Respiratory System Module
2021-2022

Microbiology Lab

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Laboratory Diagnosis of Group A Streptococcus

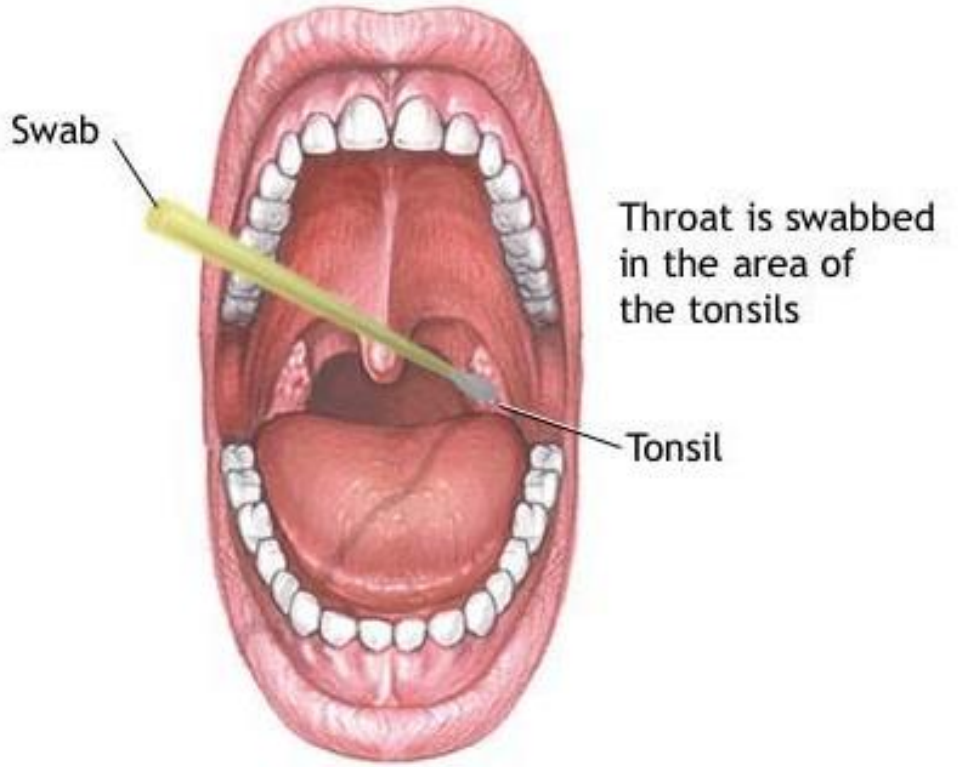
Steps of Laboratory Diagnosis of Group A Streptococcus

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:

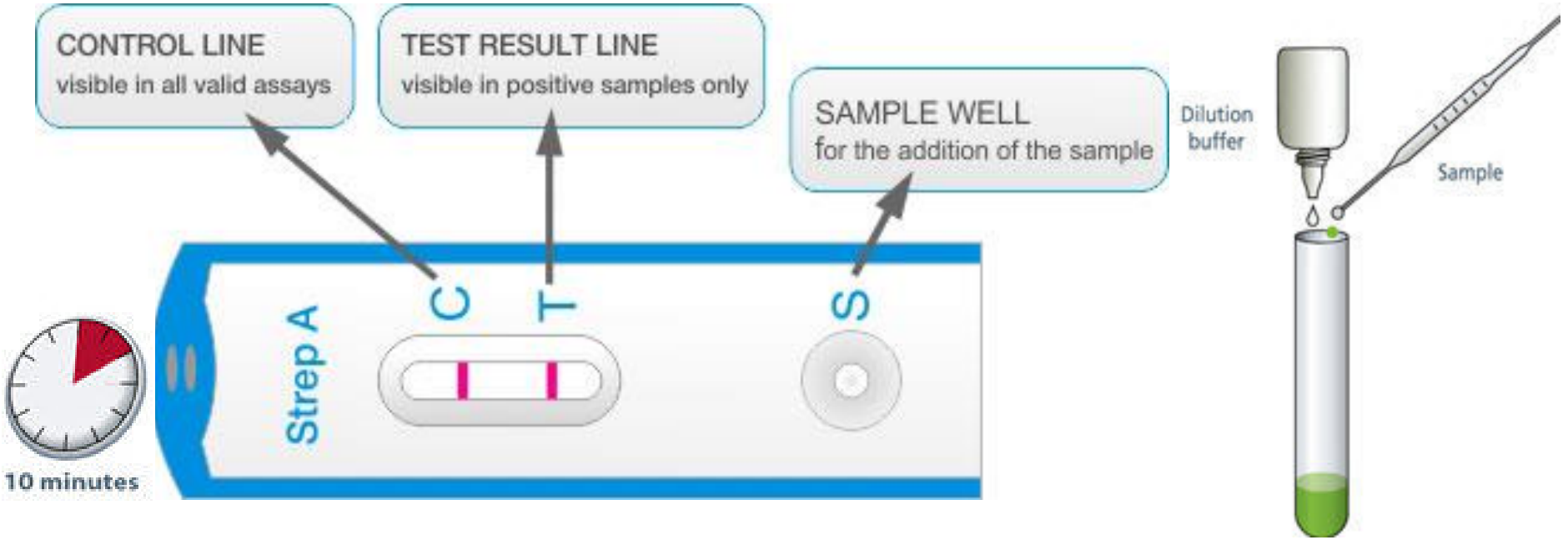
Throat swab of tonsillar area and/or posterior pharynx (Avoid the tongue and uvula)



