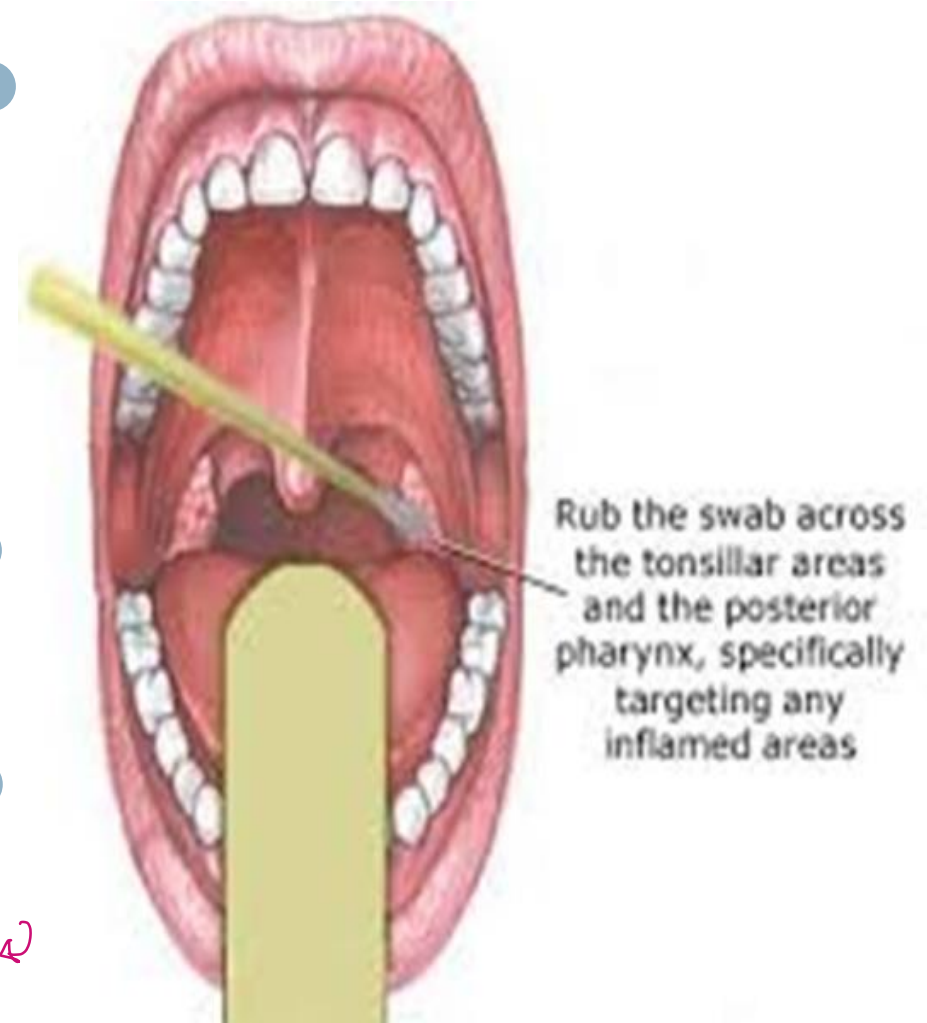
A) Collection of throat swab:

- Whenever possible throat swabs should be collected by a medical officer or experienced nurse.
- In a good light and using tongue depressor.
- Look for inflammation, membrane, exudate, or pus.
- Swab the affected area using a sterile cotton-wool swab.
- Taking care not to contaminate the swab with saliva.
- Important: For 8 hours before swabbing, the patient must not be treated with antibiotics or antiseptic mouth-washes (gargles).

① أخذ العينه من منطقة ال inflammation

①

② قبل ٨ ساعات من أخذ العينه صرع يوفد كالمضاد antibiotics او غرغرة



B) Microscopic examination:

Gram stain:

- No attempt should be made to report routinely other bacteria in a Gram stained smear from a throat swab because the throat contains a wide variety of commensals that cannot be distinguished morphologically from pathogens.
- Noting the predominant organism.
- When thrush is suspected, look for Gram positive Candida yeast cells.

C) Culture of throat swab:

- Blood agar culture.
- Blood tellurite agar if *C. diphtheris* suspected.

