Genital system infections

Level 2, semester 4

Instructor information

Contact	Official email
Prof. Niveen Adel Mohamed El-wakeel	niveen10@gmail.com
Dr.Amany Elmatbouly Elsayed	amanielmatbouly@gmail.com
Dr.Aya Ahmad Elnegery	ayaelnegery@mans.edu.eg
Dr. Nada Hamid Qandeel	nadahamid@mans.edu.eg
Dr. Lamis Mohamed Taha	Lamis_mohamed@mans.edu.eg
Dr. Aya Gamal Borham	ayagamalborham@mans.edu.eg
Dr. Azza Mohamed Mamon	Azzam2010@mans.edu.eg

Learning outcomes:

At the end of this Lecture students should be able to

- Describe normal flora (Microbiome) of the genital system
- Classify genital system infections.
- Define appropriate samples for diagnosis of genital system infections.
- Discuss clinical manifestations of gonorrhea, syphilis, and *chlamydial* genital infections
- Discuss laboratory diagnosis of *N. gonorrhea, T. pallidum, chlamydia* genital infections

Lecture outline:

1. Normal flora (Microbiome) of the genital system
2. Classification of genital system infections
3. Laboratory diagnosis of infections caused by *N. gonorrhea.*
4. Morphology of *T. pallidum and clinical stages of syphilis*
5. Laboratory diagnosis of syphilis
6. Laboratory diagnosis of chlamydia genital infections

Case scenario, Clinical Correlate, Practice points

Khaled is a 40-year-old man, presents with 2 weeks history of profuse purulent discharge from urethra. The clinician collected a sample from this discharge and transported it immediately for microbiological laboratory for bacterial culture. In the laboratory, direct smear was done and revealed intracellular Gram negative diplococci. The microbiologist reported the result of direct smear immediately to the clinician then completed the culture of the sample.

Questions:

- **What is the most likely case diagnosis and the causative organism?**
- **What is the importance of direct smear in this case?**
- **Describe the culture characters of the causative organism.**
- **What is the treatment for this case?**

Normal flora (Microbiome) of the genital system

Vagina:

- ✓ Aerobic Lactobacilli (Doderlein's bacilli) form the main flora.

- ✓ Not present at pre-puberty and diminishes after menopause (present only in the productive female life).

- ✓ Responsible for the acidic pH of vagina due to fermentation of glycogen to lactic acid.

- ✓ High acidity of the vagina produced by Lactobacillus is inhibitory to some bacteria as Streptococci and Staphylococci as well as the potentially pathogenic yeast, Candida albicans. Also, it (maintains the sperm viability)

- ✓ In addition, anaerobic Streptococci, Coliform, Diphtheroids and yeast are present as normal vaginal flora.

The flora of the anterior urethra:

- ✓ Consisting of *Staphylococcus epidermidis*, *Enterococcus faecalis* and some alpha-hemolytic *streptococci*. Their numbers are not plentiful.

- ✓ In addition, some enteric bacteria (e.g. *E. coli, Proteus*) and corynebacteria, which are probably contaminants from the skin, vulva, or rectum.

Classification of genital system infections and causative organisms in each type

1- Sexually transmitted diseases, STDs (Venereal diseases):

a) Gonorrhea caused by *N. gonorrheae*.

b) Syphilis caused by *T. pallidum*.

c) Soft sore caused by *H. ducryii*.

d) Lymphogranuloma venereum caused by *Chlamydia*.

e) Nongonococcal uretheritis (NGU) caused by *Chlamydia and Mycoplasma genitalium* .

f) AIDS caused by HIV.

g) HBV.

h) HSV-2.

2. Non-venereal diseases:

In males: Prostatitis and urethritis may be due to:

In female: Salpingitis, oopharitis and endometritis

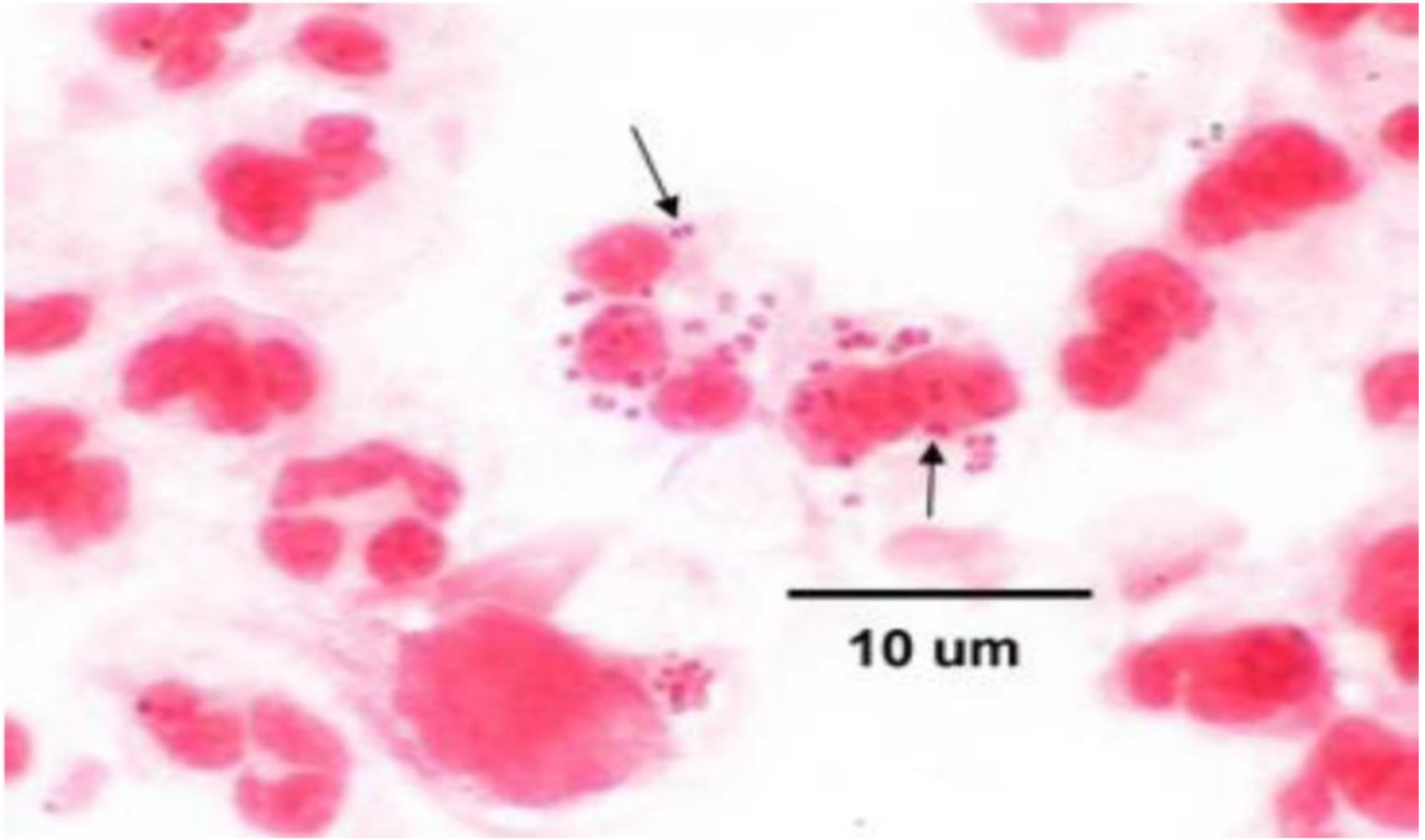
Causative organisms:

