Microbiological Diagnosis of Respiratory Tract Infections

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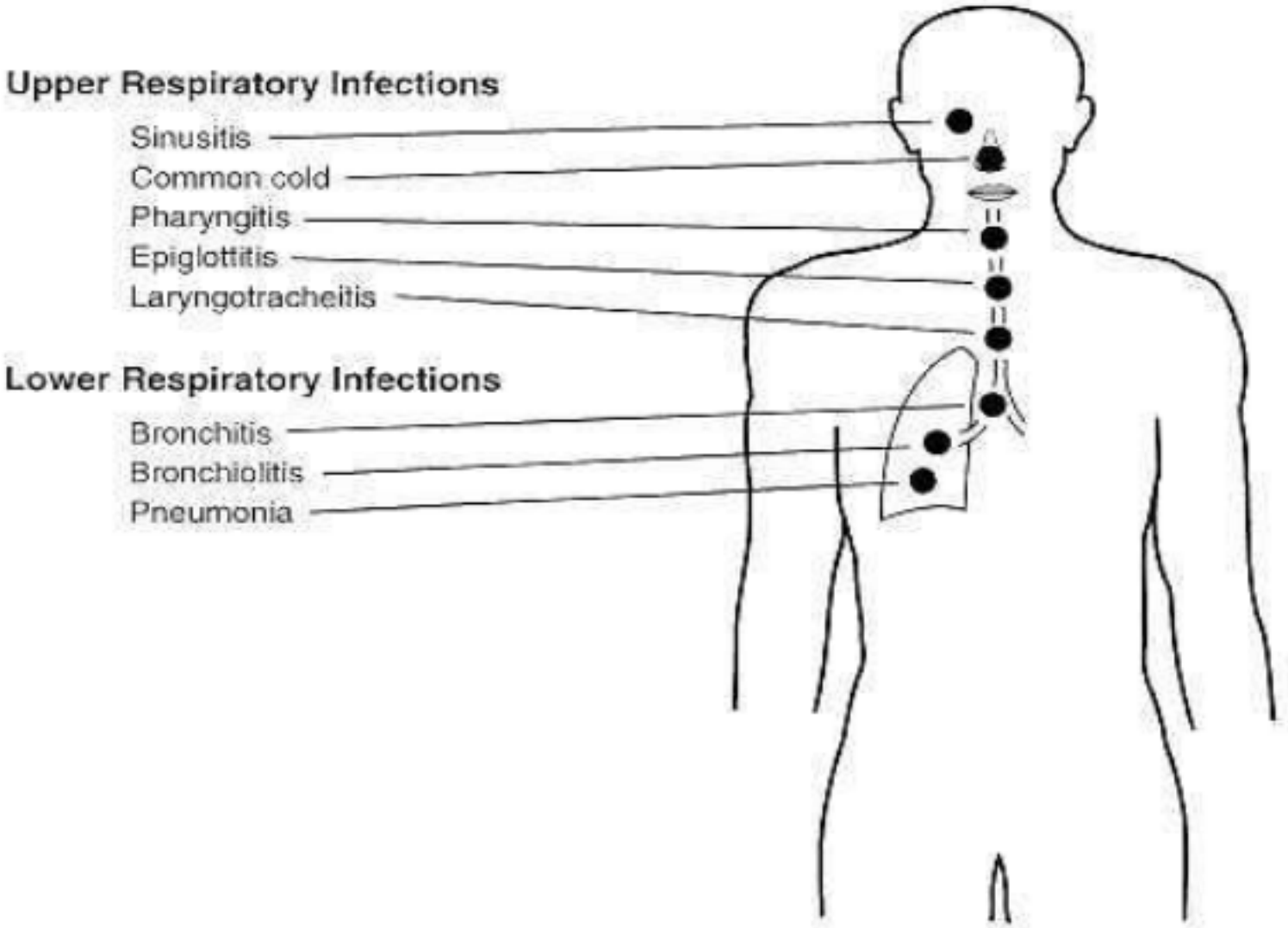
Objectives

- What are the types of respiratory tract infections?
- What are the pathogens in each type of respiratory tract infections?
- What are the specimens collected in each type for diagnosis?
- How to collect and transport specimens?
- What are the methods for diagnosis?

RTI

- Upper Resp. Tract infections
  - Common cold
  - Sinusitis
  - Pharyngitis
  - Epiglotitis
- Lower respiratory tract infections
  - Brochitis
  - Pneumonia
  - Lung abscess
  - TB
  - Empyema

RTI


Upper Respiratory Infections
Sinusitis
Common cold
Pharyngitis
Epiglottitis
Laryngotracheitis
Lower Respiratory Infections
Bronchitis
Bronchiolitis
Pneumonia

RTI Pathogens

- Bacterial
- Viral
- Fungal

RTI

- Bacterial
  - Beta haemolytic streptococcus
  - *Bordatella pertusis*
  - *Haemophilus influenzae*
  - *Streptococcus pneumoniae*
  - *Staphylococcus aureus*
  - *Moraxella catarrhalis*
  - *Atypical bacteria - Leigionella pneumophila/ Mycoplasma pneumoniae/ Chlamydia pneumoniae/ C. psittaci*
  - *Klebsiella pneumoniae*

RTI

- Viral
  - Rhinovirus
  - Coronavirus
  - Influenzavirus
  - Adenovirus
- Fungal
  - *Aspergillus spp.*
  - *Pneumocystis carinii*
  - *Crypyococcus neoformans*
  - *Candida spp.*
  - *Histoplasma capsulatum / Coccidioidis*

Common cold

- Commonly caused by viruses
    - Rhinovirus - commonest
    - Coronavirus
    - Influenzavirus and parainfluenza viruses
    - Adenovirus
    - RSV
- All of these organisms show seasonal variations in incidence
- IP-48–72 hours
- classic symptoms
    - nasal discharge and obstruction, sneezing, sore throat and cough myalgia and headache
- Fever is uncommon
- Diagnosis is usually based on the symptoms