وَأذْكُرْ رَبَّكَ إِذَا نَسِيتَ

- سبحان الله

- الحمد لله

- لا إله إلا الله

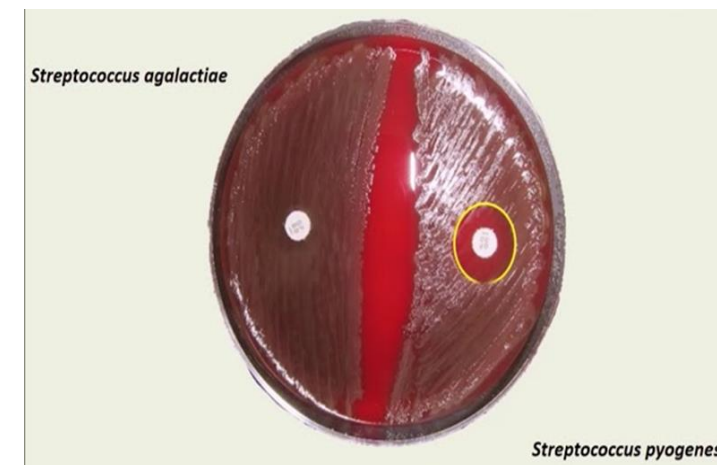
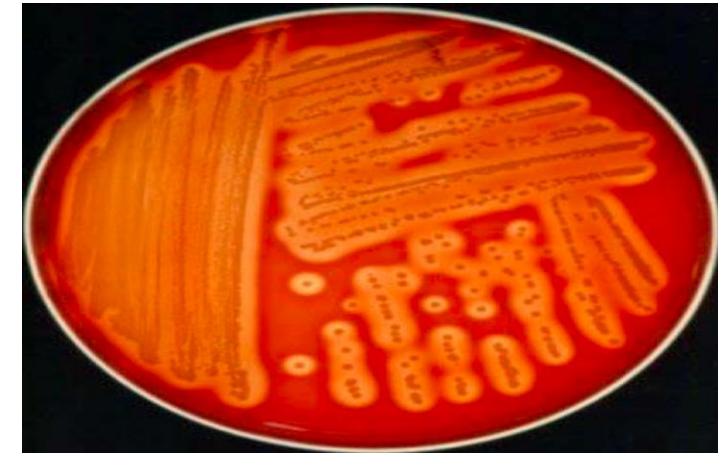
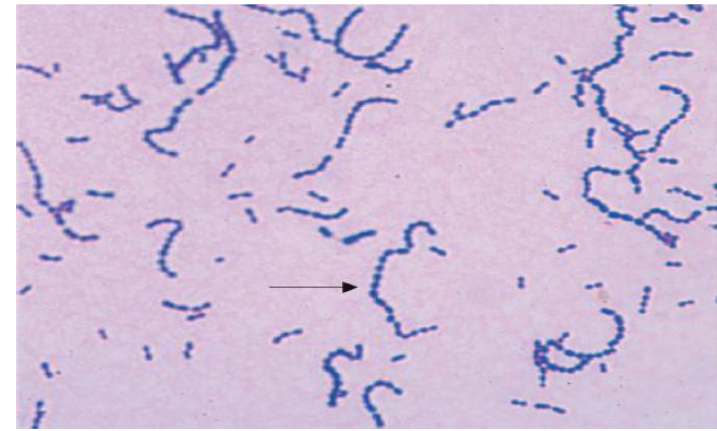
- والله أكبر

