

CHLAMYDIA

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Chlamydiae resemble viruses

- **O**bligat e intracellular
- **C**an not grow in artificial media, grow in cell lines, embryonated egg or animals
- **F**ilterable— pass through bacterial filters
- **P**roduce intracytoplasmic inclusions.

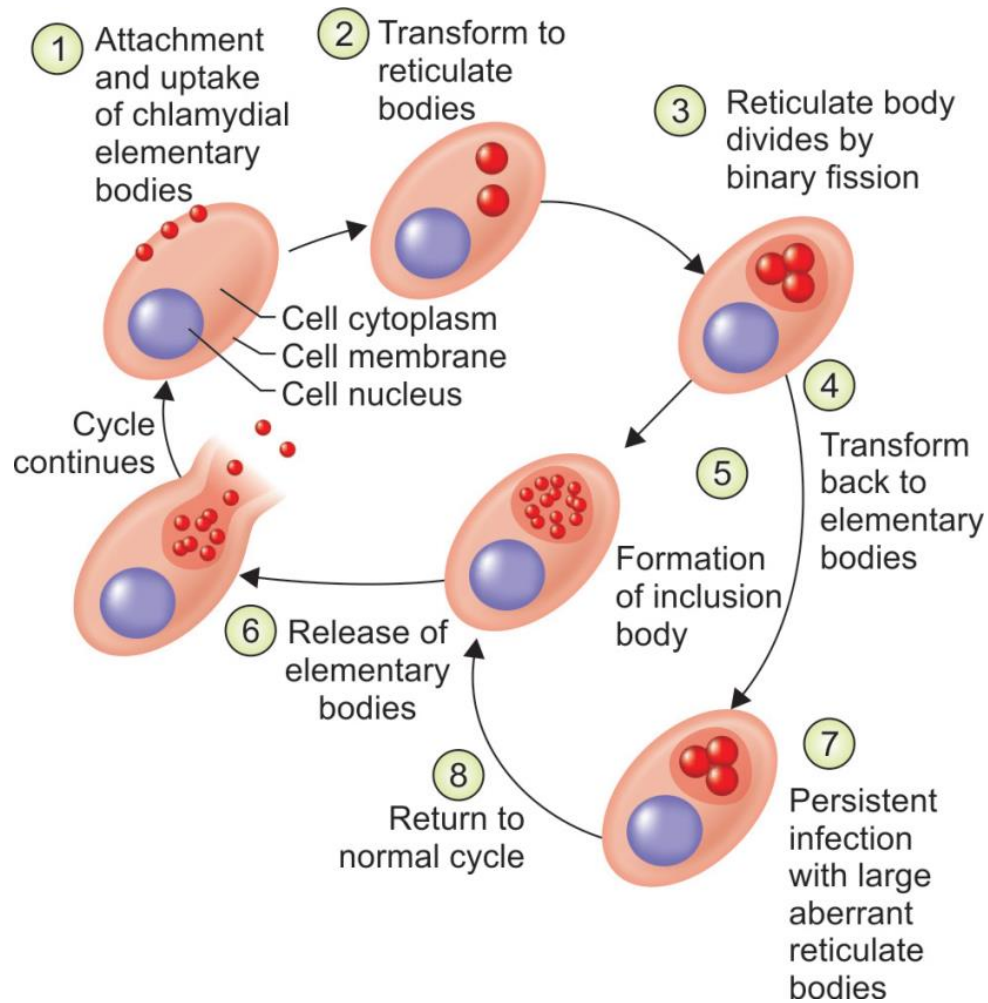
CHLAMYDIA

Chlamydiae are confirmed to be bacteria:

- Possess both DNA and RNA
- Cell wall similar to that of gram-negative bacteria
- Multiply by binary fission
- Contain prokaryotic 70S ribosomes
- Synthesize their own nucleic acid, lipids and proteins
- Susceptible to a wide range of antibacterial agents

CHLAMYDIA

- **Classification**
- Based on genetic characteristics
- *Chlamydia: C.trachomatis.*
- *Chlamydophila: C.psittaci and C.pneumoniae*
- Several non-pathogenic animal species - *C. pecorum, C. abortus, C. caviae and C. felis.*



Elementary body v/s Reticulate body

Elementary body	Reticulate body
Extracellular form	Intracellular form
Infectious form	Replicating form
Metabolically inactive	Metabolically active
Rigid cell wall	Fragile cell wall
Small size(0.20-0.30μm)	Large size (1–1.5μm)
Nucleoid is electron dense	Nucleoid is diffuse
DNA and RNA contents are same	RNA content is more than DNA