Sinusitis

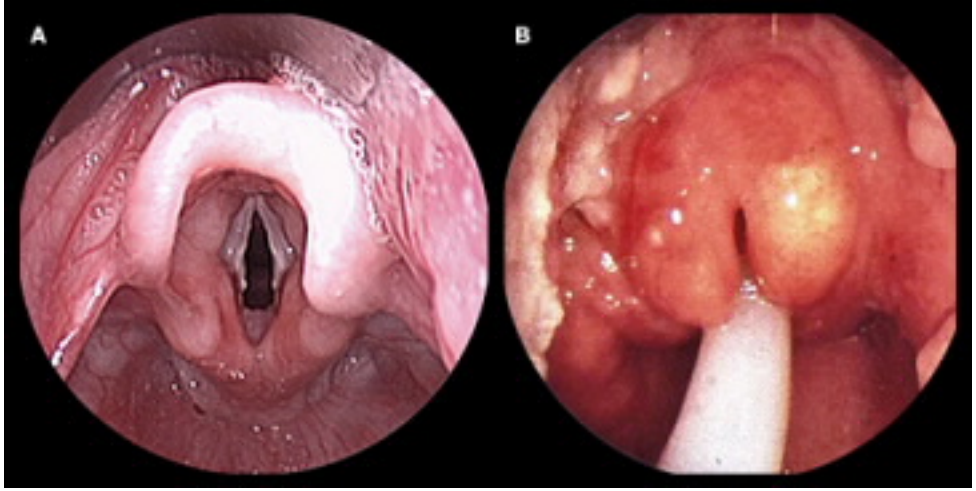
- Acute inflammatory condition of one or more of the paranasal sinuses
- Often results from infections of other sites of the respiratory tract
- Most often follows a common cold which is usually of viral etiology
- The most common bacterial agents responsible for acute sinusitis
  - *Streptococcus pneumoniae*
  - *Haemophilus influenzae*
  - *Moraxella catarrhalis*
  - Other organisms - *Staphylococcus aureus, Streptococcus pyogenes*, gram-negative organisms and anaerobes
- Chronic sinusitis is commonly a mixed infection of aerobic and anaerobic organisms.

Pharyngitis

- Most cases are due to viral infections
  - Viruses causing common cold or influenza
  - Other viruses – Coxsackie A viruses (herpangina), adenovirus, herpes simplex virus, EBV, CMV
- Bacterial causes
  - Group A beta-hemolytic streptococcus (*Streptococcus pyogenes)* is the most important
    - Cause complications – peritonsilar abscess, sinusitis ,otitis, Rheumatic fever, Glomerulonephritis
  - *Corynebacterium diphtheriae, Corynebacterium haemolyticum*

Epiglottitis

- *Haemophilus influenzae* type b is the most common cause of epiglottitis, particularly in children age 2 to 5 years
- Other – *H. parainfluenzae*, Grop A Strep, viruses
  - Life threatening, acute
  - 4D's – Dysphonia, Dysphagia, Drooling, Distress
  - Examination of throat is C/I – spasms → obstruction



Inflammed epiglottis

Tripod position

Table 93-1 Common Agents of Respiratory Infections

TABLE 93-1 Common Agents of Respiratory Infections

Clinical Illness	Bacteria	Viruses	Fungi	Other
Common cold (rhinitis, coryza)	Rare	Rhinoviruses Coronavirus Parainfluenza viruses Adenoviruses Respiratory syncytial virus Influenza viruses	Rare	Rare
Pharyngitis and tonsillitis (tonsillopharyngitis)	Group A β-hemolytic streptococci *Corynebacterium diphtheriae* *Neisseria gonorrhoeae* *Mycoplasma pneumoniae* *Mycoplasma hominis* (type 1) Mixed anaerobes	Adenoviruses Coxsackieviruses A Influenza viruses Rhinovirus, coronavirus Parainfluenza viruses Epstein-Barr virus, cytomegalovirus Herpes simplex virus	*Candida albicans*	Rare
Epiglottitis and laryngotracheitis (croup)	*Haemophilus influenzae* type b *Corynebacterium diphtheriae*	Respiratory syncytial virus Parainfluenza viruses	Rare	Rare
Bronchitis and bronchiolitis	*Haemophilus influenzae* *Streptococcus pneumoniae* *Mycoplasma pneumoniae*	Parainfluenza viruses Respiratory syncytial virus Adenoviruses	Rare	Rare

Lab diagnosis of URTI

- Specimens and methods
  - Throat swabs – culture on blood agar
  - Sinus aspirates/ purulent nasal discharge – culture aerobically and anaerobically
  - Blood for culture – in epiglottitis

  - Influenza
    - 3 swabs – 2x nasal+ throat
    - Ag detection- Immuno-fluorescence/Rapid
    - PCR
    - Ab detection – not very useful
    - Virus culture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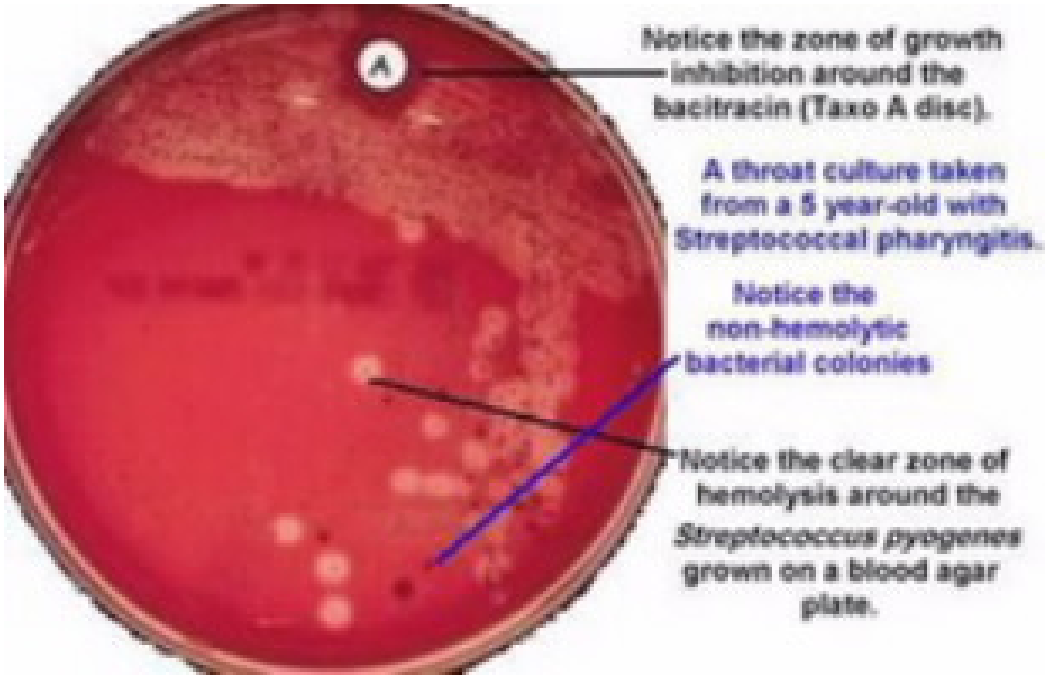
Streptococcus pyogenes on Columbia Sheep Blood Agar


A
Notice the zone of growth inhibition around the bacitracin (Taxo A disc).
A throat culture taken from a 5 year-old with Streptococcal pharyngitis.
Notice the non-hemolytic bacterial colonies
Notice the clear zone of hemolysis around the Streptococcus pyogenes grown on a blood agar plate.