- أستغفر الله العظيم وأتوب إليه

- لا حول ولا قوة إلا بالله العلي العظيم...♥

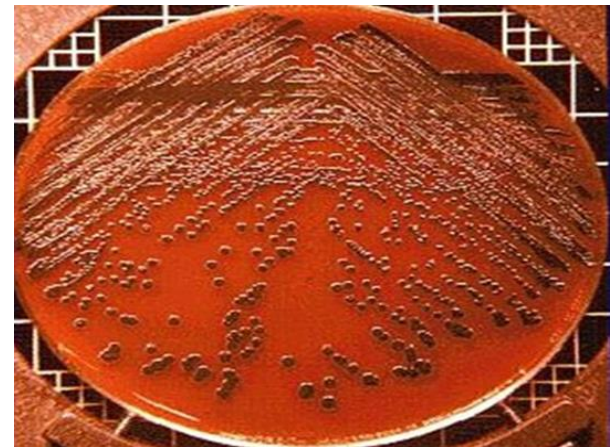
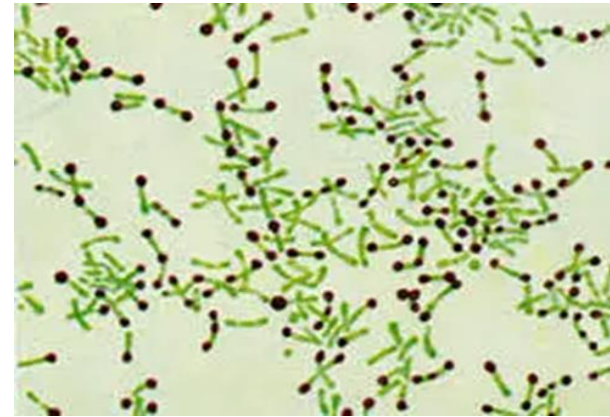
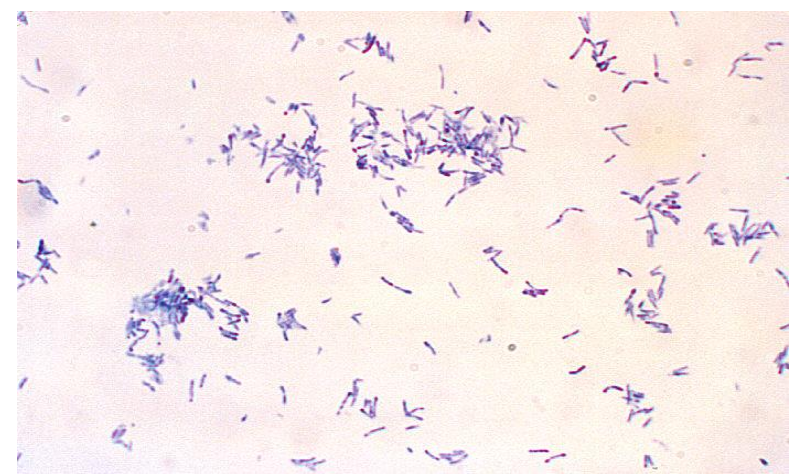
Streptococcus pyogenes

- **Gram-positive cocci**, arranged in **chains**.
- **Complete (Beta) hemolysis** on blood agar.
- **Catalase negative** (Differentiate with Staphylococci which are catalase positive).
- **Bacitracin sensitive** (Differentiate with other beta hemolytic streptococci such as *S. agalactiae* which is bacitracin resistant).



Corynebacterium diphtheria

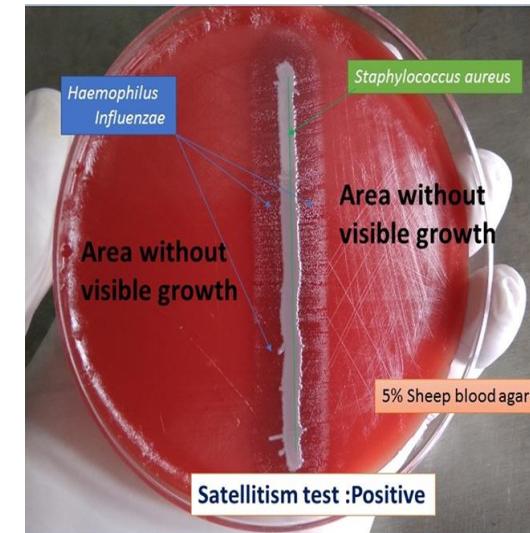
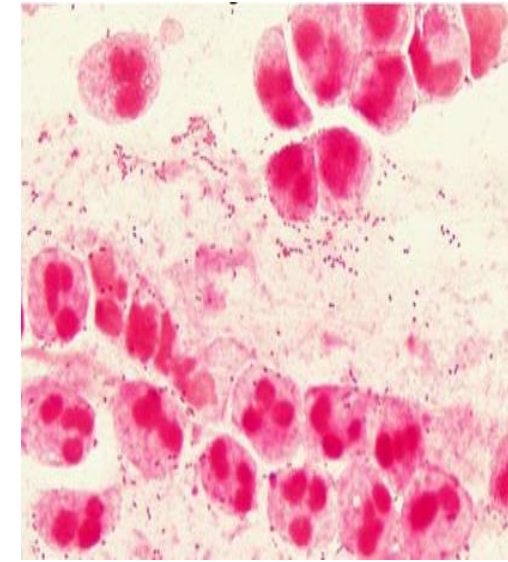
- **Gram positive rods.**
- **Non-spore-forming.**
- **Chinese-letter appearance.**
- **Metachromatic or volutin granules. Best seen by methylene blue or Neisser or Albert's stain.**
- **Black colonies on blood tellurite agar (Selective medium)**
(blood agar + 0.04% potassium tellurite)



