

Laboratory Diagnosis of Respiratory Tract Infections

Micro Lab
By Prof. Hala Tabl



Sputum (phlegm)

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



Sputum Sample Container

1**CLEAR YOUR MOUTH**

Rinse with water



Empty your mouth

2**BREATH IN AND OUT 3 TIMES****3****GIVE A SPUTUM SAMPLE**

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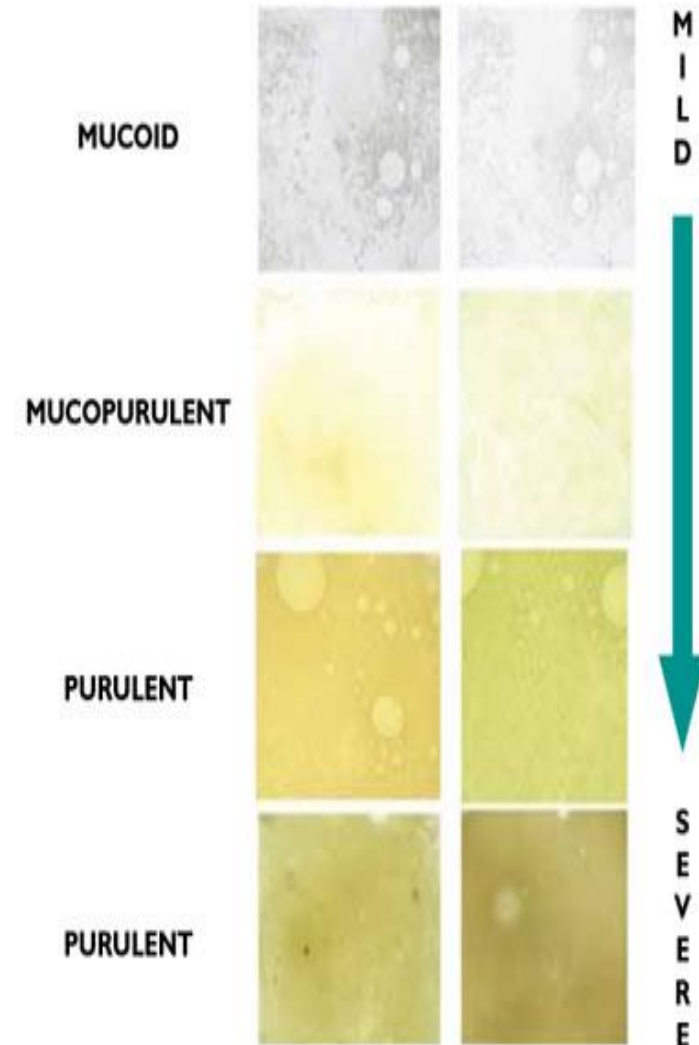


- **From lung.**
- **Thick & sticky.**
- **Contains many neutrophils and few epithelial cells.**
- **Accepted.**

- **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.
- **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:

1) Gram stained smear:

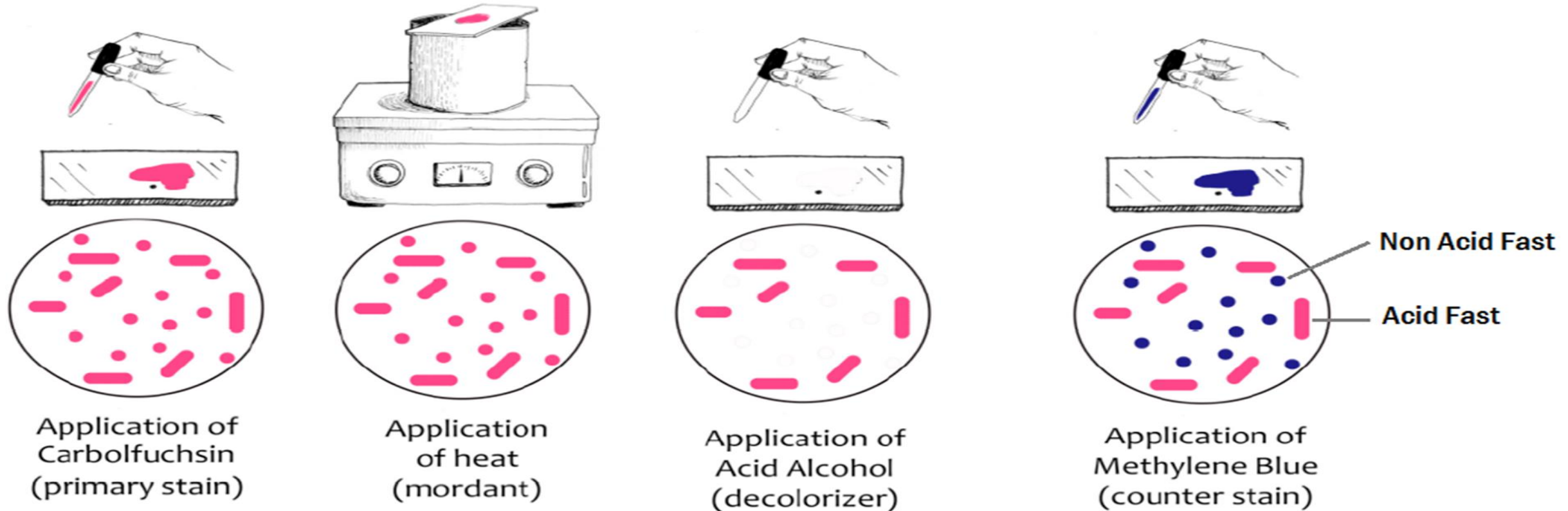
- Gram stained smears of sputum must be reported with caution. Cocci, diplococci, streptococci, and rods may be seen in normal sputum because these organisms form part of the normal microbial flora of the upper respiratory tract.
- Noting the predominant organism.
- *Note:* When pus cells are present 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