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 negative results should be followed by culture.



Laboratory Diagnosis of Group A Streptococcus

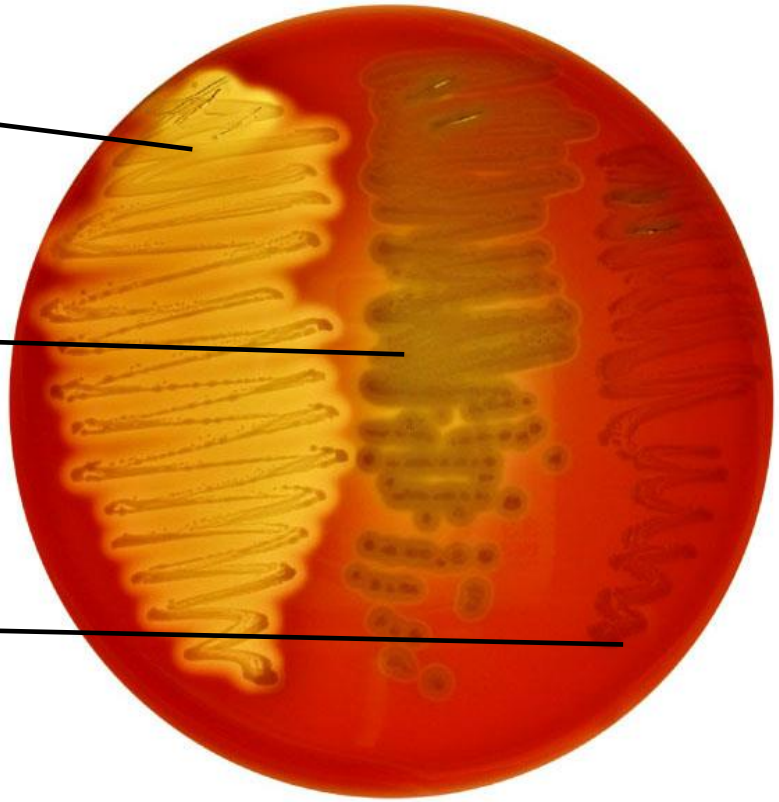
3. Group A streptococci screening culture

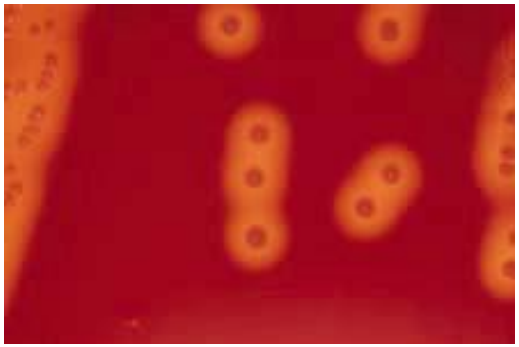
- Incubate cultures under atmospheric conditions (35^{o}C for 18-24h)
- Examine the presence of hemolytic colonies on blood agar
- Reincubate negative cultures for an additional 18-24h

