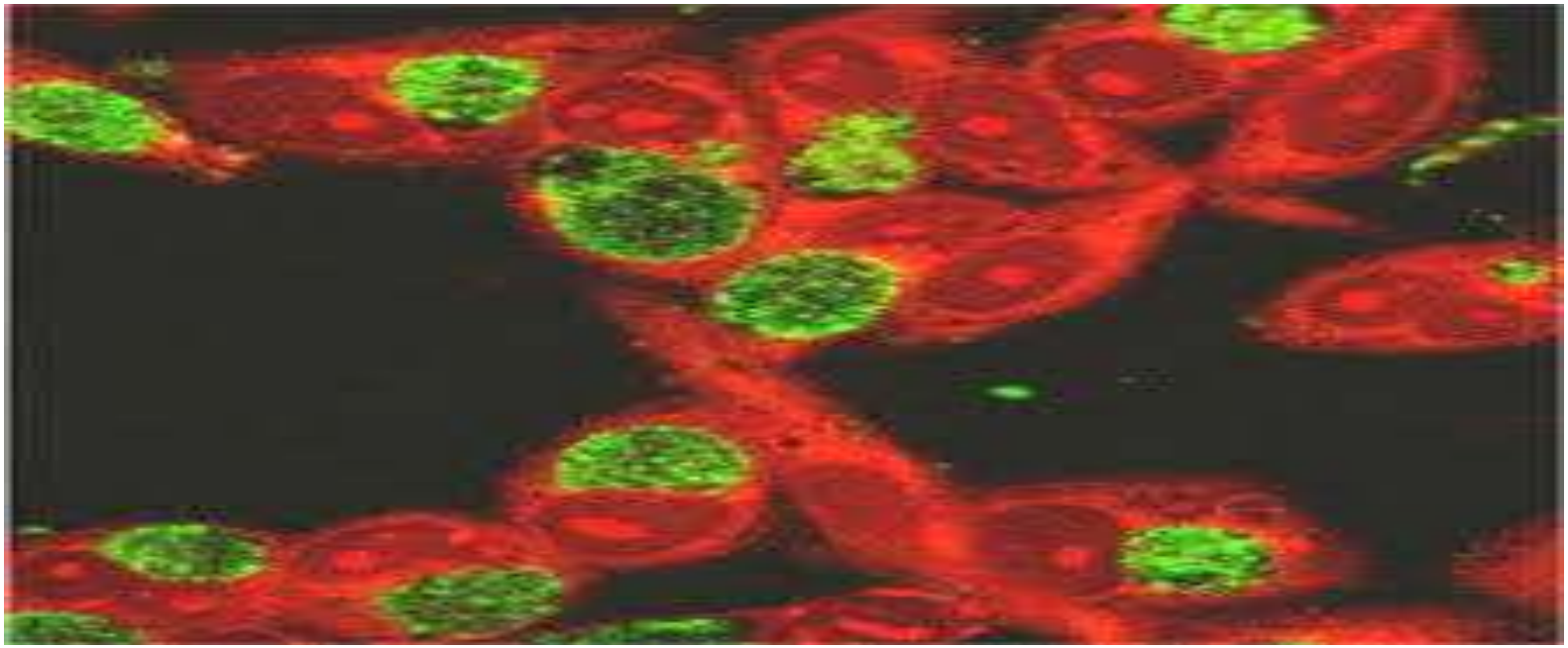
Chlamydia and Mycoplasma



Objectives

- To know about Chlamydia and Mycoplasma

✓ Classification

✓ Morphology

✓ Diseases

✓ Lab diagnosis

Classification

- Family - Chlamydiacae.
- Two genus – Chlamydia and Chlamydophila..
- Has three important human pathogens
  - C . trachomatis.
  - Chlamydophila psittaci
  - Chlamydophila pneumoniae

Chlamydiacae

- Family Chlamydiacae are **obligate intracellular** bacterial parasites.
- They have tropism for **squamous epithelial and macrophages** of the respiratory tract and GUT.
- Depend for **energy** on host cell .

General features

- Smallest **prokayotic** cell .
- Round to ovoid.
- **Two lipid bilayers** resembling a gram negative envelope.
- Non motile.
- Gram negative.
- Possess **both DNA and RNA** and ribosome .