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.

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

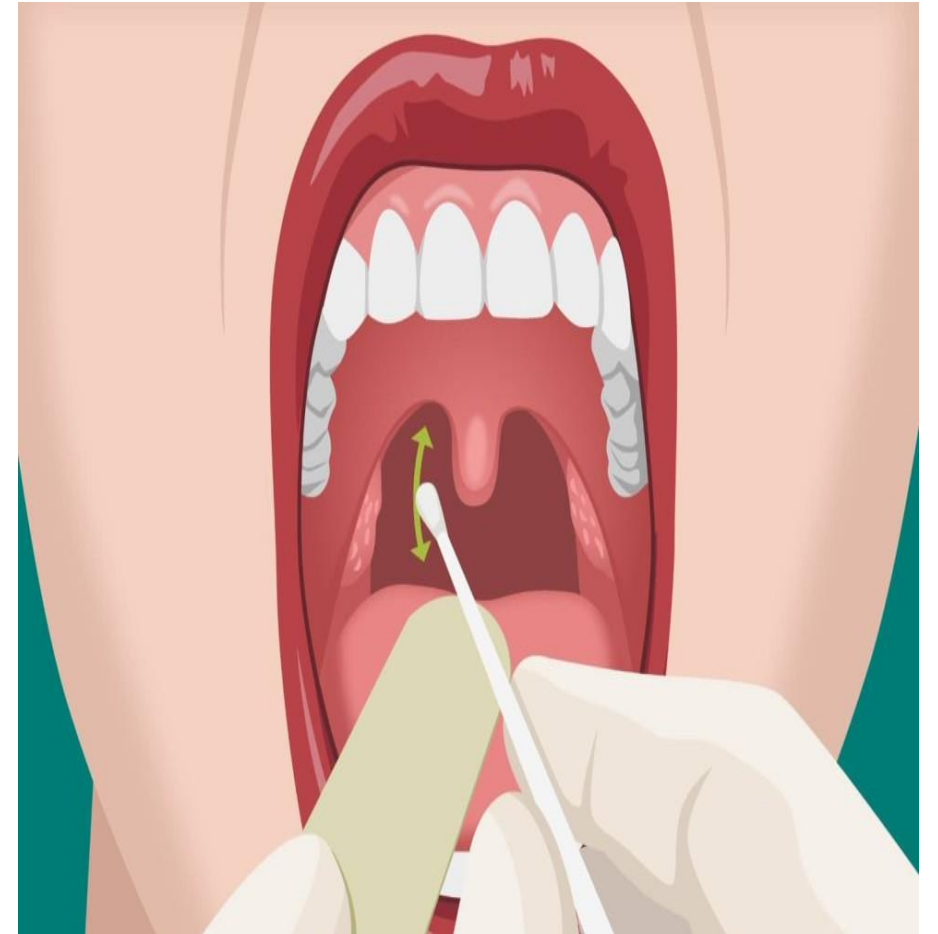
The most commonly isolated organisms

Streptococcus pyogenes

Corynebacterium diphtheria

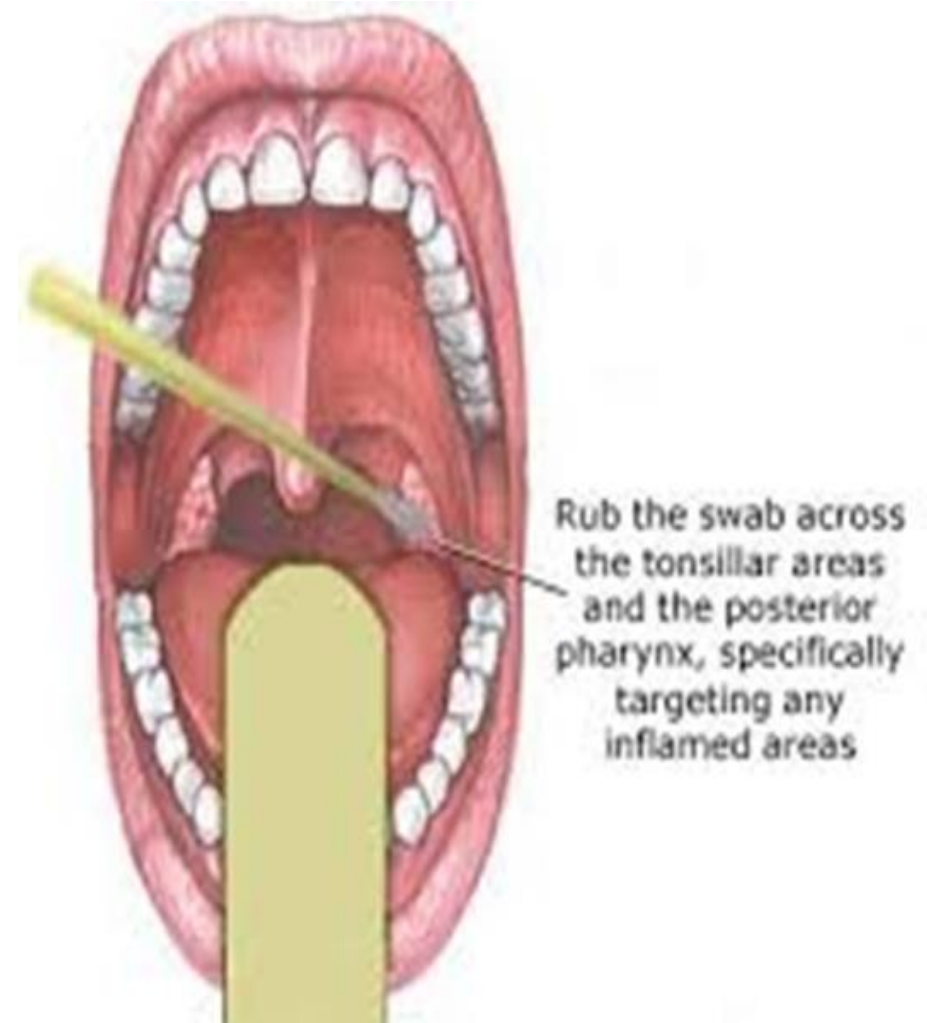
Vincent's organisms

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).



B) Microscopic examination:

Gram stain:

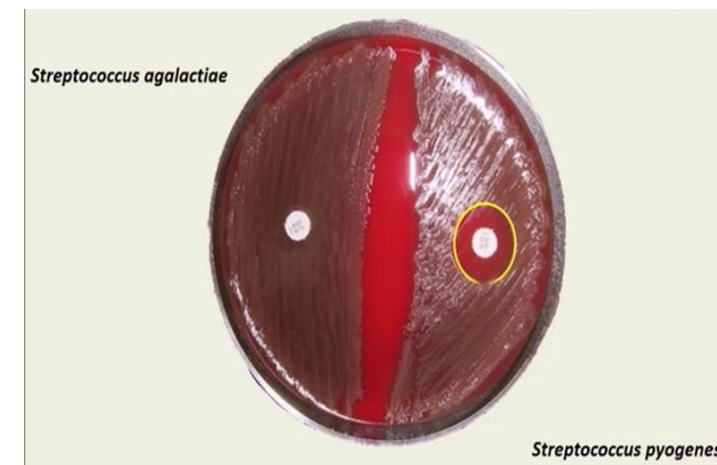
