

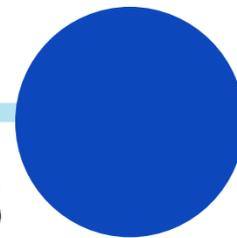


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# Diagnosis of genital system infections

Level 2, semester 3





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## Content:

- ✓ General rules for sample collection.
- ✓ Classification of genital system infections.
- ✓ Laboratory diagnosis of infections caused by *N. gonorrhoea*.
- ✓ Laboratory diagnosis of infections caused *T. pallidum*.
- ✓ Laboratory diagnosis of infections caused *chlamydia* lymphogranuloma venereum



# Learning outcomes:

At the end of this section students should be able to

- Describe general rules of samples collection
- Classify genital system infections.
- Define appropriate samples for diagnosis of genital system infections.
- Discuss laboratory diagnosis of *N. gonorrhoea*, *T. pallidum*, *chlamydia lymphogranuloma venereum*



## General rules for sample collection:

1- Hands should be washed **before and after** the collection





## 2- The samples must be:

- ✓ Taken before the start of antimicrobial therapy.
- ✓ Representative of infectious process e.g. swab from depth of wound (not from surface).
- ✓ Adequate volume.
- ✓ Collected aseptically in an appropriate, sterile container, which must be dated, appropriately labeled and the requisition form completed



- ✓ Transported rapidly to the laboratory
- ✓ As fresh as possible fearing of death of delicate organisms (Gonococci, hemophilus) or overgrowth of contaminants
- ✓ genital tract specimens are best taken at the bedside where suitable media are directly inoculated and direct smears are made.



## Genital system infection

### Anatomical classification:

#### 1- Male:

- ✓ Urethritis.
- ✓ Prostatitis.

#### 2- Female:

- ✓ Vulvitis.
- ✓ Vaginitis.
- ✓ Cervicitis.
- ✓ Endometritis.
- ✓ Salpingitis.
- ✓ Oophritis



## Sexually transmitted diseases, STDs (Venereal diseases):

- a) Gonorrhoea caused by *N. gonorrhoeae*.
- b) Syphilis caused by *T. pallidum*.
- c) Soft sore caused by *H. ducryii*.
- d) Lymphogranuloma venereum caused by *Chlamydia*.
- e) Nongonococcal urethritis (NGU) caused by *Chlamydia and Mycoplasma genitalium*.
- f) HIV.
- g) HBV.
- h) HSV-2.



## 2. Non-venereal diseases:

### In males:

Prostatitis and urethritis may be due to:

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

### In female:

Salpingitis, oopharitis and endometritis are due to:

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



## Sample collection of Genital tract infections:

### a) In male:

- Acute: urethral discharge.
- Chronic: morning drop, prostatic massage.

### b) In females:

- Cervicitis: Cervical swab
- Vaginitis: vaginal swab, discharge.



## Genus *Neisseria* contains:

### 1) Pathogenic *neisseria*: 2

- *N. gonorrhoea*: causes gonorrhoea (STD)
- *N. meningitidis*: causes acute cerebrospinal meningitis

### 2) Non pathogenic (commensal) *neisseria*:

Normal flora

## Manifestations:

➤ **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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# Laboratory diagnosis of *Gonorrhoea*



## 1- Sample:

### In acute disease:

profuse pus & secretions from the urethra or cervix.