β-hemolysis
(Group A streptococci)

α-hemolysis

γ-hemolysis

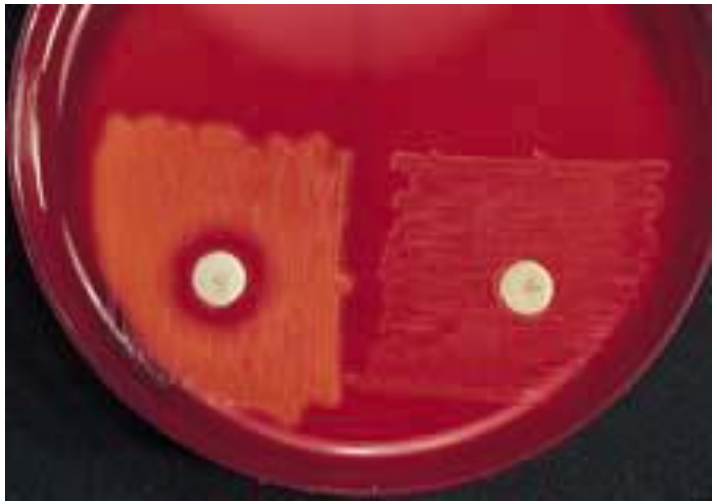




Laboratory Diagnosis of Group A Streptococcus

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

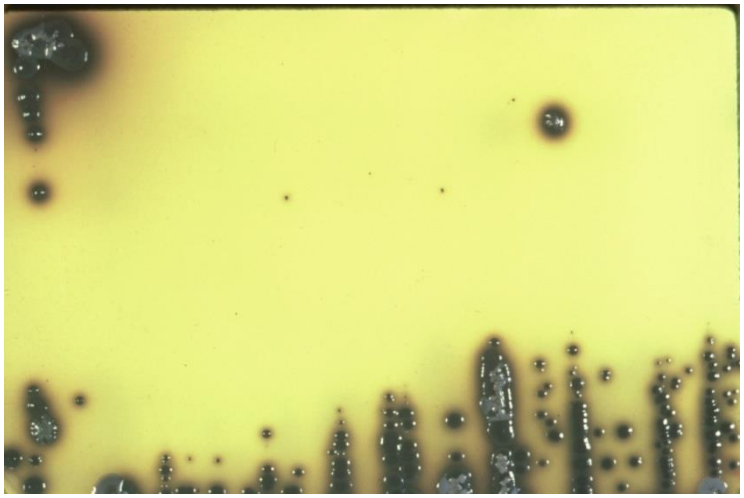
C. Culture media: cysteine-tellurite plate (Tisdale agar)

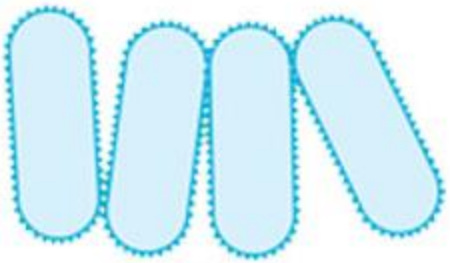
Results:

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

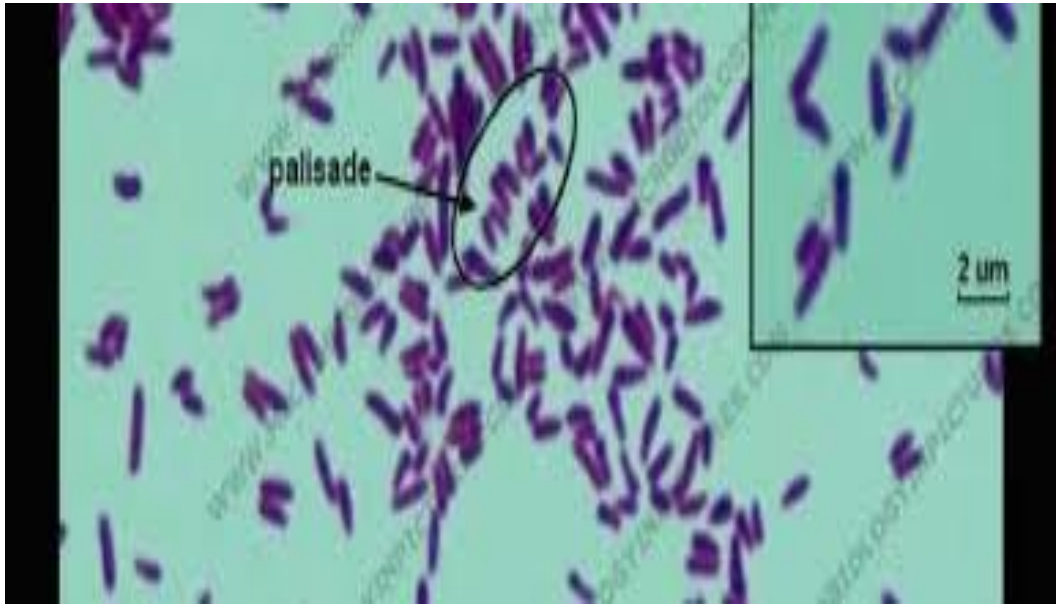
D. Urease and oxidase negative, Catalase positive