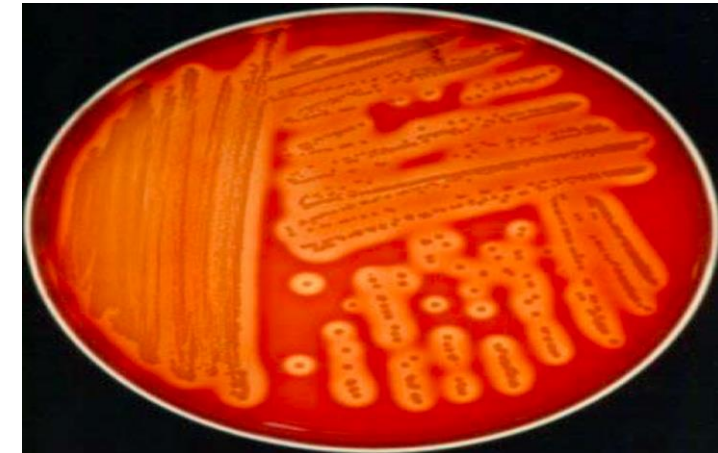
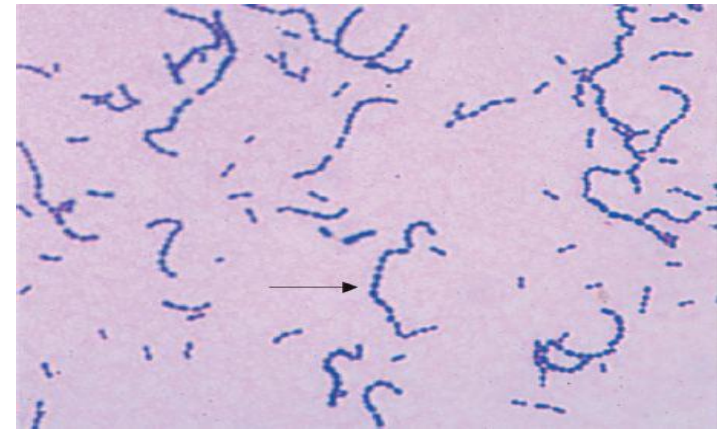
- No attempt should be made to report routinely other bacteria in a Gram stained smear from a throat swab because the throat contains a wide variety of commensals that cannot be distinguished morphologically from pathogens.
- Noting the predominant organism.
- When thrush is suspected, look for Gram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