Haemophilus influenzae

* كروية

- Gram negative coccobacilli.
- Quellung reaction positive. → يعني [Capsulated]
- Grow on chocolate agar.
- X&V factor test: requires both factors.
* كروية
- Grow close to colonies of Staph aureus.
* كروية
x/v factor فبعطي لنا blood hemolysis مؤول عن S. Aureus له & Satellitism
- Produce NO hemolysis.

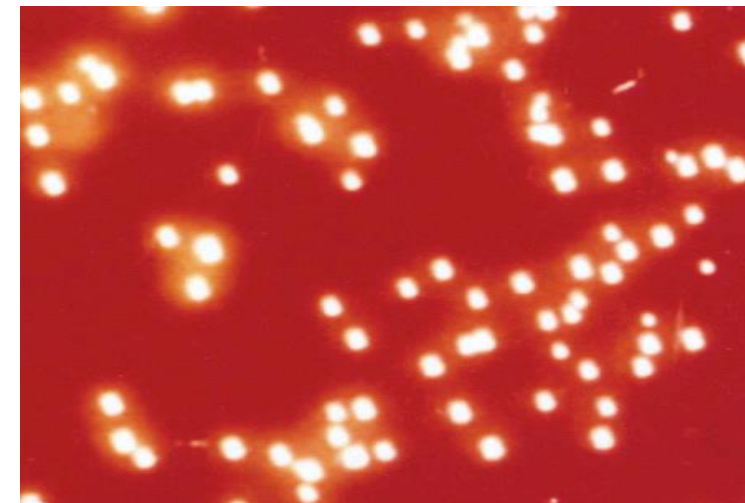


Bordetella pertussis

- Gram **negative coccobacilli**.
- **Grows on:**
 - ✓ **Bordet Gengou medium**
 - ✓ **Charcoal-cephalexin blood agar.**
- Colonies are greyish white with shiny convex surface “**Mercury drop**” appearance.
- Does **NOT** require X and V factors.



Charcoal-cephalexin blood agar

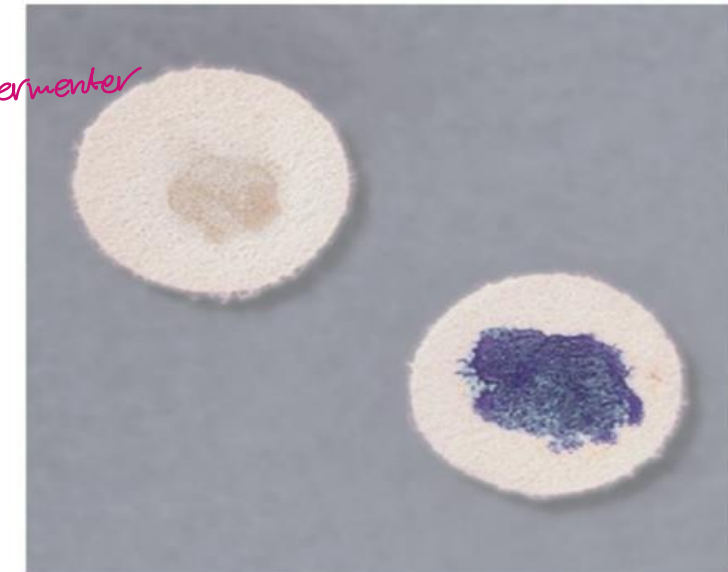
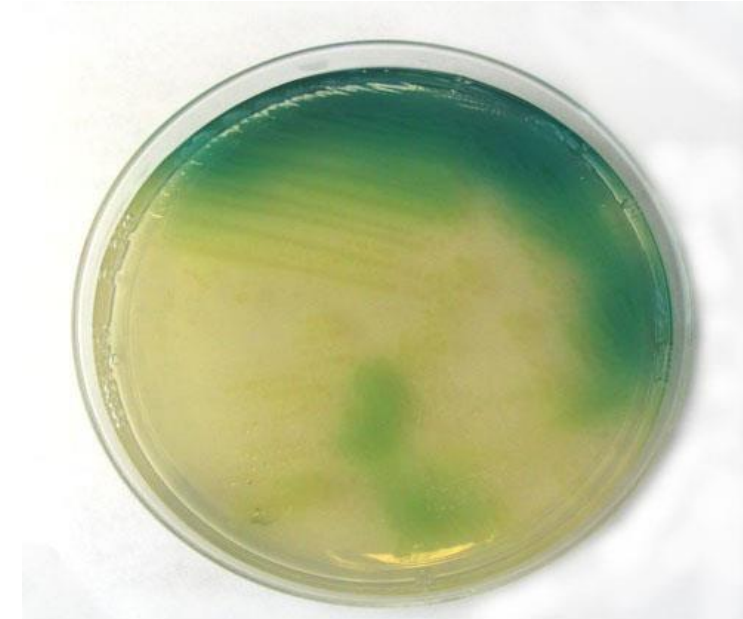