DIAGNOSIS OF DIPHTHERIA





Palisades



DIAGNOSIS OF DIPHTHERIA

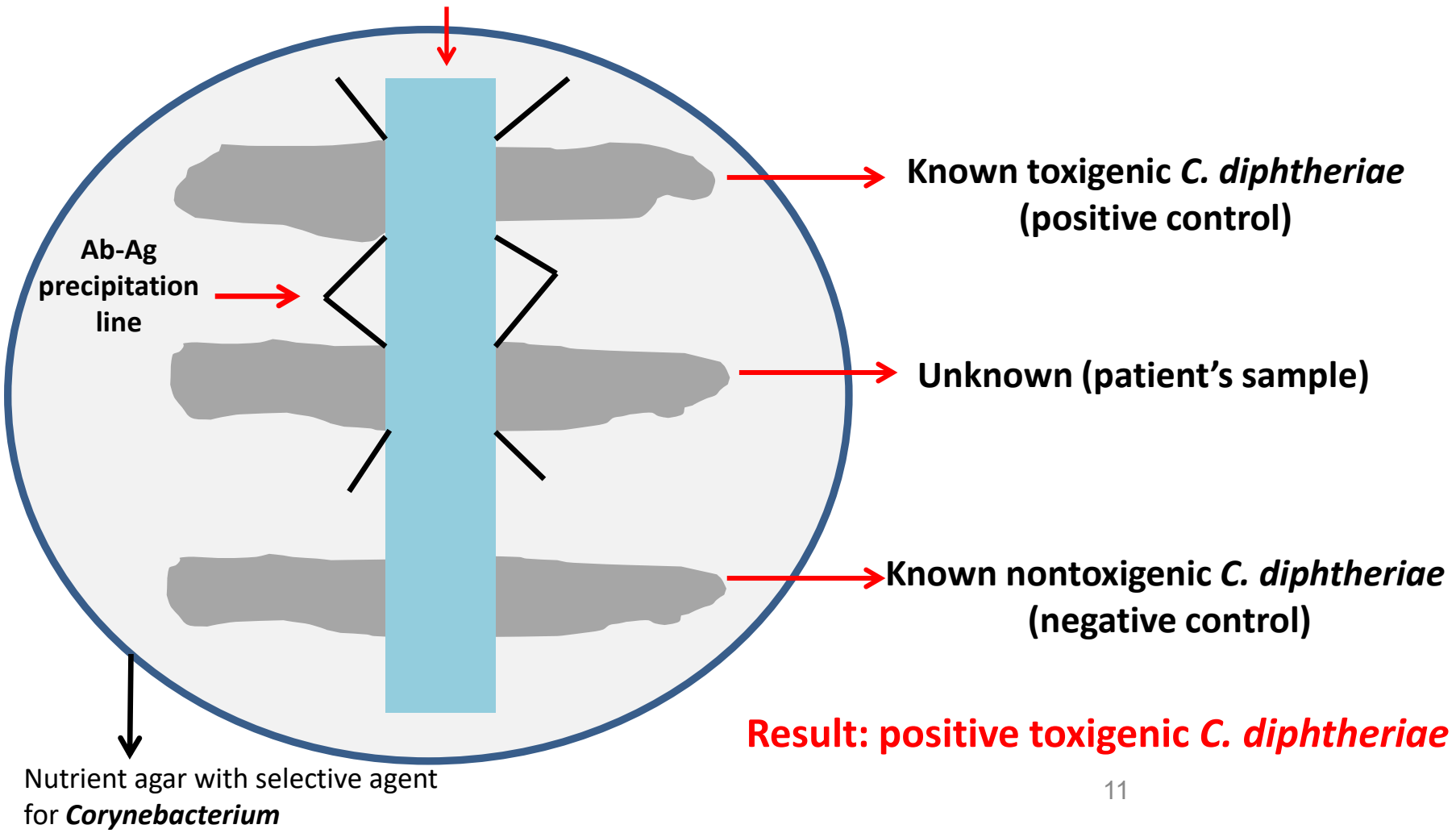
F. Toxin demonstration.