- **Differ from other bacteria -**

No Peptidoglycan content in their cell wall.

Lack enzymes of ETC.

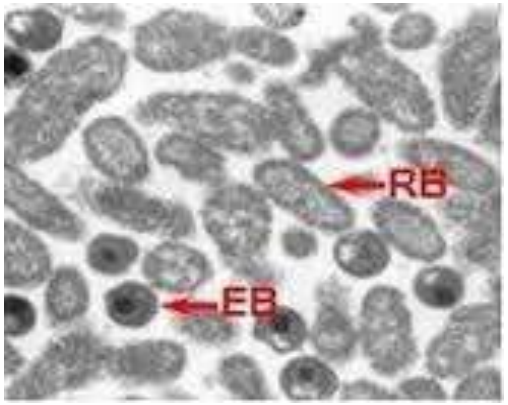
Require ATP and nutrient sources from host cells.

Morphology and growth

Chlamydia replicate by **unique complex cycle** in susceptible host cell -

They exist **in two** morphological forms

- **Elementary body-** extracellular , infectious form, spherical, rigid cellwall
- **Reticulate body** – intracellular, growing , replicative form,oval pliable cell wall,



Replicative cycle

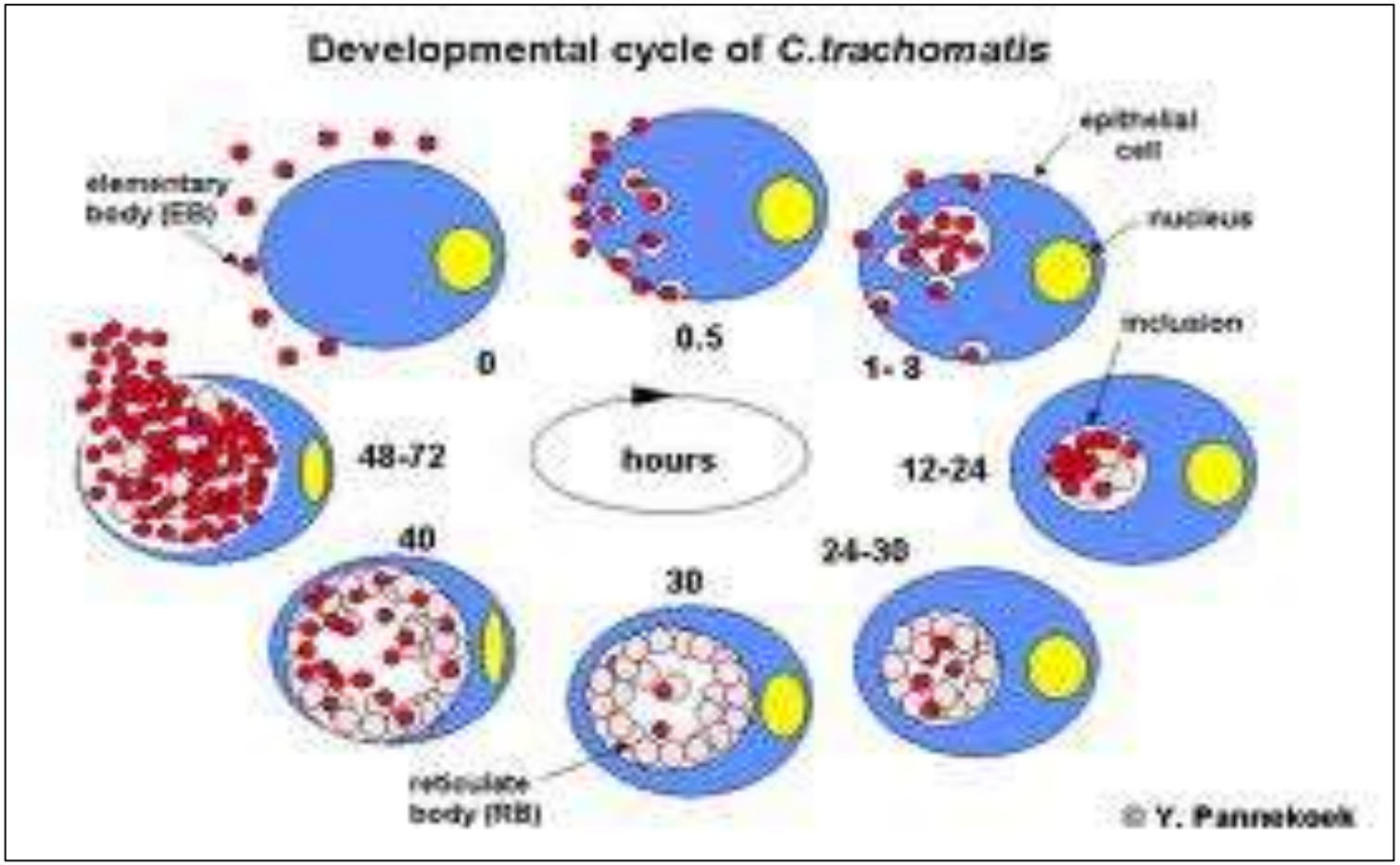
- **EB** attach to **surface** – **engulfed** by cell –