C) Culture of throat swab:

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

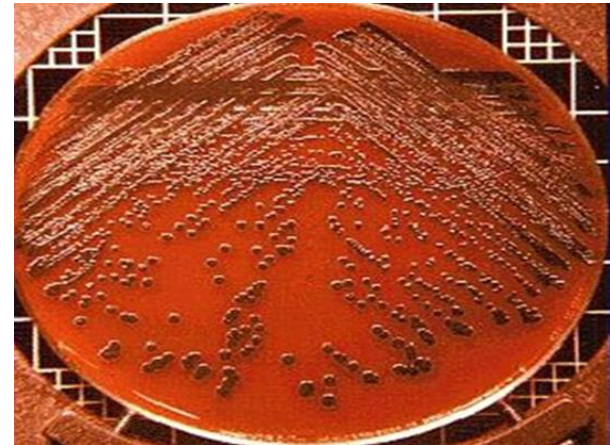
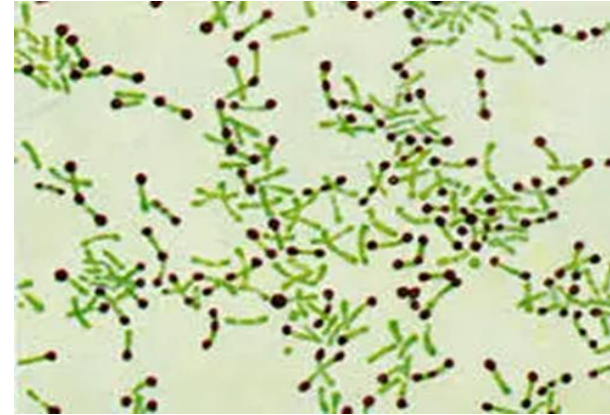
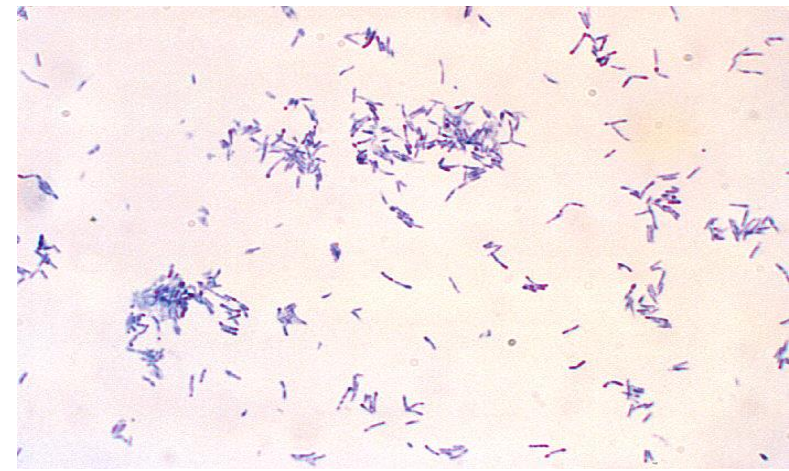
Streptococcus pyogenes

- **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).



