

RSM Lab 1&2

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Laboratory Diagnosis of Group A Streptococcus

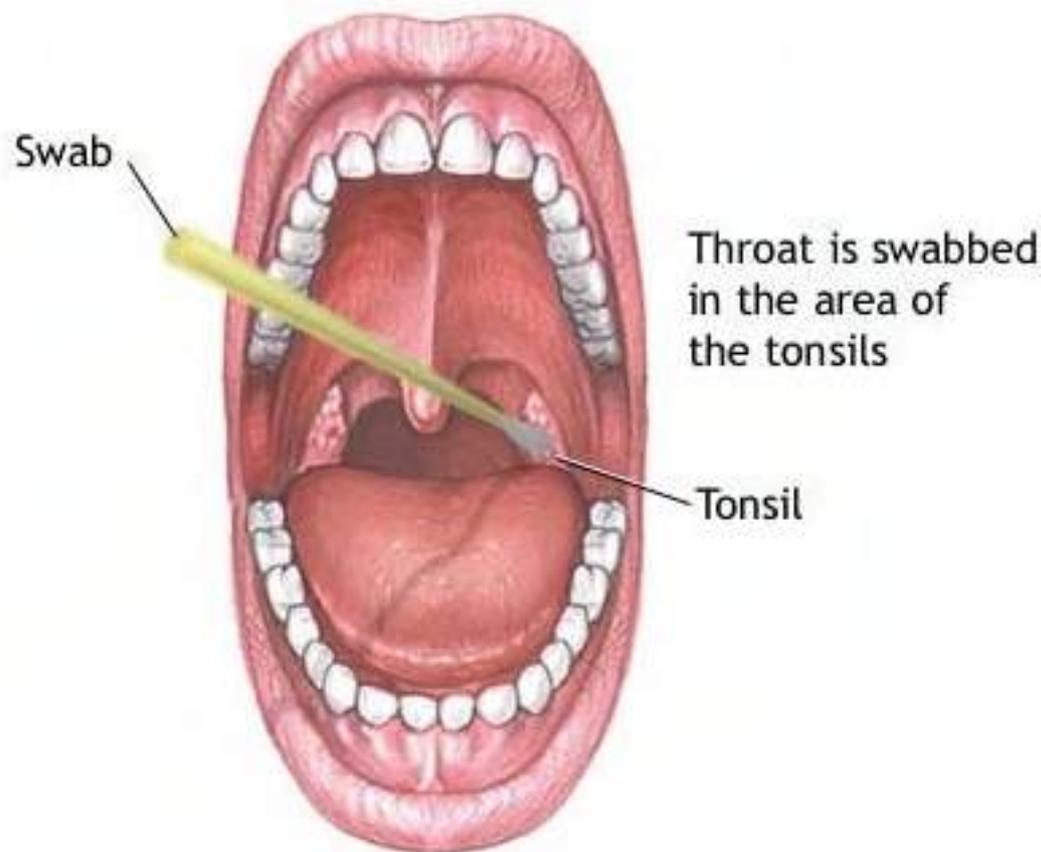
Steps of Laboratory Diagnosis of Group A Streptococcus

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:

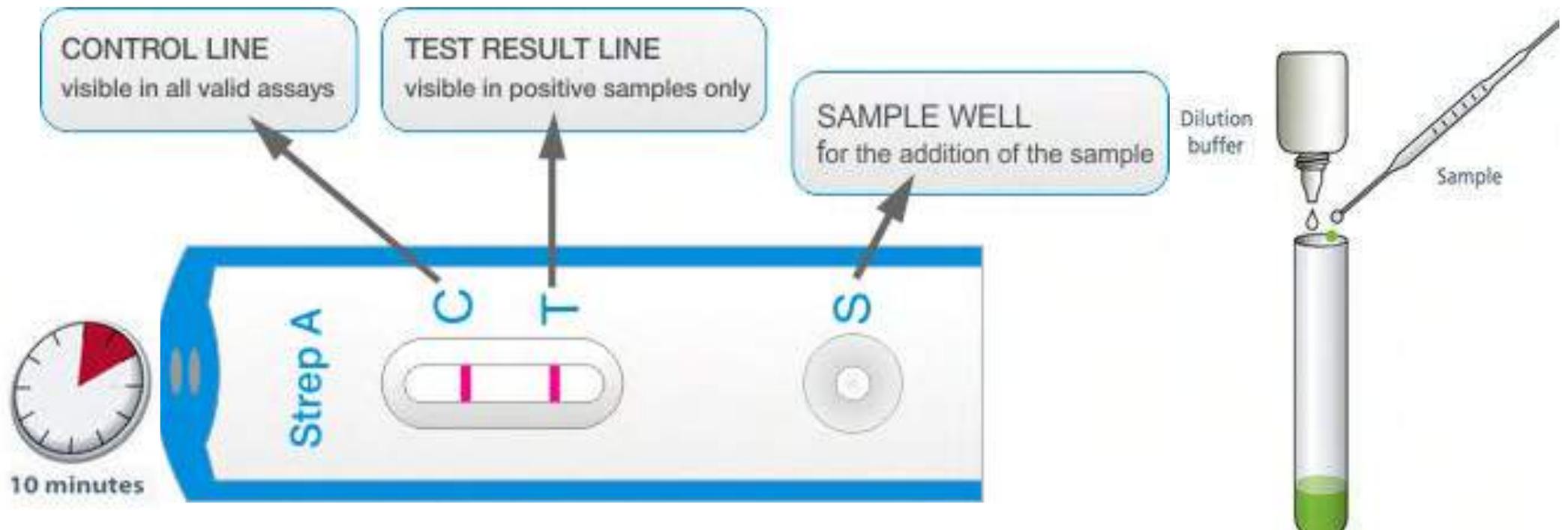
Throat swab of tonsillar area and/or posterior pharynx (Avoid the tongue and uvula)



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

2. Direct Antigen detection:

Interpretation:

The specificity of rapid strep-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

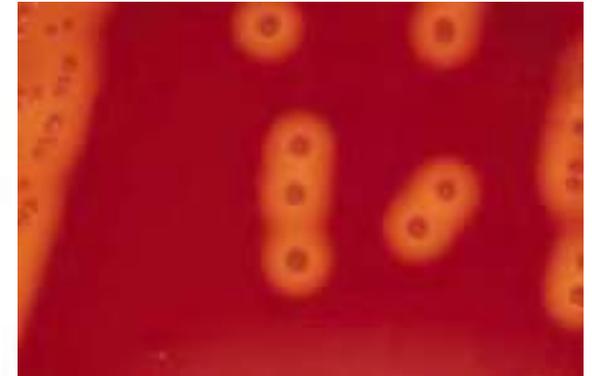
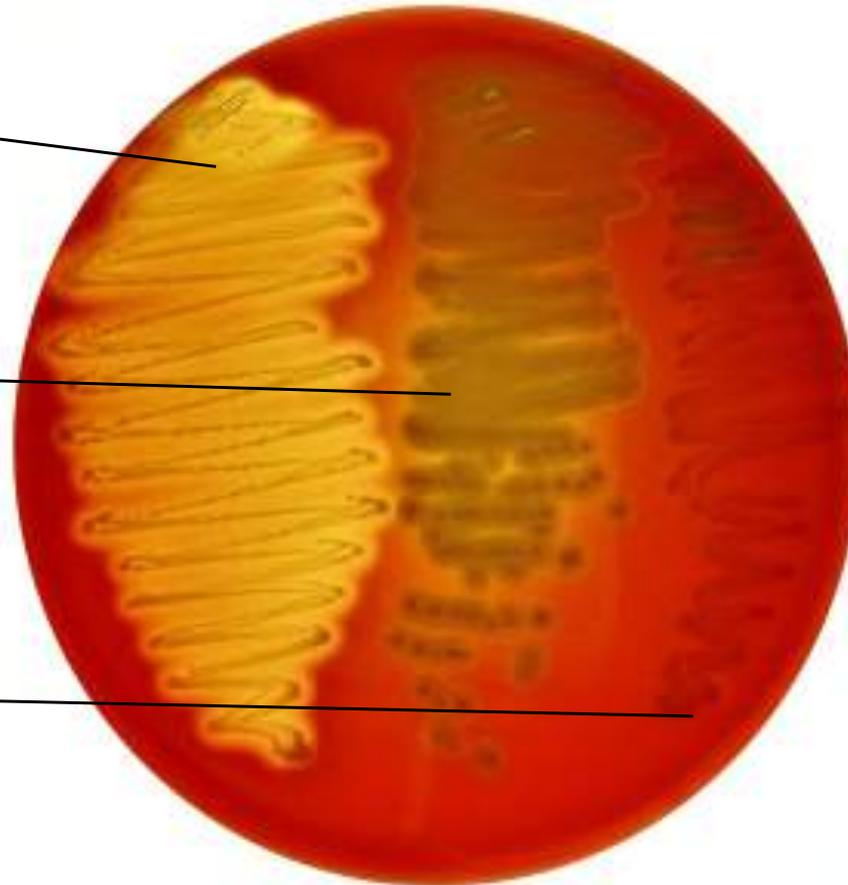
3. Group A streptococci screening culture