Bronchitis and Bronchiolitis

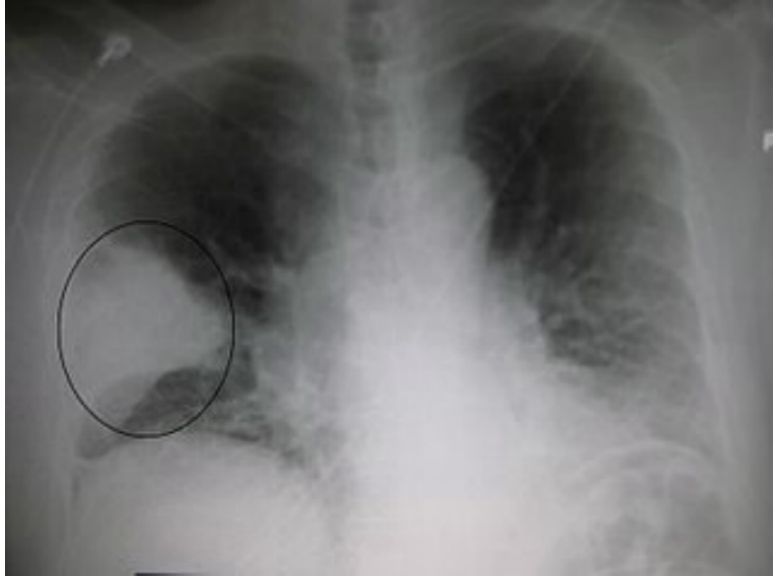
- Inflammation of the bronchial tree
- Usually preceded by an upper respiratory tract infection

- Chronic bronchitis
  - appears to be caused by a combination of environmental factors, such as smoking, and bacterial infection with pathogens such as *H influenzae* and *S pneumoniae*

- Bronchiolitis is a viral respiratory disease of infants and is caused primarily by respiratory syncytial virus

Pneumonia

- Pneumonia is an inflammation of the lung parenchyma
- Lobar pneumonia
  - alveolar process involving an entire lobe of the lung
- Bronchopneumonia
  - alveolar process occurring in a distribution that is patchy without filling an entire lobe
- Atypical pneumonias
  - those that are caused by atypical bacteria
- Aspiration pneumonia
  - caused by anaerobic organisms which usually occur in patients with periodontal disease or depressed consciousness
- CAP
- HAP



Lobar pneumonia

Bronchopneumonia

CAP

- Pneumonias occurring in usually healthy persons who become ill outside of a hospital or chronic-care facility
- Agents of CAP
  - *Streptococcus pneumoniae* - in very young and elderly
  - *Haemophilus influenzae*
  - *Staphylococcus aureus*
  - *Moraxella catarrhalis*
  - *Mycoplasma pneumoniae* - b/w 5-19 years
  - *Chlamydia pneumoniae* } Atypical
  - *Legionella pneumoniae* }

HAP

- Infections acquired 48-72 hours after admission to a health care setting
- Contributory factors
  - Hospitalised >/=2 days within 90 days preceeding this infection
  - Resides in nursing care facility
  - Recent antibiotic therapy or chemotherapy
  - Wound care within past 30 days of this illness
  - haemodyalisis

- Pneumonia is the most frequent nosocomial infection in ICUs
- Most cases are associated with mechanical ventilation – VAP
- Frequently, infections are polymicrobial

Agents of HAP

- Gram-negative bacilli 59%
  - ***Pseudomonas aeruginosa* 16%**
  - *Enterobacter* species 11 %
  - *Klebsiella pneumoniae* 7 %
  - Other enteric gram-negative bacilli 9 %
- ***Staphylococcus aureus* 17 %**
  - Mostly MRSA
- *Acinetobacter* 3 %

- *Streptococcus pneumoniae* 2-20 %
- Anaerobes 10-20 %
- Fungi 0-10%
- Mixed 13-54%

Pneumonia in immunocompromised patients

- Pneumonia is one of the most life-threatening infections in the immunocompromised host
- A broad range of pathogens needs to be excluded
    - TB
    - Fungal
    - Viral
    - Bacterial – common pathogens, gram negative coliforms

Fungal causes of Pneumonia - **SDL**

- *Pneumocystis carionii*
    - *Pathogen*
    - *Pathogenesis*
    - *Risk factors*
    - *Diagnosis and treatment*

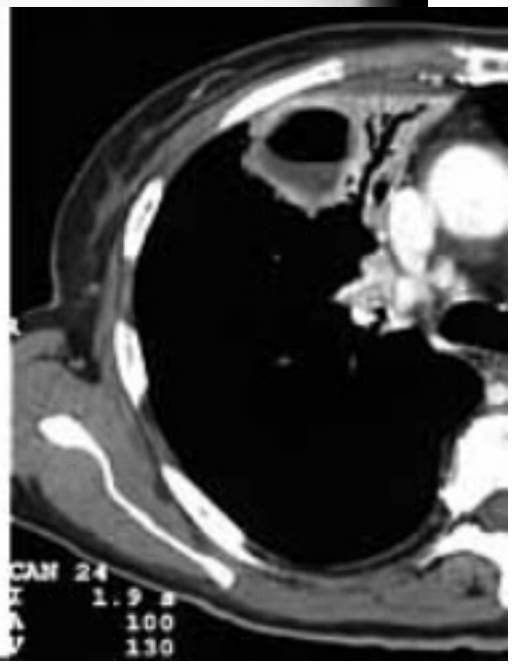
In the course of the disease increasing butterfly-shaped densities starting from the hilum.
HIV+ Pt

