

وَقُلْ رَبِّ زِدْنِي عِلْمًا



RESPIRATORY SYSTEM

HAYAT BATCH



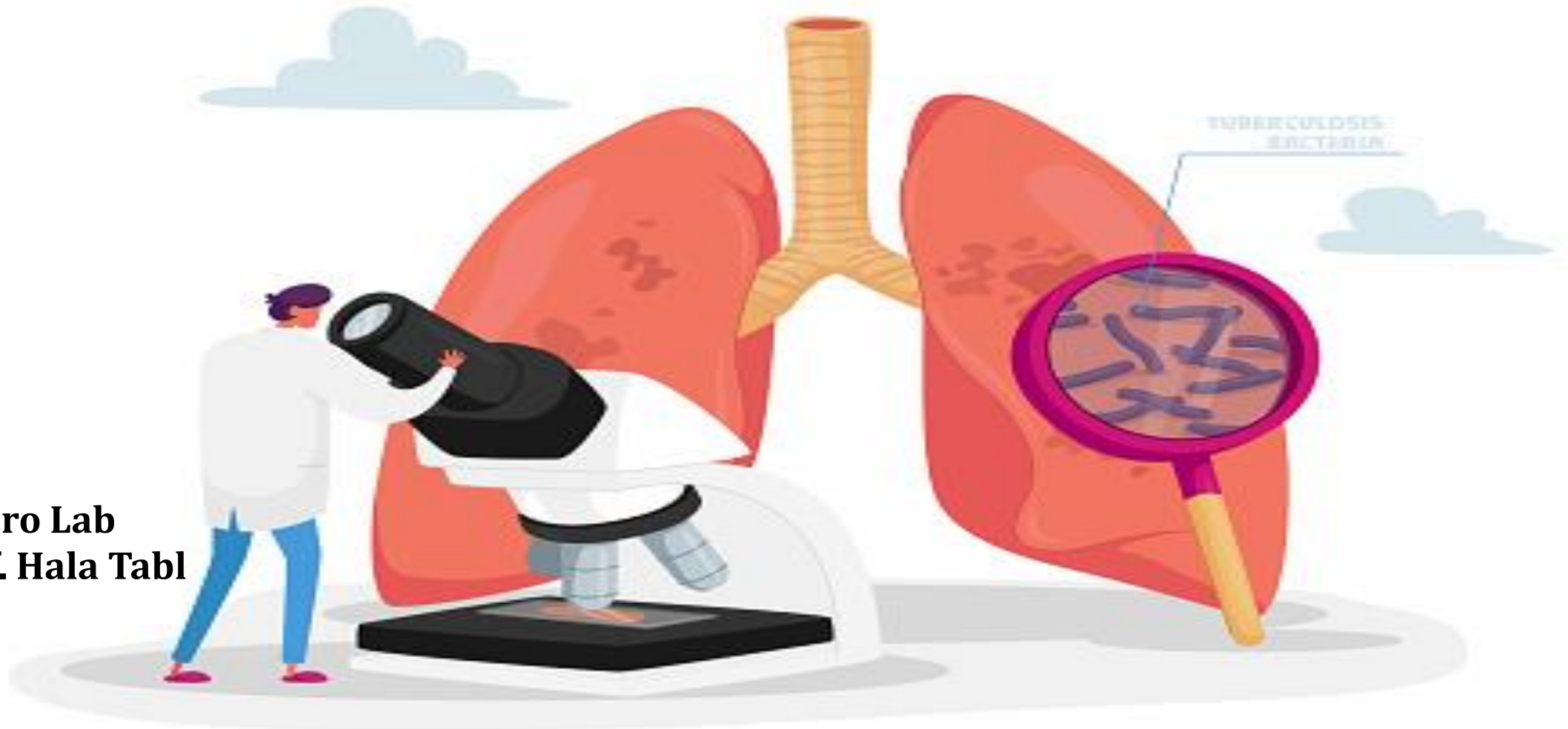
SUBJECT : _____

LEC NO. : Microbiology lab 1

DONE BY : Mahmoud Al Qusairi

Laboratory Diagnosis of Respiratory Tract Infections

Micro Lab
By Prof. Hala Tabl



اهم عينة في lower respiratory tract infection

Sputum (phlegm)

The most commonly isolated organisms

Streptococcus pneumonia

Haemophilus influenza

Staphylococcus aureus

Klebsiella pneumonia

Pseudomonas aeruginosa

البكتيريا إلي بتمل pneumonia ممكن نلاقيها في عينة sputum



مهم جداً

A) Collection of sputum sample

- Sputum either: السليمة القادمة
- **Expectorate**, patient cough deeply and spit any sputum.
- **Induced sputum**, produced by using nebulized hypertonic saline.
- 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. morning sample. تكون العينة مجمعة طول الليل
- When pulmonary tuberculosis is suspected, up to **three specimens** may need to be examined to detect AFB. لما اكون شاك ب Tb باخذ 3 عينات ب 3 ايام مختلفة يعني لو طلعت الأولى negative لازم تعيد الفحص
- 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. بيحصل لها natural autolysis فيجب فحص العينة بسرعة

