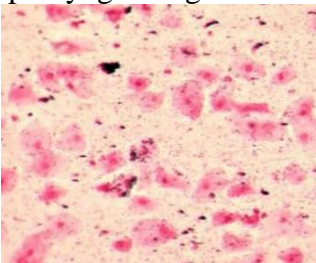




- What are the steps needed to obtain a proper sputum specimen?

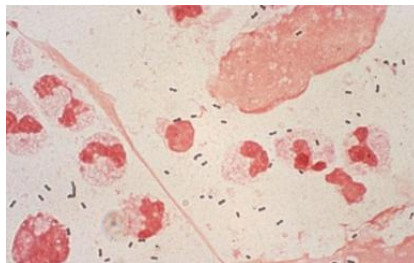
- **You need the following:**
 - ✓ A wide-mouth labeled container.
 - ✓ Gloves.
 - ✓ Water.
 - ✓ Mouth wash + tissues.
- **To avoid contamination when obtaining the specimen:**
 - ✓ Patient must blow his nose first.
 - ✓ Patient must brush his teeth and rinse with mouthwash.
- **Patient must cough deeply so sputum can be obtained.**
- **Cap the container after you collect the specimen, label it “sputum specimen” and write:**
 - ✓ Patients name.
 - ✓ Time of collection.
 - ✓ And if any antibiotics were administered to the patient.

- Interpretation criteria for sputum Gram staining:

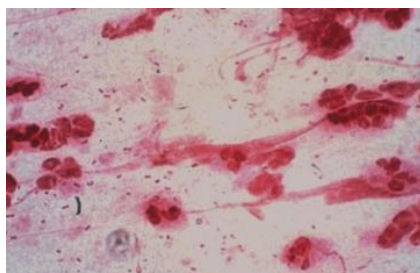
Adequacy of specimen	Quantity of PMNs	Kind and number of organisms present
<p>> 25 squamous cells under (X10) objective → sample is rejected (why?) → because it is contaminated with oropharyngeal organisms.</p> 	<p>> 25 neutrophils under (X10) objective → sample is accepted (why?) → this indicates the presence of pus (inflammation)</p>	<ul style="list-style-type: none"> • Yeast pneumonia are RARE! • Legionella, Mycoplasma and Chlamydia cannot be seen by Gram stain

- Examples on different organisms detected by Gram stain from sputum specimen:

- **Streptococcus pneumoniae:** in the specimen below you can notice several neutrophils but no epithelial cells are present (no contamination). S.pneumoniae are paired, lancet-shaped, Gram (+) cocci.

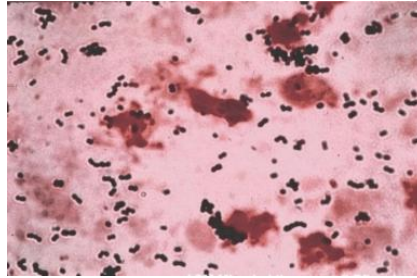


- **H.influenzae:** small, pleomorphic, Gram (-) bacteria, coccobacilli-shaped. Abundant neutrophils are present.

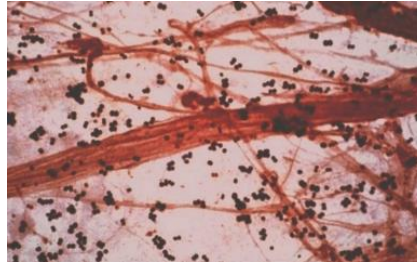




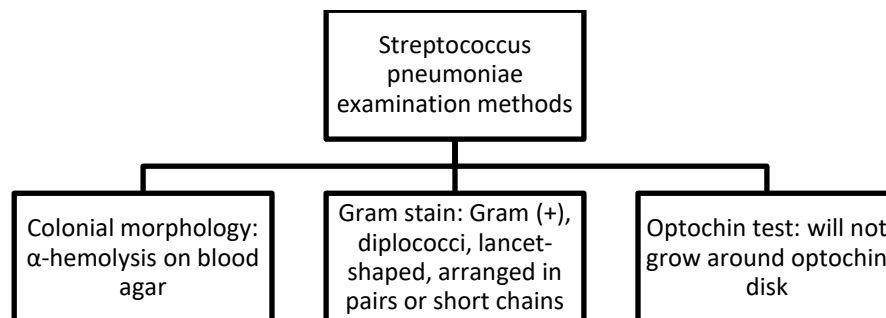
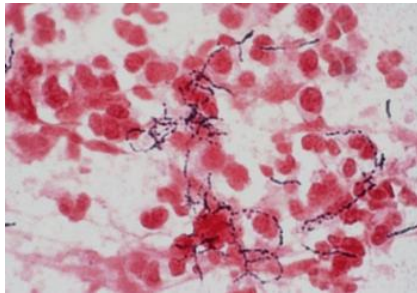
- **Streptococcus agalactiae (group B Streptococcus):** large, round, Gram (+) cocci which are found in pairs, chains and clumps. These cocci are larger and rounder than pneumococci, which rarely, if ever form chains of more than four cocci in the sputum.



