RSM Lab 1

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Laboratory Diagnosis of Group A Streptococcus

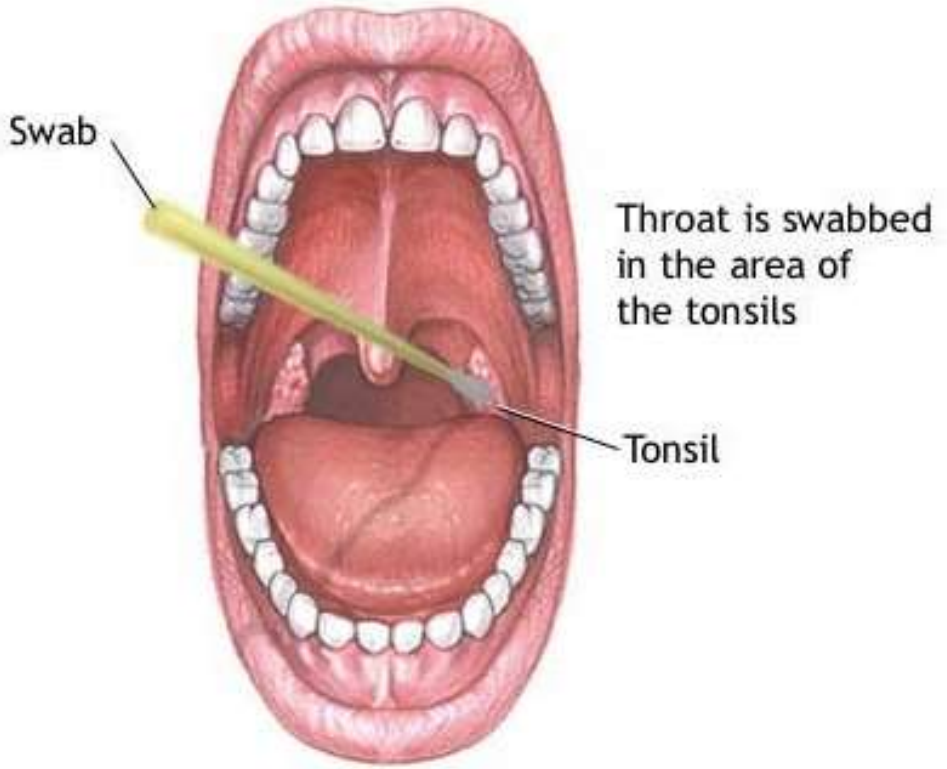
Steps of Laboratory Diagnosis of Group A Streptococcus

1. Specimen collection
2. Direct Antigen detection
3. Group A streptococci screening culture
4. Identification of GAS
5. Reporting results.

Laboratory Diagnosis of Group A Streptococcus

1- Specimen:

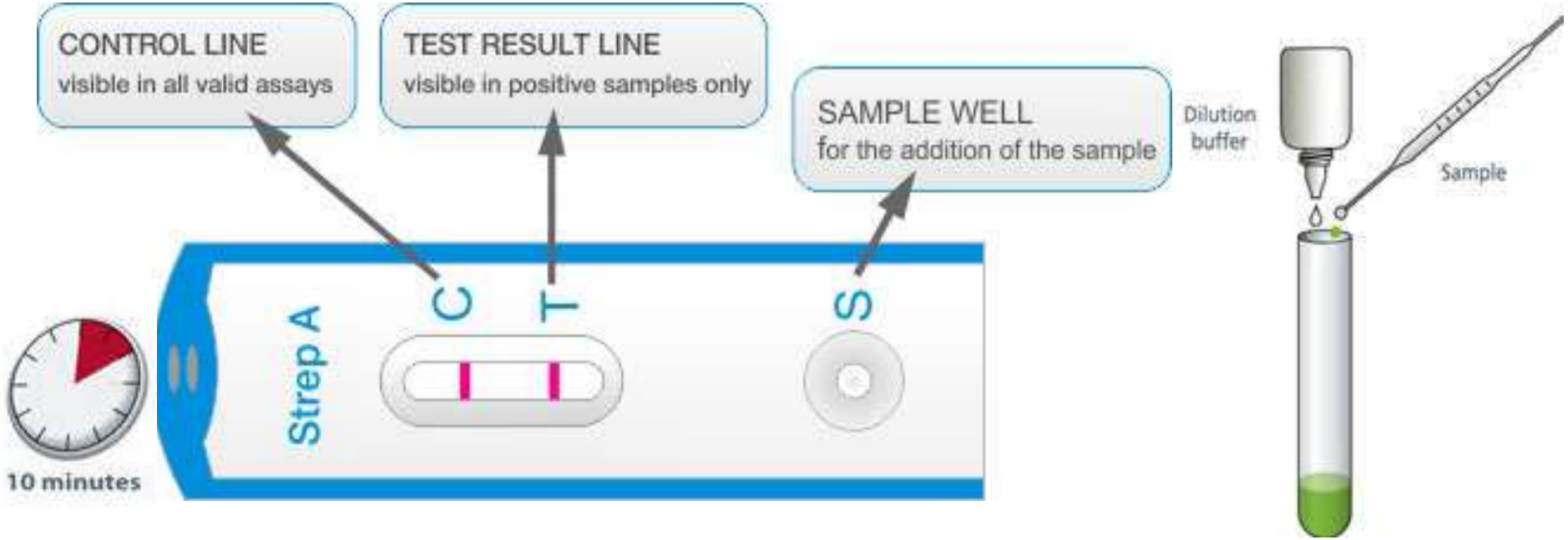
Throat swab of tonsillar area and/or posterior pharynx (Avoid the tongue and uvula)