في ناس بيكون عندهم صعوبة بإخراج ال sputum ال thick او scanty او المريض مش قادر يكح(عضلاته مش مساعده ب cough

فإحنا بنساعده في اخراج ال sputum بيشرب مي كثير او بيستنشق من جهاز بي عمل evaporation of hypertonic saline هذا بيساعد على induction of sputum ويمكن نعمل percussion على الصدر والظهر عشان بيساعد على اخراج sputum طيب لو لسا مش عارف؟ ممكن نستخدم bronco alveolar lavage ندخل ب endoscope ونحقن normal saline وبنوني السائل الي طلع



Sputum Sample Container

1

CLEAR YOUR MOUTH



Spit into water



Empty your lungs

2

BREATH IN AND OUT 3 TIMES



3

GIVE A SPUTUM SAMPLE



Priority of the project: The first step is to clear your mouth. Then, breathe in and out three times. Then, cough into your hand. Then, give a sputum sample. Copyright © 2010, Center for Communications Programs, Johns Hopkins University. All rights reserved.

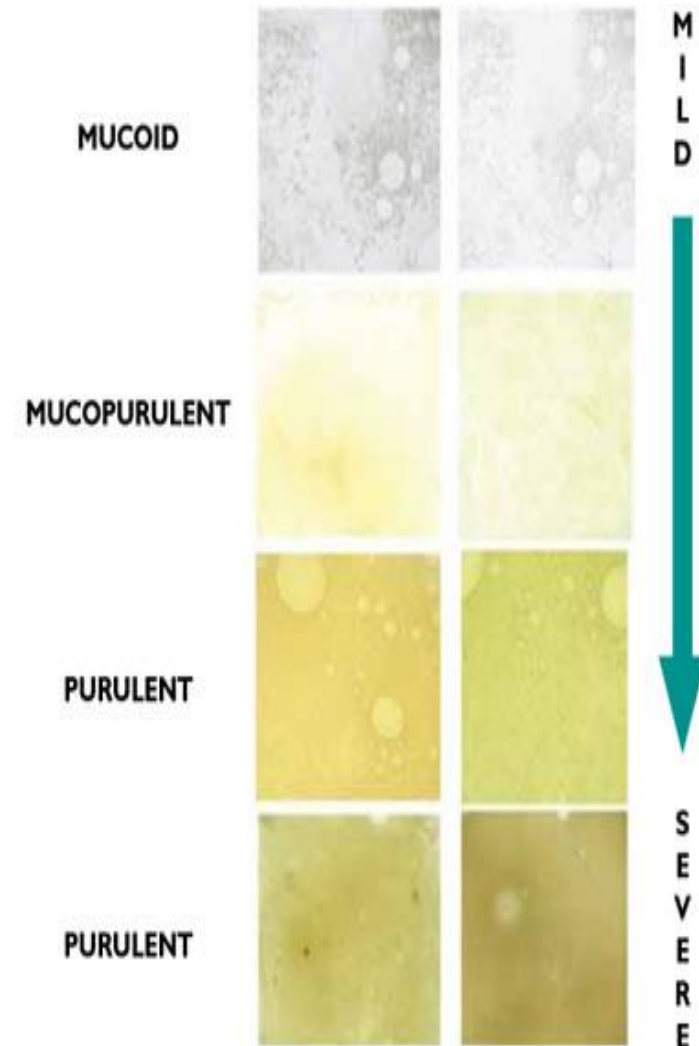


- **From lung.**
- **Thick & sticky.**
- **Contains many neutrophils and few epithelial cells.**
- **Accepted.**

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

B) Macroscopic examination of the sputum

- **Normal sputum:** Thin and clear colorless mucus.
- **Mucopurulent:** Yellow / green with pus and mucus. زيادة في pus و neutrophils
- **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. بيصير معها hemoptysis
 - ✓ **Currant jelly sputum:** K. pneumonia.
- **Rusty sputum** (due to decomposed Hemoglobin): it is typical for S. pneumoniae. ال bleeding يكون بسيط و scanty و بيتراكم فترة في lung فياخذ لون غامق
- **Green / blue sputum** (due to exopigment): P. aeruginosa.



C) Microscopic examination of sputum:

1) Gram stained smear:

ممکن يكون في العينة bacteria as normal flora فالي بيخليني اشك انه هاي البكتيريا
(upper hand) predominant في العينة pathogenic انها بتكون

- 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.
- *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.