Life Cycle

- **Attachment:** Elementary bodies attach to specific receptors on host cells → endocytosed
- **Intracellular survival:** Elementary body resides inside the vacuole (phagosome), to complete entire growth cycle
 - Prevent phagosome-lysosome fusion
- **Elementary bodies transform to reticulate bodies**
- **Replication:** Reticulate bodies divide by binary fission
 - Synthesizing their own nucleic acid, lipids and proteins except ATP - **energy parasites**

Life Cycle

- **Transform back to elementary bodies**
- **Inclusion body:** Vacuoles gradually increase in size to form inclusion body
- **Release:** Mature inclusion body contains 100–500 elementary bodies → released from the host
- **Persistent infection:** Sometimes, the development is arrested at the reticulate body stage

Classification of Chlamydia

	Biovar	Serotype	Disease
C.trachomatis	TRIC	A, B, Ba, C	Trachoma
		D, Da, E,F,G,H,I,Ia,J,Ja,K	Genital chlamydiasis Inclusion conjunctivitis Infant pneumonia
	LGV	L1,L2,L2a,L3	Lymphogranuloma venereum
C. psittaci	Nil	Only 1 serotype	Community-acquired atypical pneumonia
C.pneumoniae TWAR agent	Nil	Many serotypes	Psittacosis

Trachoma

- Chronic keratoconjunctivitis (*C.trachomatis* serovars A, B, Ba and C)
- **Mode of transmission:** direct contact (fingers and fomites) with discharges
- Indirect contact through contaminated clothes or flies
- **Age:** Infection acquired by 2–3 years and active disease among young children

Trachoma

- **Acute infection presents as:**
 - Follicular conjunctivitis (inflammation of conjunctival
 - lymphoid follicles) and papillary hyperplasia
 - Follicles rupture to leave shallow pits termed
 - Herbert's pits
 - Cornea gets infected (keratitis).

Trachoma

- **Late stage (cicatrization):**
 - Recurrent infection → limbal scarring or on palpebral conjunctival scarring
 - New vessel formation - pannus
 - Eyelashes become wet and turn inward – entropion → which may rub on the corneal surface (trichiasis)
- opacity and blindness

C. trachomatis Serovars D–K

- **Genital Infections**
- **Nongonococcal urethritis (NGU):** most common cause of nongonococcal urethritis (30–50%)
 - Differs from gonococcal urethritis (GU) by:
 - Incubation period is 7–10 days, compared to 2–5 days for GU
 - **S**ymptoms: Mucopurulent discharge is followed by dysuria and urethral irritation (GU has purulent discharge).

C. trachomatis Serovars D–K

- **Postgonococcal urethritis (PGU)** - 2nd commonest cause
- GU treated with penicillin/cephalosporin alone without adding any antichlamydial drugs.
- **Epididymitis and proctitis:** Commonest cause of epididymitis in males
- **Reactive arthritis (Reiter's syndrome):** Conjunctivitis, urethritis, arthritis & characteristic mucocutaneous lesions
- Men : women =10:1

C. trachomatis Serovars D–K

- Commonest cause of **peripheral inflammatory arthritis** in young men
 - Large joints of legs, or sacroiliac joints
 - HLA-B27 haplotype
 - **Mechanism:** Immune-mediated inflammatory response to an infection at a distant site. Self limiting and Relapse
- **In females:**
 - Mucopurulent cervicitis commonest manifestation → endometritis, salpingitis, PID & pelvic peritonitis
 - Perihepatitis (Fitz–Hugh–Curtis syndrome)

C. trachomatis Serovars D–K

- **Inclusion Conjunctivitis**
- **Ophthalmia neonatorum (or inclusion blennorrhoea)** - new borne
 - *C. Trachomatis* more common than gonococcus
- **Adult inclusion conjunctivitis:** Acute follicular conjunctivitis following swimming (swimming pool conjunctivitis)
- **Infant Pneumonia**
 - Interstitial pneumonia that develops within 3 weeks to 3 months of birth

C. trachomatis Serovar L1, L2, and L3

□ Lymphogranuloma venereum – STD

- **First stage: Painless papule, ulcer or vesicle on penis or vulva**
- **Second stage:**
 - **Bubo** - Enlarged, tender & soft Inguinal lymph nodes
 - **Fistulae** - buboes may breakdown and discharge may spread externally as chronic fistulae
 - **Systemic symptoms** - fever, headache and myalgia

C. trachomatis Serovar L1, L2, and L3

- **Third stage:** in untreated cases
 - Rectal stricture or rectovaginal and rectal fistulae
 - **Elephantiasis**—edematous granulomatous hypertrophy of vulva, scrotum or penis
 - **Elephantiasis** of the vulva or scrotum
- **Epidemiology:** Incidence decreasing
 - Endemic in Southeast Asia, South America and Caribbean