### In chronic disease: (Scanty or no discharge)

#### a) In males:

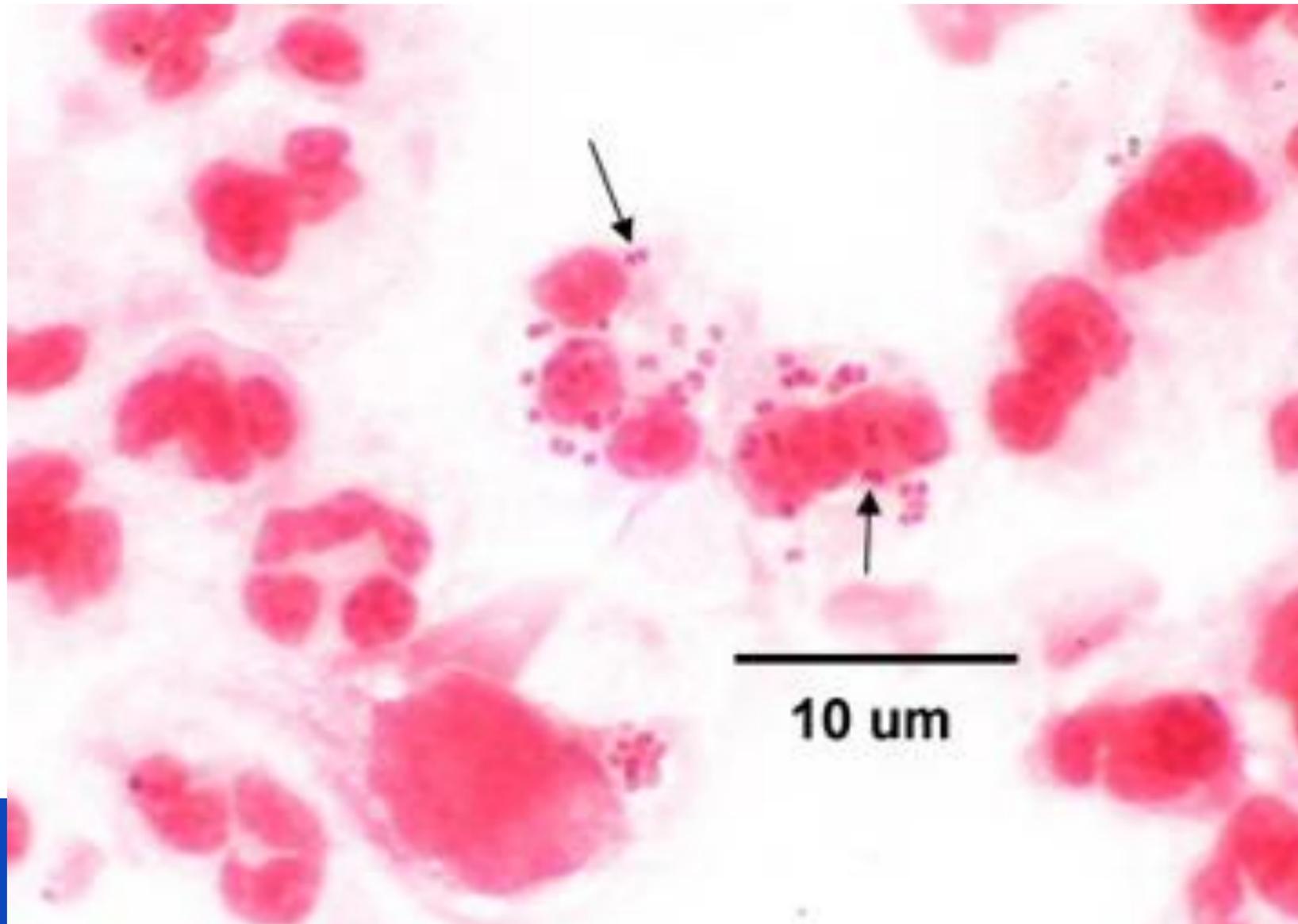
- 1- morning drop from the urethra
- 2- centrifuged deposit of urine
- 3- prostatic massage

#### 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**



### 3.Culture:

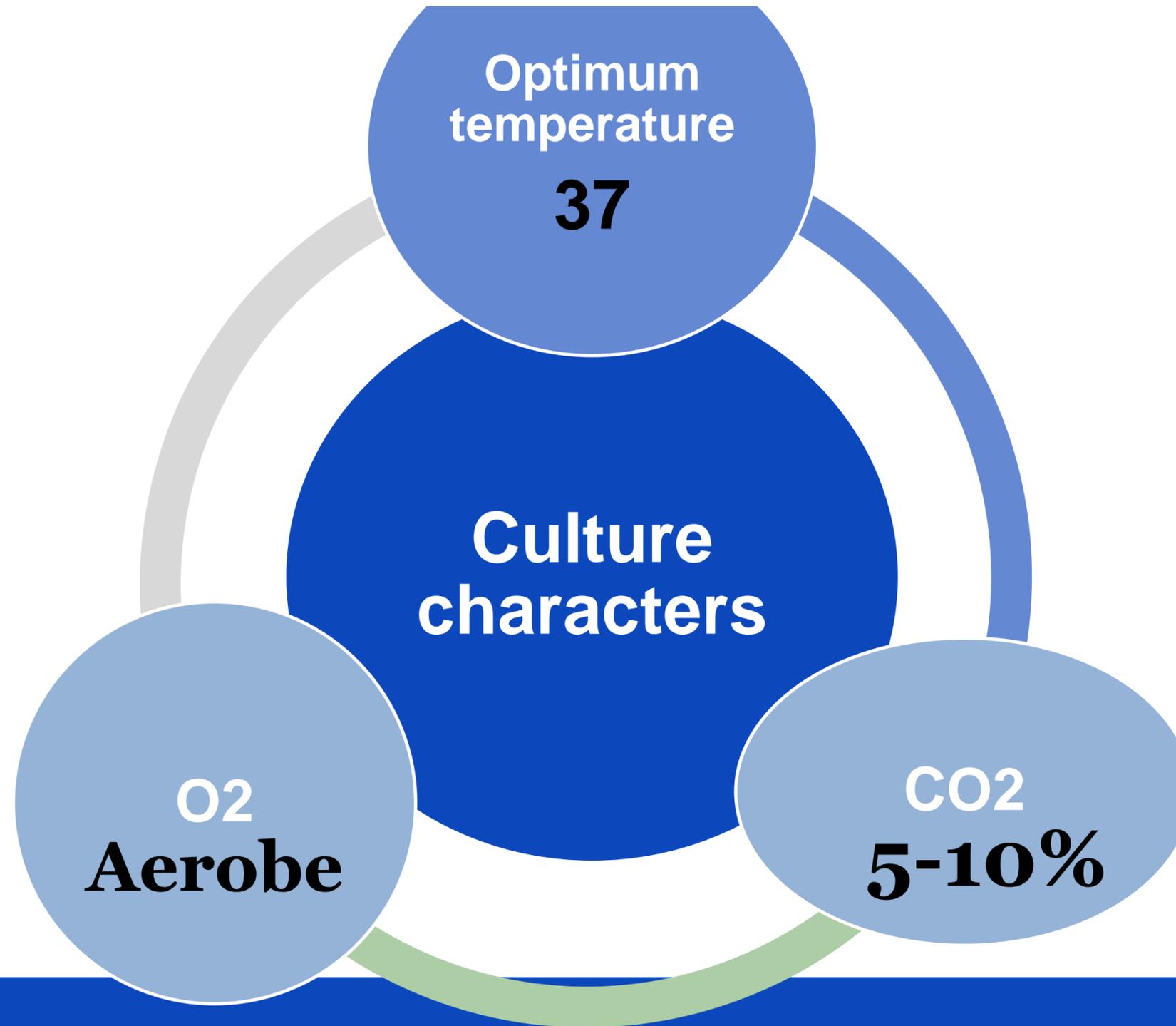
**A- Media:** complex requirements:

- **Ordinary media:** No growth
- **Enriched media:**
  - Chocolate agar:** Grow
- **Selective media:**
  - Thayer Martin medium:** Grow

### B- Requirements:

- Aerobic, 37°C, 10% Co<sub>2</sub>







**Enriched  
Chocolate agar**



**Media**

**Ordinary  
No growth**

**Selective  
Thayer-Martin**





## C- Identification of the obtained 'colonies' by:

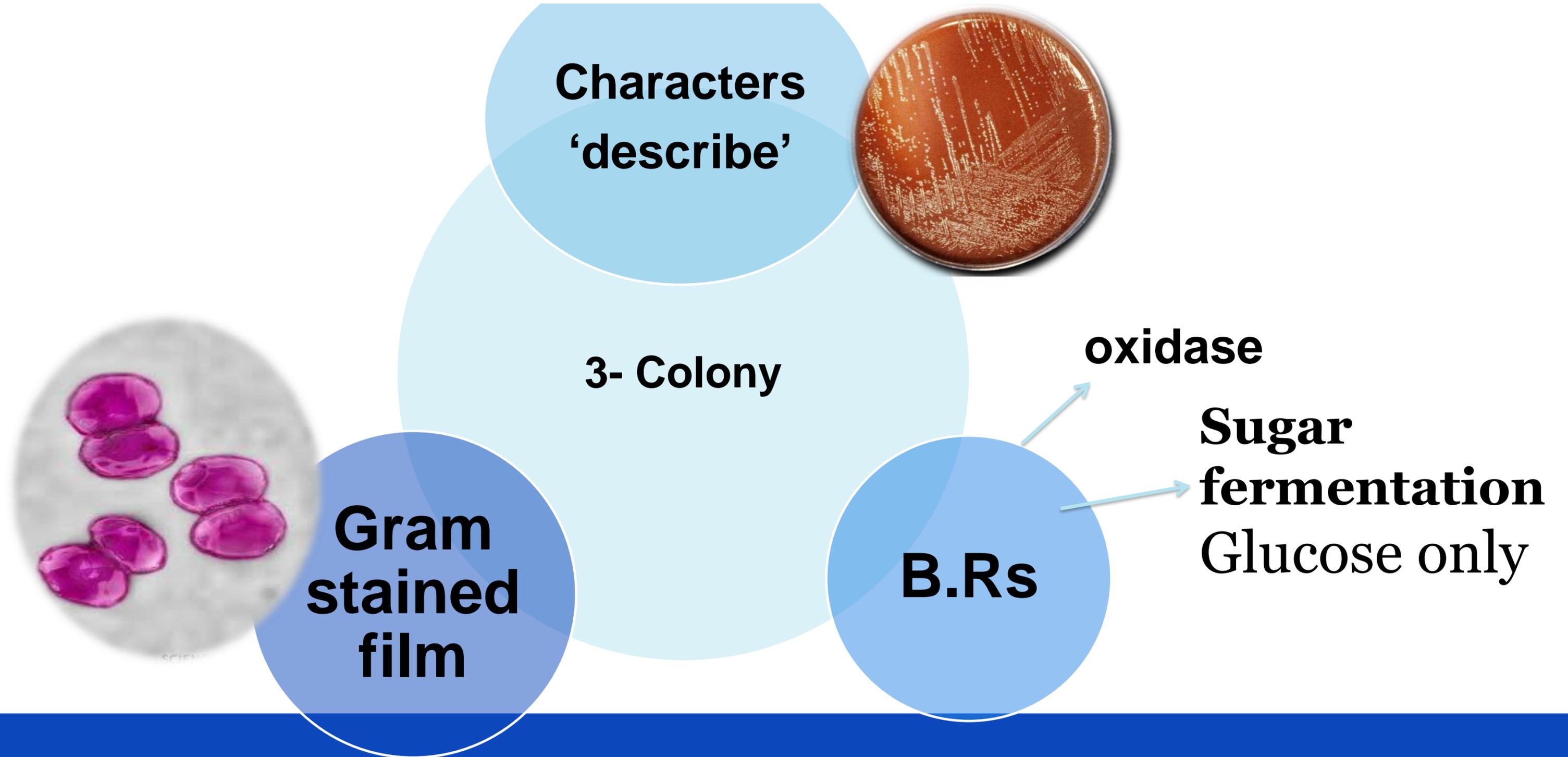
✓ Film stained with Gram stain to show characteristic morphology