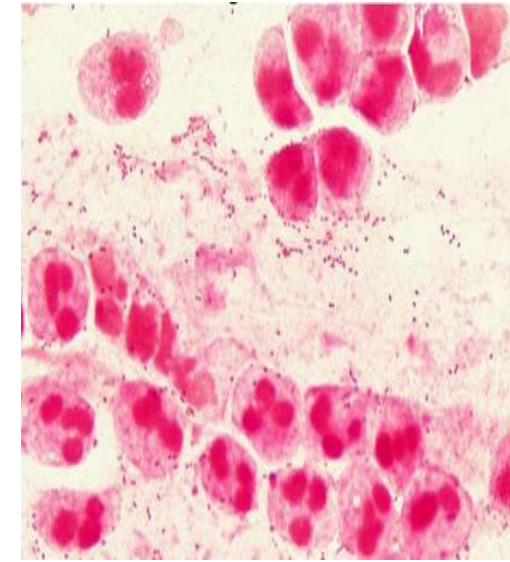
Corynebacterium diphtheria

- 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

- Gram negative coccobacilli.
- Quellung reaction positive.
- Grow on chocolate agar.
- X&V factor test: requires both factors.
- Grow close to colonies of Staph aureus.
- Produce NO hemolysis.

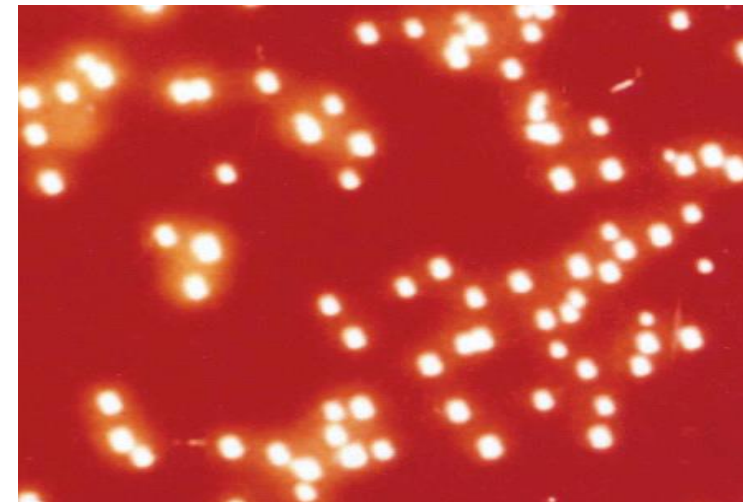


Bordetella pertussis

- **Gram negative coccobacillilli.**
- **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.**



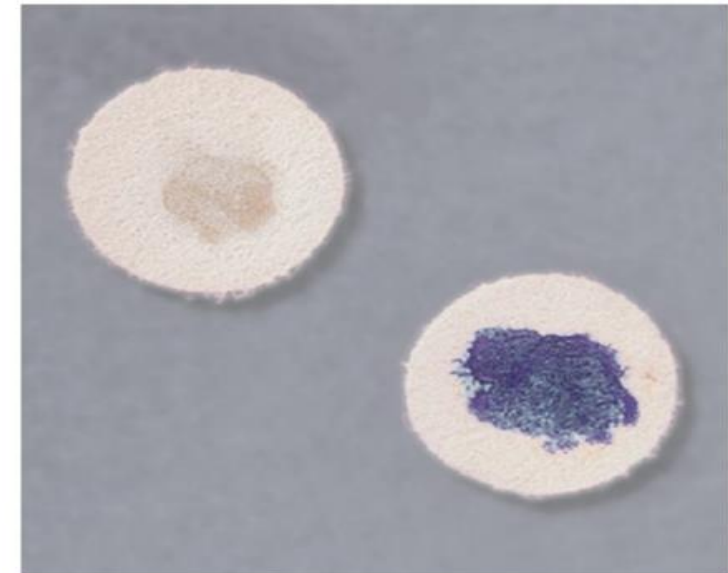
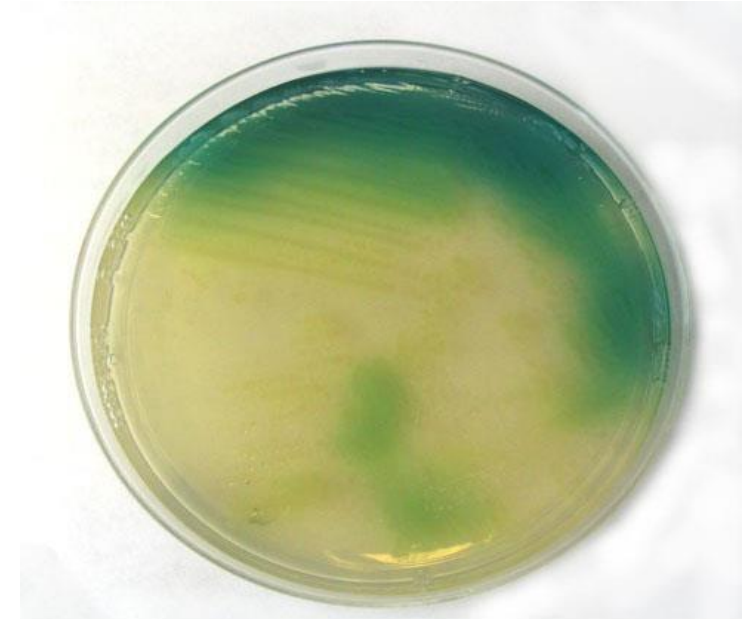
Charcoal-cephalexin blood agar