- Incubate cultures under atmospheric conditions (35°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

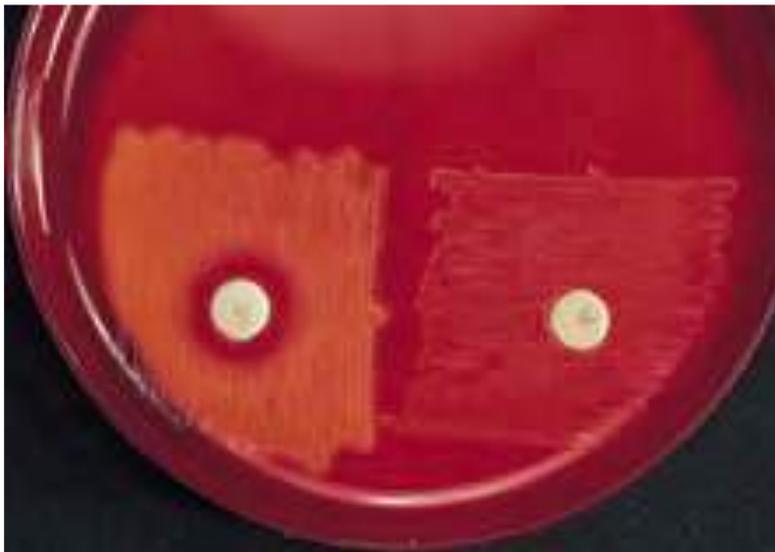
γ -hemolysis



Laboratory Diagnosis of Group A Streptococcus

4. Identification of GAS:

- Catalase test
- Bacitracin susceptibility
 - 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

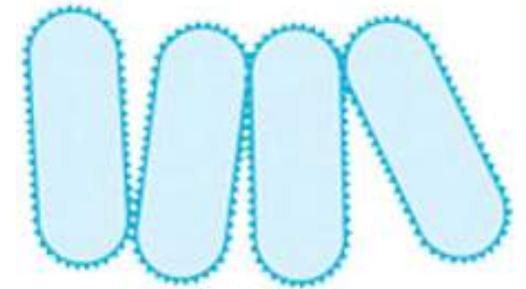
- *S. pyogenes* group A isolated
- beta hemolytic streptococci, not group A streptococci isolated
- No *S. pyogenes* or beta hemolytic streptococci

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 arrangement
 - 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

3. Toxin demonstration.

As the pathogenesis is due to diphtheria toxin, mere isolation of bacilli does 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

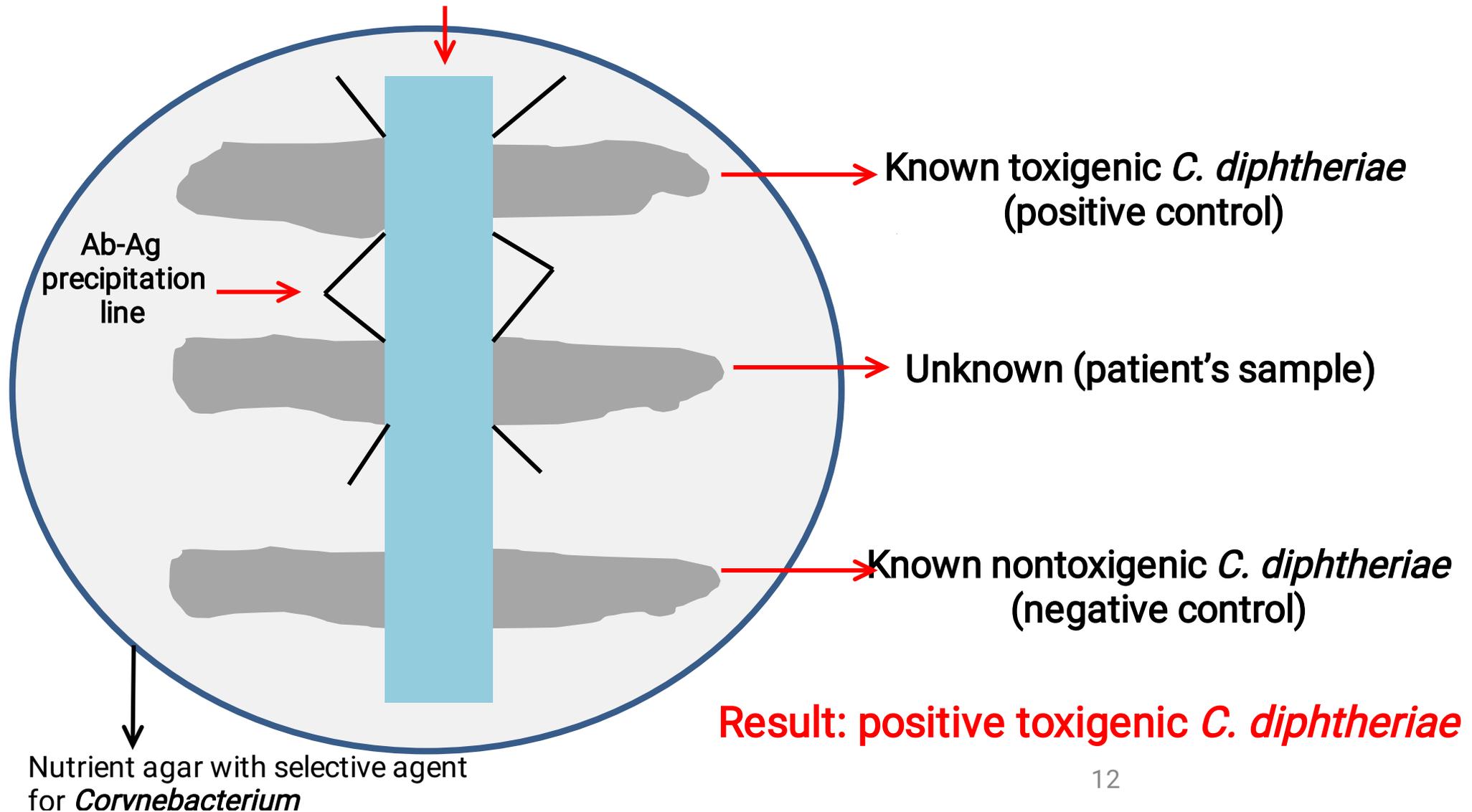
Results: if the strain is virulent, the unprotected animal will die within four days

