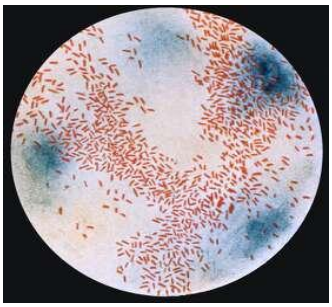
Unit II – Problem 1 – Microbiology lab

- **What are the characteristics of Hemophilus bacteria?**

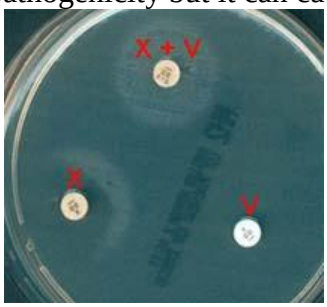


  - It is pleomorphic متعددة الأشكال
  - Small, gram negative (red/pink stain under microscope).
  - Coccobacilli (oval in shape).
  - Non-mobile (no flagella).
  - Capsulated.
  - Capnophilic: depends on CO_2 for its growth.

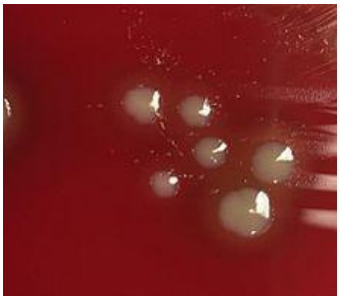
- **Laboratory investigations for Hemophilus bacteria:**
  - **Hemophilus has 3 species. In order to differentiate between them, we culture them in a petri dish which has no blood but we add filter papers with factor X, factor V and a third filter paper containing both factors X and V. Then, we check where the bacteria replicates.**

	Factor X (heme)	Factor V (NAD)	Factors X+V
H.influenzae			√
H. parainfluenzae		√	√
H.ducreyi	√		√

  - **Notes:**
    - ✓ H.influenzae grows in enriched media (chocolate agar mixed with blood).
    - ✓ H.influenzae will not grow in nutrient agar because factors X and V are not present.
    - ✓ H.influenzae causes: meningitis (inflammation of the meninges), epiglottitis (inflammation and swelling of epiglottis resulting in respiratory distress) and otitis in children. Transmission; airborne route.
    - ✓ H.parainfluenzae is normally present in oral cavity and pharynx with low pathogenicity but it can cause endocarditis.

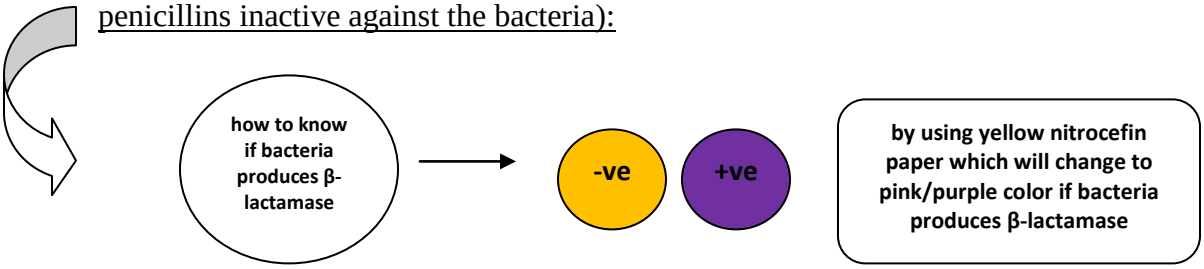


Chocolate agar

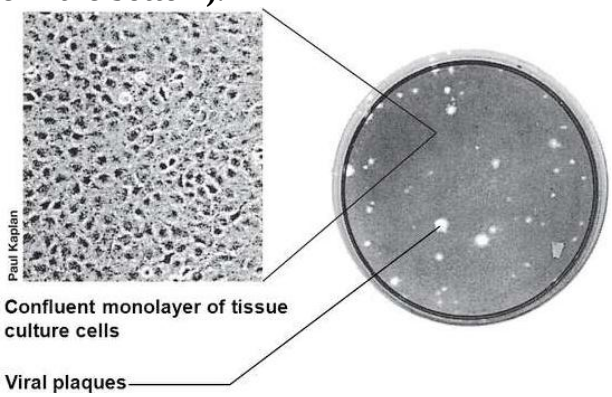


Blood agar

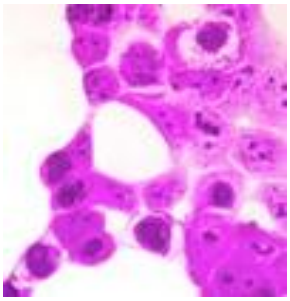
- **Treatment of Hemophilus bacteria:**
  - **Ampicillin or amoxicillin (if isolates are susceptible).**
  - **Cephalosporins**.
    Notice that some bacteria can produce β-lactamase enzyme (which will render penicillins inactive against the bacteria):



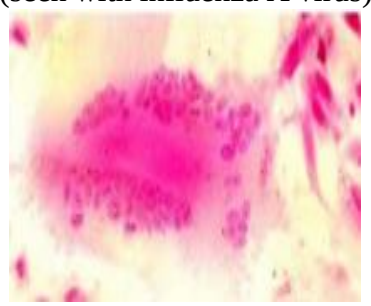
- **Viral culture:**
  - **A sample for viral culture is obtained by nasopharyngeal aspirate and cultured inside viable cells (because viruses are intracellular organisms) to produce a monolayer. This can be visualized under the inverted microscope (in which objective lenses are in the bottom).**



  - **Cytopathic effects**: are the changes which will occur to the monolayer cells when a virus is present. Examples include:
    - ✓ Formation of giant cells.
    - ✓ Lysis of the cells (seen with Herpes virus).
    - ✓ Rounding (seen with ECHO virus).
    - ✓ Intranuclear inclusions (seen with influenza A virus).



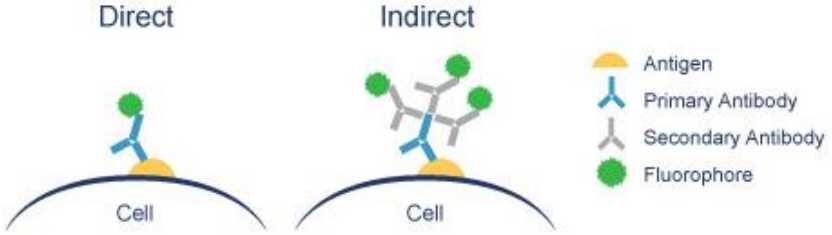
Inclusion bodies



Giant cell formation

Cell rounding

  - **Trypsinization**: when cultured cells produce more than one layer of cells, trypsin is used to detach these cells from each other.
- **Immunofluorescence**:
  - **Direct**: antibodies attached to fluorescent dye are added to the sample. If virus is present in the sample, the slide will appear green under the microscope.
  - **Indirect**: a blood sample is obtained. anti-antibodies attached to fluorescent dye are added. If antibodies against a specific virus are present in the blood sample, the slide will appear green under the microscope.



- **Enzyme-Linked Immunosorbent Assay (ELISA):**
  1. Add antigen (sample).
  2. Add antibody.
  3. Add anti-antibody with enzyme.
  4. Add chromogen (substrate with color).