  Form **phagosome** – after 6-8 hrs **reorganise into RB** and replication continues for 18 - 24 hrs.

Inclusion body – intracytoplasmic vacuole filled with RB. Can be detected by histologic stains.

After 48 hrs – multiplication stops and change to EBs.

By 72 hrs – host cell rupture releasing infected EBs.


Developmental cycle of C.trachomatis
elementary body (EB)
0
0.5
epithelial cell
nucleus
1- 3
inclusion
12-24
24-30
30
40
48-72
hours
reticulate body (RB)
© Y. Pannekoek

Resistance

- Being heat labile

  Inactivated **at 56 deg within minutes.**

  – Susceptible to **chemicals** like ether ,ethanol formalin .

  – Can be stocked **at 4 deg C.**

  – Culture can be preserved **frozen at -70 deg.**

Antigenic structure

- **Two major antigens -**

Genus specific – lipopolysaccharide.(3-deoxy-manno-octulosonic acid). Important in scarring and fibrosis by inducing cytokines.

Antibodies can be detected **by IF and CF tests**.

Species specific- outer membrane proteins .

– Elicits protective immunity.

– IF

Serotype specific - serovars on basis of **MOMP.**

Serological typing

- **Chlamydia-** 3 species

  C.trachomatis, C.suis , C. muridarum

C.trachomatis divided **into biovars**

TRIC- trachoma inclusion conjunctivitis

LGV - lymphogranuloma venerum

BIOVARS are further divided into many serotypes .

Chlamydophila

Has 6 species-

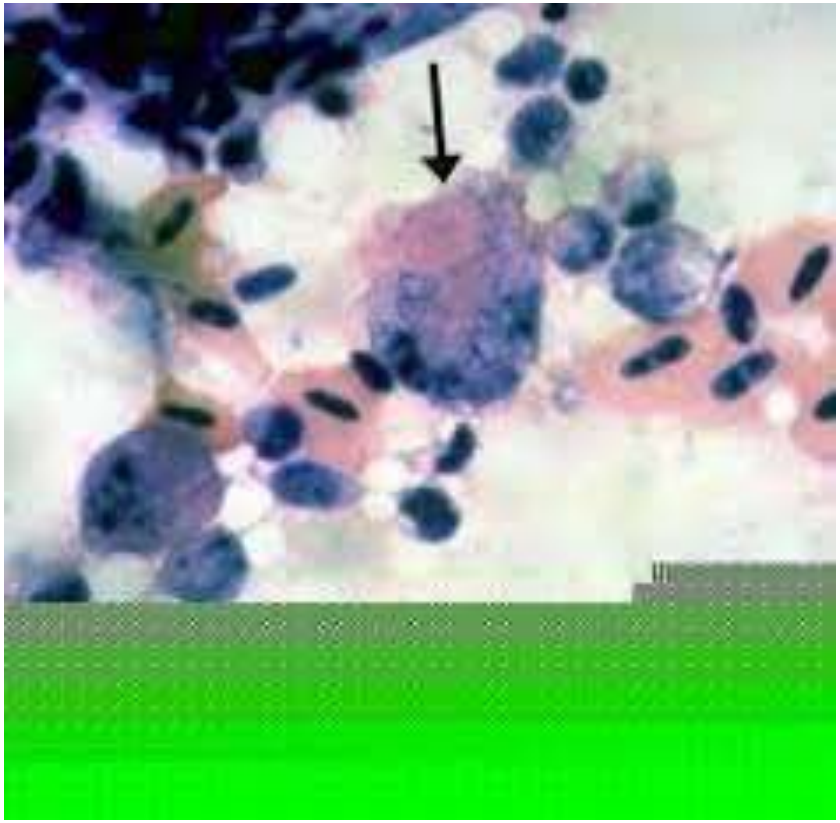
- **C.pneumonia-** no biovars ,no serotypes.
- **C.psittaci -**no biovars ,many serotypes
- C.pecorum
- C.caviae
- C.felis
- C.abortus