- *S. aureus*
- *S. pyogenes*
- *E. coli.*
- *P. pyocanea.*
- *Proteus.*

Neisseria gonorrhea

Morphology of N. gonorrhea (Gonococci):

- ✓ Shape & Gram stain: Gram negative cocci (kidney-shaped)
- ✓ Arrangement: Diplococci
- ✓ Motility: Non motile
- ✓ Spore formation: Non spore forming.
- ✓ Capsule: not capsulated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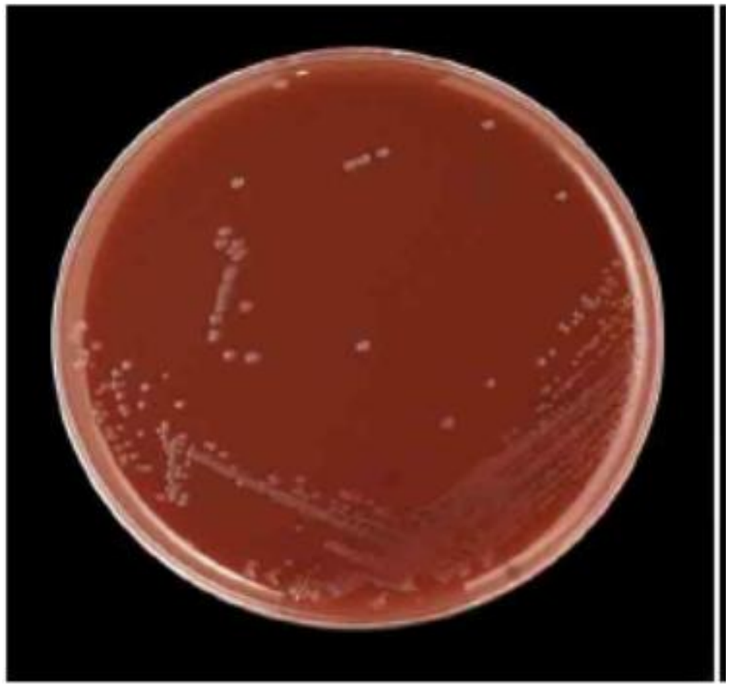
Culture characters:

- ✓ O2: Aerobic
- ✓ Optimum temperature is 37ºC
- ✓ CO2:10% CO2 is required for growth.

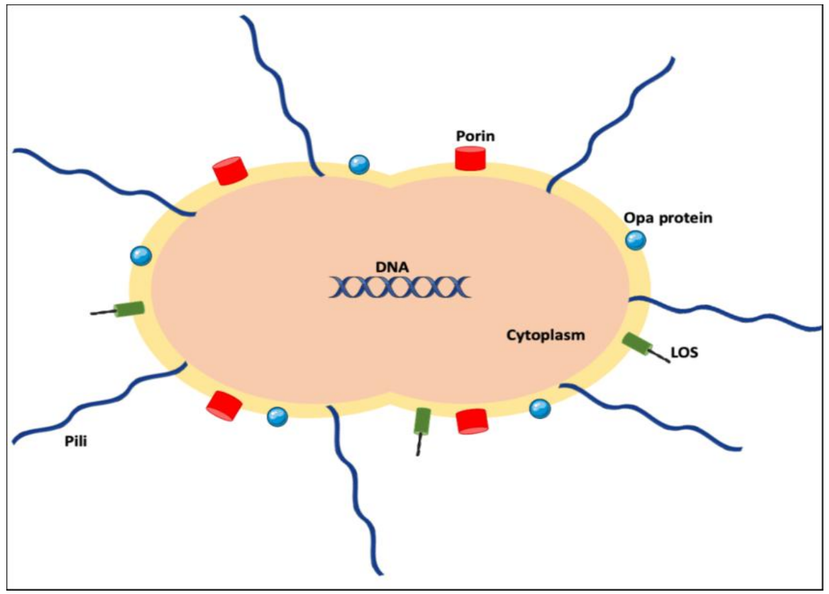


Culture media :

- ➢ Ordinary media: Cannot grow on it
- ➢ Enriched media: Grow on chocolate agar
- ➢ Selective media: Grow on Thayer-Martin media

Virulence factors:

- ✓ Pili (fimbriae) and outer membrane proteins: adhesion.
- ✓ Endotoxin: Lipooligosacchrides.
- ✓ IgA proteases: inactivate human IgA.



Manifestations: (Gonorrhea)

- **Male**: **Acute urethritis:** Characterized by **profuse** purulent discharge

- **Female**:

Cervicitis

Characterized by **profuse** vaginal mucopurulent discharge & sometimes acute urethritis & dysuria.