Bordet Gengou medium

Pseudomonas aeruginosa

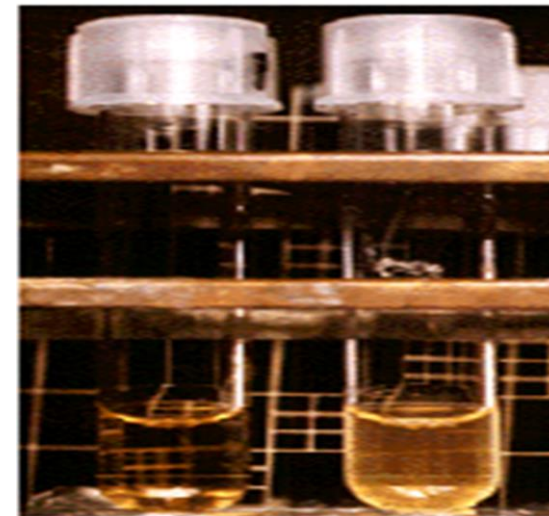
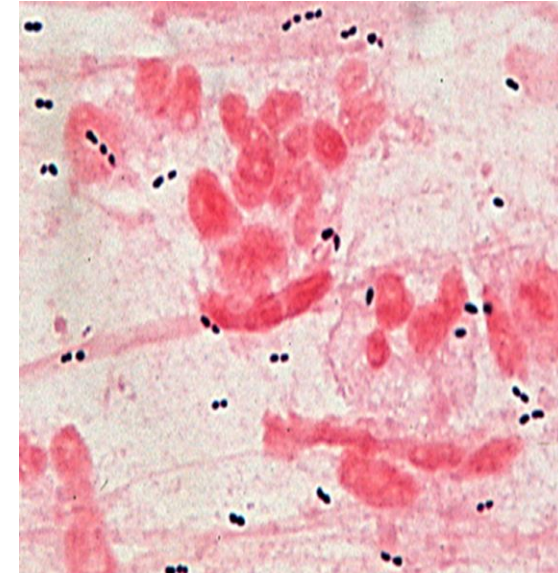
سالمونيلوز

- Gram negative bacilli.
- Motile. سالمونيلوز
- Growth at 42°C. سالمونيلوز
- Sweet or grape like odor (fruity aroma).
- Pale yellow colonies on MacConkey's. → Non lactose fermenter
- Produce exopigments. سالمونيلوز
- Oxidase positive.



Streptococcus pneumoniae

- Gram-positive, diplococci.
- Capsulated, capsule appears as unstained halo.
- Quellung reaction positive. *H. influenzae*
S. pneumoniae
- Alpha haemolysis on blood agar.
- Optochin sensitive. *Killed by it*
- Ferment Inulin.
- Soluble in bile. *تذوب في المرارة* → *يعني لا ينجس عليه* (bile) *Clearness*
- Catalase-negative.