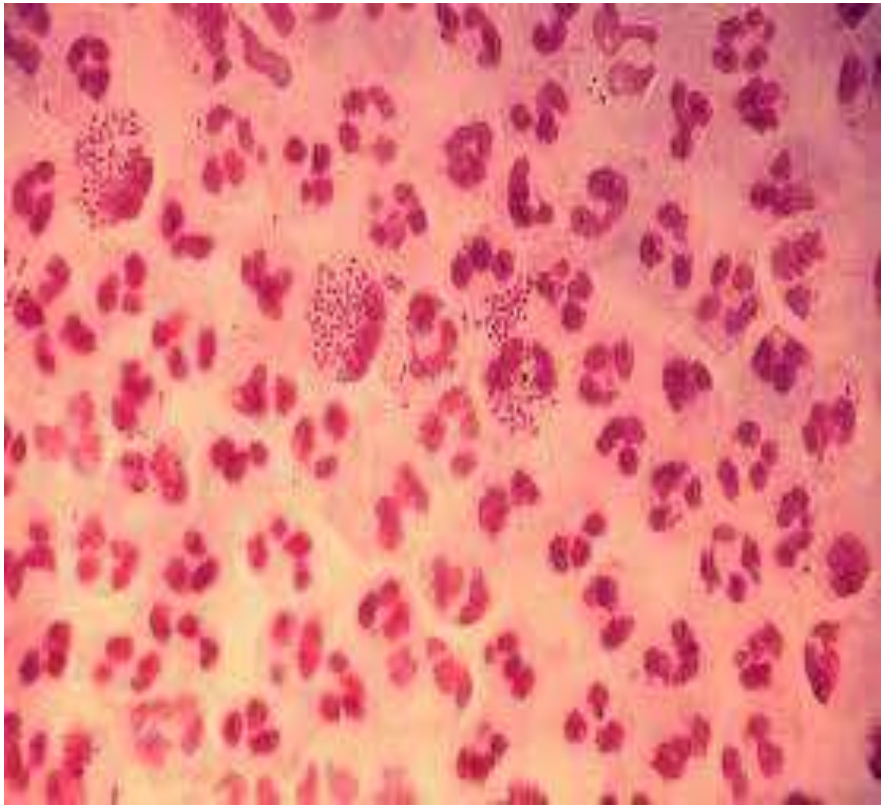
DISEASES

- **C.trachomatis** – genitourinary infection and eye infection.
- **C.psittaci** – respiratory infection.
- **C. pneumoniae** – atypical pneumonia.

Laboratory diagnosis

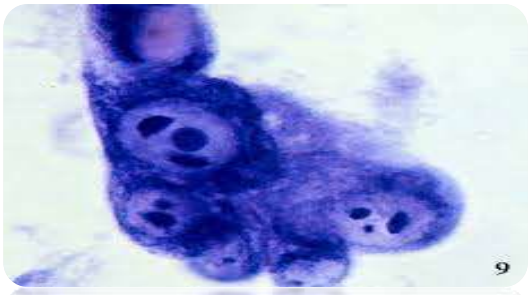
- **Specimens-** depend on type of lesion (urethra cervix,conjunctiva, lung) –scraping , swabs, blood.
- **Cytological examination.**
- **Culture and isolation-** cell culture/ yolk sac / mice
- **Detection of chlamydia antigen -** Direct Fluoroscent antiody/Elisa
- **Serodiagnosis -** microimmunofluorescence





Chlamydia trachomatis

- **Trachoma** – keratoconjunctivitis. (**A,B,Ba,and C.)** Conjunctival scraping- inclusion bodies known as **Helberstaedter- prowazek body**
- Lymphogranuloma venerum
- Inclusion conjunctivitis in adults
- Non gonococcal urethritis
- Perinatal infections
    - Neonatal conjunctivitis
    - Infant pneumonia



LGV

- Sexually transmitted disease characterised by **suppurative inguinal adenitis .**
- C. trachomatis **serovars L1,L2 and L3.**

INCLUSION CONJUNCTIVITIS

Sexually active adults it may transfer to eye through **genital secretions** and lead to **paratrachoma a**nd is caused by **D to K** also known as **swimming pool conjunctivitis**.