❑ In vitro test: Elek's test

DIAGNOSIS OF DIPHTHERIA

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

Sterile filter paper with *C. diphtheriae* antitoxin

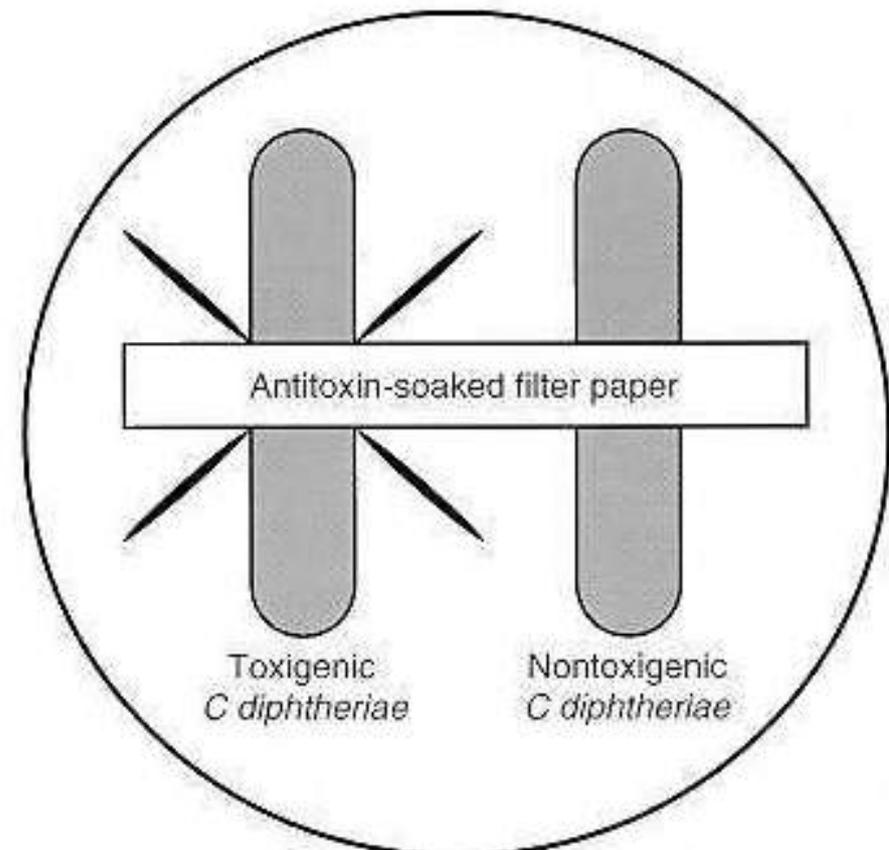
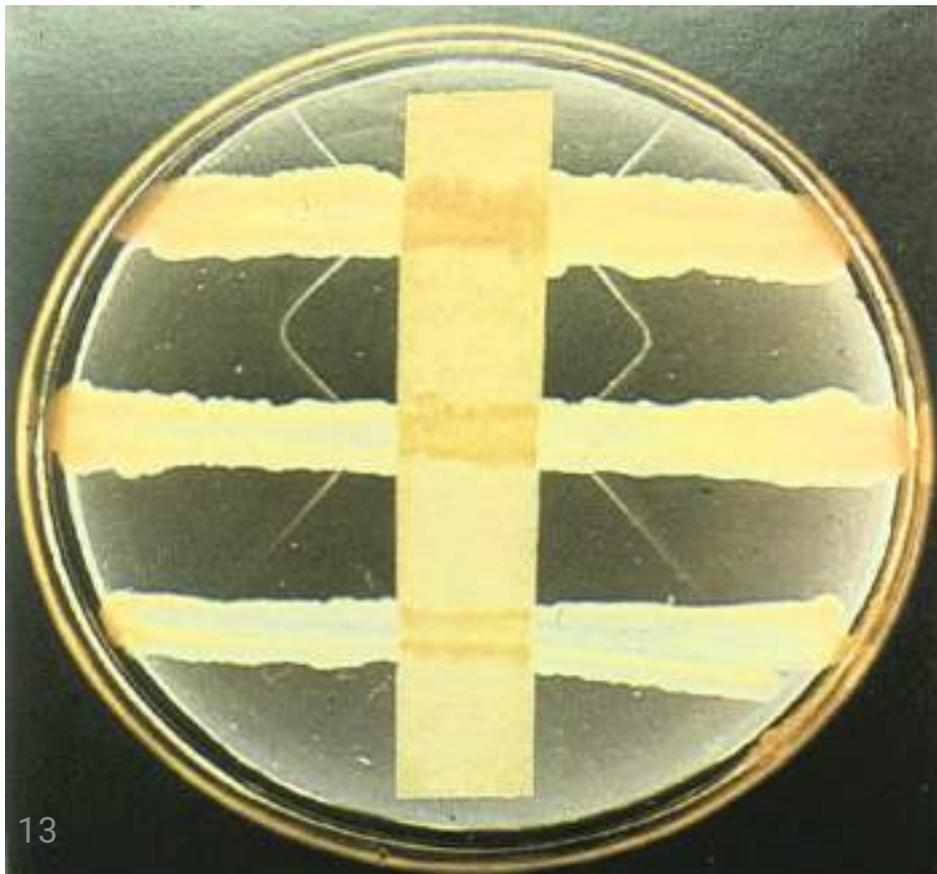


DIAGNOSIS OF DIPHTHERIA

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

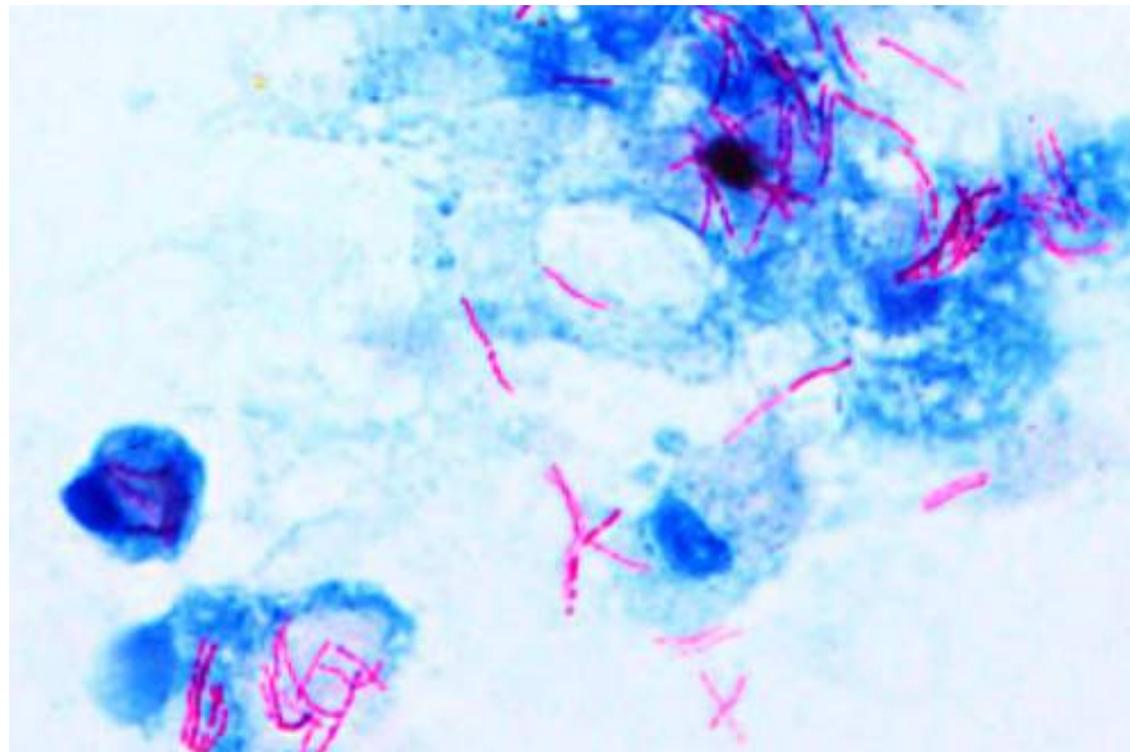
Laboratory Diagnosis Lower Respiratory Infection

Diagnosis of active Tuberculosis:

1- Direct microscopy by Ziehl-Neelsen staining:

Results of ZN staining

- Negative results: AT least 100 oil immersion field should be examined before giving negative results
- Positive results: *M. tuberculosis* appears 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 (ELISA, Latex agglutination....)