Decontamination & concentration (Petroff's method):

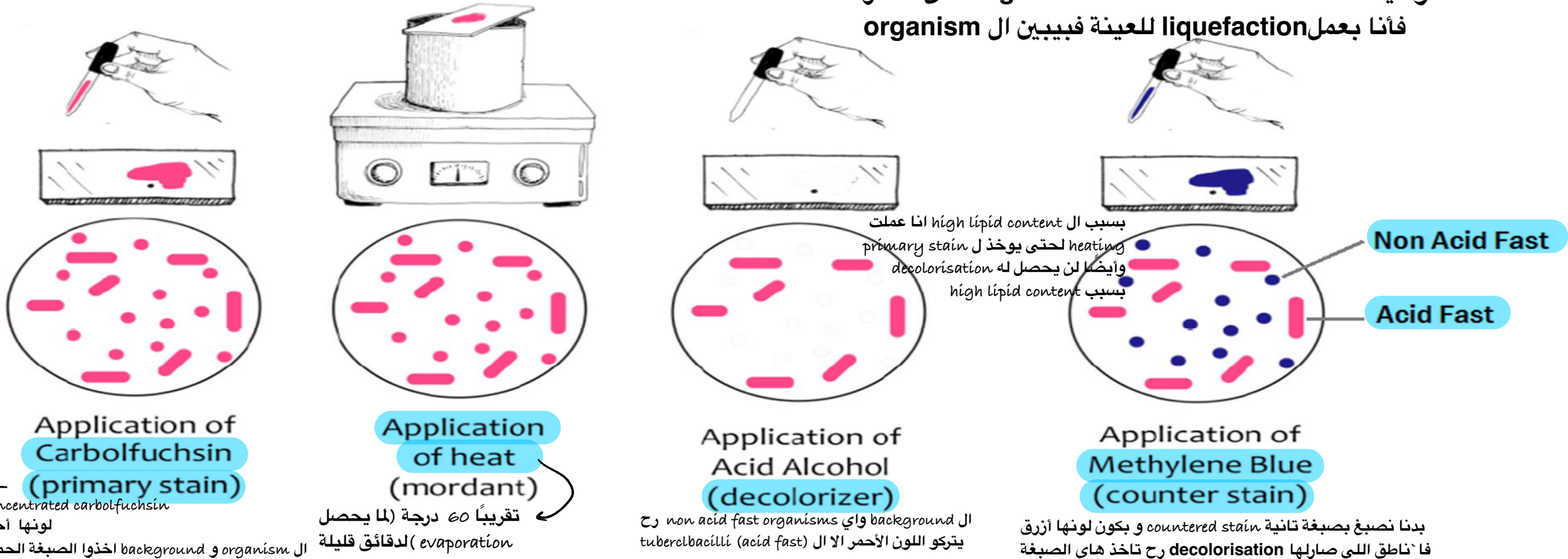
1. Liquefies the specimen, so release the tubercle bacilli.
2. Destroys bacteria other than T. B bacilli.

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

Petroff's method يعمل Ziegler-Neelsen stain قبل ما اصبغ العينة ب alkaline and acidic يعمل decontamination باستخدام مجموعة من normal flora (كما نعلم ان tb compounds عشان اتخلص من لعظم resistant acidic and alkaline media)

معظم عينات sputum من مرضى tb بتكون thick and purulent ومليانه caseous material ف organism مش ظاهر فإنا بعمل liquefaction للعينة فبيبين ال organism

سهج جداً



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

When Aspergillus infection is suspected.

4) Saline preparation:

When paragonimiasis is suspected.

5) Giemsa stained preparation:

When histoplasmosis or Pneumocystis infection is suspected.

D) Cultivation of sputum:

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.

streptococcus ممكن
pneumonia

homophiles ممكن
influenza

Klebsiella ممكن
pneumoniae

↳ caused by anaerobic
organism
لأنه

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.