Non gonococcal urethritis

- Caused by serotypes **D-K.**
- Inflammation of **genital organs.**
- **Perinatal infection**

  Neonatal conjunctivitis

  Infant pneumonia

Laboratory diagnosis

- **Smears-** inclusion body by Giemsa.
- **Isolation-** cell culture HeLa Cells ,McCoy and BHK.
- **Antigen detection-**DirectFluoroscent antibody/ Elisa .
- **Nucleic acid probe/PCR/LCR.**
- **Serological test** –CFT and micro IF.

Treatment

- Sulphonamide and Tetracycline.

Chlamydophila pneumoniae

- **Clinical findings-** bronchitis and pneumonia,pharyngitis ,coronary heart disease, asthma.
- **Laboratory diagnosis** – same.

- demonstration of elimentary bodies.

- culture.

- antigen , antibodies detection.

- **Treatment-** macrolides.

Chlamydia psittaci

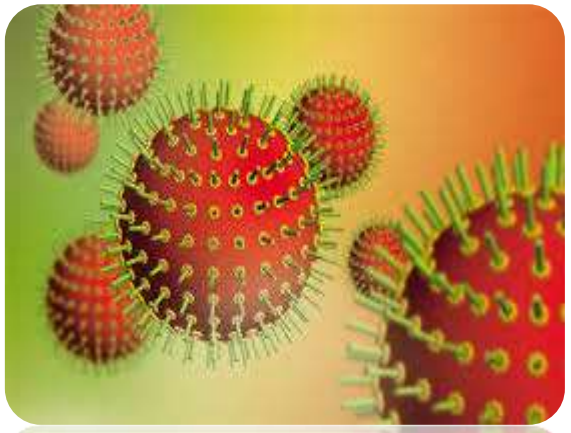
- **Source** – infected birds .
- **Clinical presentation** – influenza like syndrome ,pneumonia.
- **Epidemiology** – occupational.
- **Lab diagnosis** – culture – levinthal colelillie bodies.
- **Antigen detection** –DIF , EIA
- **Treatment-** tetracycline ,macrolide.

Mycoplasma

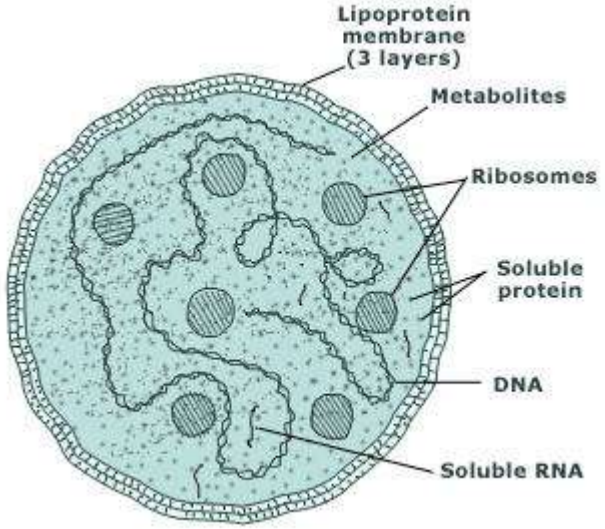
- **Smallest free living** bacteria ,pass bacterial filter.

Morphology – gram negative ,pleomorphic. Also known as **PPLO** (pleuropneumonia like organism)

- Lack cell wall .



- Enclosed in **trilayered** membrane having sterol .



Reproduction

- Divide by **binary fission.**
- Grow on **cell free media.**
- Adhere to **epithelium of respiratory and urogenital tract**.

Classification

- **Mycoplasmatacae**

  Mycoplasma do not hydrolyse urea.

  Ureaplasma hydrolyse urea.