Empyema

- Pus in the pleural space
- It typically is a complication of pneumonia (when para-pneumonic effusion gets infected)
  - Pathogens - *S.pneumoniae, Streptococcus milleri, Haemophilus influenzae*, *S. aureus* (MSSA/MRSA*)*, gram-negative organisms and anaerobes
- It can also arise from
    - penetrating chest trauma
    - esophageal rupture
    - complication from lung surgery

Lung abscess

- A localized area of pus formation in the lung parenchyma
- As the abscess grows the tissue becomes necrotic and collapsed creating a cavity
- Causes
    - Post pneumonia - *S. aureus* (MSSA/MRSA*)*, Klebsiella , *S. pneumoniae, Pseudomonas*
    - Aspiration – anaerobes and aerobes mixed (Fusobacteium , Bacteroides, Peptostreptococcus)
    - Endobronchial obstruction – tumour ,FB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Lab diagnosis of LRTI

- Specimens
  - Respiratory specimens
    - Sputum (coughed up or induced)
    - Endotracheal secreations (ET)
    - Bronchoscopic specimens
      - Bronchial washing
      - Broncho-alveolar lavage (BAL)
      - Protected specimen brush (PSB)
    - Lung biopsy (FNA/ transbronchial/ open)
    - Pleural aspirate
    - Pleural biopsy
    - Empyema aspirate

Lab diagnosis of LRTI

- Specimens
  - Other specimens
    - Blood
    - urine

Sputum collection and transport

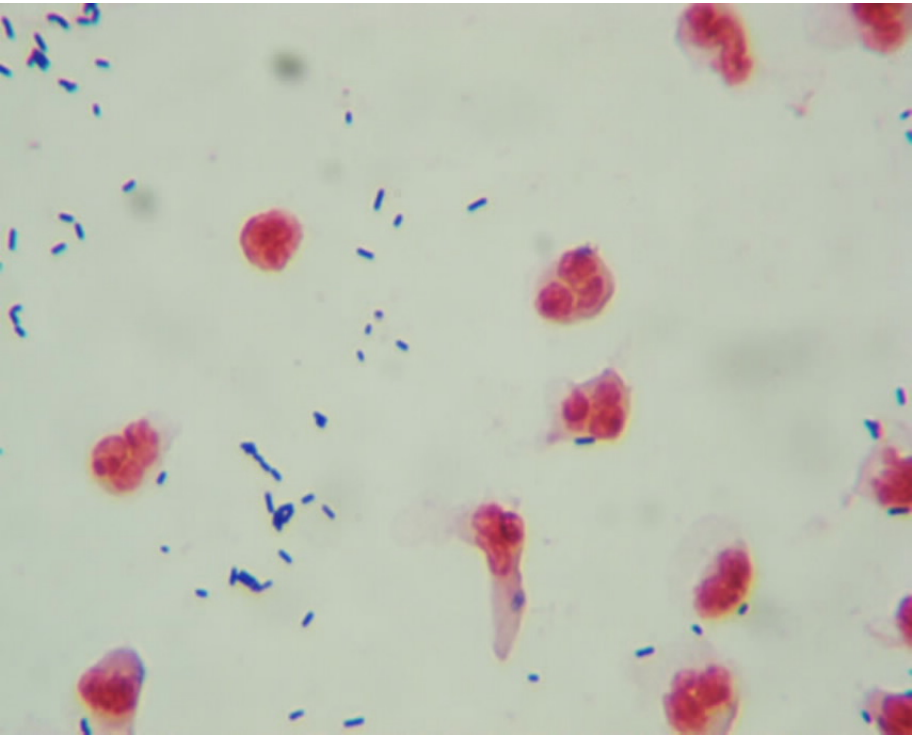
- Proper instructions should be given to the patient to collect a good quality sample deep from the lungs (not saliva)

- Collected into a sterile container

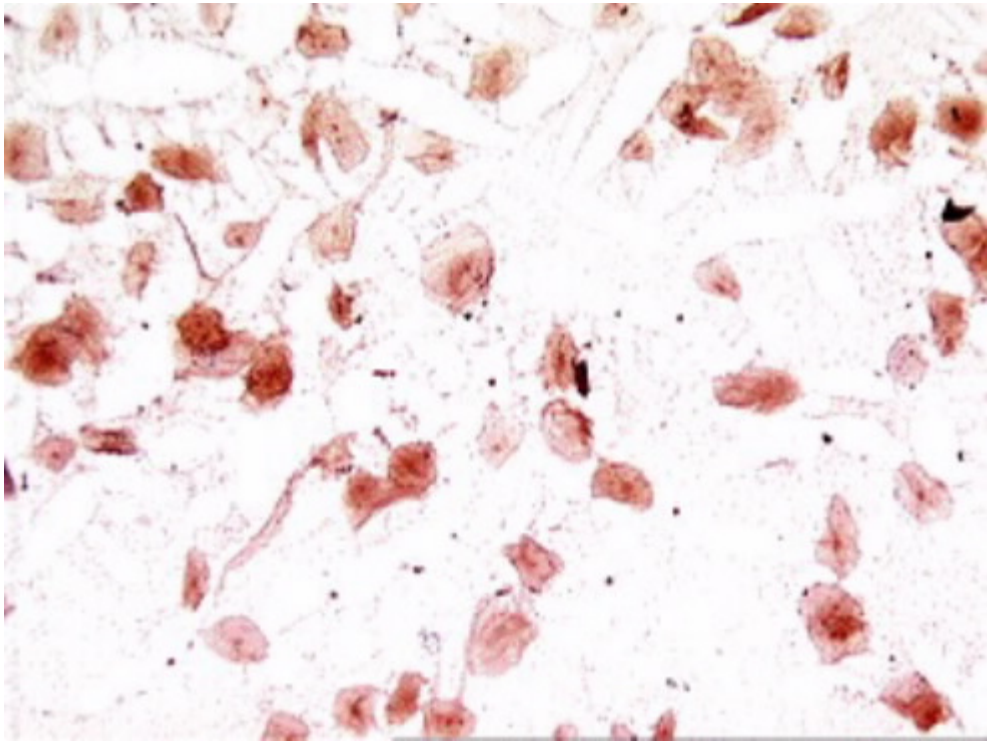
- Transported within 2 hours of collection
- Refrigerate if a delay is anticipated

Quality of sputum specimen

- Only good quality specimens are accepted and cultured
- Quality of the specimen is assessed by the presence of pus cells and epithelial cells in the Gram stained smear under low power
  - Pus cells >25/LPF with epithelial cells <10/LPF
    → good quality
    → represent a deeply coughed up sputum
    → accepted for culture
  - Pus cells <10/LPF with epithelial cells >25/LPF
    → poor quality
    → rejected (not cultured)
    → " unsatisfactory specimen for culture. Please repeat."