- **Staphylococcus aureus:** numerous Gram (+) cocci, round and large which can be single, paired or grape-like clusters. The clusters strongly suggest that the organisms are Staphylococci.

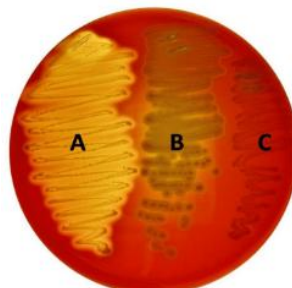


- **Nocardia asteroides:** branching, beaded, Gram (+) bacilli. Actinomyces look the same and Streptomyces species are also long, branching Gram (+) rods but are usually not beaded.



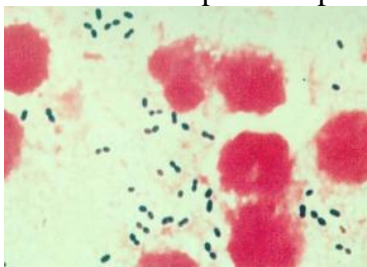
- **Colonial morphology:**

- β-hemolysis on blood agar:** complete breakdown of RBCs.
- α-hemolysis on blood agar:** partial breakdown of RBCs.
- γ:** no hemolysis on blood agar.

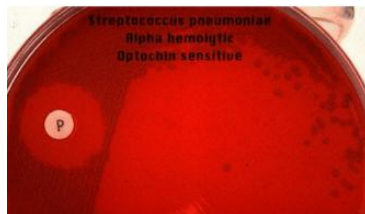




- **Gram stain**: Gram (+) diplococci and neutrophils in sputum sample.



- **Optochin test**: blood agar showing the inhibitory zone >14 mm obtained with *S.pneumoniae* to optochin which exceeded that obtained with other α -hemolytic viridians Streptococci (< 14mm).



- **Bile solubility test for Streptococcus pneumoniae**: it distinguishes *S.pneumoniae* from all other α -hemolytic Streptococci (where *S.pneumoniae* is bile soluble).

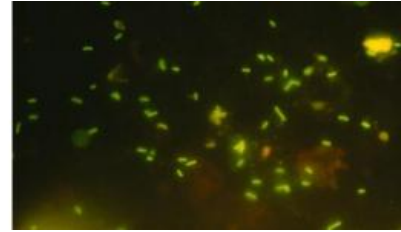
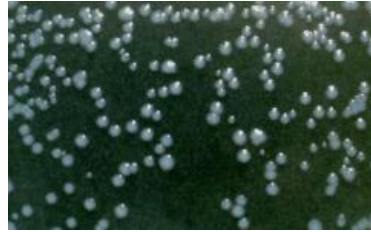


- **Streptococcus pneumoniae microbiological properties**:
 - Gram (+), diplococci, lancet-shaped, arranged in pairs or chains.
 - Catalase (-), sensitive to optochin, α -hemolytic.
 - Lysis by addition of bile.
 - Capsule: it is a major pathogenic factor.
 - C-substance (quellung reaction) and M-protein.
- **Clinical features of S.pneumoniae**:
 - Fever and chills.
 - Cough and copious mucopurulent sputum.
 - Pleuritis and chest pain.
 - Hemoptysis.
- **Treatment of S.pneumoniae**:
 - Penicillins (penicillin G) whereas macrolides are given for patients with atypical pneumonia.
- **Vaccination**: multivalent vaccine for:
 - Infants and elderly.
 - Immunocompromised patients.
 - Patients with sickle cells disease.