Vulvovaginitis: Occurs in girls 2-8 years of age.

- **Newborn: Ophthalmia neonatorum:**

Occur in newborns who are exposed to infected secretions in the birth canal.

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N.gonorrhea in Gram stained film are arranged in:

a) Clusters

b) Long chains

c) Dilococci

d) Short chains

Laboratory diagnosis of *N. gonorrhea*

1- Sample:

In acute disease:

profuse pus & secretions from the urethra or cervix.

In chronic disease: (Scanty or no discharge)

a) In males:

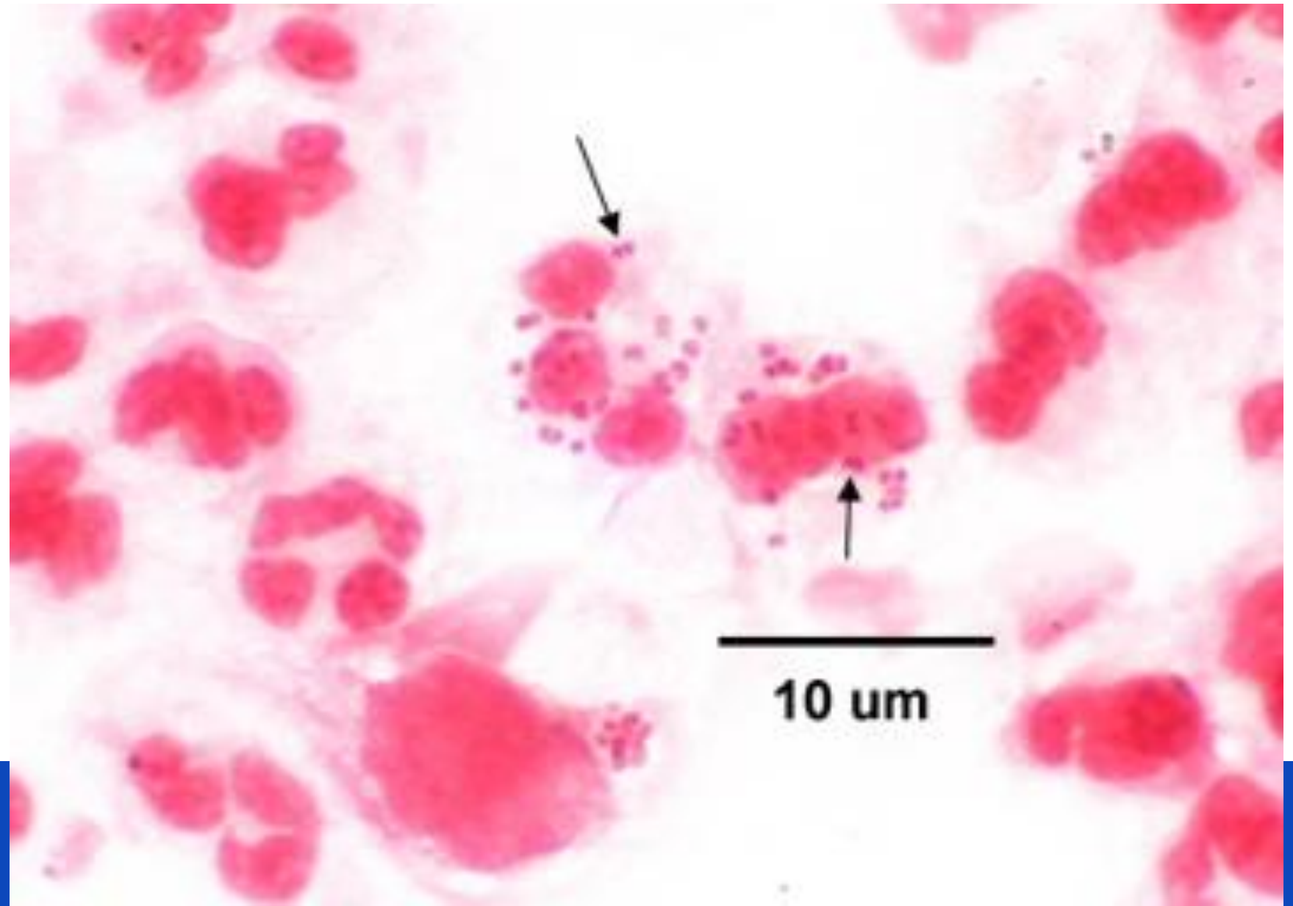
1- morning drop from the urethra
2- centrifuged deposit of urine
3- prostatic massage

b) In females:

- cervical secretion is obtained after cervical irritation.

2- Direct film stained with Gram stain:

Detection of intracellular Gram negative diplococci is **diagnostic**



A- Media: complex requirements:

- **Ordinary media:** No growth
- **Enriched media**:

  Chocolate agar: Grow
- **Selective media:**

  Thayer Martin medium: Grow

B- Requirements:

- Aerobic, 37°C, 10% Co2

C- Identification of the obtained ‘colonies’ by:

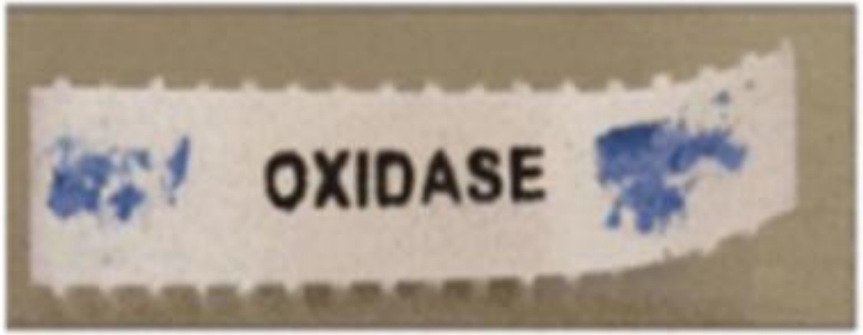
✓ Film stained with Gram stain to show characteristic morphology

(Gram negative diplococci, Non motile, Non spore forming)