Suitable for culturing



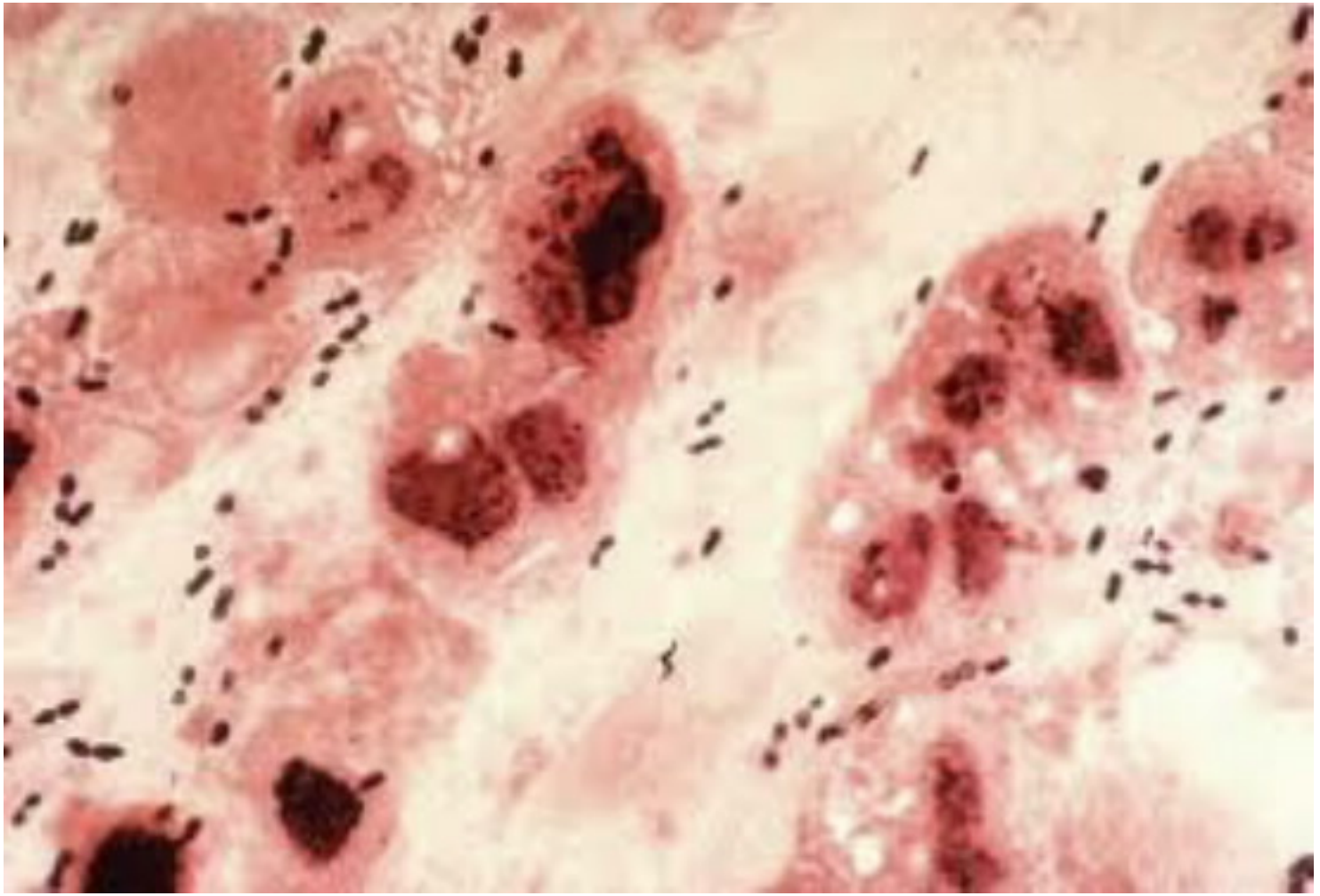
Unsuitable for culturing

If sputum can not be produced

- Induced sputum
  - By allowing patient to breath aerosolized droplets of saline for ~ 10 min.
  - Do not attempt in suspected TB patients
- Gastric aspirates
  - Used exclusively for isolation of TB
  - May be collected in very young children when it is difficult to collect sputum

Methods

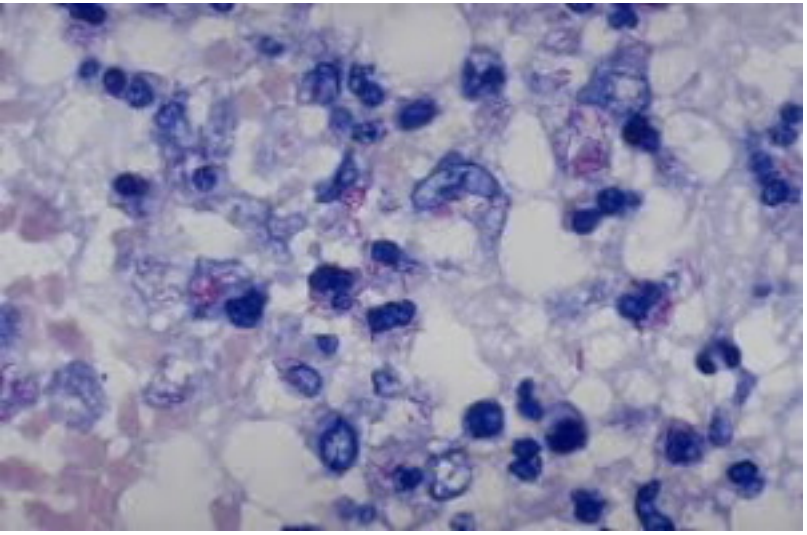
1. Sputum Gram stain
   - Utility controversial for diagnosis of pneumonia
   - Can not diagnose the eatiological agent with certainty
   - Important to recognize the suitability of specimen for culture
   - Look for morphology , staining characteristics and predominant organism
     - Predominance of lanceolated GPDC – *S. pneumoniae* sen 62%, spe 88%
     - Small gram negative coccoacilli – *H. influenza*