As the pathogenesis is due to diphtheria toxin, 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 vitro test: Elek's test**

DIAGNOSIS OF DIPHTHERIA

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

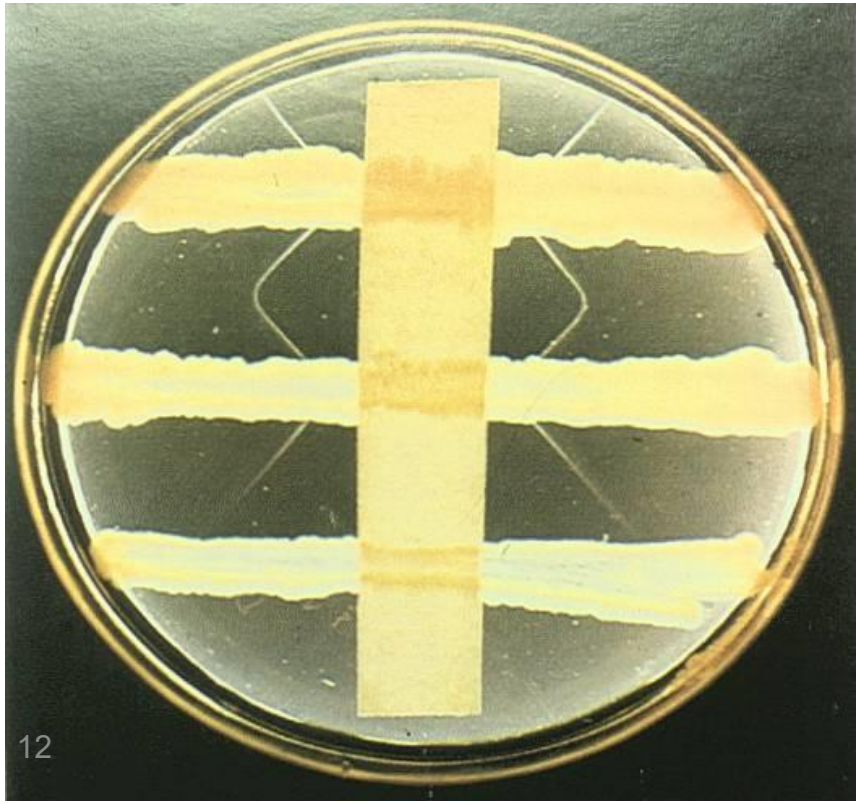