CHLAMYDOPHILA PSITTACI

- A pathogen of parrots and other psittacine birds causing psittacosis
- **Reservoirs: Pet birds (parrots, parakeets, macaws, and cockatiels) and poultry (turkeys and ducks)**
- **Mode of transmission:** Inhalation of aerosols from avian nasal discharges and from infectious avian fecal or feather dust
- **D**irect contact with infected birds
- **N**o person to person transmission

Psittacosis

- **Clinical manifestations:** Incubation period 5–19 days
- **Respiratory** manifestation - most common form, mild influenza-like syndrome to a fatal pneumonia
- **Septicemia** → meningoen­cephalitis, endocarditis, pericarditis, arthritis and gastrointestinal symptoms
- **Typhoid-like syndrome**

CHLAMYDOPHILA PNEUMONIAE

- Exclusive human pathogen
- **Transmission** - person to person by inhalational
- **Atypical pneumonia:** 10% community-acquired pneumonia
- **F**ever, non-productive cough and absence of leukocytosis
- **U**pper respiratory tract - pharyngitis and sinusitis
- **A**therosclerosis: **A**ntibodies elevated and *C. Pneumoniae* recovered from atheromatous plaques
- **A**utoimmune reaction - Antibodies to OMP cross-react

Laboratory diagnosis

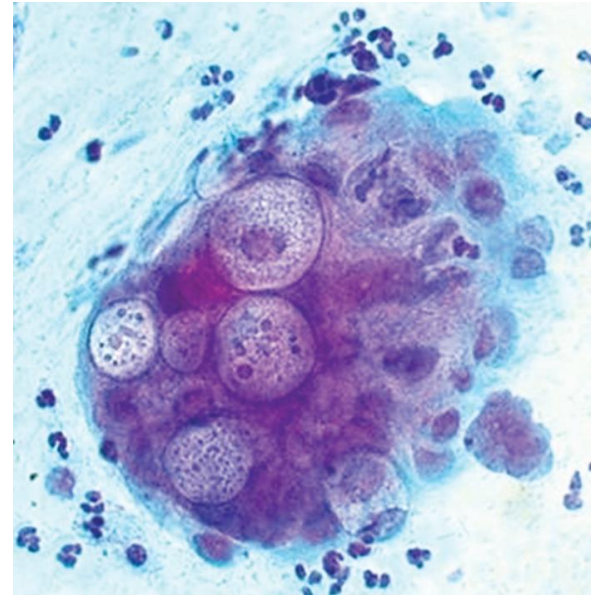
- **Specimen**

- Scrapings or swabs - sample must contain cells
- Urethral swab for NGU
- Endocervical swab for cervicitis
- Conjunctival swabs for ocular infections
- First catch urine samples in the morning
- Nasopharyngeal aspirate
- Bubo aspirate for LGV.

Microscopy

- **Gram staining:** Though gram-negative poorly stained
- **Presumptive diagnosis: Sterile pyuria.** Presumptive diagnosis is usually made based on neutrophil count:
 - NGU, post gonococcal urethritis, epididymitis, reactive arthritis more than 4 neutrophils per oil immersion field (OIF)
 - Cervicitis more than 20 neutrophils per OIF
 - Proctitis more than 1 neutrophils per OIF.

- **Castaneda, Machiavello or Gimenez stains**
- **Lugol's iodine:** *C.trachomatis* glycogen matrix stained with Lugol's iodine
- **Inclusion bodies:**
 - Halberstaedter–Prowazek (H–P) body in trachoma
 - Miyagawa corpuscle in LGV
 - CL body (Levinthal-Cole-Lillie) body in psittacosis