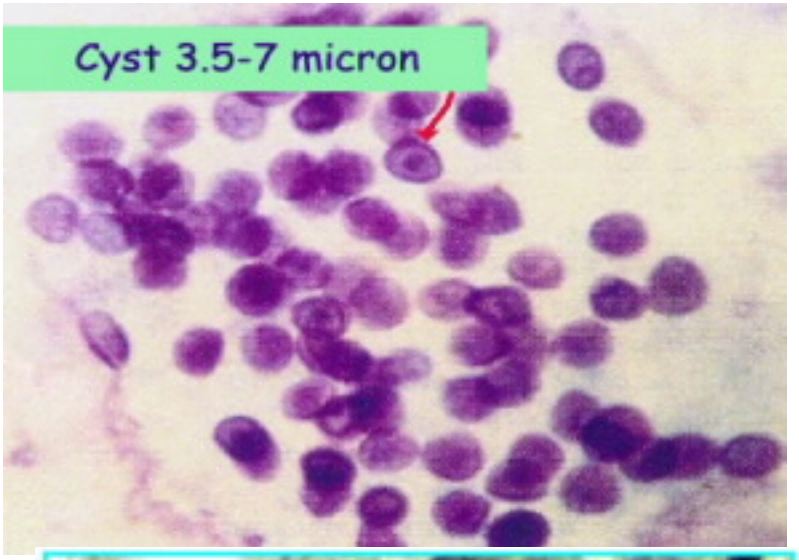
Methods

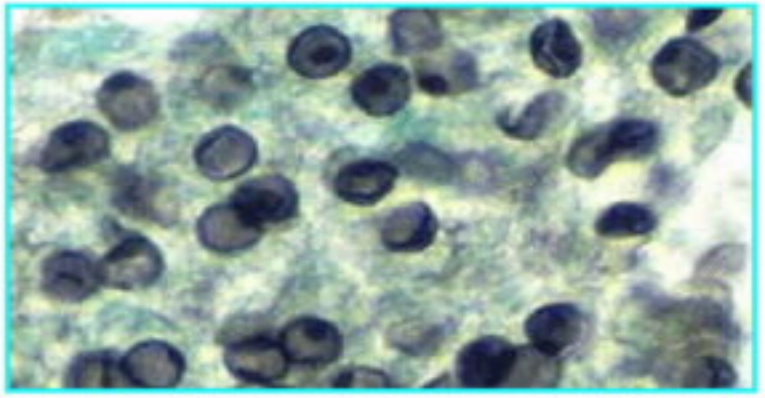
2. Other staining techniques

Z-N staining → AFB



Giemsa/ Toluidine O/
Gomari's methamine silver



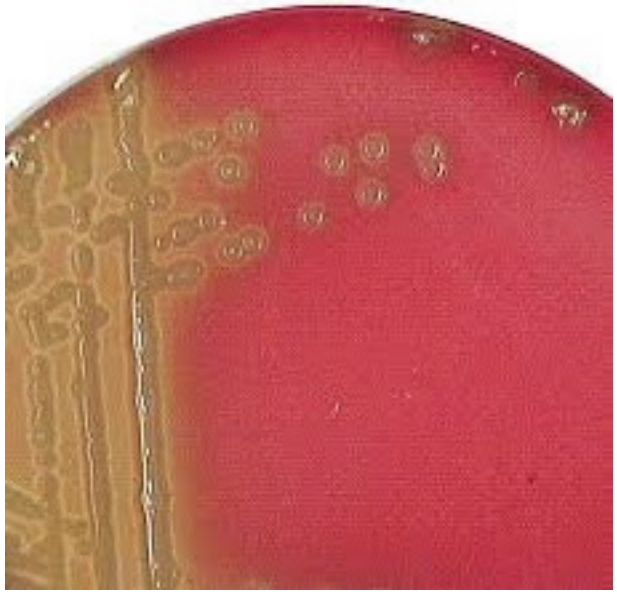


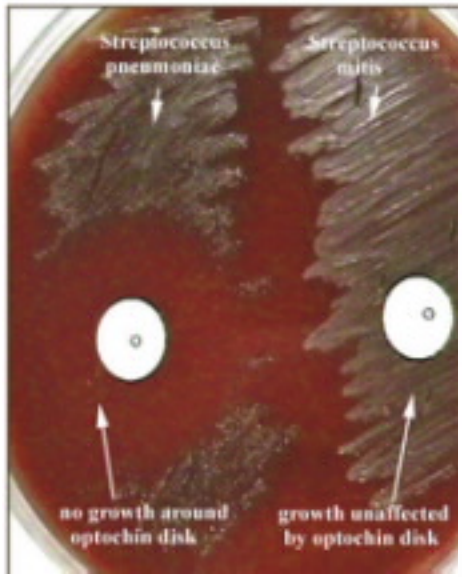
Methods

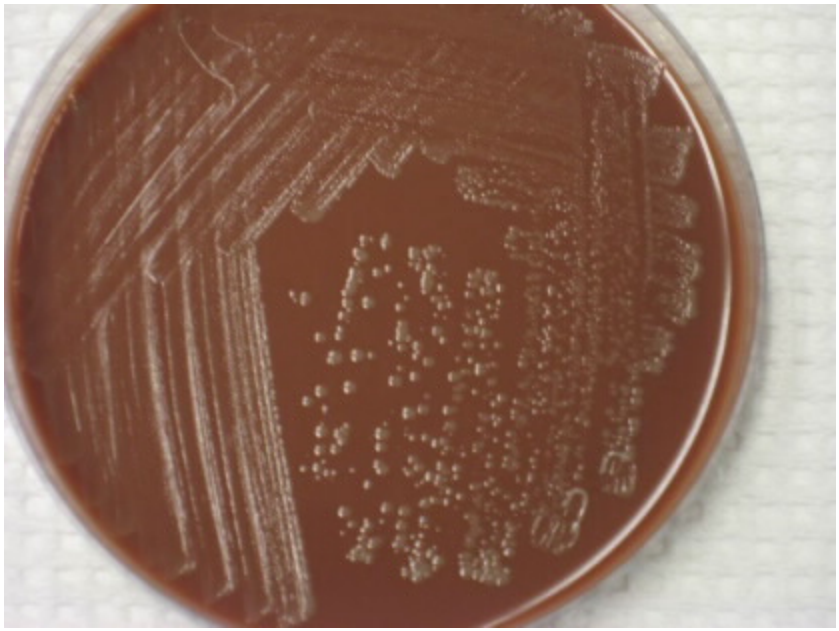
3. **Sputum Culture and ABST**
   - **Routinely inoculated on**
     - **Sheep blood agar**
     - **Chocolate agar**
     - **MacConkey agar**
   - **If suspecting legionnaires disease**
     - **Buffered charcoal yeast extract (BCYE) media**
   - **If suspecting TB**
     - **LJ media**
   - **If suspecting pertusis**
     - **Bordet Gengou medium**
   - **If suspecting fungal infection**
     - **Sabourauds Dextrose medium**