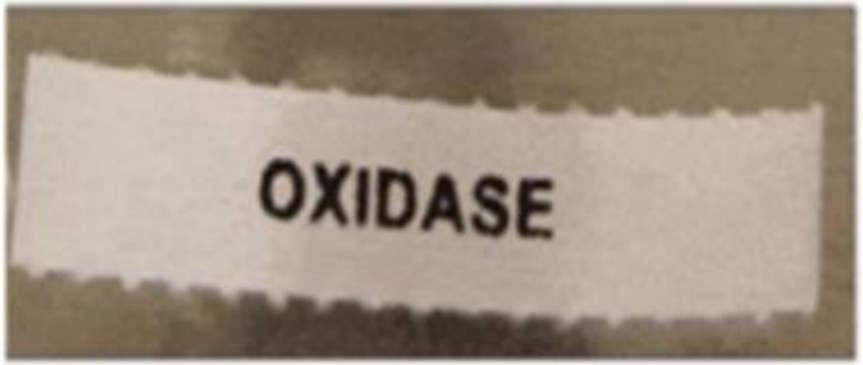
✓ **Oxidase test:** positive.

✓ **Sugar fermentation:**

*N. **g**onorrhea* ferment **g**lucose only (with acid production only)



Oxidase positive



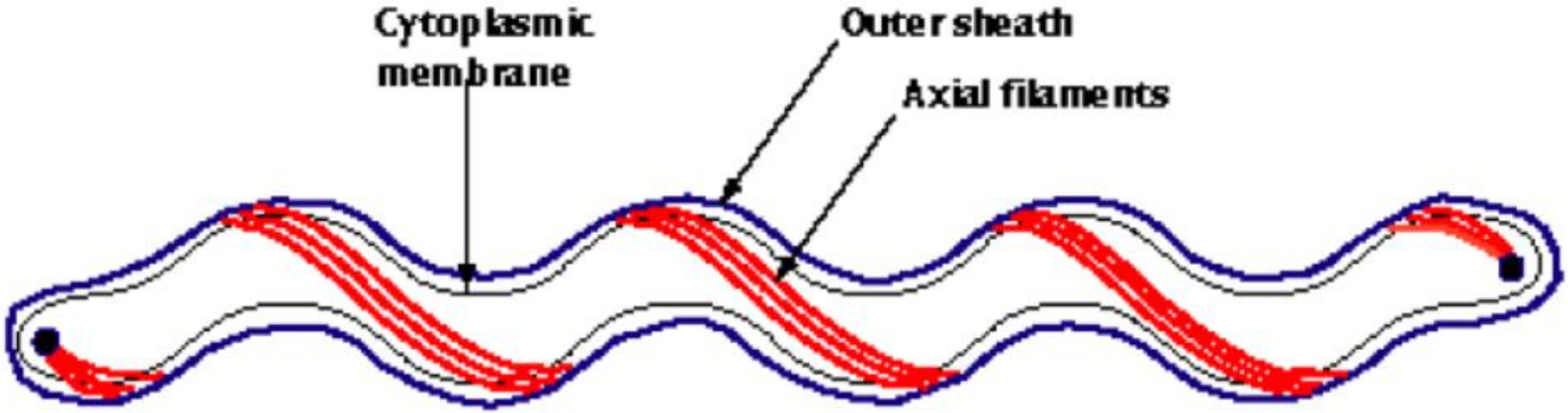
Oxidase negative

Treponema pallidum

Morphology:

- ✓ Slender, Spiral filaments with regular coils (coils are small and large in numbers), motile.
- ✓ Can not be seen by light microscopy but can be seen unstained by dark-field microscopy.
- ✓ Can not be stained by Gram stain, can be stained by Giemsa and Fontana stains.
- ✓ In tissues can be visualized by silver impregnation methods.

- ✓ Have axial filaments, which are otherwise similar to bacterial flagella
- ✓ Filaments enable movement of bacterium by rotating in place.



Culture characters:

- ✓ Pathogenic Treponema have not yet been cultured in vitro on artificial media.
- ✓ Pathogenic strains : can grow in testicles of rabbit or cultured rabbit epithelial cells
- ✓ The cells have a high lipid content (cardiolipin, cholesterol), which is unusual for most bacteria.
- ✓ Microaerophilic,
- ✓ Fastidious organism with narrow optimal ranges of pH (7.2 to 7.4), and temperature (30 to 37°C).

Pathogenesis: Syphilis:

- ✓ Causative agent: *T. pallidum,* strictly a human disease.
- ✓ Mode of transmission: by sexual contact. Organisms penetrate mucous membranes or enter minute breaks in the skin.
- ✓ Less than 10 organisms are capable of producing infection.

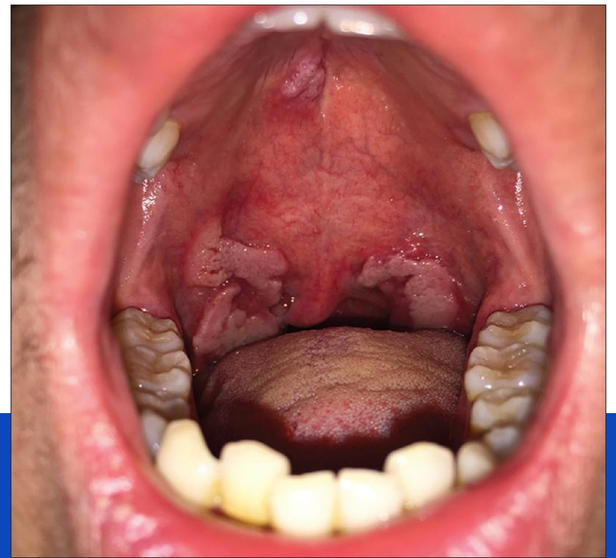
The disease passes into 3 clinical stages:

✓ **Primary Syphilis**: **chancre**

(painless, indurated ulcer) develops at the site of inoculation and associated with regional lymphadenopathy. This lesion is filled with treponemes and is, therefore, highly contagious

- ✓ **Secondary Syphilis:** mucocutaneous rash. mucous patches on mucous membranes and wart-like lesions called condyloma lata in moist intertriginous areas.