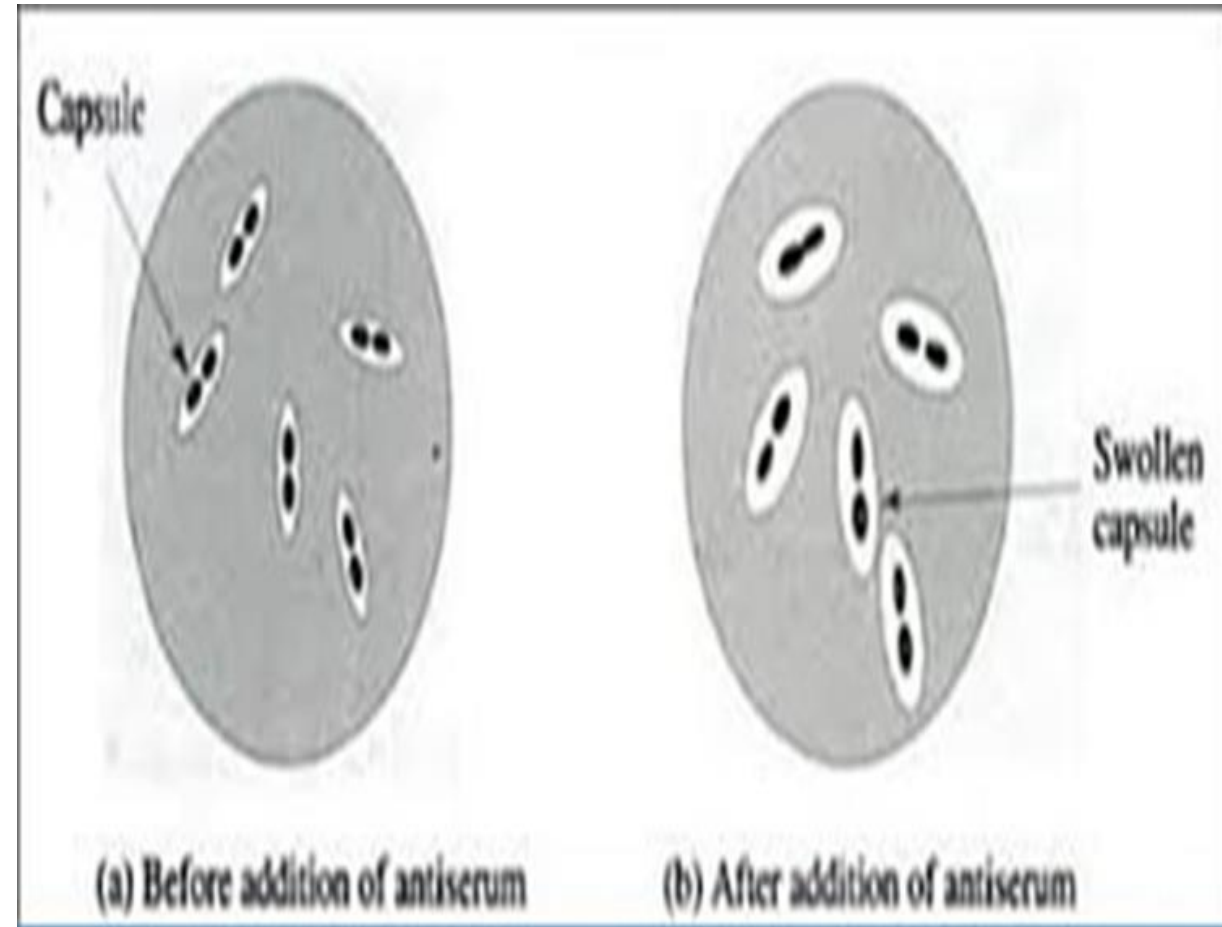