Laboratory Diagnosis of Group A Streptococcus

2. Direct Antigen detection:

1. The patient's throat is first swabbed to collect a sample of mucus.
2. The sample is applied to a strip of nitrocellulose film and, if GAS antigens are present, these will migrate along the film to form a visible line of antigen bound to labeled antibodies
3. Because a common problem is the low sensitivity. All negtive results should be followed by culture.



Laboratory Diagnosis of Group A Streptococcus

2. Direct Antigen detection:

Interpretation:

The specificity of rapid sterp-test for the presence of GAS is at least 95%, with some studies finding close to 100% specificity. Therefore, if the test result is positive, the presence of GAS is highly likely. However, 5% to 20% of individuals carry GAS in their throats without symptomatic infection, so the presence of GAS in an individual with pharyngitis does not prove that this organism is responsible for the infection

Laboratory Diagnosis of Group A Streptococcus

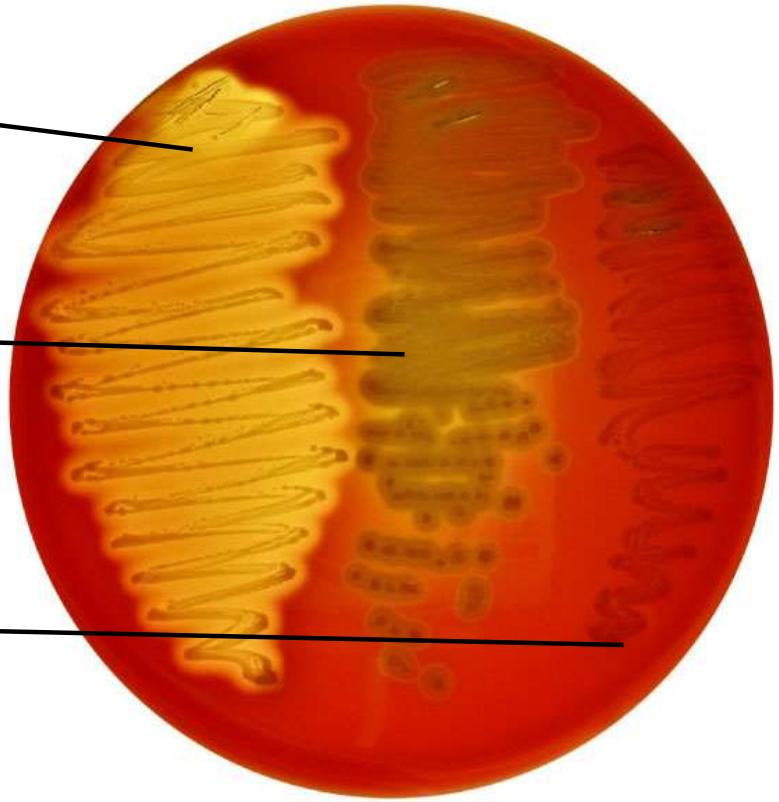
3. Group A streptococci screening culture

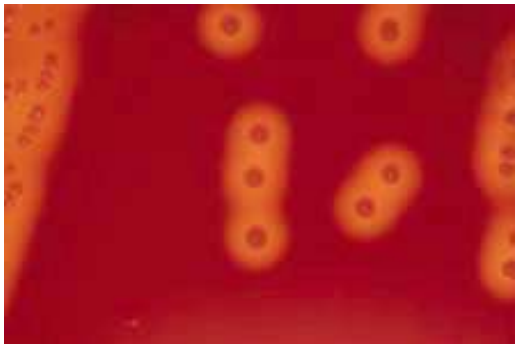
- Incubate cultures under atmospheric conditions (35ºC for 18-24h)
- Examine the presence of hemolytic colonies on blood agar
- Reincubate negative cultures for an additional 18-24h

β-hemolysis
(Group A streptococci)

α-hemolysis

γ-hemolysis

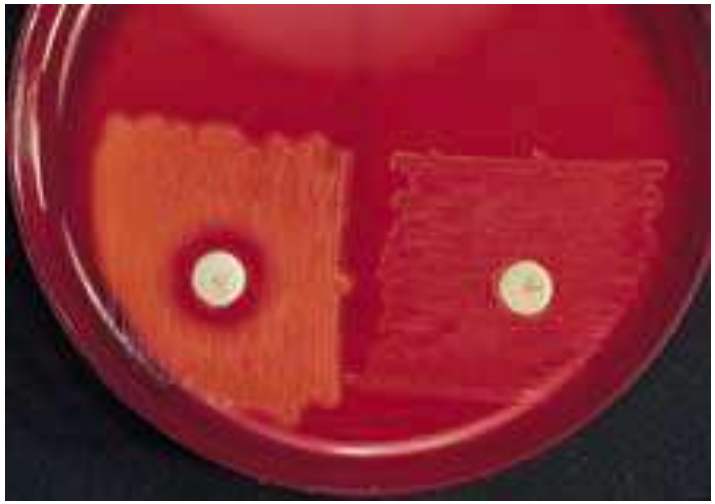




Laboratory Diagnosis of Group A Streptococcus

4. Identification of GAS:

- Catalase test
- Bacitracin susceptibilty
  - Principle:
    - For identification of group A
    - distinguish between *S. pyogenes* from other beta hemolytic streptococci
    - *Strep. pyogenes* is sensitive to Bacitracin giving zone of inhibition around disk