All these lesions are highly contagious.

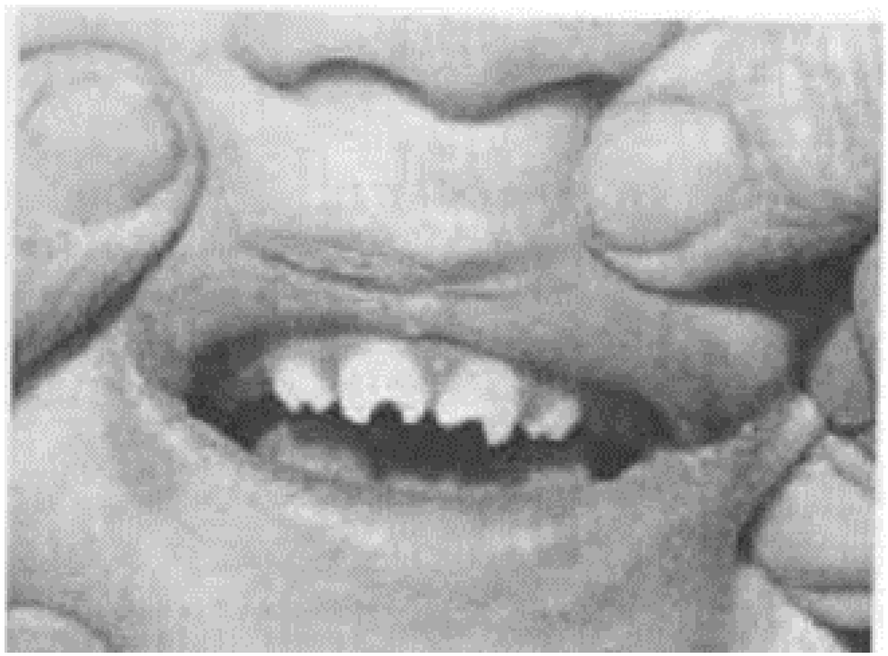


✓ **Tertiary syphilis:** is characterized by long-term complications: gumma (granulomatous-like lesion), cardiovascular and neurosyphilis.

Can affect all areas of the body and be fatal.

Congenital syphilis:

through placenta: fetus may <u>die</u> or <u>borne live with congenital anomalies</u>



Laboratory diagnosis of Syphilis

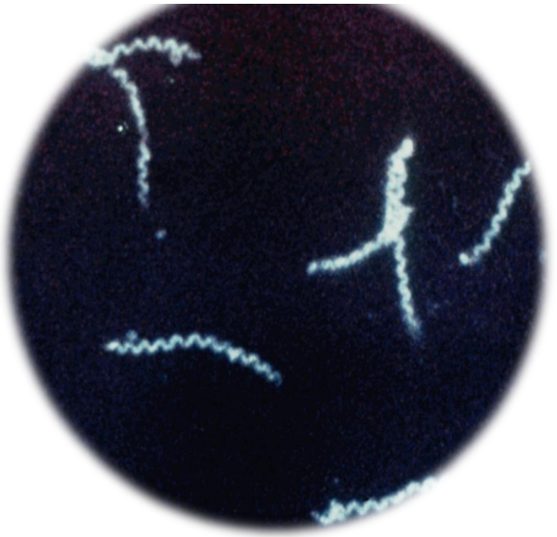
1- Samples:

-(primary stage): **Exudate** from **chancre**-

-(secondary stage): from **Mucous patches and skin eruptions**-

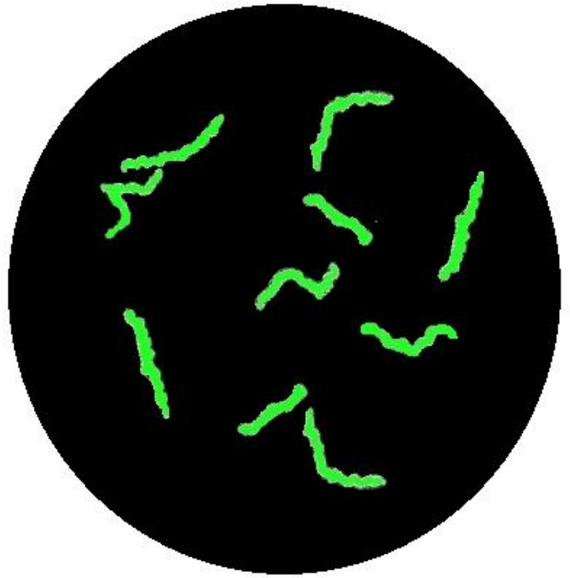
- **Blood** for serology (Antibodies)

2- Direct smear



A- Unstained ‘fresh wet’ film

Examined with dark field microscope for motile *treponemas*



B- Direct immune fluorescence (IF) Using fluorescein-labelled anti-*treponemal* antibodies examined by florescent microscope

3- serological tests

Syphilitic patients produce 2 types of antibodies:

1st (specific): reacts only with ***treponemal* antigens**

2nd (Non specific): **reagin antibodies** react with lipid antigens

e.g., **cardiolipin**

Serologic tests fall into 2 general categories:

1. Treponemal antigen tests

2. Non treponemal antigen tests

1. *Treponemal* antigen tests

Detect specific antibodies (antibodies directed against protein constituents of *T. pallidum*).

Examples are:

a) Fluorescent *T. pallidum* antibody absorption (FTA- ABS).

b) *Treponema pallidum* immobilization (TPI) test

c) Micro-hemagglutination *for T. pallidum (MHA-TP).*

Use: Confirmation.

2. Non *treponemal* antigen tests

Which measure non specific antibodies (directed against lipid antigens, principally cardiolipin).

Examples:

1- Venereal Disease Research Laboratory (VDRL)

2- Rapid plasma reagin test (RPR)

3- Wassermann test [Complement fixation test]

Use:

- ✓ Screening.
- ✓ Epidemiological purposes.
- ✓ Evaluate the effect of treatment

- ✓ The sensitivity of the tests varies with the stage of the disease.
- ✓ The results of non-treponemal tests usually parallel the extent of infection; titers tend to be highest during secondary syphilis and subside during subclinical infection (latency) or following antibiotic therapy.
- ✓ The treponemal tests often remain reactive for life.