4- Molecular methods (PCR)



M. tuberculosis

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 transport

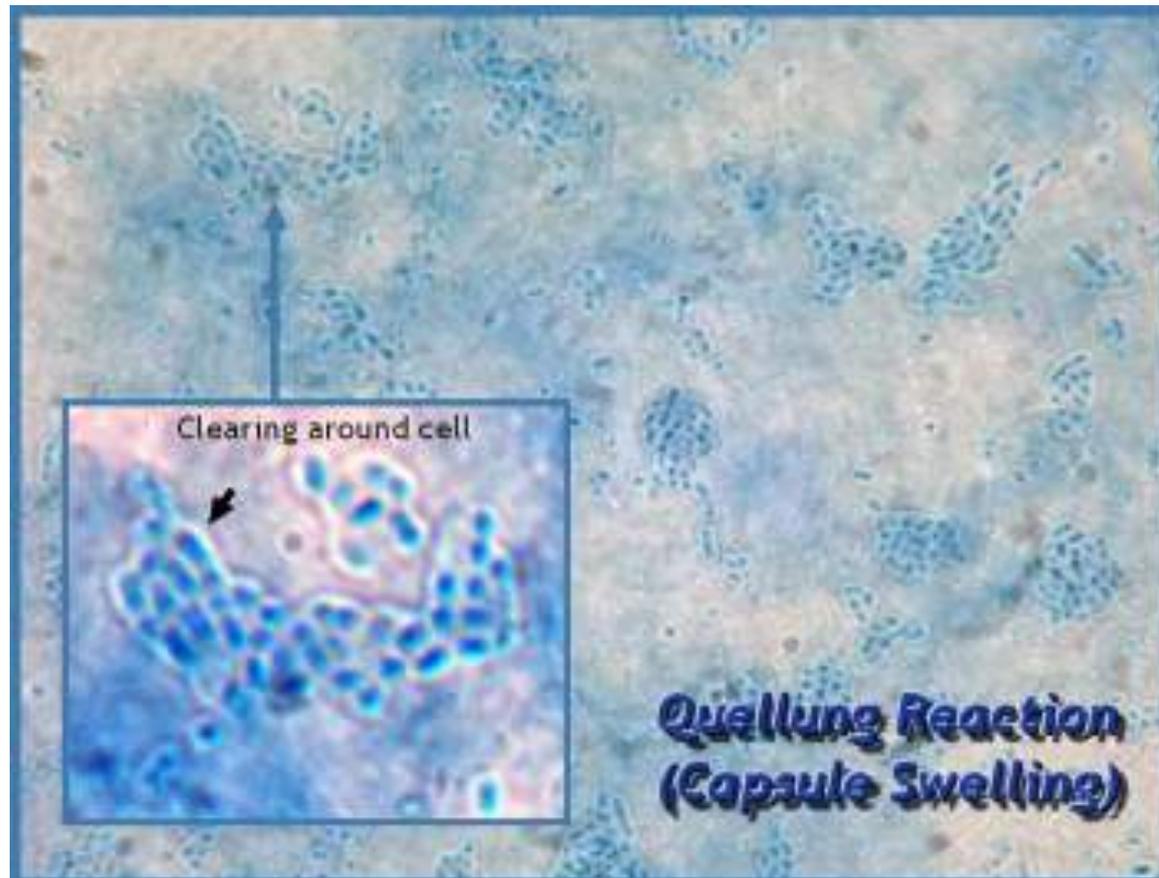
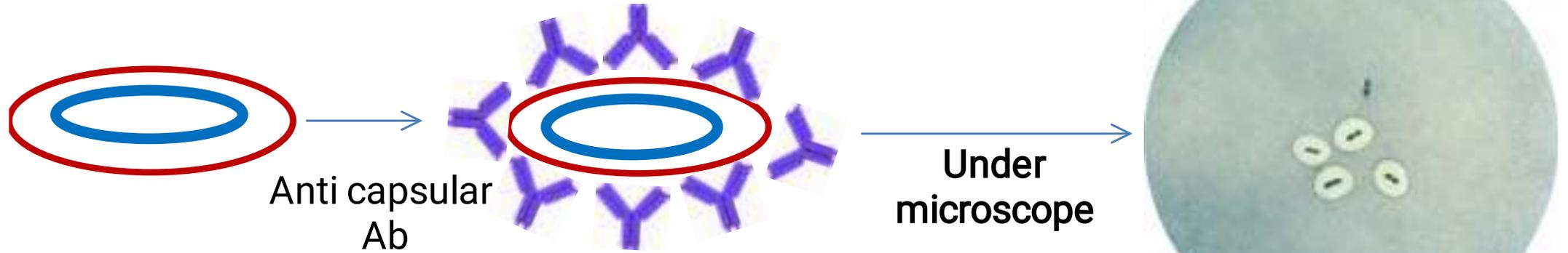
- ❑ Depending on the site of infection, various specimens may be collected such as CSF, blood, respiratory tract sputum, throat swabs, middle ear, and sinuses
- ❑ As *H. influenzae* is highly sensitive to low temperatures, the specimen should never be refrigerated
- ❑ Sample should be transported and processed immediately without any delay.

2. Direct detection:

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

Laboratory Diagnosis of *H. Influenzae*

Capsule detection (Quellung reaction)



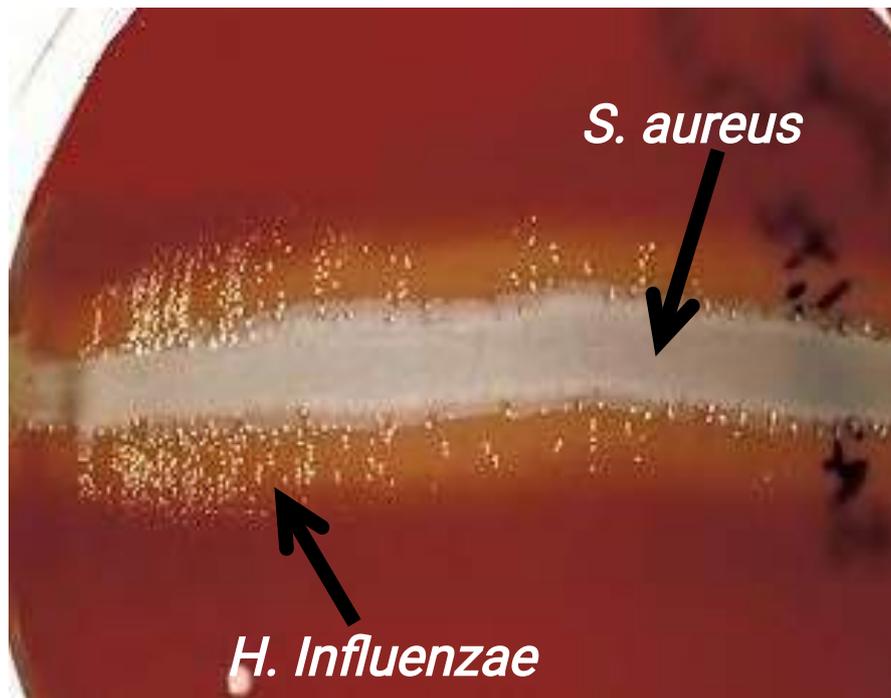
Laboratory Diagnosis of *H. Influenzae*

3. Culture:

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

B. Culture media used are as follows:

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



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



H. Influenzae grown on Chocolate 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.

Paragonimus westermani adult

Shape: coffee bean.

Color: pinkish - brown .

Suckers: The oral and ventral suckers are equal in size.

Short oesophagus and two long simple intestinal caeca.