- **Pathogenic to humans**

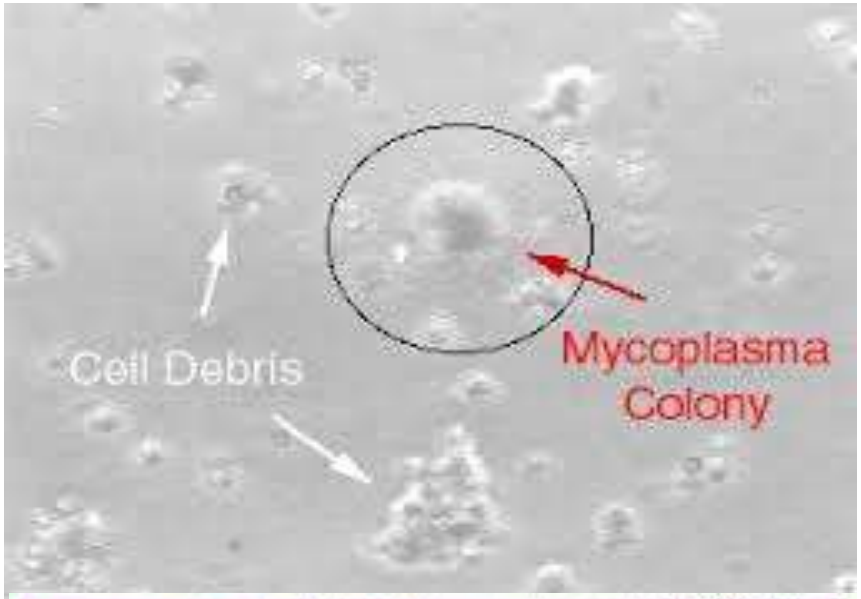
  M.hominis

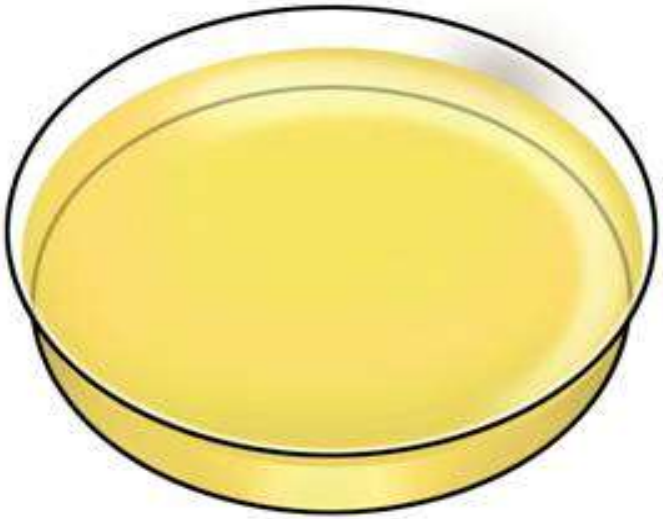
  M.pneumoniae

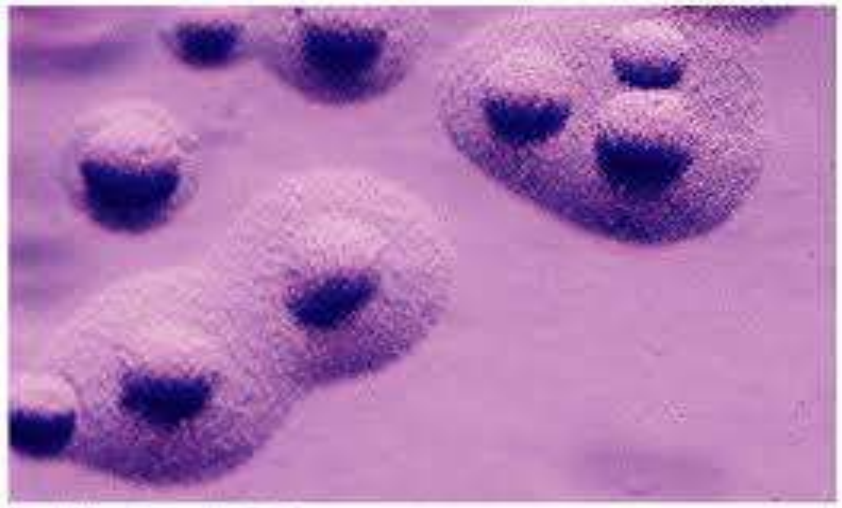
  U.urealyticum

Cultivation

- **Facultatively anareobic**.
- For primary isolation **95 %nitrogen and 5% CO2**
- 22-41 deg c.
- **Media** – enriched with 20 percent horse serum and yeast extract.
- **PPLO broth** -yeast extract , serum, glucose ,phenol red.
- Solidified by agar , **SP-4 media , Mycotrin RS.**
- **Fried egg colony .**
- Seen by **Dienes method**