Group A streptococci is susceptible to Bacitracin disk (left); The right shows resistance

Laboratory Diagnosis of Group A Streptococcus

5. Reporting results:

The results on the microbiology request form may include

- *S. pyogenes* group A isolated
- beta hemoltyic streptococci, not group A streptococci isolated
- No *S. pyogenes* or beta hemolytic steptococci

DIAGNOSIS OF DIPHTHERIA

Diagnosis

1. The initial diagnosis of diphtheria is entirely clinical
2. Laboratory diagnosis

A. Specimen: from the nose and throat and any other mucocutaneous lesion. A portion of membrane should be removed and submitted for culture along with underlying exudate

B. Direct smear:

- Gram stain: club shaped Gram positive bacilli with chinese letter arrangment

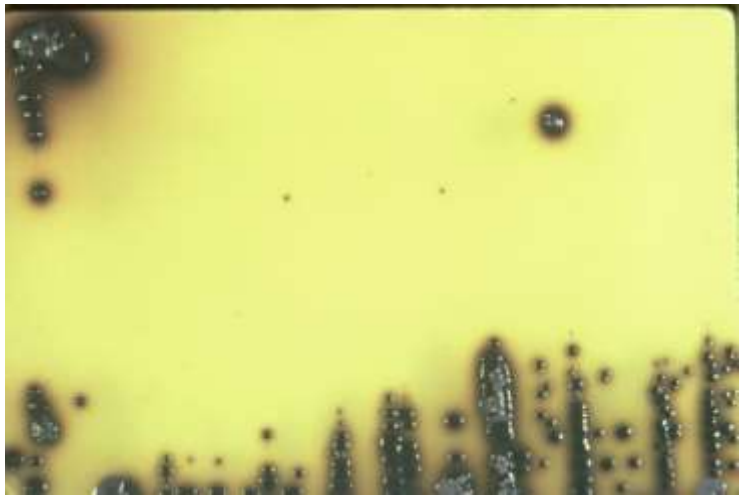
C. Culture media: cysteine-tellurite plate (Tisdale agar)

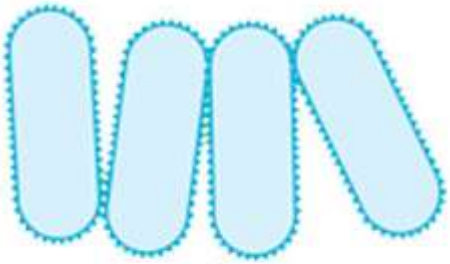
Results:

- C. diphtheriae : produce grayish-black colonies, surrounded by a brown/black halo.

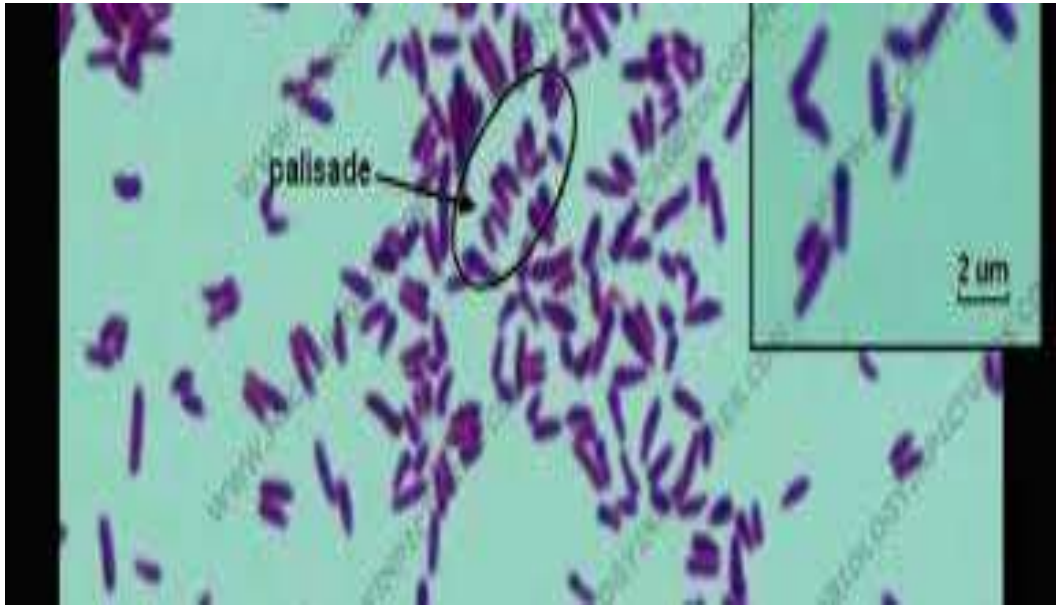
D. Urease and oxidase negative, Catalase posiive