Which one of these serological tests is an example of non-treponemal antigen tests:

a) Fluorescent T. pallidum antibody absorption (FTA-ABS).
b) Treponema pallidum immobilization (TPI) test
c) Micro-hemagglutination for T. pallidum (MHA-TP).
d) Rapid plasma reagin test (RPR)

Treatment:

- ✓ Penicillin is the drug of choice in the treatment of syphilis.
- ✓ Penicillin was found to be effective in eradicating syphilis of all clinical stages as well as the congenital infection.

Chlamydia trachomatis

Chlamydial infections in genital system:

- ✓ **Lymphogranuloma venereum**, is a venereal disease spread sexually characterized by genital lesions and regional lymph node involvement (buboes).

- ✓ **Non gonococcal urethritis** in men and **acute salpingitis and cervicitis** in women.

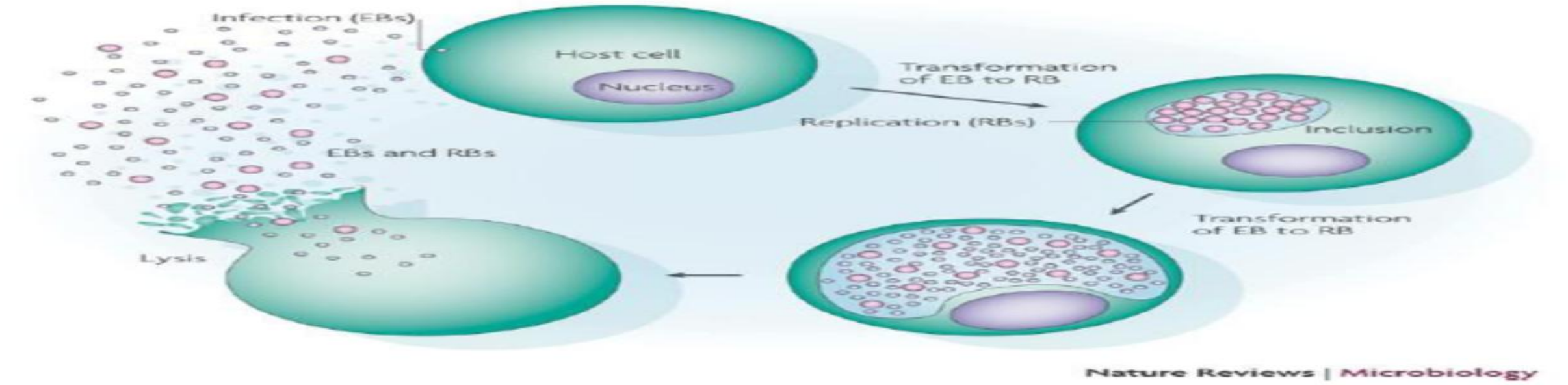
Chlamydiae resemble viruses in the following:

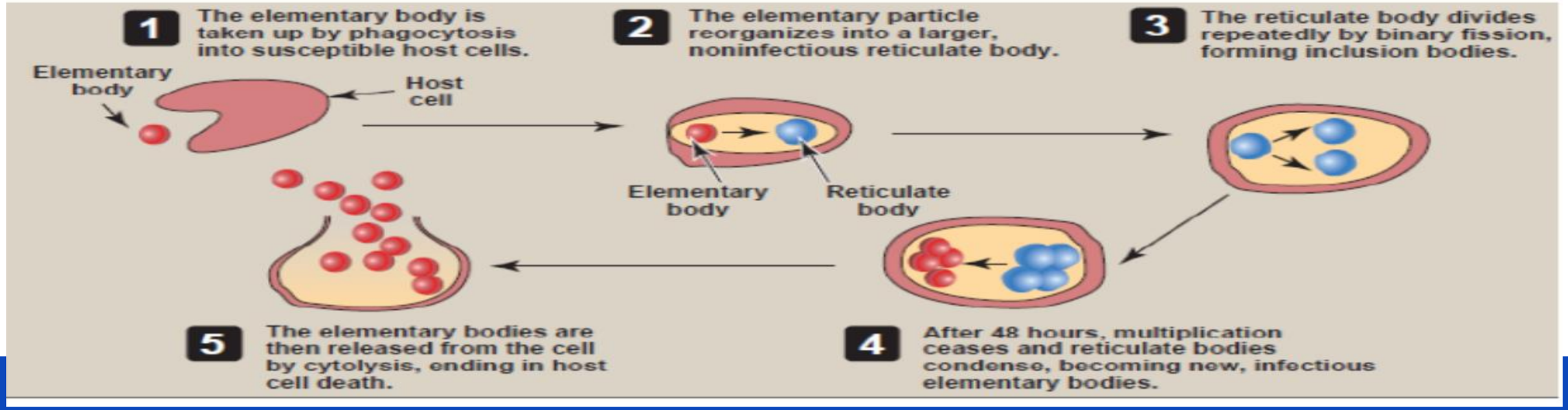
- ✓ Obligate intracellular
- ✓ Cannot make ATP
- ✓ Grow in cell culture
- ✓ No peptidoglycan

Chlamydiae resemble bacteria in the following:

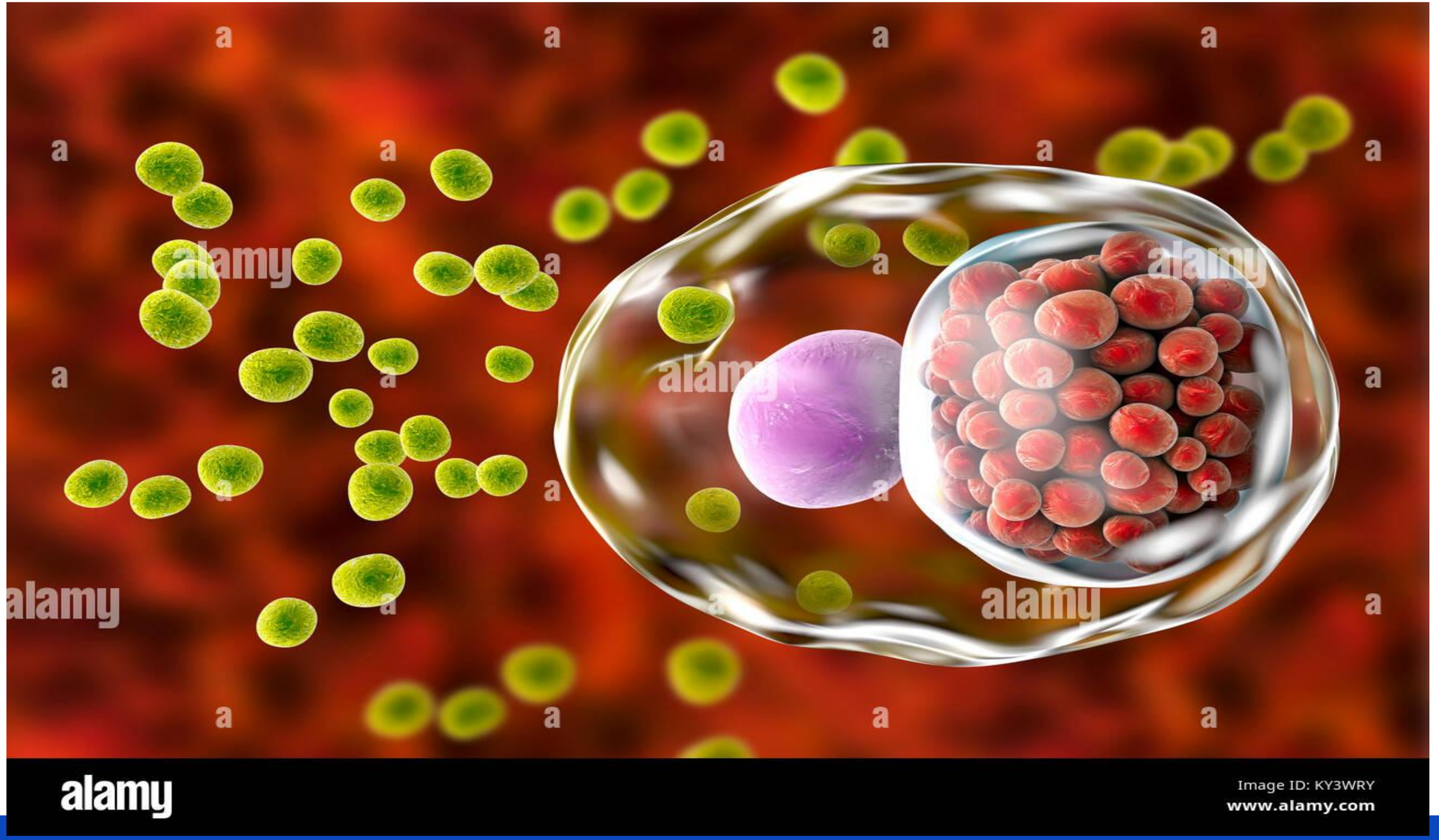
- ✓ Have DNA, RNA, ribosome.
- ✓ Have Inner and outer membrane & cell wall.
- ✓ Susceptible to Antibiotics
- ✓ Multiply be binary fission.

Life cycle of *chlamydiae*





Elementary body	Reticulate body (RB)
Small	Large
Extracellular	Intracytoplasmic
Infectious form	Non-Infectious form
Non-replicating	Metabolically active and replicating
Released from ruptured infected cell	Within cells, the site of replication appears as an inclusion body, which can be stained and visualized microscopically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Laboratory diagnosis:

1.Specimens:

✓Scrapping from the urogenital tract.

✓Urethral or Cervical exudates.

2.Microscopic examination:

Inclusion bodies in scraped tissue cells are detected by staining with Giemsa or iodine or by staining with fluorescent monoclonal antibodies.

3.Culture:

a) **On McCoy cells**: after incubation, typical cytoplasmic inclusions are seen.

b) **Yolk sac of embryonated egg**: have been used to isolate *Chlamydia*.