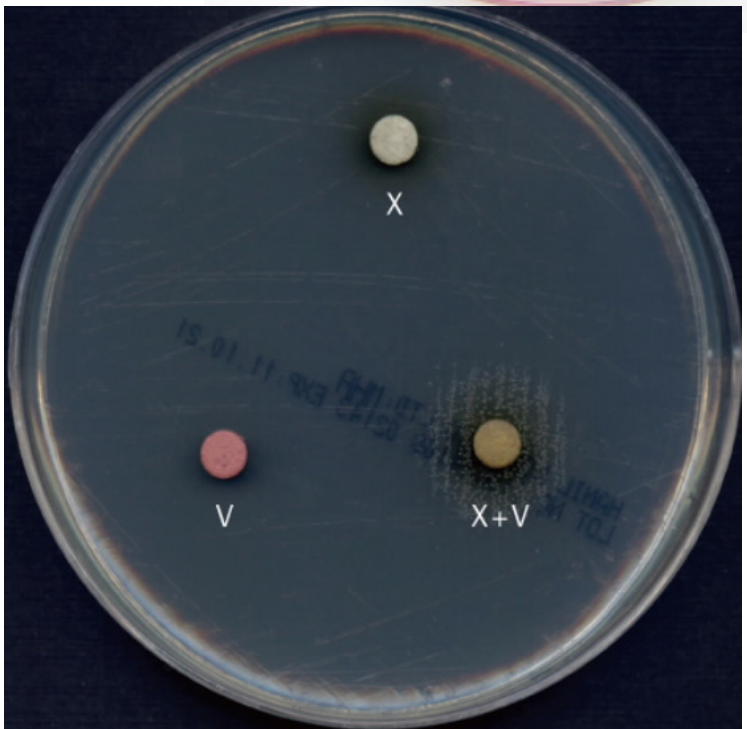
Methods

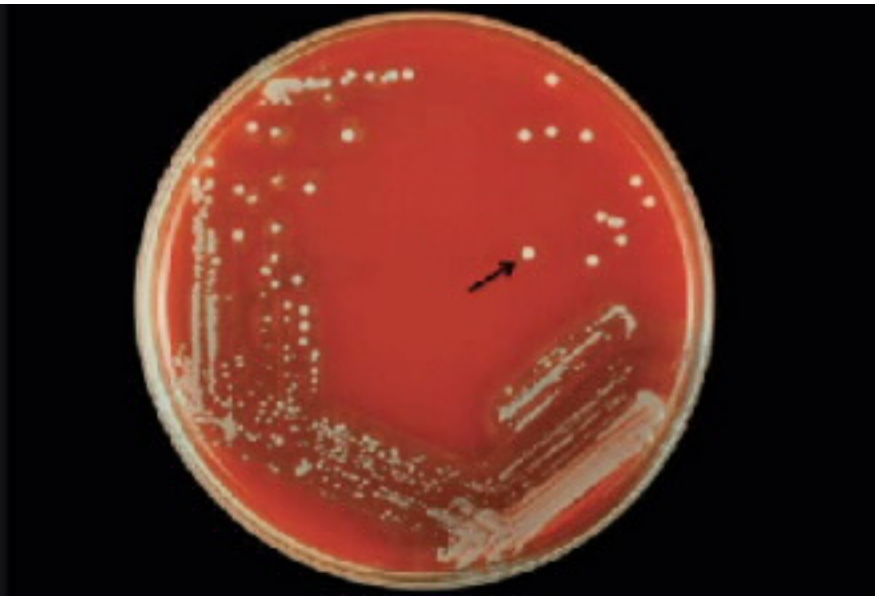
4. Culture of other specimens
   - Blood cultures are done in patients with pneumonia who are ill to be hospitalized (not for OPD patients), Lung abscess, Empyema
   - BAL and PSB specimens are superior specimens to diagnose LRTI
   - Quantitative cultures of ET, BAL, PSB are better than qualitative cultures to determine the significance of isolates



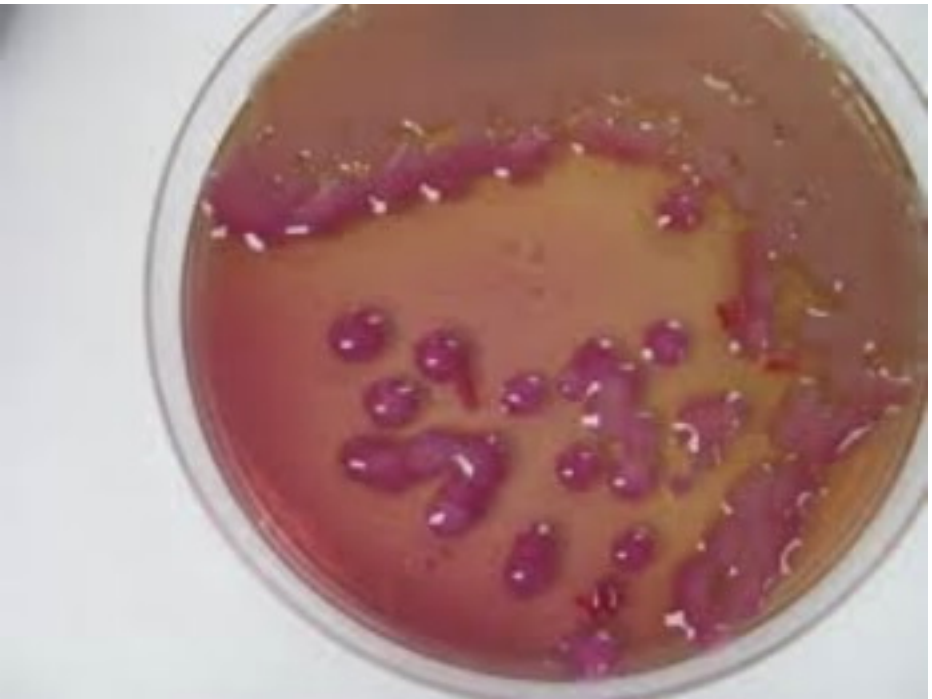

Streptococcus pneumoniae
Streptococcus mitis
no growth around optochin disk
growth unaffected by optochin disk