DIAGNOSIS OF DIPHTHERIA





Palisades



DIAGNOSIS OF DIPHTHERIA

3. Toxin demonstration.

As the pathogenesis is due to diphtheria toxin, mere isolation of bacilli dose not complete the diagnosis. Toxin demonstration should be done following isolation, which can be of two types, in vivo and in vitro

- In vivo tests (animal inoculation):

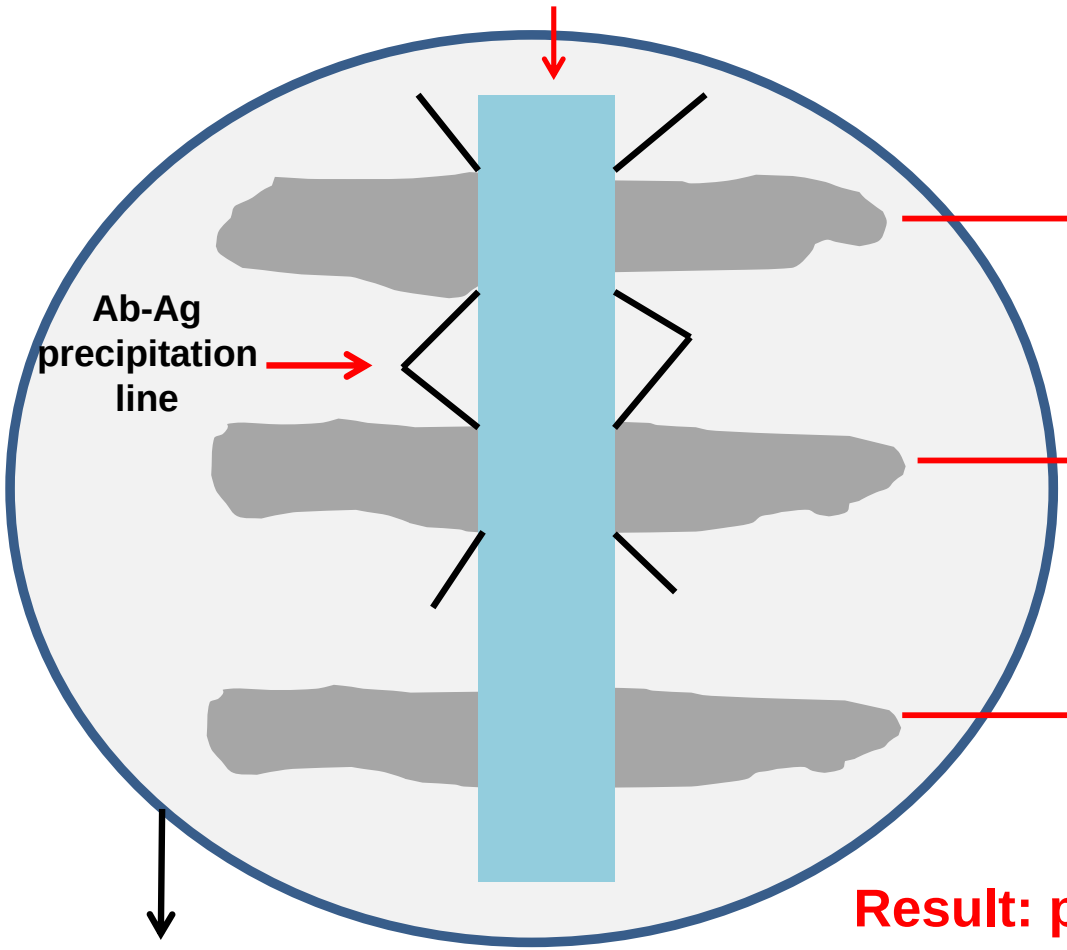
0.8ml of the culture broth of the test strain is injected subcutaneously into two guinea pigs, one of which has been protected with 500 units of the diphtheria antitoxin on the previous day

Results: if the strain is virulent, the unprotected animal will die within four days

- In vitro test: Elek's test

DIAGNOSIS OF DIPHTHERIA

Elek's test: rapid diagnosis (16-24 hrs)



Known toxigenic *C. diphtheriae* (positive control)

Unknown (patient's sample)

Known nontoxigenic *C. diphtheriae* (negative control)