Bordet Gengou medium

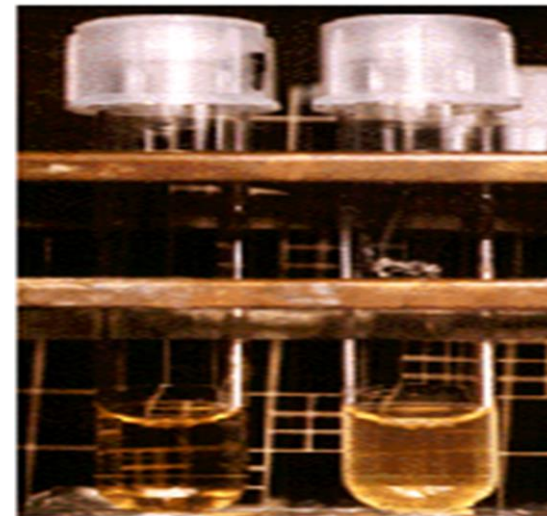
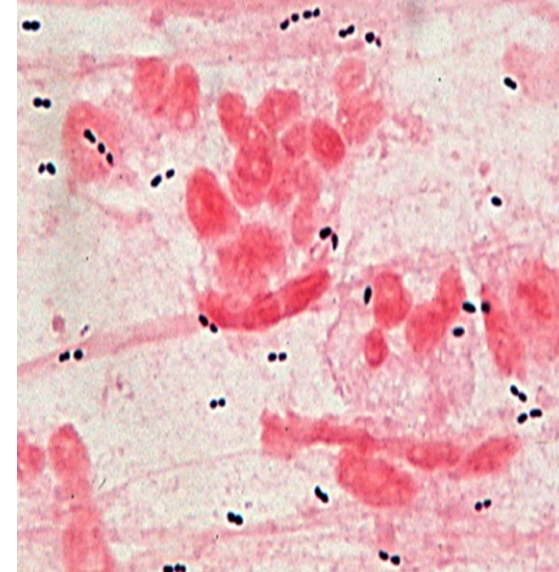
Pseudomonas aeruginosa

- **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.**



Streptococcus pneumoniae

- Gram-positive, diplococci.
- Capsulated, capsule appears as unstained halo.
- Quellung reaction positive.
- Alpha haemolysis on blood agar.
- Optochin sensitive.
- Ferment Inulin.
- Soluble in bile.
- Catalase-negative.