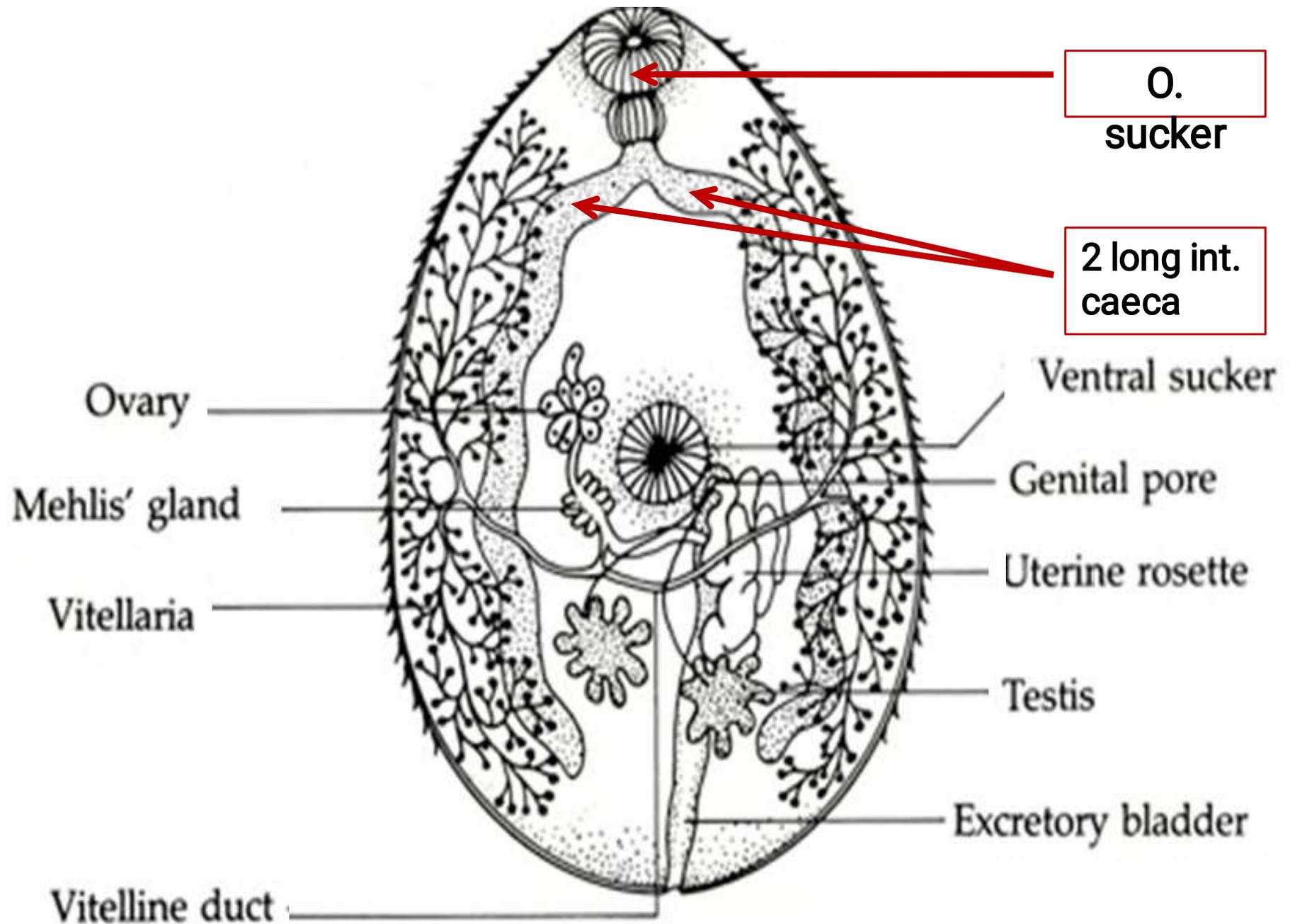
Two lobulated testes in the posterior 1/4.

One branched ovary in front of testes.

Coiled rosette shaped uterus.



Paragonimus westermani adult



Paragonimus westermani Egg

Size : 90 x 50 μm .

Shape : Oval.

Shell : Thick shell with operculum .

Color : Golden brown.

Content : Immature ovum.



Paragonimus westermani cercaria

- Oval in shape.
- Has small knob like tail (micro-cercous cercaria)
- The body covered with spine.
- Has oral & ventral suckers.
- Has 7 pairs of penetration glands.



Encysted metacercaria of *Paragonimus* (I.S)

- Spherical in shape.
- The cercaria loses its tail & secretes a thick cyst wall.
- Present in the viscera & muscles of crabs & cray fish.
- The I.S to Man, fish eating animals & carnivorous.



Echinococcus granulosus adult

- Size: 3-6 mm.
- Morphology:
- Scolex.
- 3 segments:
- Immature segment.
- Mature segment (longer than broad).
- Gravid segment (longer than broad).



Echinococcus granulosus scolex



- Globular in shape.
- Has 4 suckers.
- Has rostellum with 2 rows of hooks.

Echinococcus granulosus

Egg

(I.S to man & herbivorous).

Size: 30-40 um.

Shape: Spherical.

Shell: Thick, radially striated
embryophore.

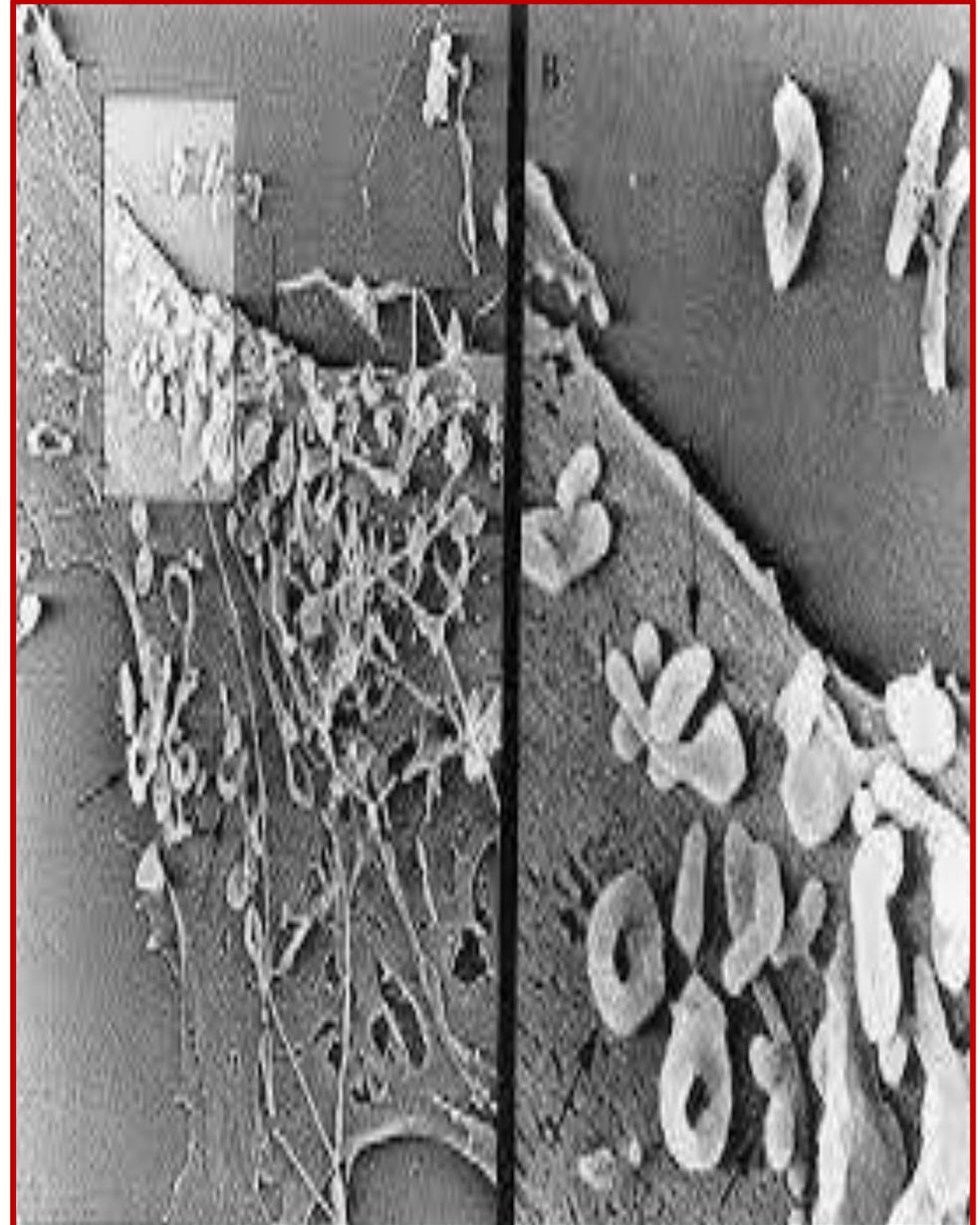
Color: brownish.