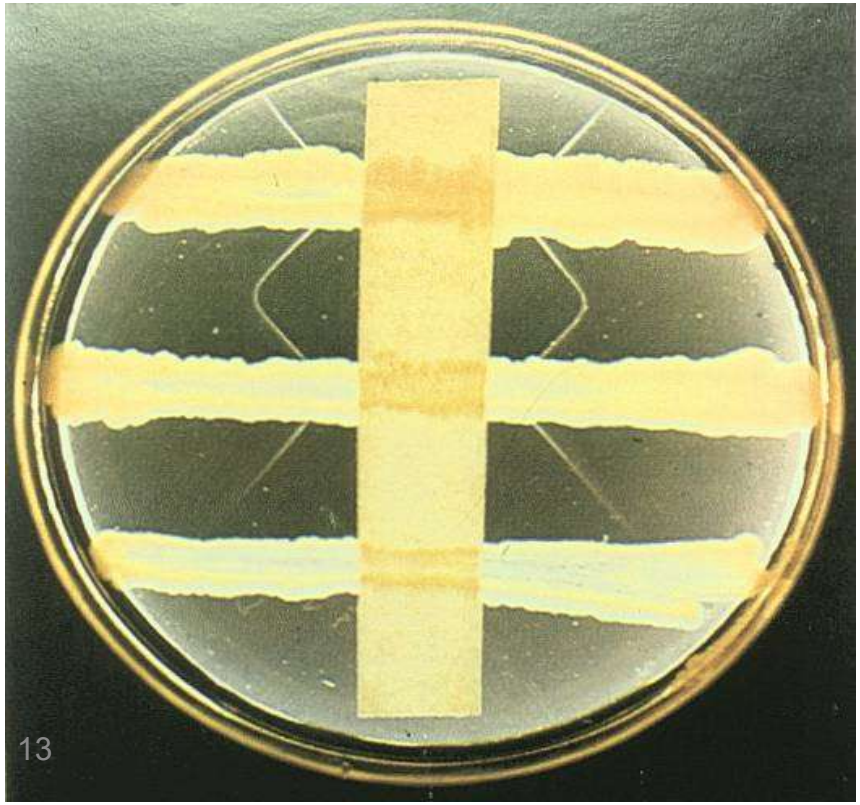
Result: positive toxigenic *C. diphtheriae*

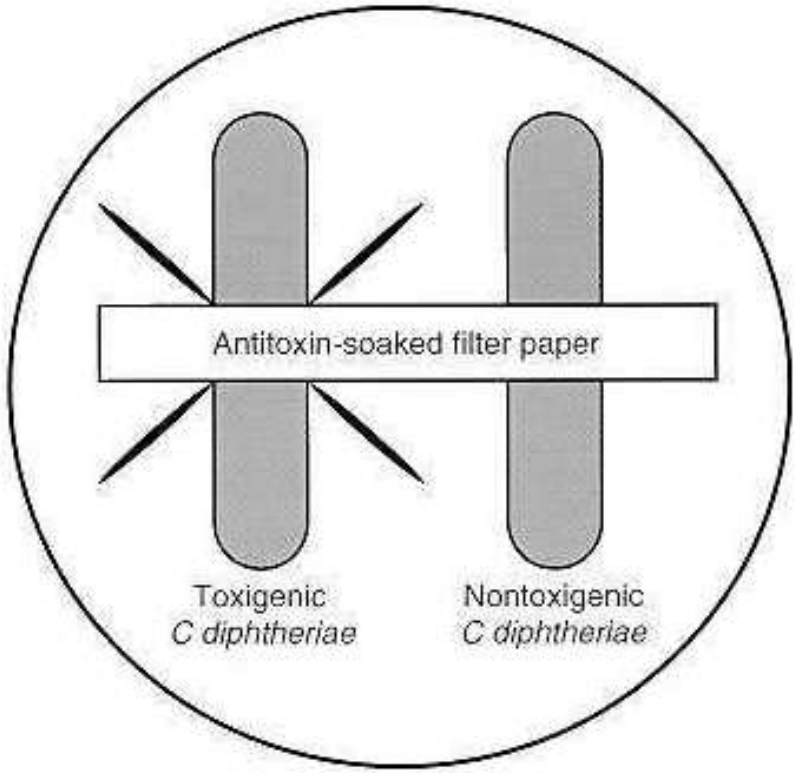
DIAGNOSIS OF DIPHTHERIA

Elek ‘s test: rapid diagnosis (16-24 hrs)

Results:

Positive test: formation of four radiating lines resulting from the precipitation reaction between exotoxin and diphtheria antitoxin.





Laboratory Diagnosis Lower Respiratory Infection

Sputum culture

The sputum culture is an important part of the diagnostic evaluation of potential lower respiratory tract infections. However, expectorated sputum specimens are variably contaminated by colonizing oropharyngeal flora, making results hard to interpret. Proper collection of the specimen is crucial to the recovery of the etiological agent.

Specimen criteria:

- If possible, specimen should be collected before antimicrobial treatment.
- First morning specimen is best.
- Specimen must be collected in a sterile container.
- If multiple cultures are ordered they should be collected at least 24 hours apart.