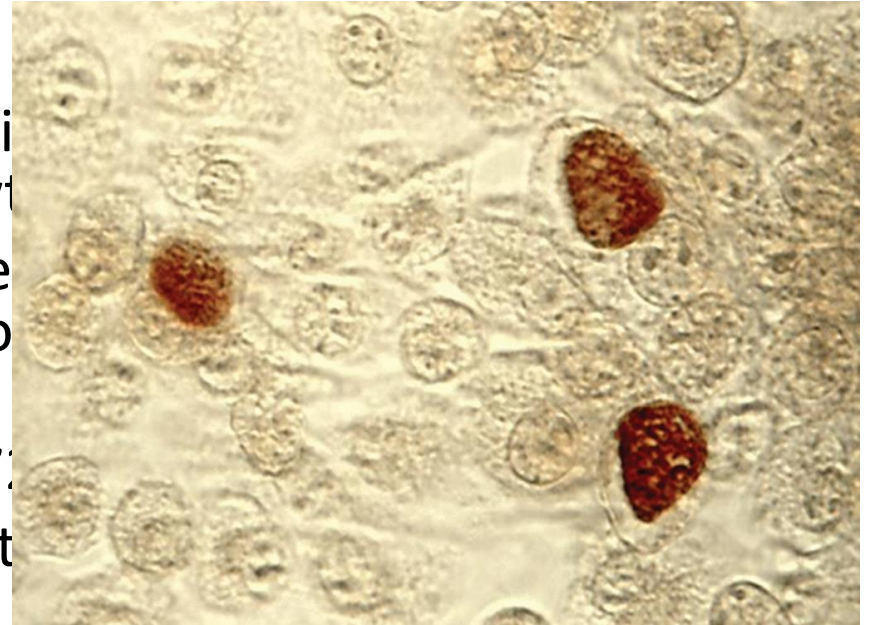
Antigen Detection

- **Direct Immunofluorescence Test (DIF)**
 - For direct detection of inclusion bodies in clinical material
 - Fluorescent tagged monoclonal antibodies directed against group-specific LPS antigen or species-specific MOMP antigens are used
- **Enzyme Immunoassays (Antigen Detection)**
 - Detects chlamydial group specific antigens (LPS)

Culture

- Do not grow in artificial media. Grow in embryonated egg (yolk sac), animal (mice) and cell line
- **Cell line** - highly specific, less sensitive (90% compared with NAATs), time consuming, technically demanding
 - *C. trachomatis* - McCoy, HeLa 229, buffalo, green monkey and baby hamster kidney (BHK-21) cell lines
 - *C. pneumoniae* - Hep2 or human fibroblast cell line
 - *C. Psittaci* - isolation should not be attempted in the routine laboratory because of the risk of laboratory infection

- **Procedure:**
- γ -radiation or idoxuridine or cycloheximide – To bring Cell li their stationary phase of growth
- **Promote contact:** Pre-treatment lines with diethylaminoethano dextran or centrifugation
- **Incubation:** 10% CO₂ for 48–72
- **Detection:** stained to demonstrate inclusions



Nucleic Acid Amplification Tests (NAAT)

- **Advantages:**
 - Highly sensitive and specific, Rapid
 - Differentiate the species and serovars
- NAATs are currently the diagnostic assays of choice for chlamydial infection
- **Methods available are:**
 - Polymerase chain reaction (PCR)
 - Real time PCR
 - FilmArray respiratory panel.

Serology (Antibody Detection)

- Useful for LGV, infant pneumonia and psittacosis (systemic infections)
- **Complement fixation test (CFT) using LPS antigen**
 - Group specific and cannot distinguish between species
 - Titer of $\geq 1:64$ is considered significant
- **ELISA** – Antibodies against LPS antigen

Microimmunofluorescence (MIF)

- **Uses** species & serovar specific **MOMP** (major outer membrane protein) antigen
- Detects IgM and IgG separately
- **N**ot widely used - procedure is highly technically demanding and labor intensive
- **S**ingle high titer of $\geq 1:512$ is diagnostic
- Fourfold rise of titer at 2–3 weeks interval is more significant

Summary of Lab Diagnosis

Clinical presentation	Specimen	Recommended laboratory test
Genital infections		
Urethritis (NGU) and cervicitis (<i>C. trachomatis</i> D–K)	Urethral swab or endocervical swab	NAAT, direct detection (EIA and DIF)
	First-catch urine in the morning	NAAT, direct detection (EIA and DIF)
Pelvic inflammatory disease/ Fitz–Hugh–Curtis syndrome (<i>C. trachomatis</i> D–K)	Endocervical swab, fallopian/ peritoneal swab	NAAT, antigen detection (EIA and DIF), culture
Lymphogranuloma venereum (<i>C. trachomatis</i> L1–L3)	Serum	Antibody detection (ELISA, MIF, CFT)
	Scraping from ulcer base	Direct detection (EIA and DIF), culture
	Lymph node aspirate	Culture
Ocular infections		
Trachoma (<i>C. trachomatis</i> A–C)	Conjunctival swab (upper)	NAAT,
Ophthalmia neonatorum (<i>C. trachomatis</i> D–K)	Conjunctival swab (lower)	direct detection (EIA and DIF), culture
Pulmonary infections		
Infant pneumonia (<i>C. trachomatis</i> D–K)	Serum	IgM antibody detection (EIA, MIF)
	Nasopharyngeal aspirate	NAAT, direct detection (EIA and DIF), culture
Psittacosis (<i>C. psittaci</i>)	Serum	Antibody detection (MIF, CFT)
Community-acquired pneumonia (<i>C. pneumoniae</i>)	Serum	Antibody detection (MIF)
	Respiratory secretions	Direct detection (EIA and DIF)

THANK YOU..!