Content: Mature hexacanth
embryo (onchosphere)

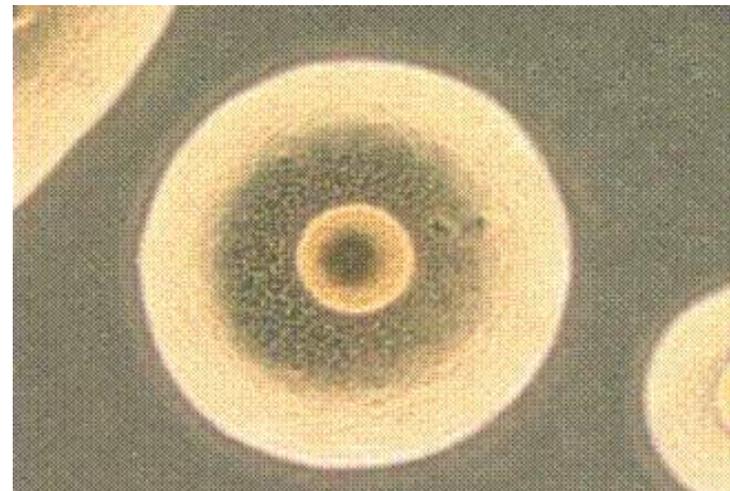


Mycoplasma pneumoniae

- The smallest prokaryotic organism.
- Size: 0.2-0.3 μm .
- Polymorphic:
 - Spherical.
 - short rod.
 - pear shaped.
 - filamentous.

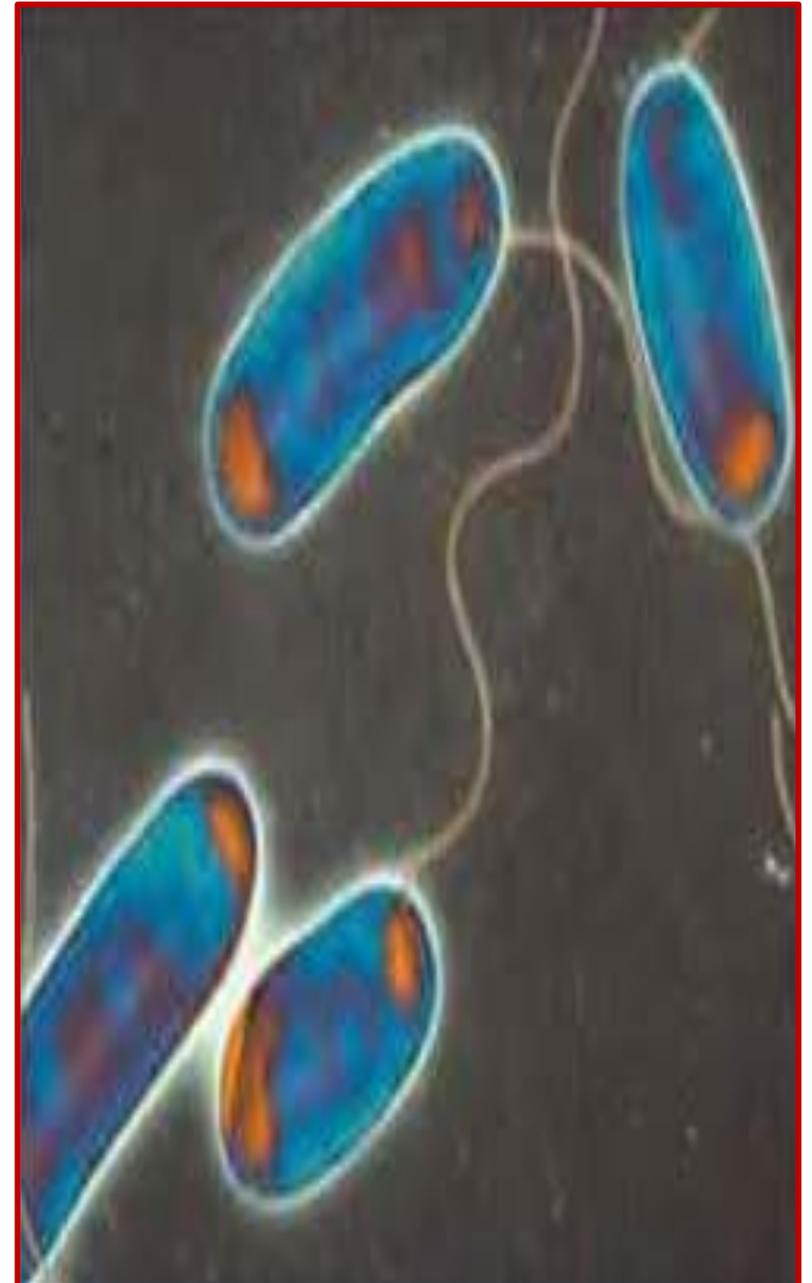


Fried egg appearance colonies of *Mycoplasma* in culture



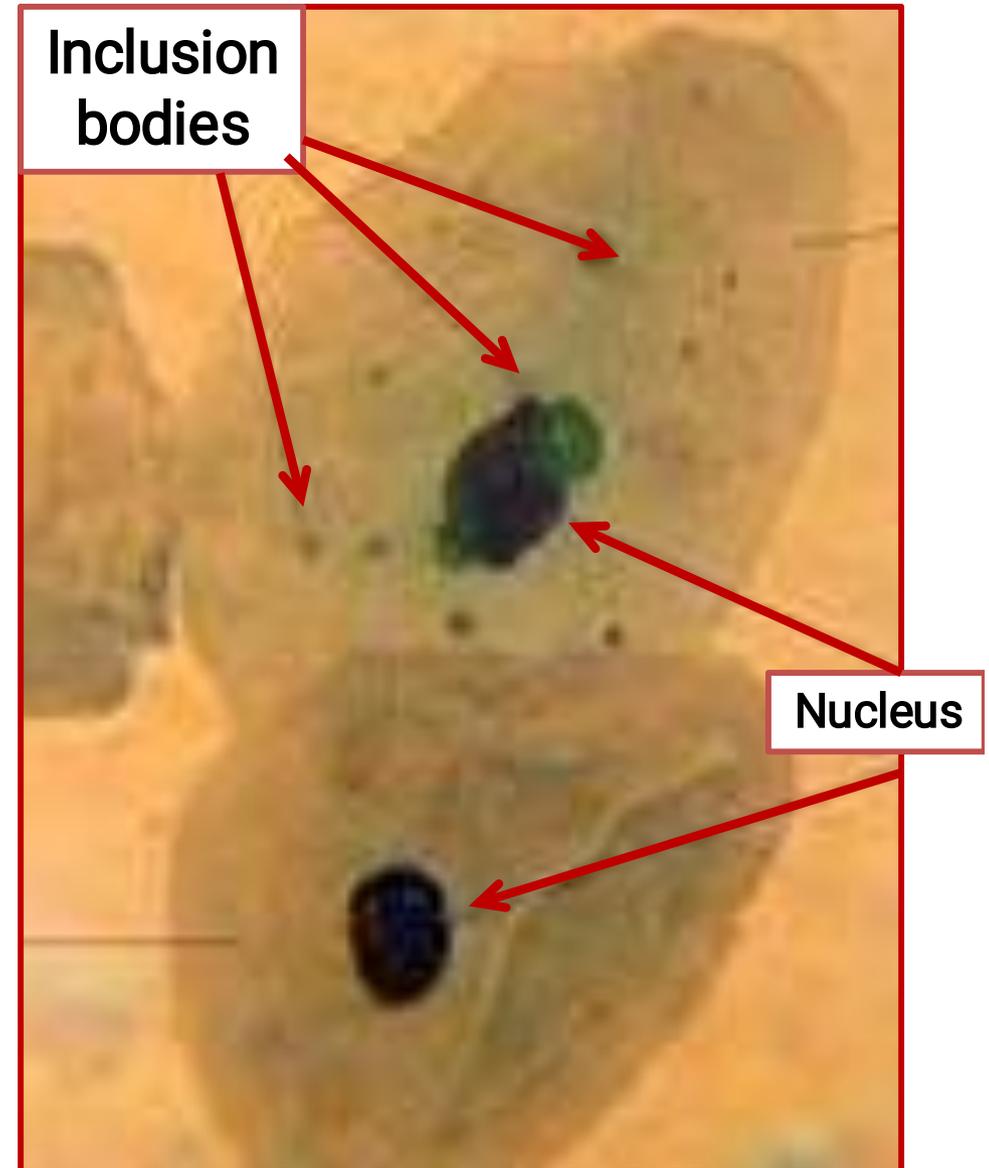
Leigionella pneumophila

- Gram negative rods
- Has pointed ends and wrinkled surface.
- Motile with polar flagella.
- Strict aerobe.
- Require for growth, media containing L- cysteine & iron.