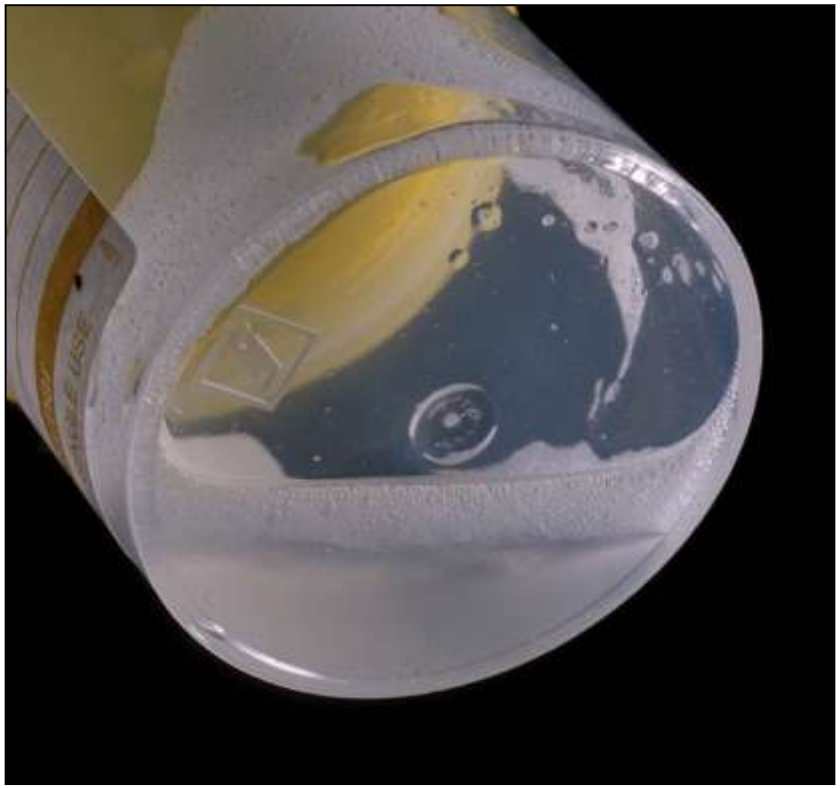
Laboratory Diagnosis Lower Respiratory Infection

Expectorated sputum

- Specimen collection should be supervised by a trained professional.
- Request the patient to remove any dentures and to rinse the mouth or gargle with plain water before specimen collection.
- Tell the patient to provide a specimen from a deep cough, avoiding, as much as possible, mixing the specimen with saliva or nasal secretions.
- Make sure the patient understands the difference between saliva (from mouth) and sputum (from chest).

Laboratory Diagnosis Lower Respiratory Infection

Specimen Quality



Poor quality sputum

Better quality

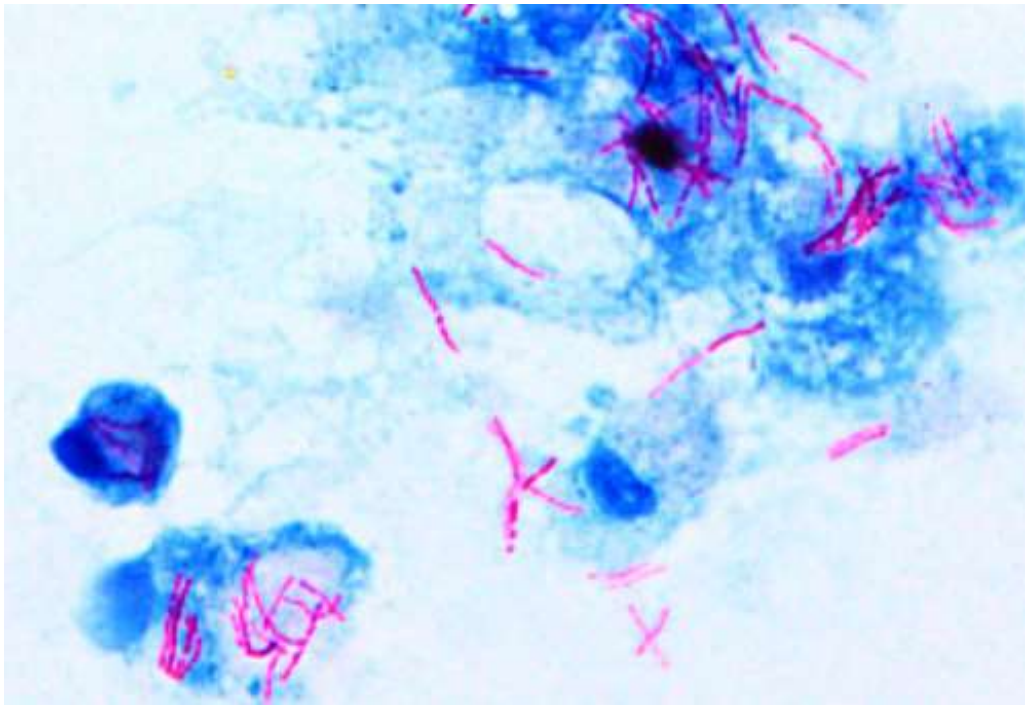
Laboratory Diagnosis Lower Respiratory Infection

Diagnosis of active Tuberculosis:

1- Direct microscopy by Ziehl-Neelsen staining:

Results of ZN stainng

- Negative results: AT least 100 oil immersion field should be examind before giving negtive results
- Positive results: *M. tuberculosis* appers as long slender red colored acid fast bacilli

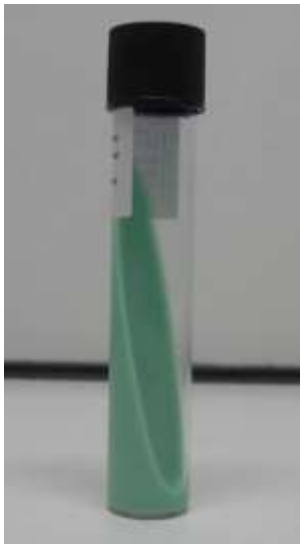


Laboratory Diagnosis Lower Respiratory Infection

Diagnosis of active Tuberculosis:

2- Culture :