upper respiratory tract infection

Throat swab

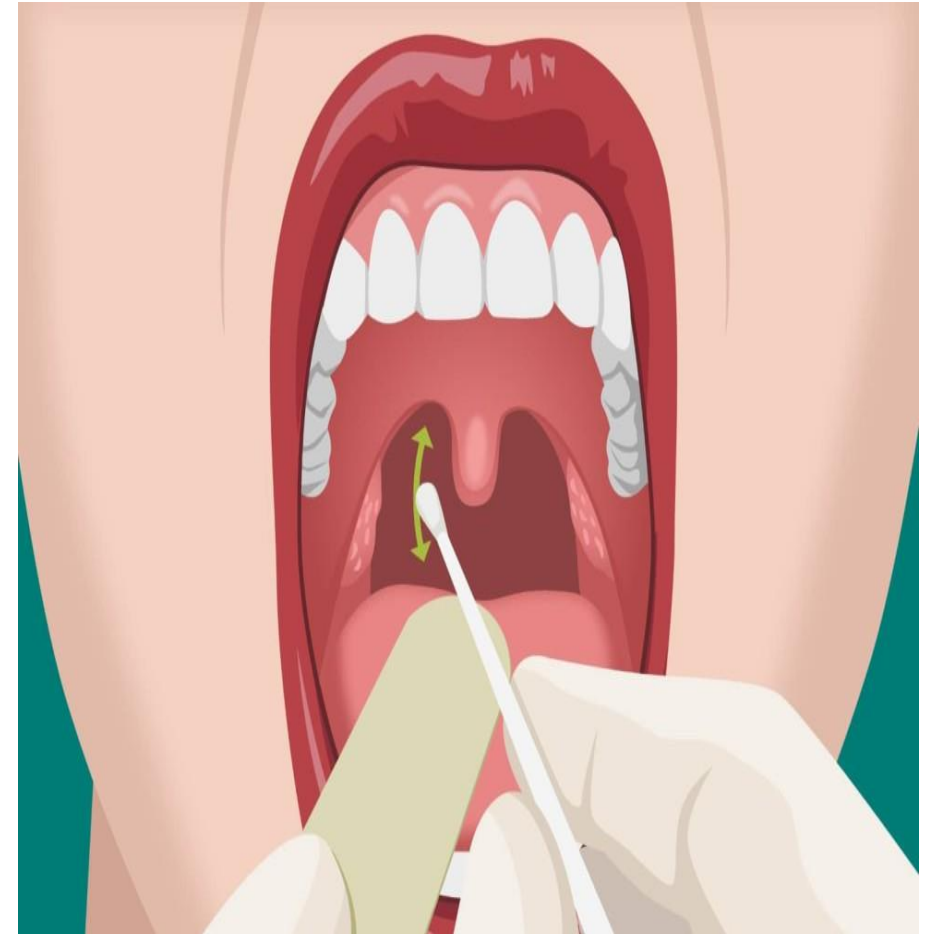
The most commonly isolated organisms

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).

قبل اخذ العينة ب ٨ ساعات يجب عدم اخذ antibiotic او antiseptic gargle

لكشف منطقة pharynx



بأخذ منه swap

وبطلع بسرعة




Rub the swab across the tonsillar areas and the posterior pharynx, specifically targeting any inflamed areas

B) Microscopic examination:

Gram stain: → sputum نفس المنطق في ~

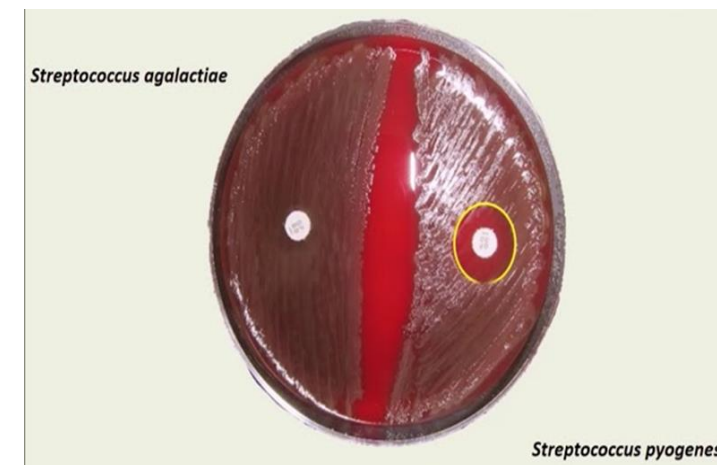
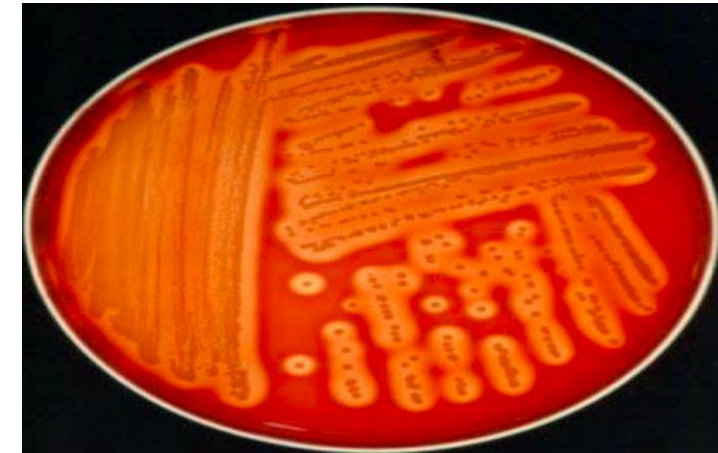
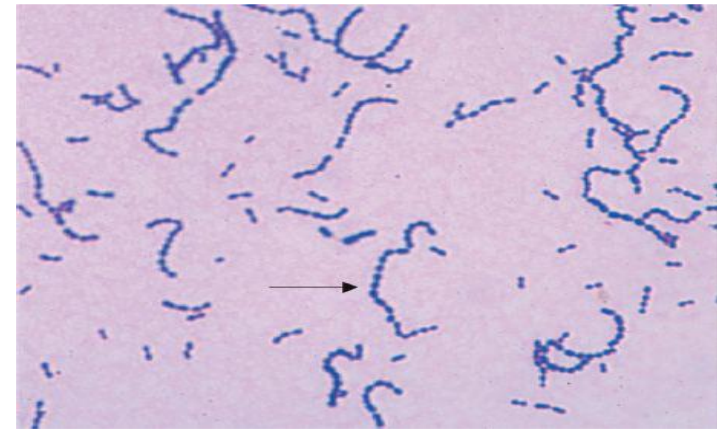
- No attempt should be made to report routinely other bacteria in a Gram stained smear from a throat swab because the throat contains a wide variety of commensals that cannot be distinguished morphologically from pathogens.
- Noting the predominant organism.
- When thrush is suspected, look for Gram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

C) Culture of throat swab:

- Blood agar culture.  If STREPTOCOCCUS PYOGENES suspected
- Blood tellurite agar if C. diphtheris suspected.