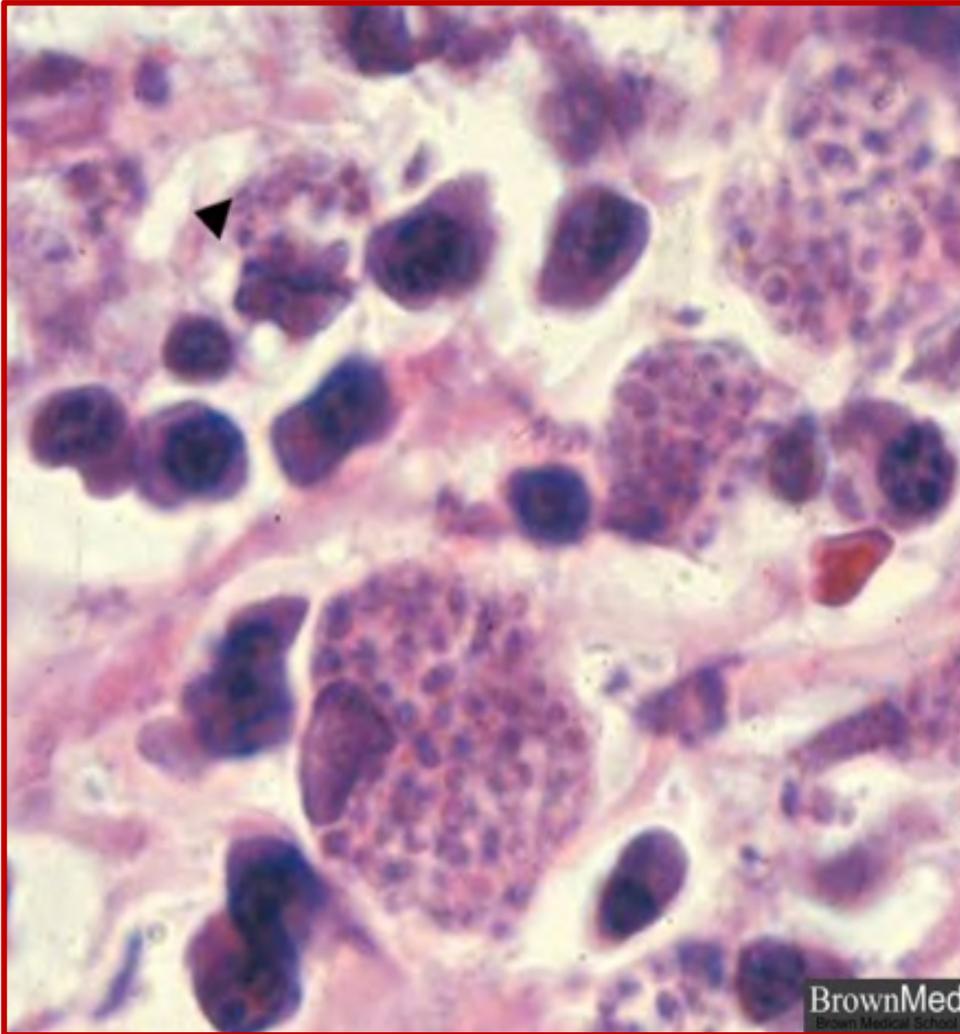


Chlamydia pneumoniae inclusion bodies inside respiratory epithelial cells

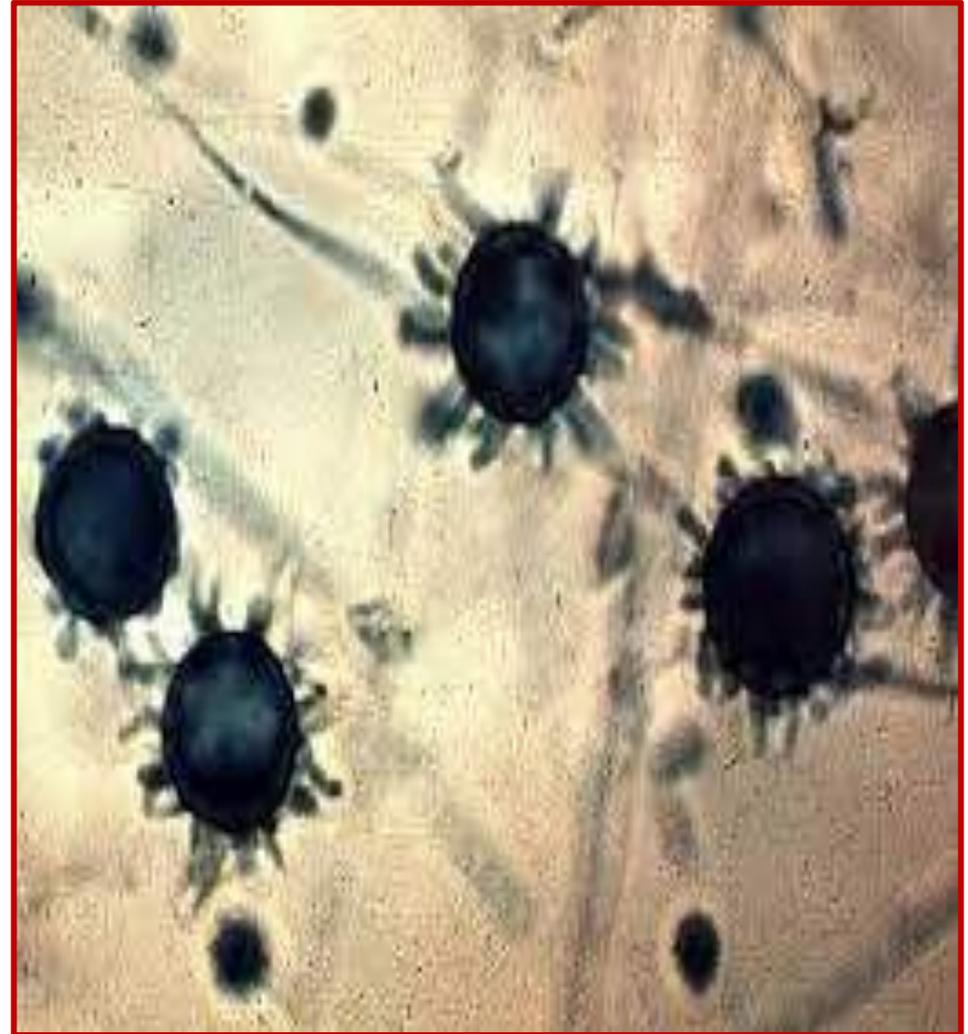
- An obligate intracellular Gram –ve bacteria that infects human.
- Small in size.
- Can not synthesize ATP for energy.
- Has both RNA and DNA.
- Multiply by binary fission.
- Has a rigid cell wall.
- Has ribosomes & synthesize their own proteins.
- Has metabolically active enzymes.