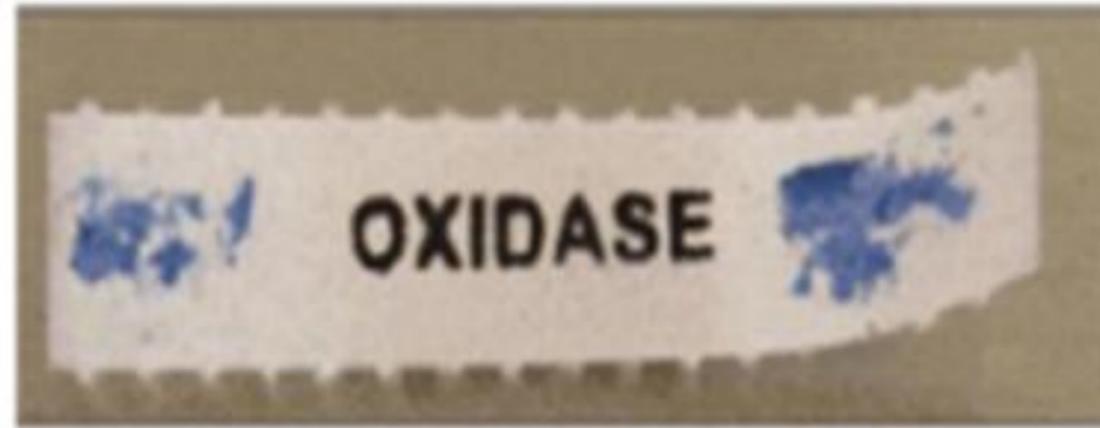
(Gram negative diplococci, Non motile, Non spore forming)

✓ **Oxidase test:** positive.

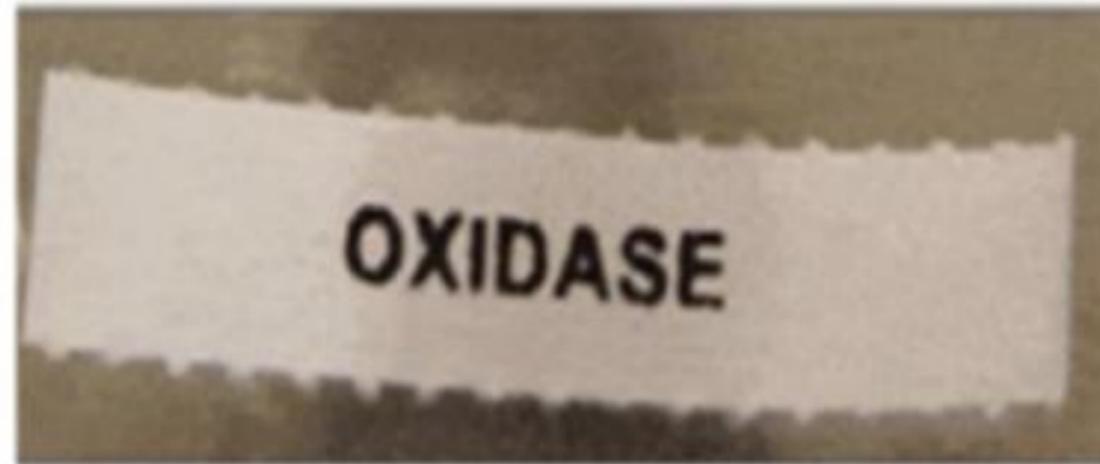
✓ **Sugar fermentation:**

*N. gonorrhoea* ferment glucose only (with acid production only)