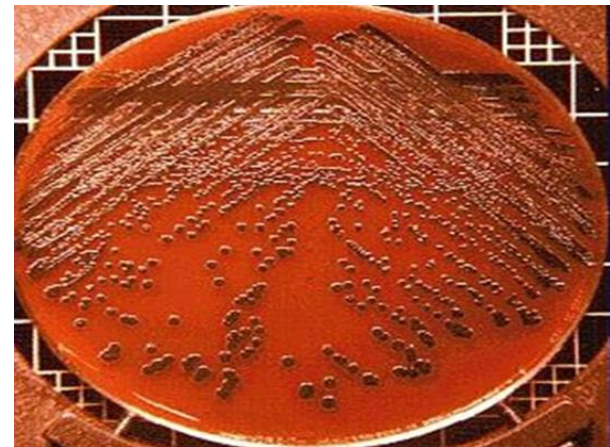
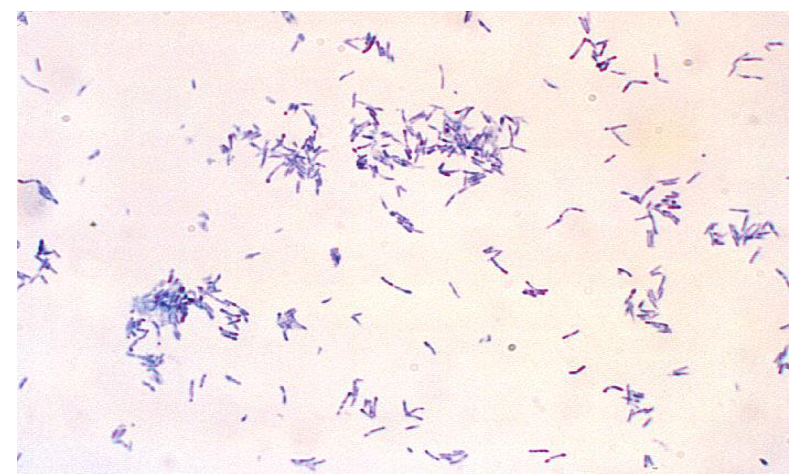
Streptococcus pyogenes

- **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 → ^{عندنا} capsule

- **Gram negative coccobacilli.**

- * **Quellung reaction positive.**

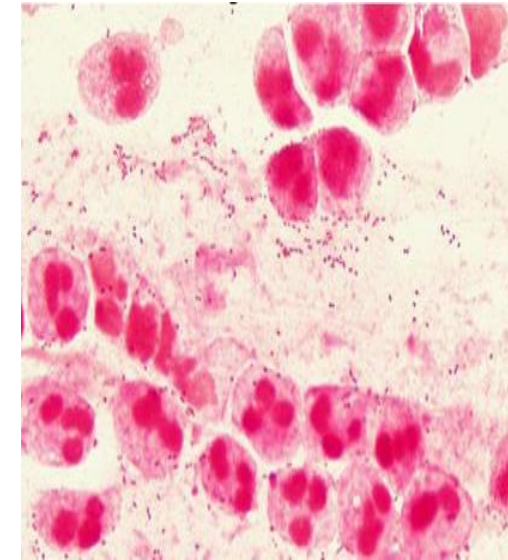
Quellung reaction positive → Capsulated organism

- **Grow on chocolate agar.**

- **X&V factor test: requires both factors.**

- **Grow close to colonies of Staph aureus.** (Satellitism).