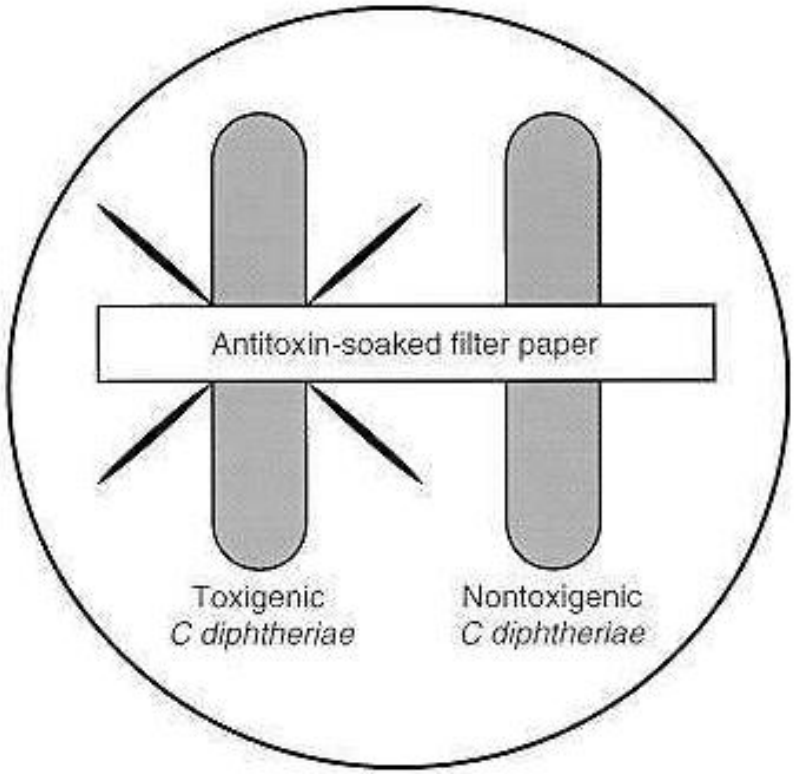
DIAGNOSIS OF DIPHTHERIA

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.

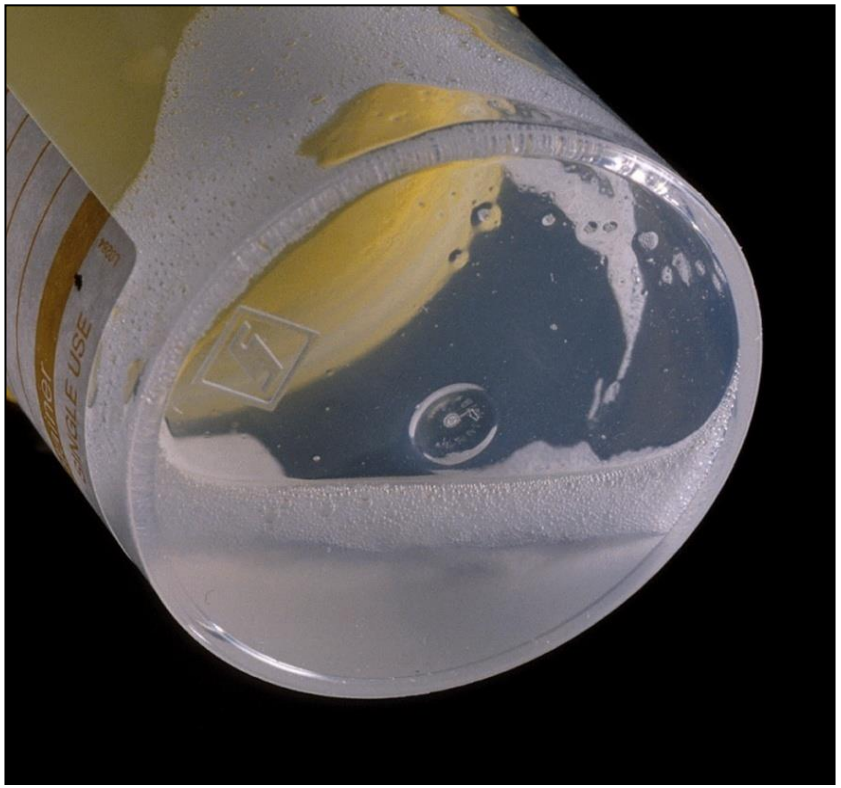
Laboratory Diagnosis Lower Respiratory Infection