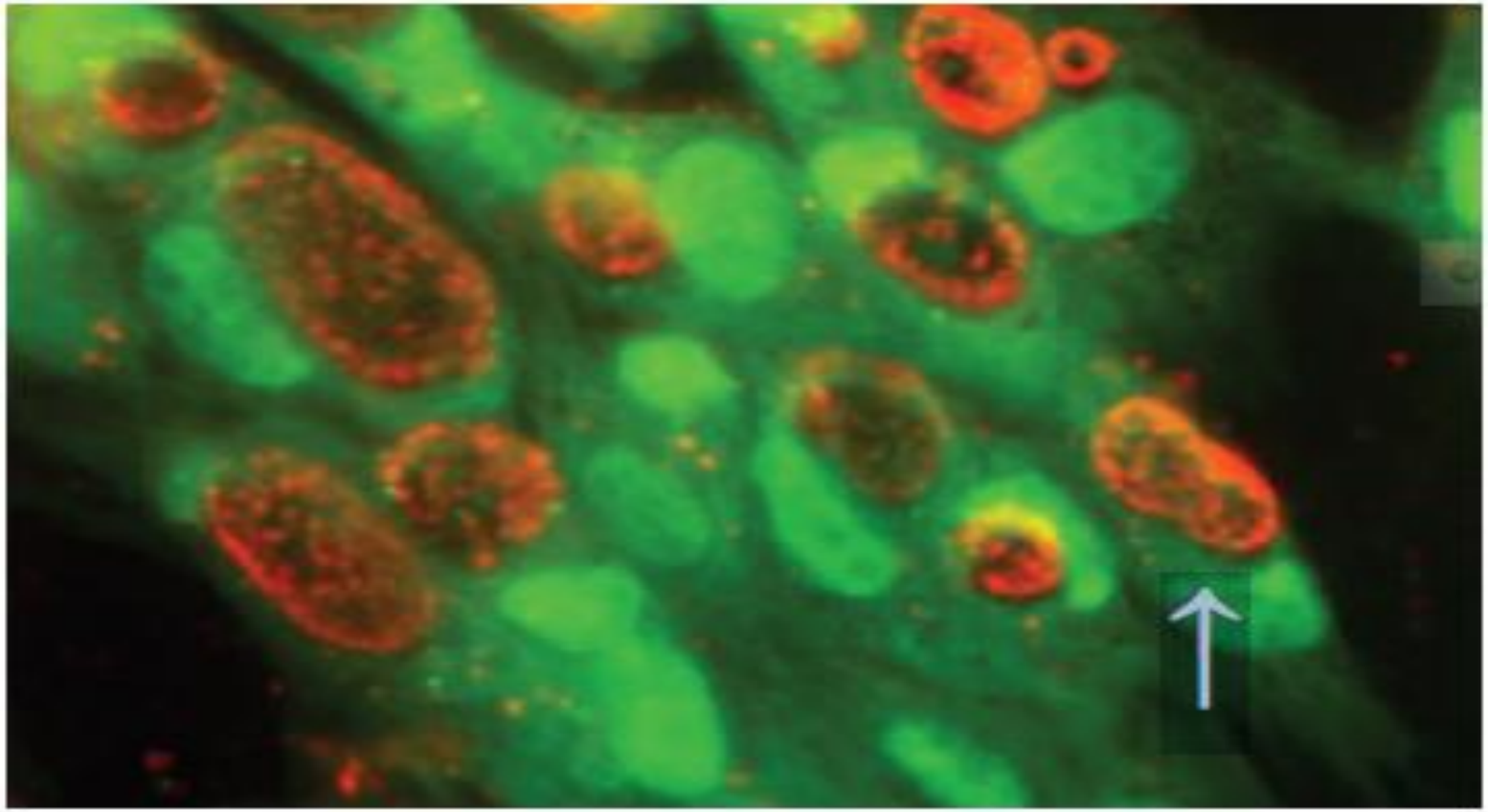
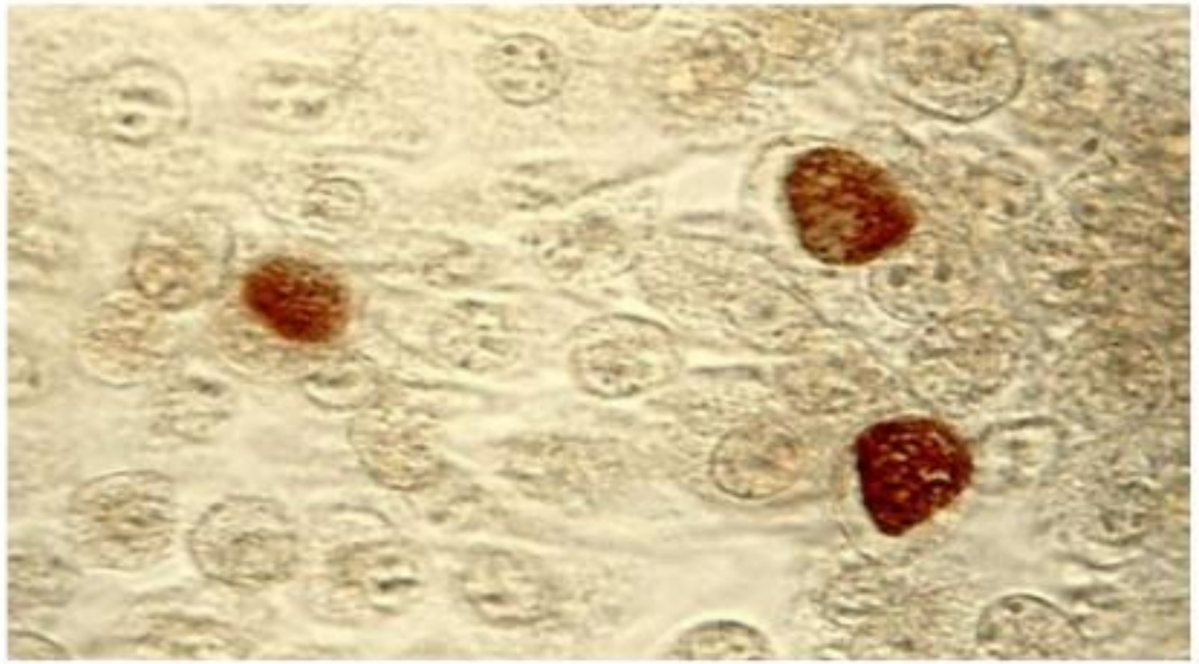


Fig 3. Direct fluorescent antibody – C. trachomatis using Chlamydia monoclonal antibody conjugate (×1000). Red spots representing EBs. The arrow points to an inclusion body.

Isolation of chlamydia is possible by **yolk-sac** inoculation method and tissue culture in **McCoy cells (synovial carcinoma cell line)**



Chlamydia trachomatis **in McCoy cells brown colored**

4. Serological tests:

a) Detection of *chlamydial* antigen directly in specimens by using specific immunofluorescent antibodies prepared against *C.trachomatis*.

b) Detection of anti-*Chlamydia* antibodies in sera or tears from infected humans by the complement fixation or immunofluorescence tests.

5. Molecular techniques:

- DNA probes: It is possible to diagnose C -trachomatis in tissue specimens by hybridization with a specific DNA probe.
- PCR: is used for diagnosis.

Treatment:

- ✓ There is no available vaccines
- ✓ It is important to take precautions against sexually transmitted infections because chlamydia is so common and it doesn't produce symptoms.
- ✓ Tetracycline and Erythromycin are the drugs of choice.
- ✓ Penicillin is not effective.

Case scenario, Clinical Correlate, Practice points

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Questions:

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References:

- Cheesbrough M. (2000): Microbiological tests. Cited by Cheesbrough, M., (ed.) District Laboratory Practice in Tropical Countries, Part 2, Microscopical techniques used in microbiology, Cambridge University Press, UK

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Thank you