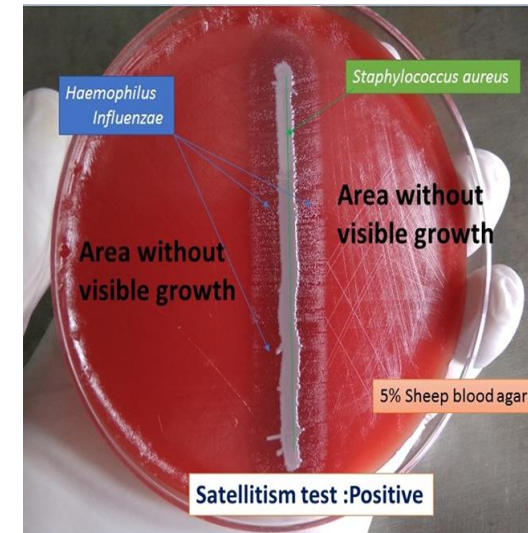
- **Produce NO hemolysis.**



Haemophilus influenzae

عندنا capsule لذلك

الوحيدة

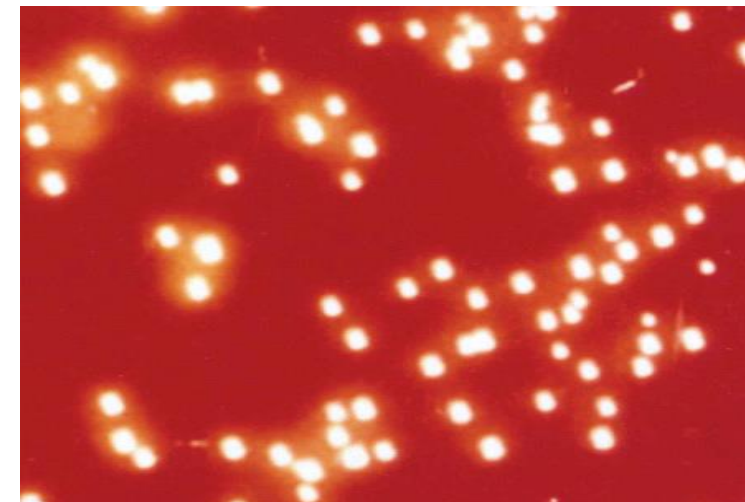


Bordetella pertussis

- **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

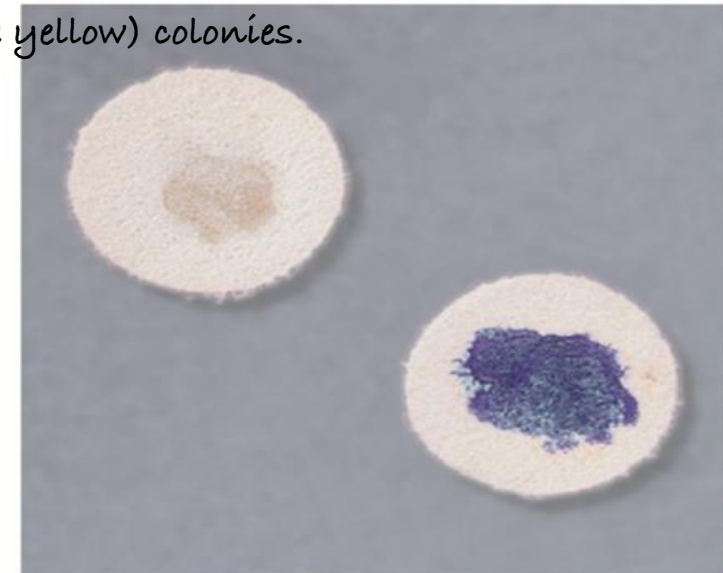
Pseudomonas aeruginosa

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

Pyocyanin, blue-green pigment.



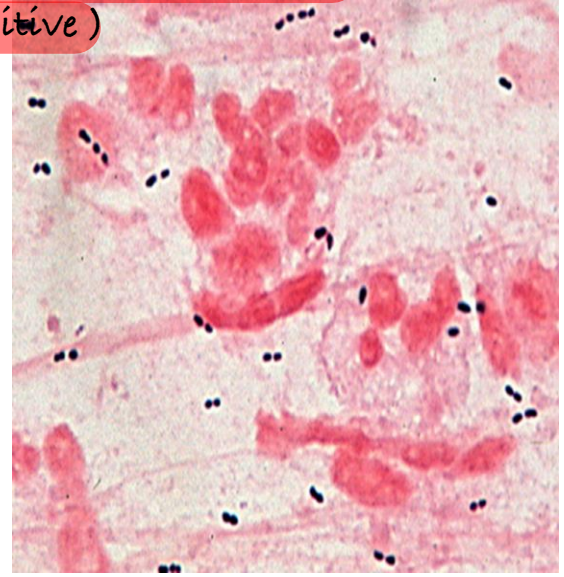
non-lactose-fermenting (pale yellow) colonies.



Streptococcus pneumoniae

- Gram-positive, diplococci.
- Capsulated, capsule appears as unstained halo.
- Quellung reaction positive.
- Alpha haemolysis on blood agar.
- Optochin sensitive. = Killed (inhibited) by optochin = Optochin disk surrounded by clear zone
- Ferment Inulin.
- Soluble in bile. → clear عند وضع bile الانبوية صارت insoluble معناته turbid لو ضل
- Catalase-negative.