X
V
X+V




COAGULASE
TEST
POSITIVE
NEGATIVE

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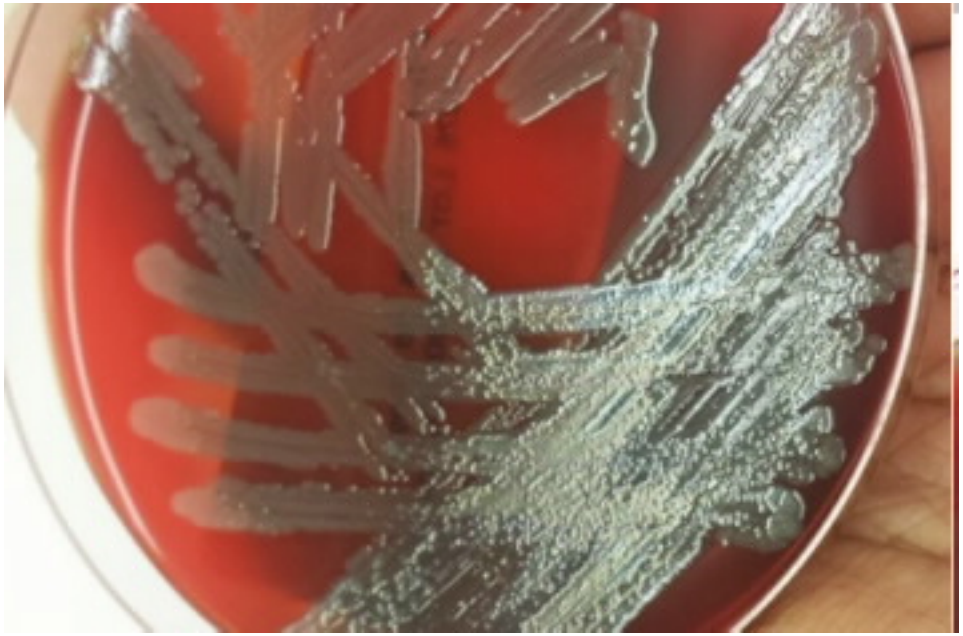

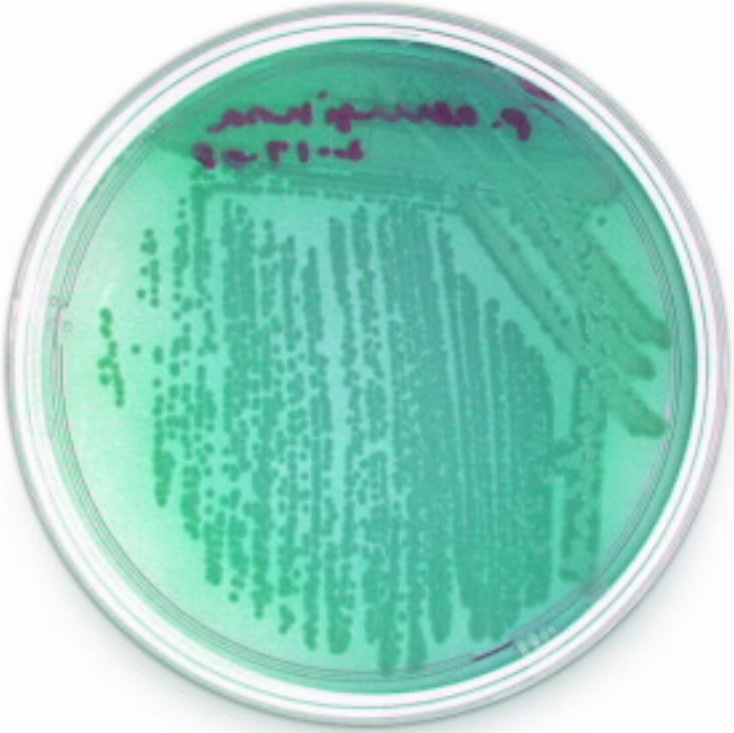

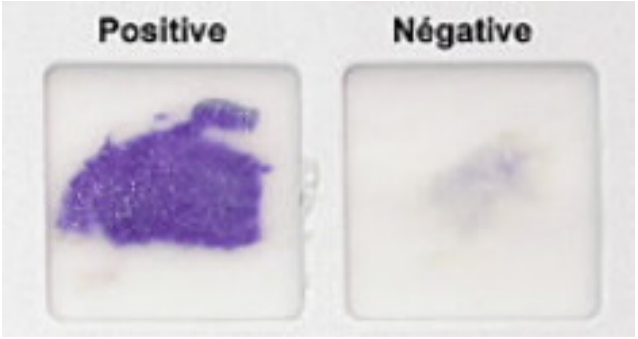
Positive
Négative

Methods

5. Antigen detection
   - In urine
     - *Leigionella pneumoplila* serogroup 1
       - Non invasive, high sensitivity & specificity, rapid
       - Ag persists in urine for weeks- months after treatment
     - *Streptococcus pneumoniae*
       - ICT
       - Detects C polysaccharide
   - In serum
     - Not done routinely

Methods

6. Serology – Abs
   - *Mycoplasma pneumoniae*
   - *Chlamydia pneumoniae*

   } IgM 1:16 or 4 fold rise in titre

   - *Leigionella*
   - *Coxiella burnetti*

   } Usefulness is limited

Methods

7. Molecular
   - NAAT/ Hybridization
   - Useful for uncultivable or fastidious organisms
     - Viral
     - TB
     - Leigionella, Mycoplasma, Chlamydia spp.
     - *Pneumocystis jiroveci*

Other diagnostic tests

- Radiological – CXR, CTS
- Cytokine assays – CRP
- Haematological – WBC/DC, BP

Summary

- Upper and lower respiratory tract infections needing microbiological investigations
- Pathogens to look for
- Collections and transport of specimens
  - Proper selection of specimen
  - Sterile container
  - Proper instructions to patients
  - Proper transport conditions
- Types of tests available, selection of appropriate test in each patient