Expectorated sputum

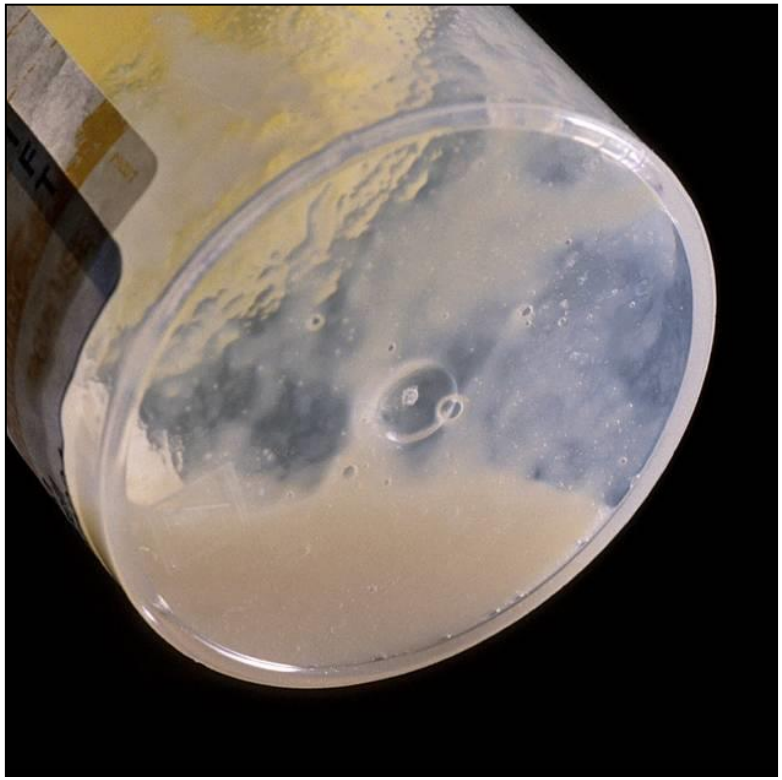
- Specimen collection should be supervised by a trained professional.
- Request the patient to remove any dentures and to rinse the mouth or gargle with plain water before specimen collection.
- Tell the patient to provide a specimen from a deep cough, avoiding, as much as possible, mixing the specimen 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 of *H. Influenzae*

1. Specimen collection and transport

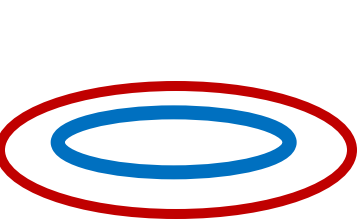
- Depending on the site of infection, various specimns may be collected such as CSF, blood, respiratory tract sputum, throat swabs, middle ear, and sinuses
- As H. influenze is highly sensitive to low tempertures, the specimen should never be refragutrated
- Sample should be trasported and proscessed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

2. Direct detection:

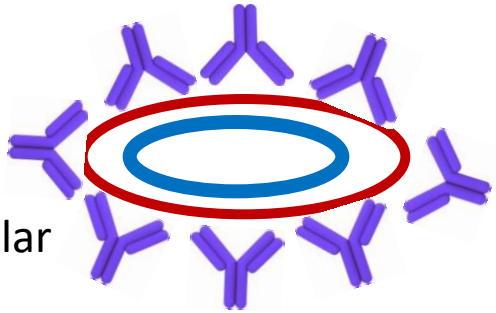
- Gram staining: preparation from different sampls may show gram-negative coccobacilli
- Capsule detection (Quellung reaction)
- Antigen detection: The type b capsular antigen can be detcted in CSF, urine, or other bdy fuids by
  - latex agglutination using particles coated with antibodies to type b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

Laboratory Diagnosis of *H. Influenzae*

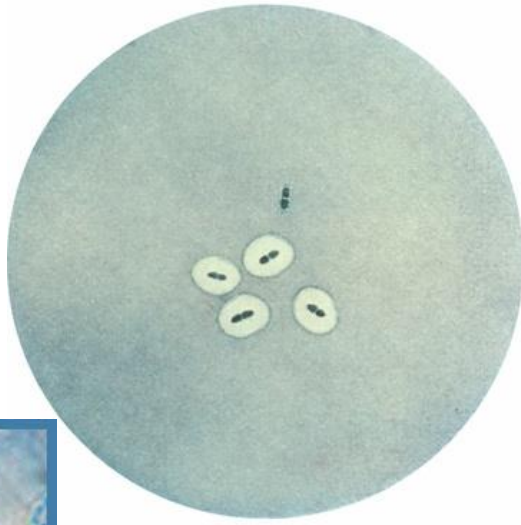
Capsule detection (Quellung reaction)