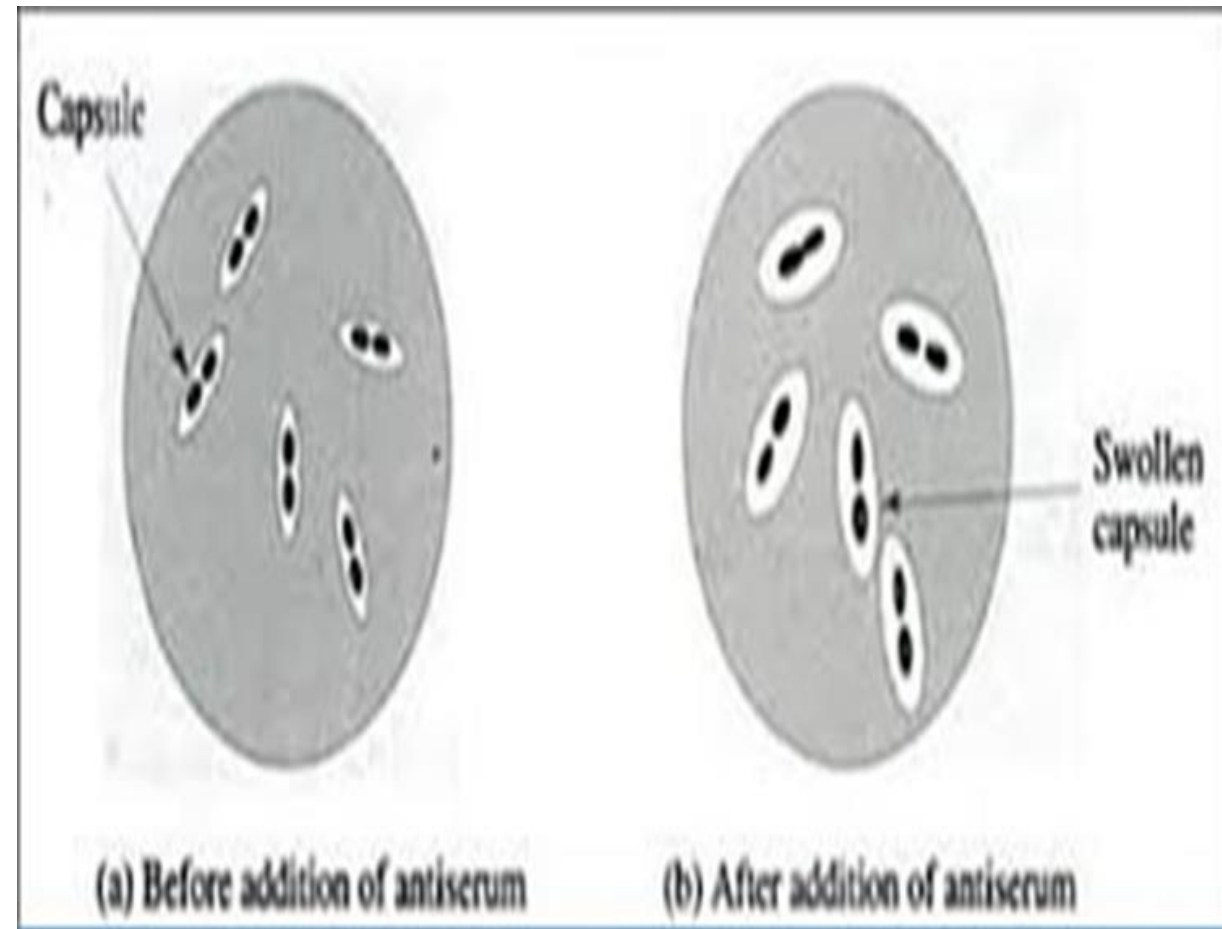
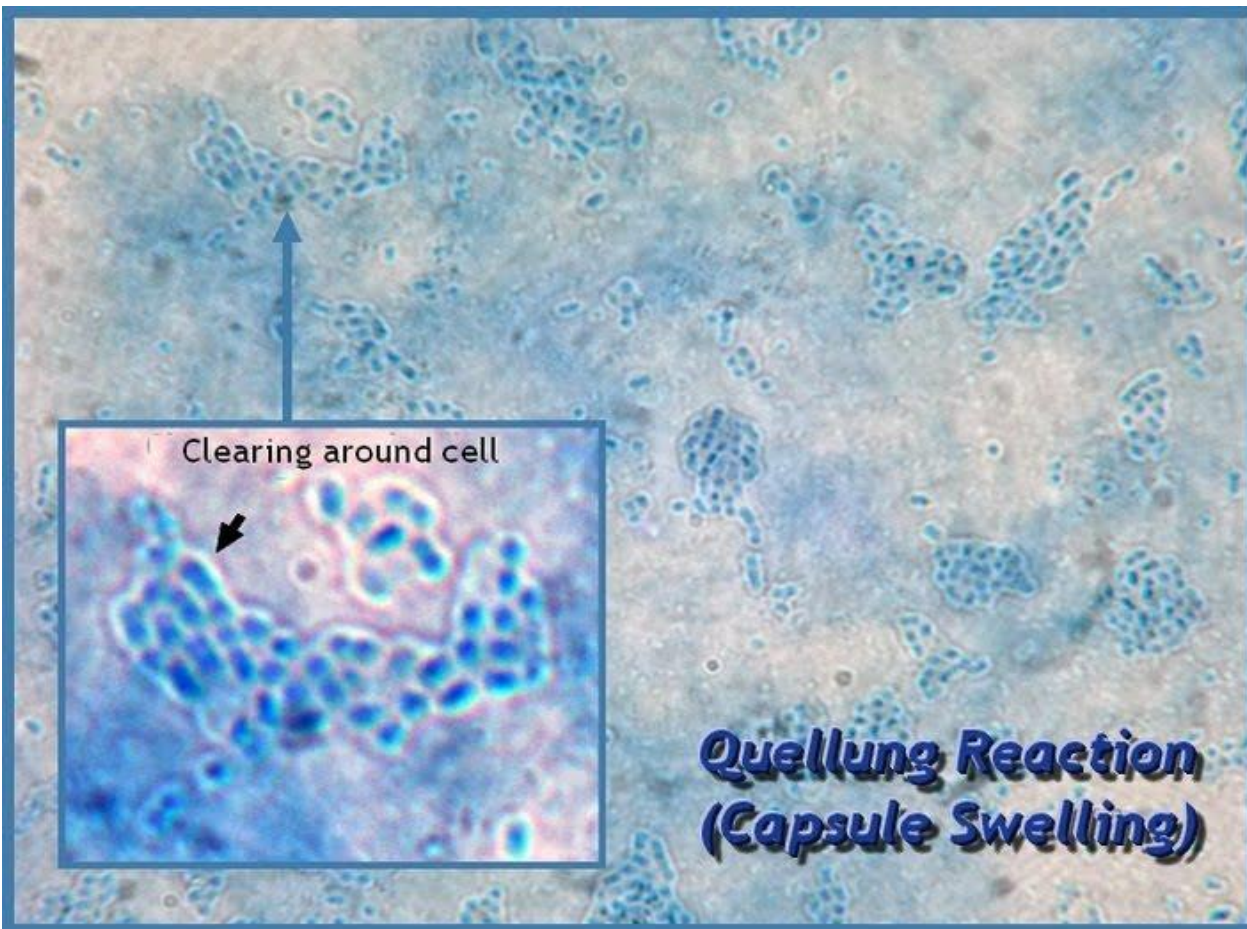
Streptococcus pneumoniae
 Haemophilus influenzae → Quellung reaction positive (capsule swelling test positive)