Oxidase positive



Oxidase negative



Oxidase +ve

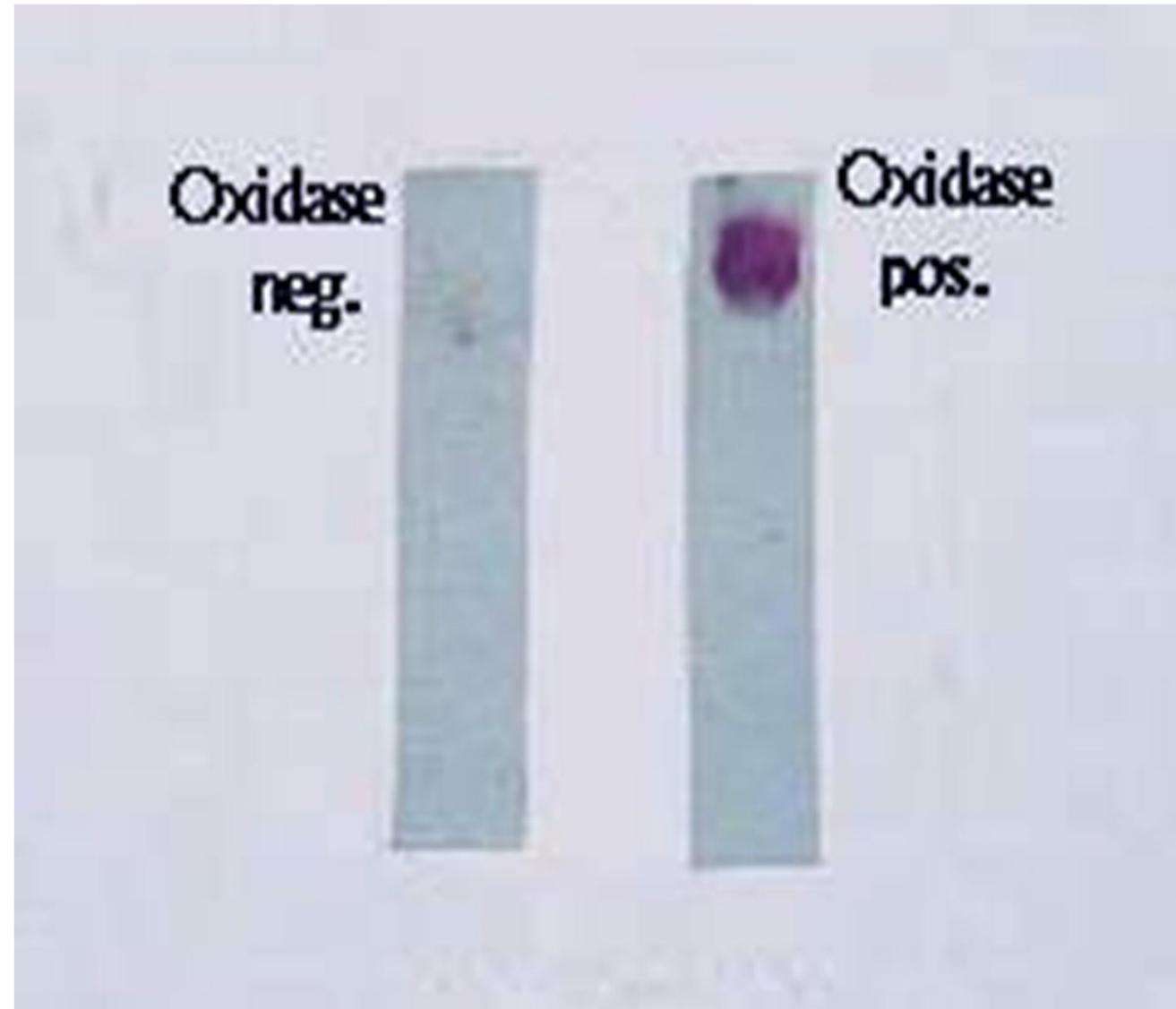
Oxidase -ve



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## Oxidase test





# Diagnosis of Syphilis

## 1- Samples:

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

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

- **Blood** for serology (Antibodies)