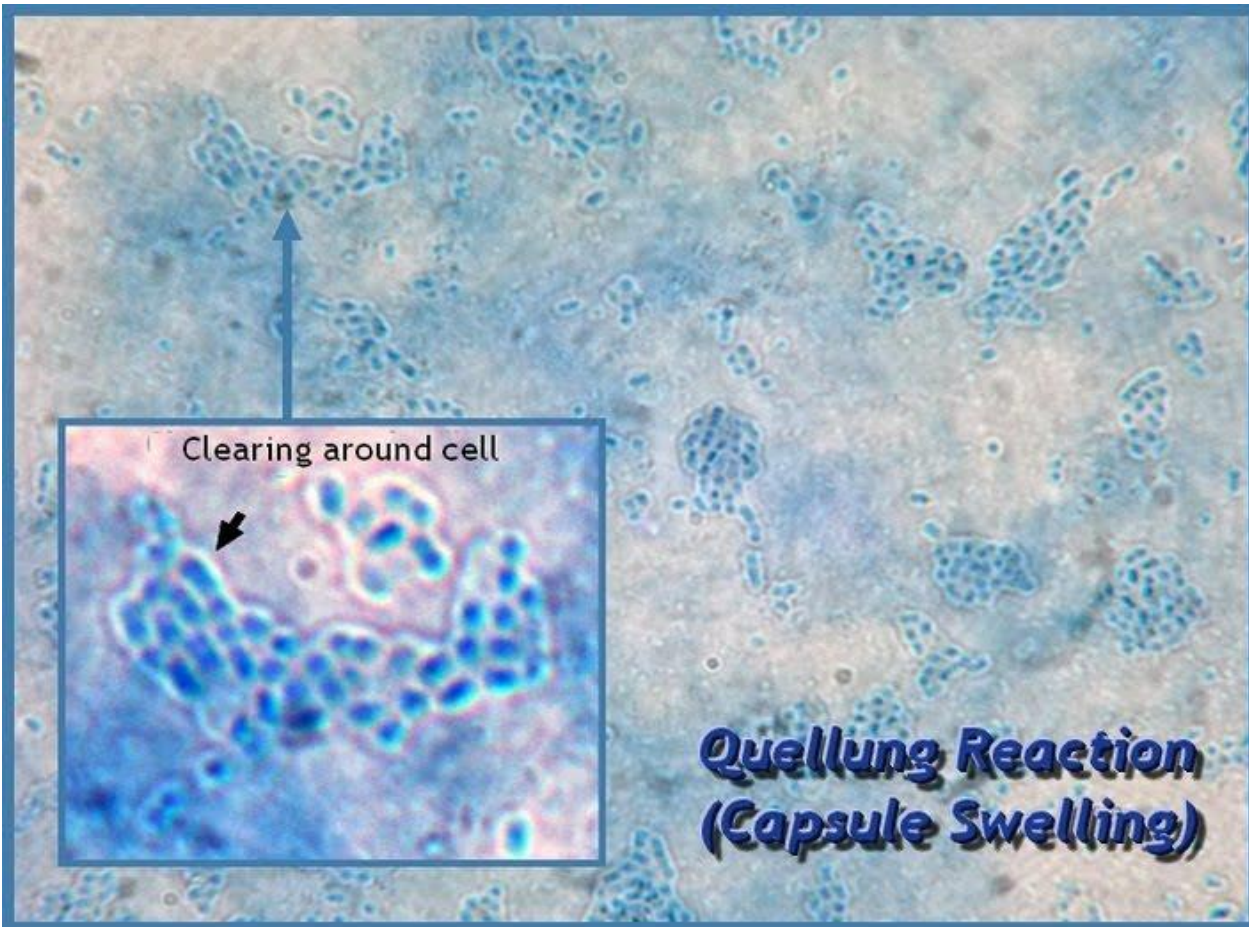