- **Legionellosis:**

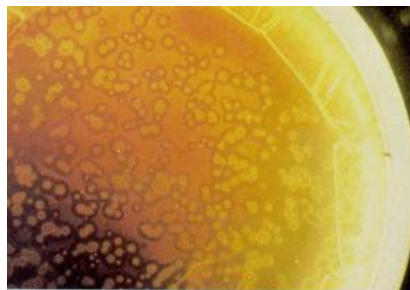
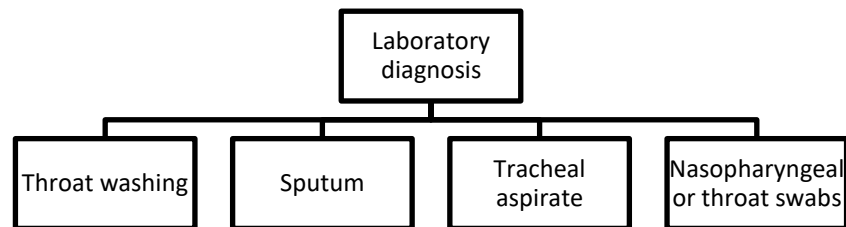
- **Etiological agent:** Legionella pneumophila (which are Gram-negative rods).
- **Disease:** legionellosis.
- **Source:** water sources.
- **Clinical features:**
 - ✓ Fever and chills.
 - ✓ Productive or non-productive cough.
 - ✓ Radiographic evidence of pneumonia.
 - ✓ Multi-system involvement (diarrhea, hematuria... etc).
- **Laboratory diagnosis:**
 - ✓ Charcoal-yeast agar with iron and cysteine.
 - ✓ Direct immunofluorescence assay.



- **Treatment:** erythromycin or rifampin.
- **Prevention:** hyperchlorination of heat treatment of water sources in hospitals.

- **Mycoplasmosis:**

- **Etiological agent:** Mycoplasma pneumoniae (lacks peptidoglycan).
- **Disease:** atypical pneumonia.
- **Source:** contact.
- **Clinical features:**
 - ✓ Fever.
 - ✓ Unproductive cough.
 - ✓ Headache and malaise.



Mycoplasma pneumoniae colonies with sheep red cell overlay



Mycoplasma pneumoniae colonies showing the fried egg appearance

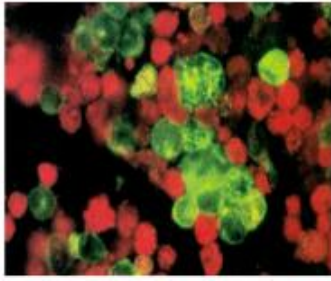
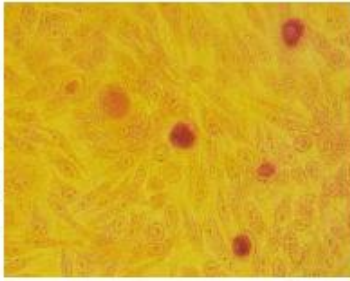
- **Treatment:** erythromycin or tetracycline.

- **Chlamydiae:**

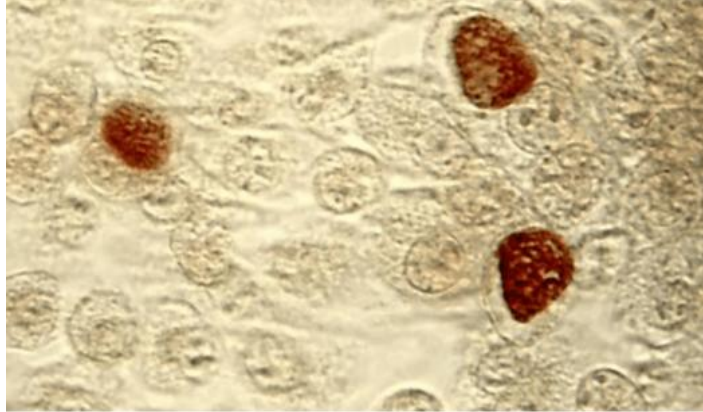
- Gram (-), intracellular, contains both DNA and RNA.
- **Infectious form:** elementary body → it infects a host cell and then will be converted to → reticulate body → binary fission and production of colonies.
- **Laboratory diagnosis:** specimen is obtained → cultured → then detected by iodine-stain or fluorescent antibody stain.



**Iodine-stained
inclusions in
McCoy cell
monolayer
infected with:
*Chlamydia
trachomatis*.**



**Fluorescent-
antibody-stained
inclusions in
McCoy cell
monolayer infected
with:
*Chlamydia
trachomatis*.**



Chlamydia trachomatis inclusion bodies (brown) in a McCoy cell culture