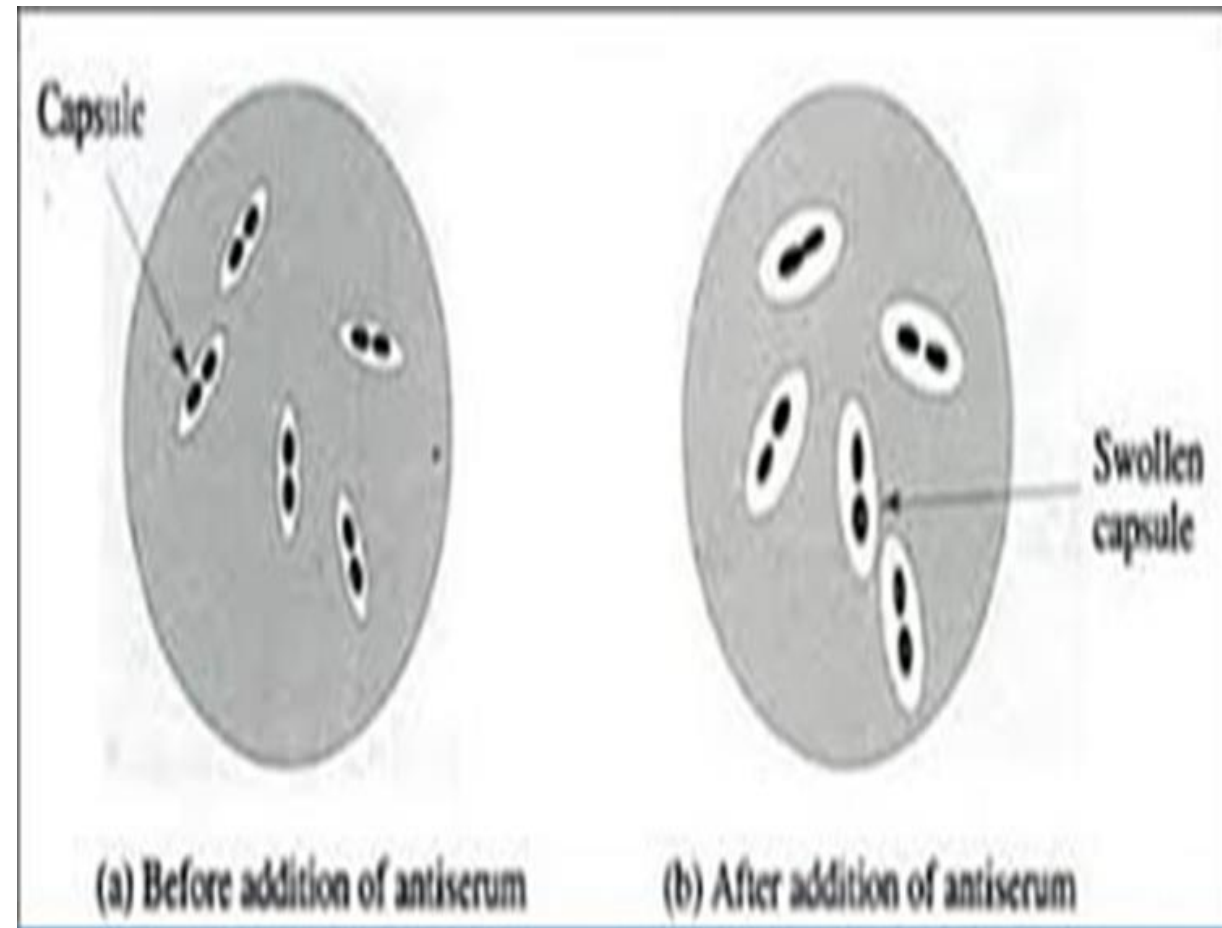
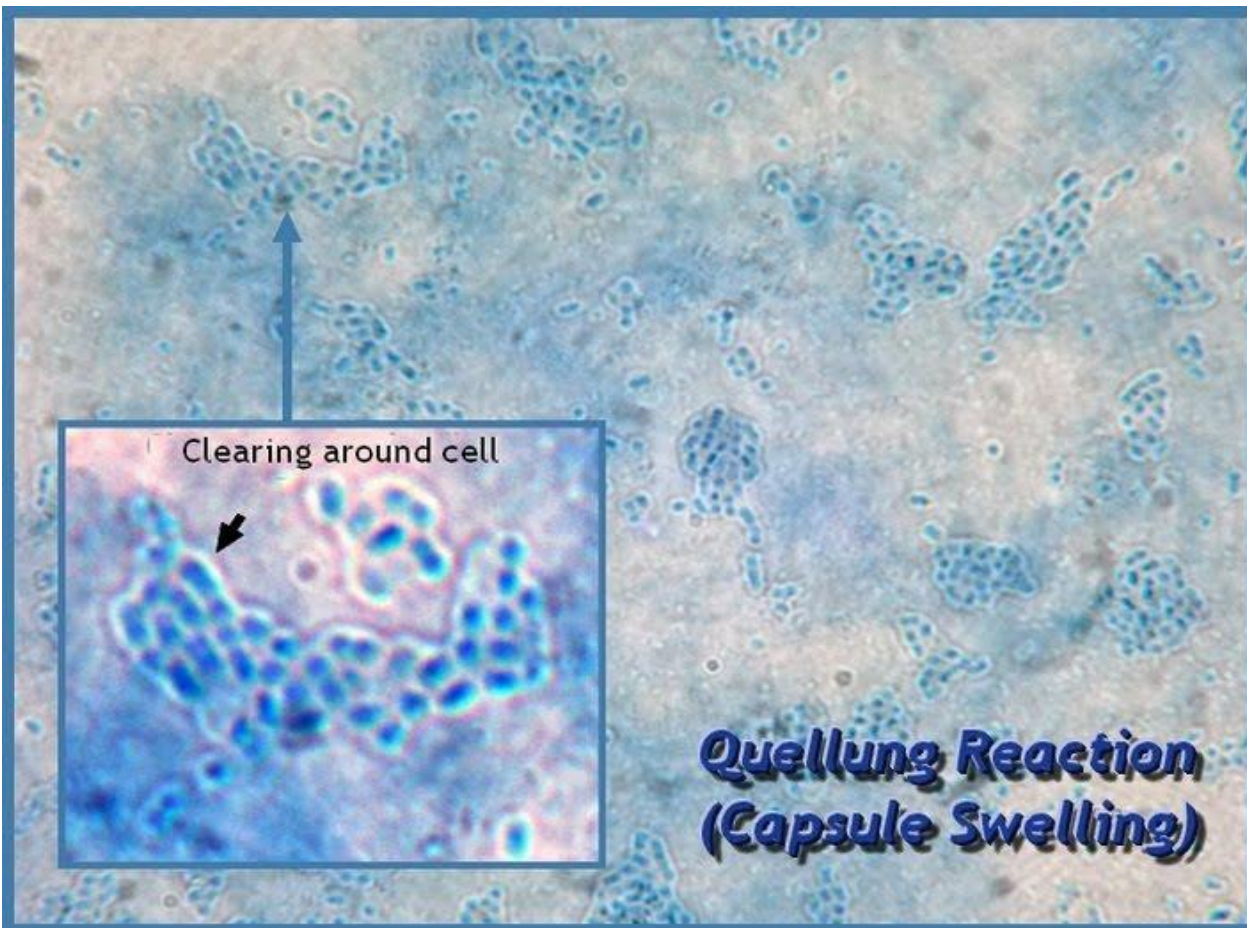
Pneumo + bile Pneumo + broth