Pneumo + bile
Pneumo + broth

Quellung reaction (Capsule swelling test)

CSF or Sputum + Specific antiserum + Methylene blue stain

→ The capsule become swollen.



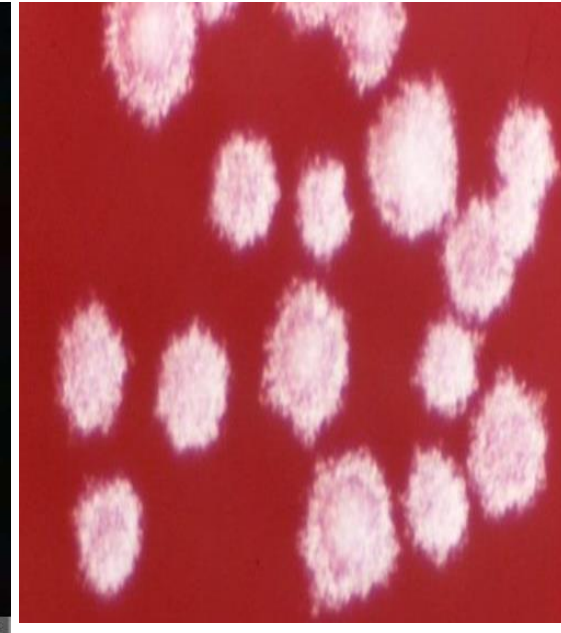
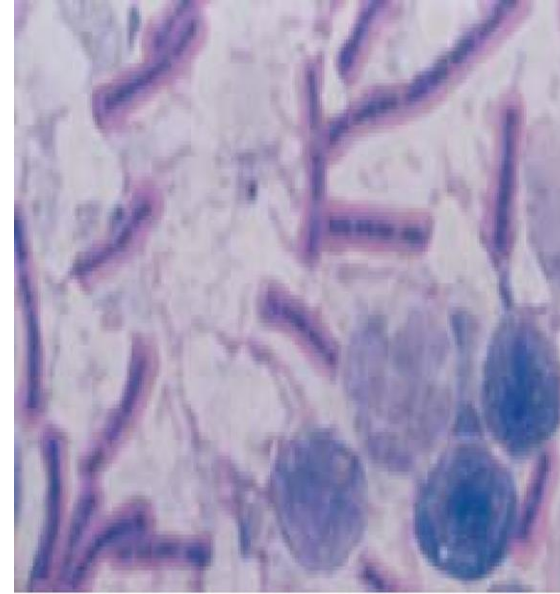
Klebsiella pneumoniae

- Gram-negative bacilli.
- Non-motile.
- Rose pink colonies on MacConkey's (lactose fermenter).
- Colonies are big, high convex with mucoid appearance.



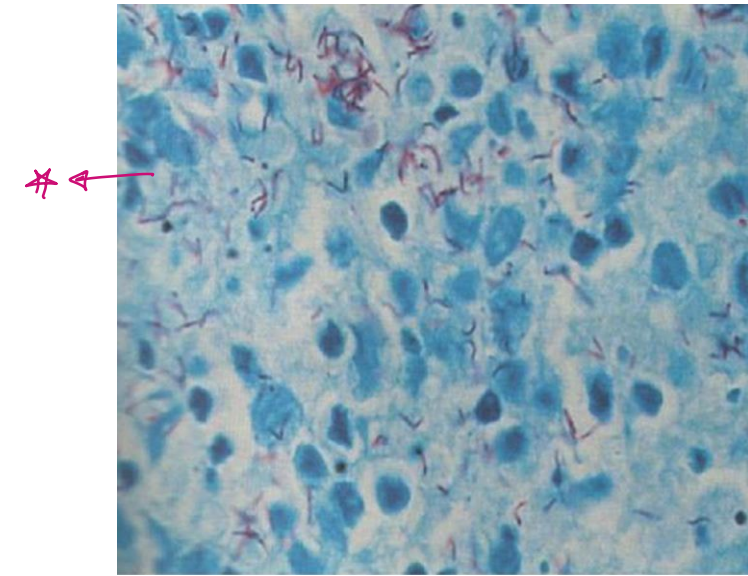
Bacillus anthracis

- Gram positive bacilli arranged in chains.
- Sporulated, the spores are oval, central.
- With polychrome methylene blue, the organism stains blue while the capsule purplish. (McFadyean's reaction)
- Colony is large opaque with rough surface and fimbriate edge (medusa head).
- Liquefies gelatin (inverted fire tree).



Mycobacterium tuberculosis

- Acid fast bacilli (Pink rods against blue background) by Ziehl-Neelsen stain.
- Obligate aerobe.
- Slow growers, growth appears after 4-6 weeks.
- Selective medium, Lowenstein-Jensen (L-J).
- Alternative media, Middlebrook's 7H10, 7H9.



Thank

you

* التريزي
TB ٣ عينات نوض ، Morning sample ، Z.N stain ،
↓
the best
de Commination and Concentration ، نكزي البناي

Case + صورة عليها
بكون واضحة

سُورَةُ الْقَصَصِ
بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ
رَبِّ إِنِّي لِمَا أَنْزَلْتَ إِلَيَّ مِنْ خَيْرٍ فَقِيرٌ ﴿٢٤﴾
"My Lord, I am truly in need of
any good that you would send down to me."
[Surah Qasas, Ayah 24]



و بس والله
موفقين يا رب