Quellung reaction (Capsule swelling test)

CSF or Sputum + **Specific antiserum** + Methylene blue stain

→ **The capsule become swollen.**



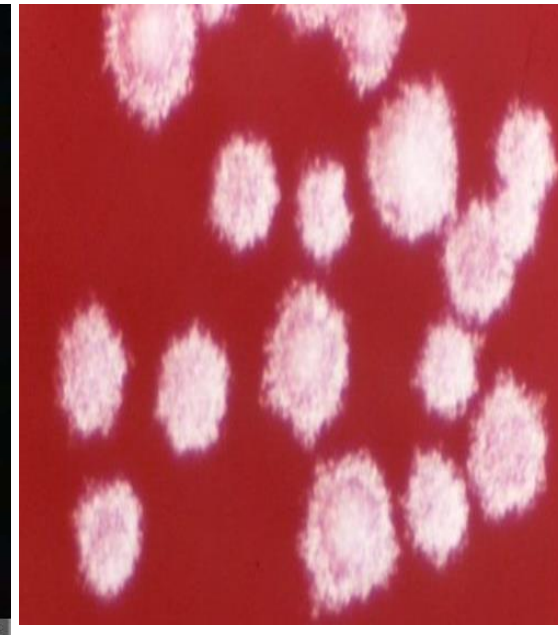
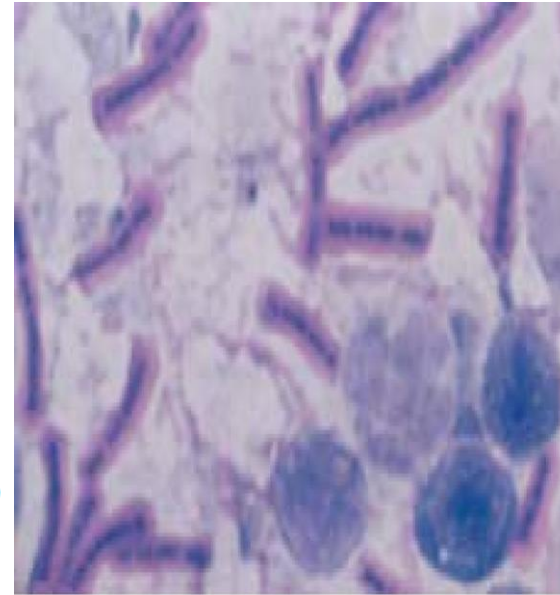
Klebsiella pneumoniae

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



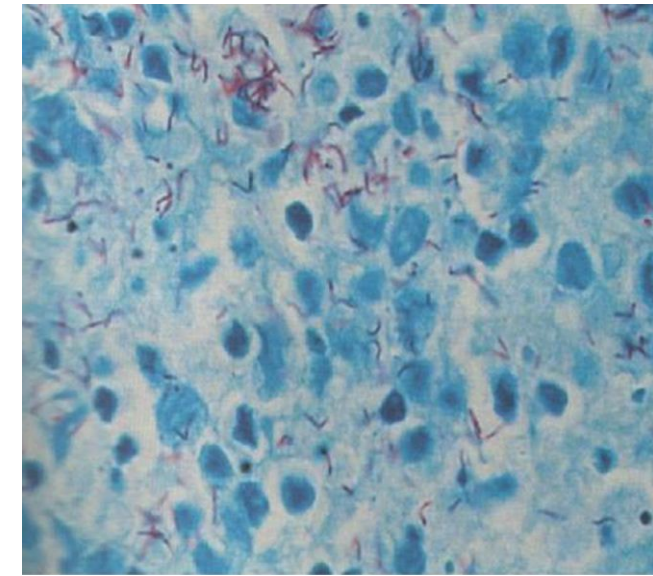
Bacillus anthracis

- 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