Quellung reaction (Capsule swelling test)

CSF or Sputum + Specific antiserum + Methylene blue stain

→ The capsule become swollen.



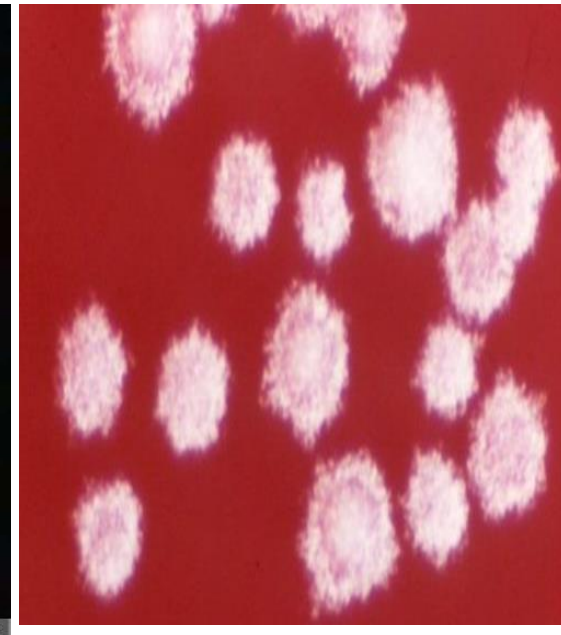
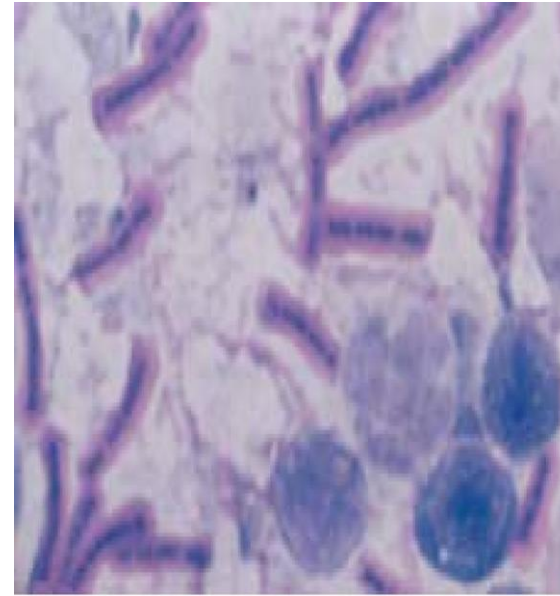
Klebsiella pneumoniae

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



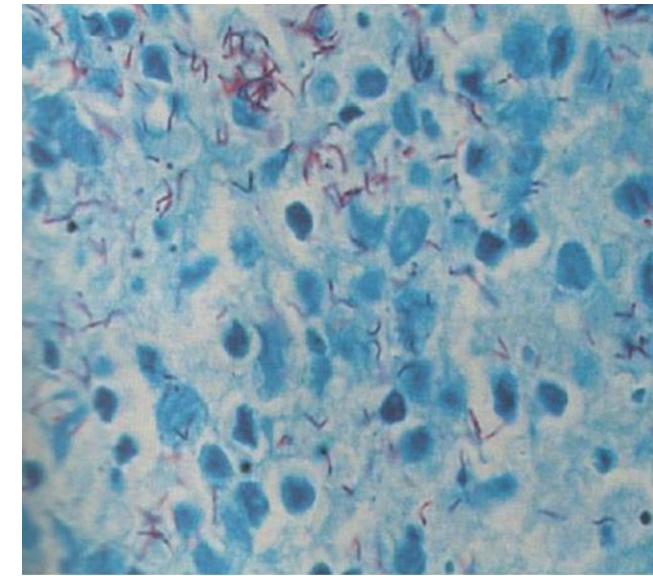
Bacillus anthracis

- **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