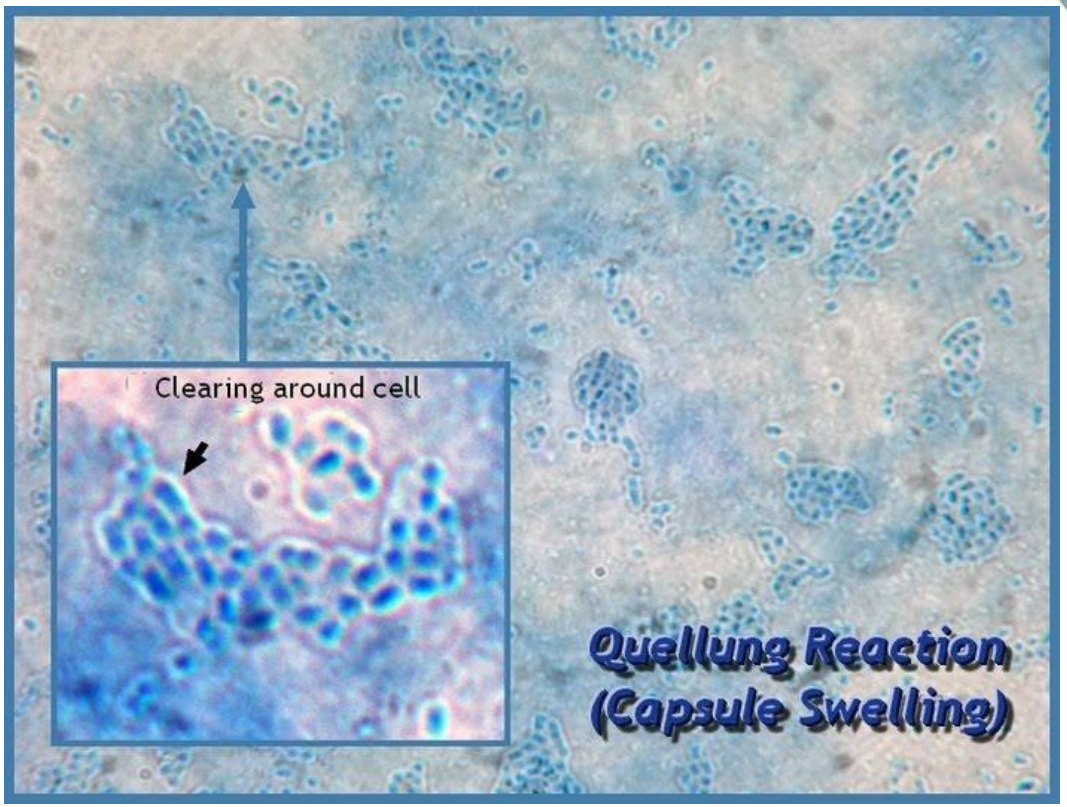


Anti capsular Ab



Under microscope





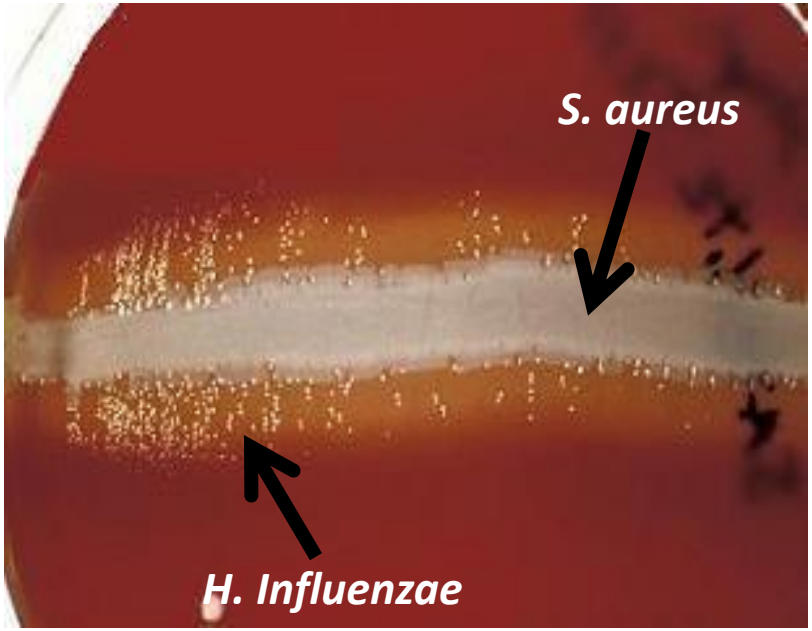
Laboratory Diagnosis of *H. Influenzae*

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*

Growth requirements

Laboratory Diagnosis of H. Influenzae

4. Biochemical tests:

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