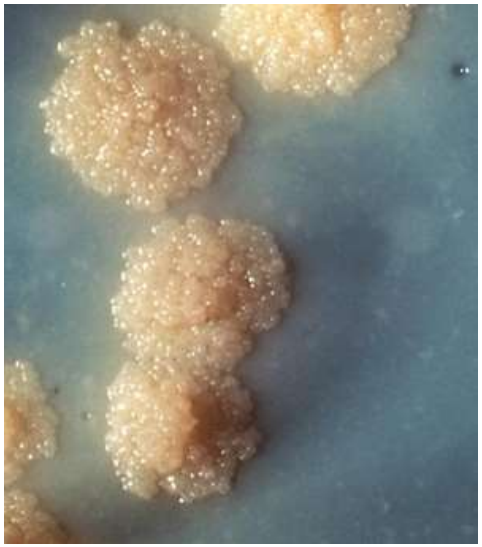
1. Incubation conditions: under aerobic conditions for 6-8 weeks
2. Culture media: Lowenstein-Jensen Agar (L J agar
3. Results: *M.tuberculosis* produces rough, and tough colonies



(L J agar)

3- Serology (ELIZA, Latex agglutination…..)

4- Moleculr methods (PCR)



***M.tuberculosis* colonies**

Laboratory Diagnosis Lower Respiratory Infection

Reasons to request mycobacterial culture:

- Patient previously on anti-TB treatment
- Still smear-positive after intensive phase of treatment or after finishing treatment
- Symptomatic and at high-risk of MDR-TB
- To test fluids potentially infected with M. Tuberculosis
- TB in health workers

Laboratory Diagnosis of *H. Influenzae*

1. Specimen collection and ransport

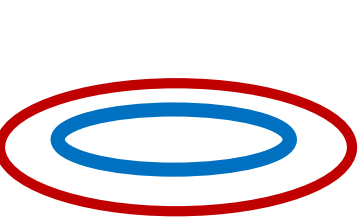
- Depending on the site of infection, various specimns may be collected such as CSF, blood, respiratory tract sputum, throat swabs, middle ear, and sinuses
- As H. influenzeis highly sensitive to low tempertures, the specimen should never be refragutrated
- Sample should be trasported and proscessed immdediatly without any delay.

2. Direct detection:

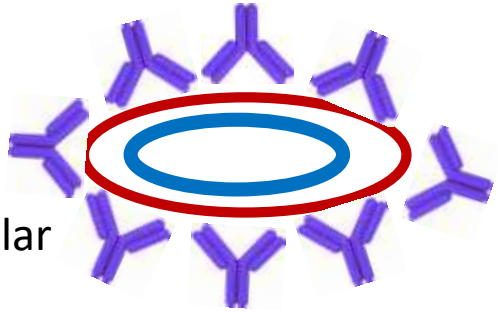
- Gram staining: preparation from different sampls may show gram-negative coccobacilli
- Capsule detection (Quellung reaction)
- Antigen detection: The type b capsular antigen can be detcted in CSF, urine, or other bdy fuids by
  - latex agglutination using particles coated with antibodies to type b antigen or
  - Direct immunofluresence test.

Laboratory Diagnosis of *H. Influenzae*

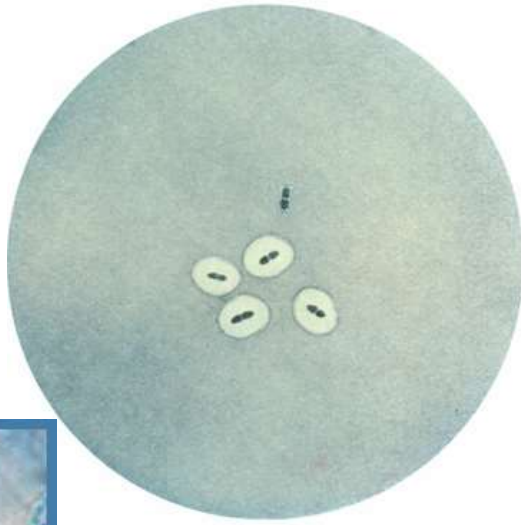
Capsule detection (Quellung reaction)