Histoplasma capsulata

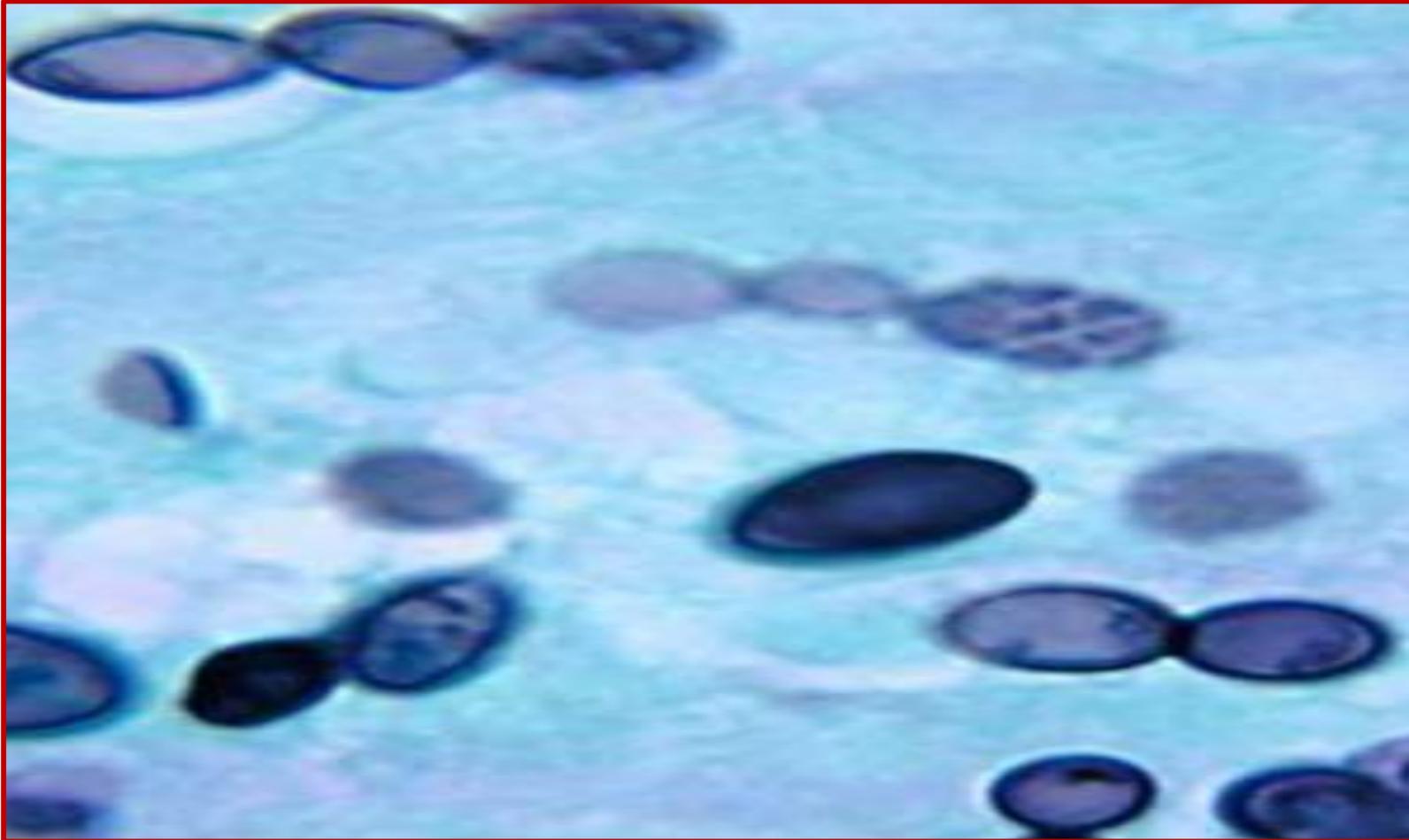


Yeast form inside alveolar macrophage



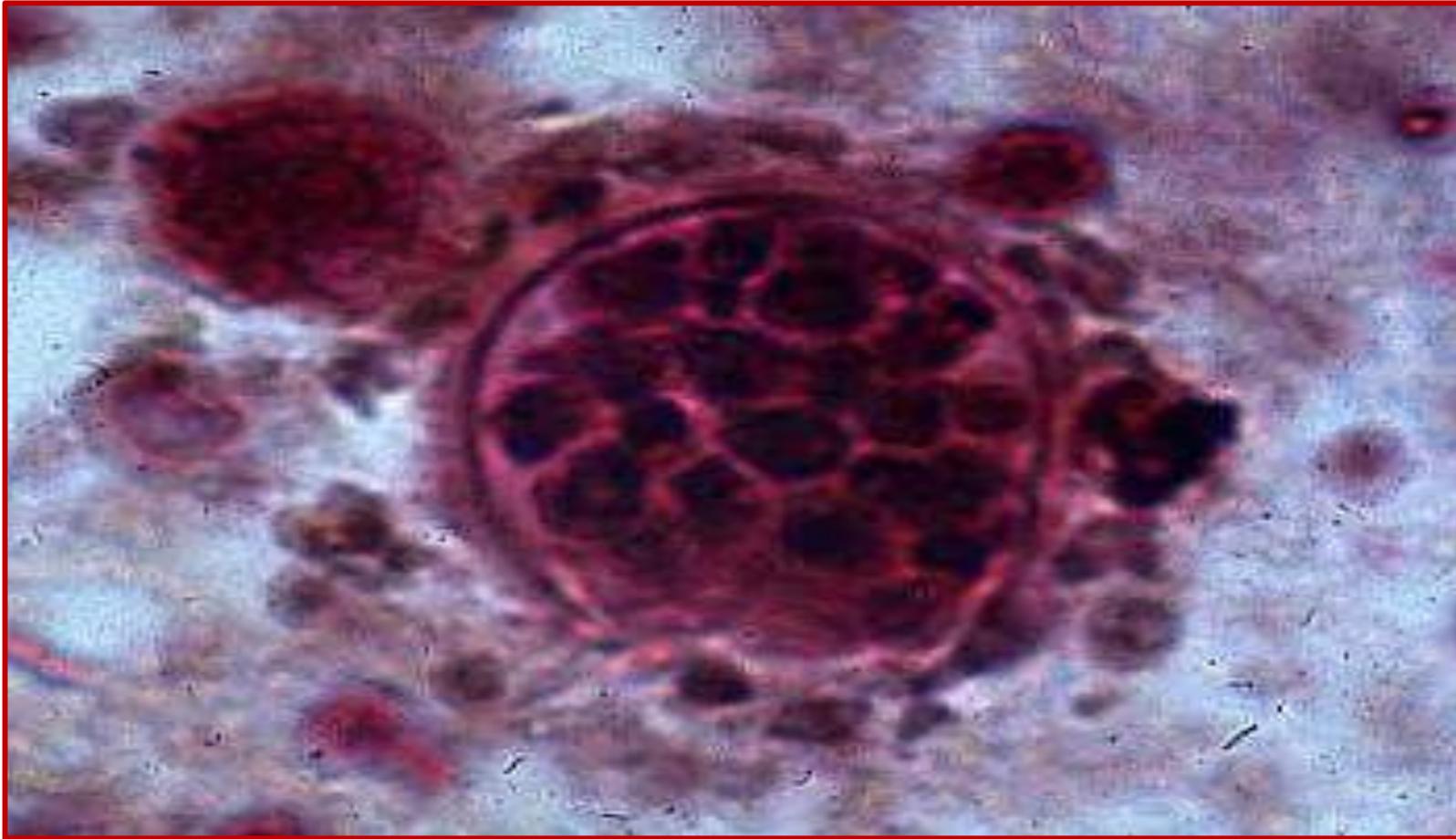
Filamentous form in culture

Yeast



- Oval in shape.
- Reproduce by budding.

Coccidioides immitis spherule



Spherule containing endospores in the lung

Aspergillus fumigatus fungus

- Filamentous septate hyphae with a flask shaped head containing spores.
- On sabouraud's agar gives white filaments with green spores.
- Airborne found in soil, water, contaminate starchy food, on decaying organic vegetation, on pillow or bedding, and air conditions.



Conidial head
containing
spores