Cell Debris
Mycoplasma
Colony





Biochemical reaction

- Mycoplasma – mostly fermentative.
- Urea – hydrolysed by ureaplasma.
- Not proteolytic.

Resistance

- Heating at 56 deg for 30 min.
- Resistant to pencillin and cephalosporin.
- **Resistant to UV light .**
- Sensitive to tetracycline, **cholorohxidine and cetrimide.**
- **Lyophilization / freezing** broth cultures at -70 deg.

Antigenic structure

- Cell membrane constituents

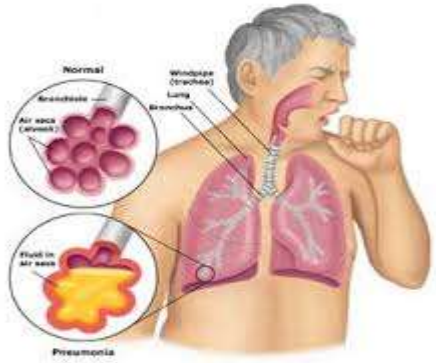
  - **glycolipids** act as antigen in vitro in CFT.

  - **protein -**P1.

Antibodies are found in convalescent serum and respiratory secretion.

M. pneumoniae

- Mild URTI.
- LRTI.
- Tracheobronchitis and pneumonia (primary atypical pneumonia or walking pneumonia)
- School age children and young adults.
- Complications may occur



M. genitalium

- Non gonococcal urethritis and PID .

- **U. urealyticum** causes genital infection.
- Transmitted by sexual contact.
- **M. hominis** – lower genital tract.
- Cause non gonococcal urethritis .

Laboratory diagnosis

- **Specimen** – depends on lesion .
- **Culture** –PPLO medium .
- Isolation and identification
- **Colonies-** mulberry shaped /fried egg
- Species identification

  Haemadsorption test(G.pig RBC adheres to colonies of M.pneum and not hominis)
- Tetrazolium reduction test- M.pneumoniae appear red.
- **Serological technique**-ELISA and IFA
- **PCR and DNA probes** – high sensitivity

Antigen detection – IF , EIA ,IMMUNOBLOTTING

DNA probes – 16SrRNA sequence in resp. exudate.

Serological tests

Specific test using mycoplasmal antigens

- CFT
- ELISA

Non specific test

Streptococcus MG test- unheated serum and heat killed str. MG suspension

Cold Agglutination TEST-antibodies that bind to human erythrocytes at low temp 4 deg

Treatment

- Tetracycline.
- Macrolides (azithromycin).
- Fluoroquinolones.

Further reading

- Essentials of medical microbiology. Apurba S Sastry.
- Textbook of Microbiology . Ananthannarayan and Paniker.

Mcqs

1. All of the following are true except

a. Elementary body is metablically active

b. Reticulate body is the replicating form

c. Reticulate body is intracellular form

d. Elementary body is infectious form

2 . The most commonly used method for isolation of Chlamydia:

a. Culture on artificial media

b. Culture on Vero cell line

c. Inoculation into guinea pig

d. Culture on McCoy cell line

3. The most sensitive and specific test for Chlamydia diagnosis:
a. Direct immunofluorescence test (DIF)
b. Culture on McCoy cell line
c. Nucleic acid amplification tests (NAAT)
d. Micro-immunofluorescence (MIF) test

4. Which is not a property of Mycoplasma?
a. Susceptibility to beta lactams
b. Have both DNA and RNA
c. Mycoplasmo pneumonioe
d. Streptococcus pneumoniae

5. Fried egg colonies are produced by:
a. Bacillus cereus
b. Haemophilus influenzae
c. Neisseria subflavo
d. Mycoplosma pneumoniae