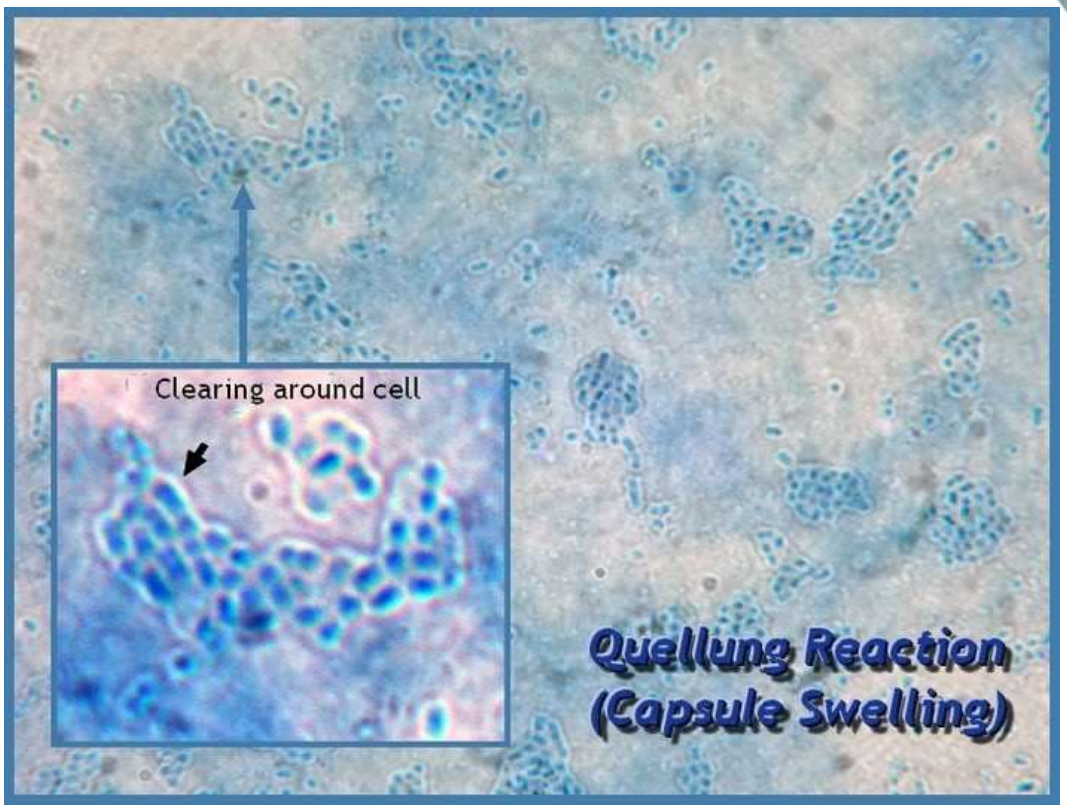


Anti capsular Ab



Under microscope





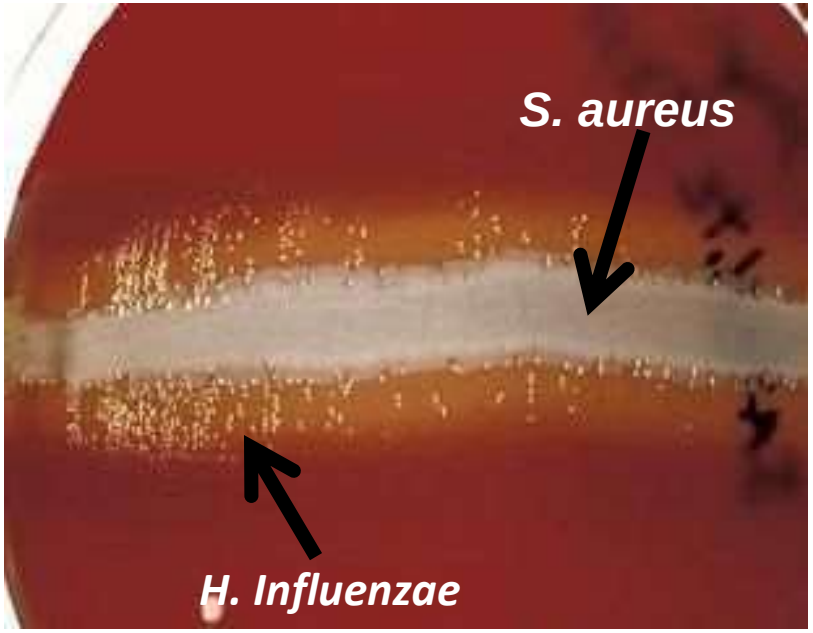
Laboratory Diagnosis of *H. Influenzae*

3. Culture:

A. Culture conditions: aerobic with 5-10 % CO2.

B. Culture media used are as follows:

- Blood agar with S. aurues streak line: Colonies of *H. influenzae* grow adjacent to S. *aurues* streak line (phenomenon is known as satellitism)
- Choclet agar



H. Influenzae grow around *S. aureus* utilizing X & V factors released from hemolyzed RBCs

H. Influenzae grown on Choclet agar

Laboratory Diagnosis of *H. Influenzae*

4. Biochemical tests:

- Reduces nitrate to nitrite.
- Catalase and Oxidase positive
- Fermentation of sugars: Glucose (+), Sucrose (-), Lactose (-) Mannitol (-).

Bordetella pertussis

Diagnosis

1. Types of samples:
   - Per-nasal swab
   - Cough plate
   - Post-nasal secretions
   - Post-nasal swab.

Bordetella pertussis

Diagnosis

2. Culture: clinical diagnosis of pertussis is best confirmed by isolation of B. pertussis from nasopharyngeal secretions or swabs. The preferred media are modified Bordet-Gengou medium and charcoal blood agar to which cephalexin has been added. The colonies should be first examined by Gram staining and then confirmed by slide agglutination test using specific antiserum. The final confirmation can also be done by subjecting the isolate to fluorescent antibody test.

3. Direct microscopy. Ordinary staining methods do not help in diagnosis of pertussis. A rapid diagnosis can be made by the fluorescent antibody technique applied directly to nasopharyngeal secretions
4. PCR

Bordetella pertussis

Diagnosis

Bordetella pertussis on **Bordet-Gengou agar**.

Colony morphology: small, shiny, round colonies, mercury-silver in color.