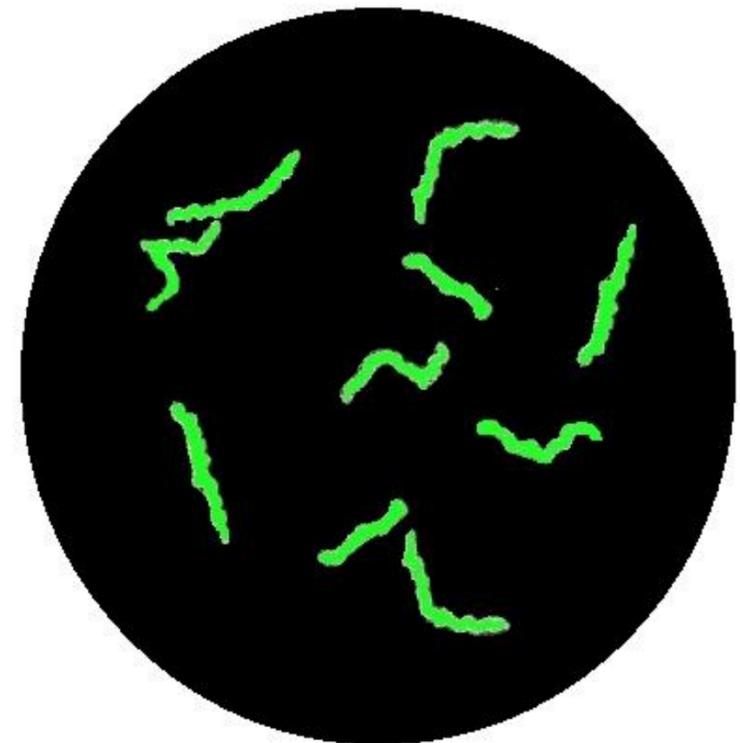
## 2- Direct smear

### 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 fluorescent microscope





## Staining:

- **Not** stained by Gram stain  
So, **Can not** be seen by light microscope
- Stained by **Giemsa**, **fontana** stains

## 3-Culture:

Pathogenic *treponema* **have not** yet been cultured in vitro



## 4- PCR:

For detection of *spirochaetes* in clinical materials confirms the diagnosis

## 5- serological tests

Syphilitic patients produce 2 types of antibodies:

**1<sup>st</sup> (specific):** reacts only with *treponemal* antigens

**2<sup>nd</sup> (Non specific):** **reagin antibodies** react with aqueous suspension of **cardiolipin**



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Serologic tests fall into 2 general categories:

- 1. Treponemal antigen tests**
- 2. Non treponemal antigen tests**



# 1. *Treponemal* antigen tests

Detect specific antibodies

Examples are:

- a) Fluorescent *T pallidum* Antibody (FTA) test
- b) *Treponema pallidum* immobilization (TPI) test
- c) *Treponema pallidum* haemoagglutination (TPHA) test



## 2. Non treponemal antigen tests

Which measure non specific antibodies

### Examples:

- 1- Venereal Disease Research Laboratory (VDRL)
- 2- Rapid plasma reagin test (RPR)
- 3- Wassermann test [Complement fixation test]



## Wassermann test:

### Principle:

Classic **complement-fixation test** using blood serum or cerebrospinal fluid.

### Complement:

- Protein constituent of normal blood serum.
- Source: guinea pig serum.
- Complement is fixed during the interaction of antigen and antibody.



## Materials required :

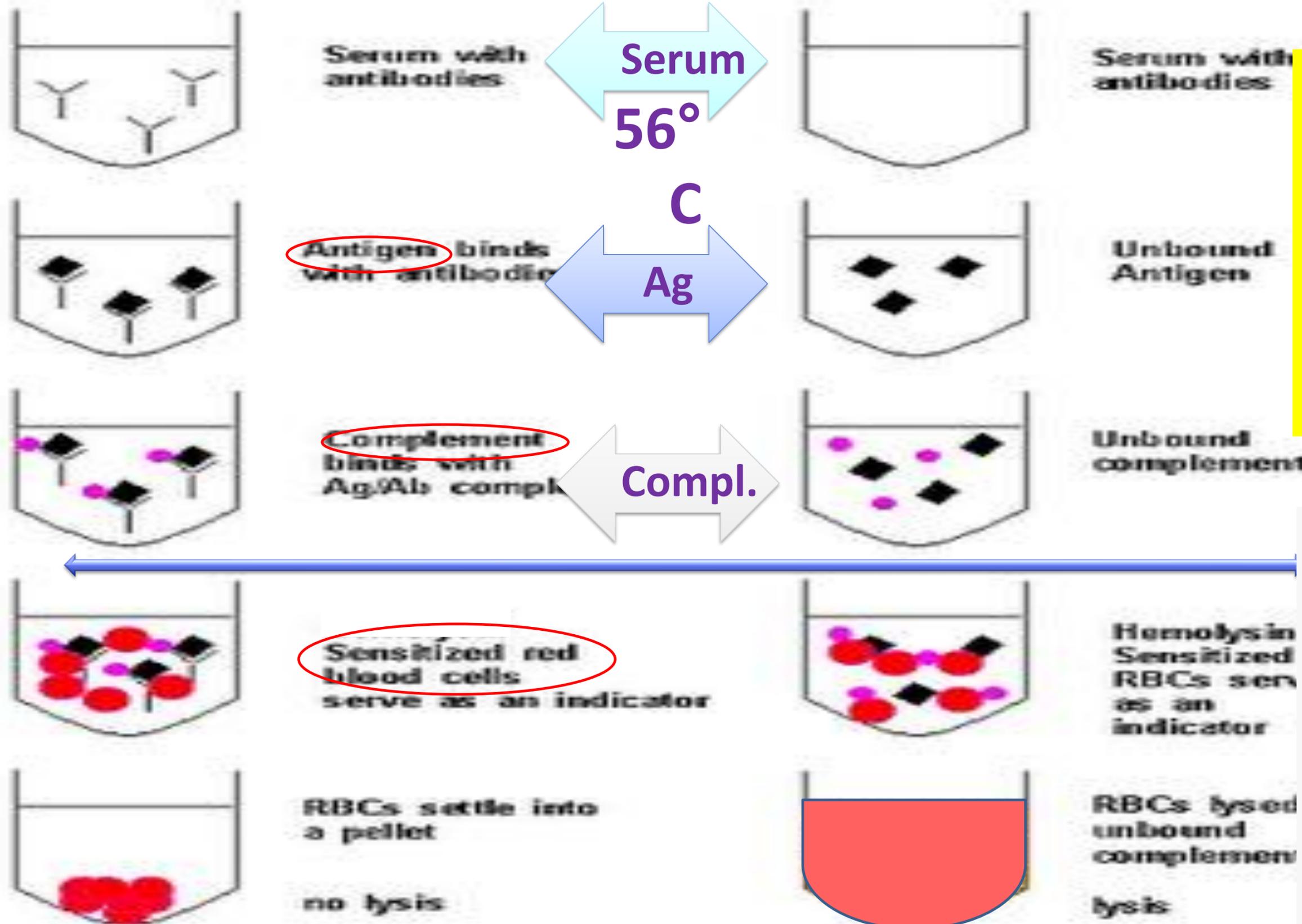
### 1- Patient serum:

heated up to **56°C for 20 - 30 minutes** to destroy complement normally present in serum

2- **Antigen (cardiolipin)** it is alcoholic extract of beef heart muscle + cholesterol + lecithin

3- **Complement:** from guinea pig serum

# Complement Fixation Test



**Test system**

**Indicator system**

No hemolysis = Positive

Hemolysis = Negative



## Interpretation of Wassermann test:

### Controls:

**Controls (Ag, Ab) → Hemolysis → Read the test-**

-

**Any control with No Hemolysis →-**

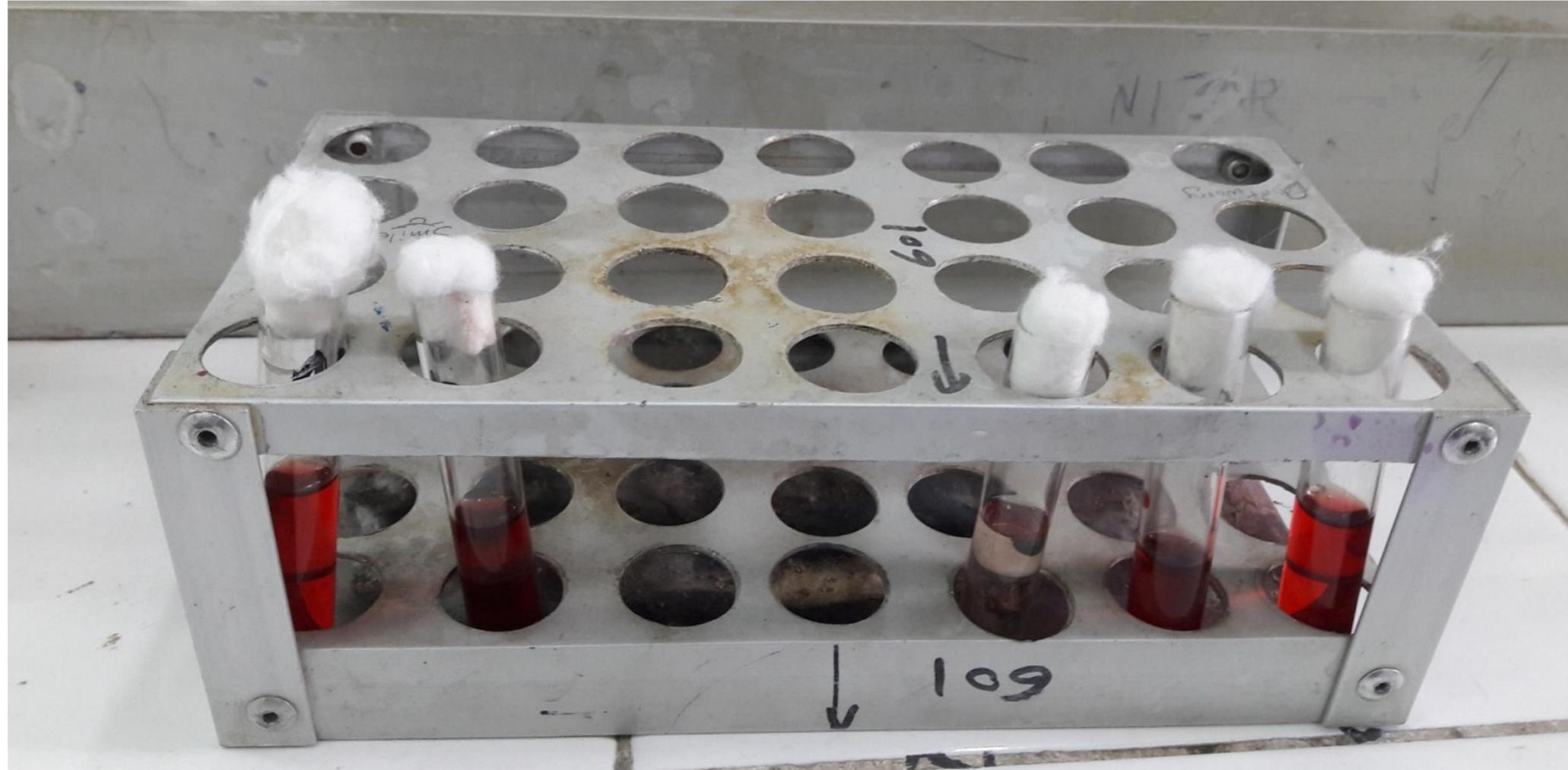
**\*Antigen Anti-complementary**

**\*Antibody Anti-complementary**

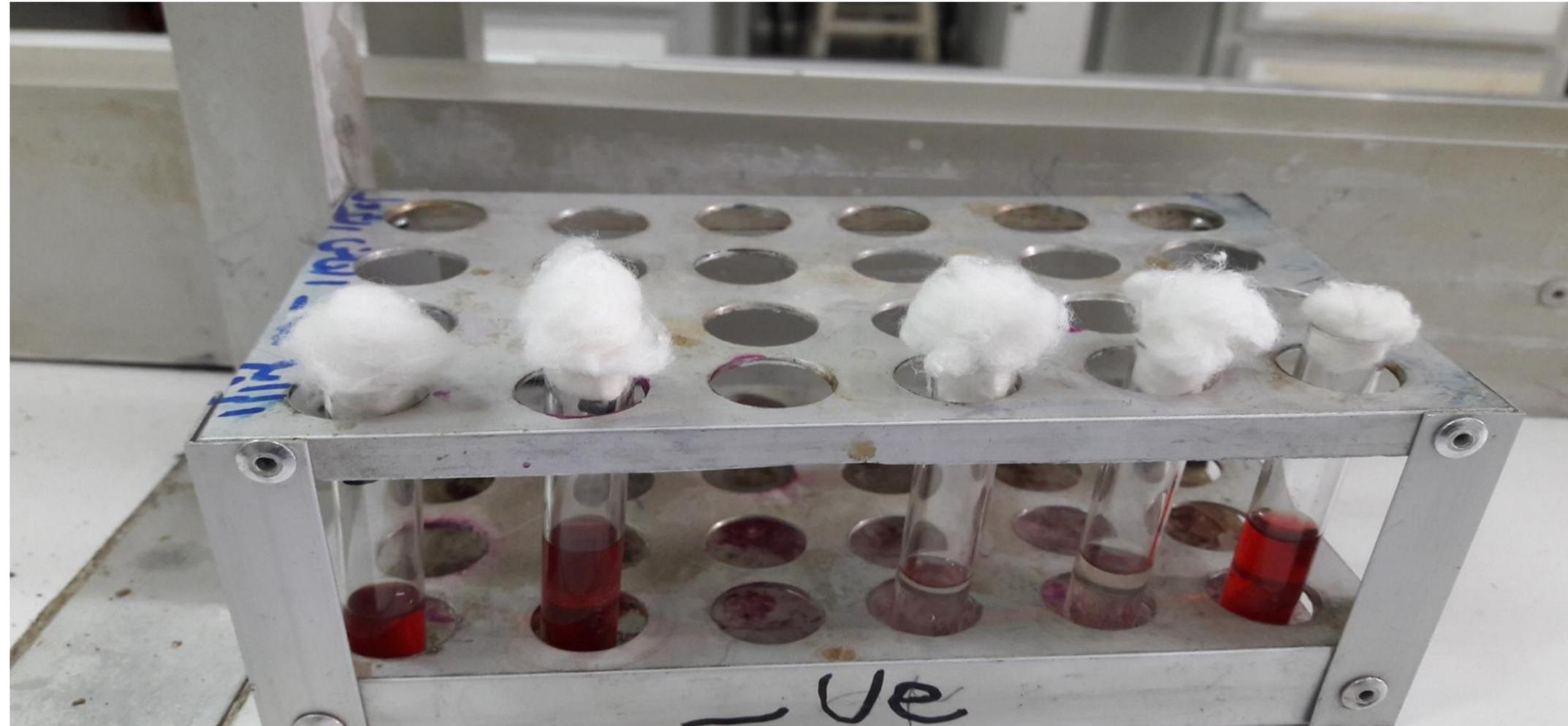
### Test:

**- No hemolysis → Positive**

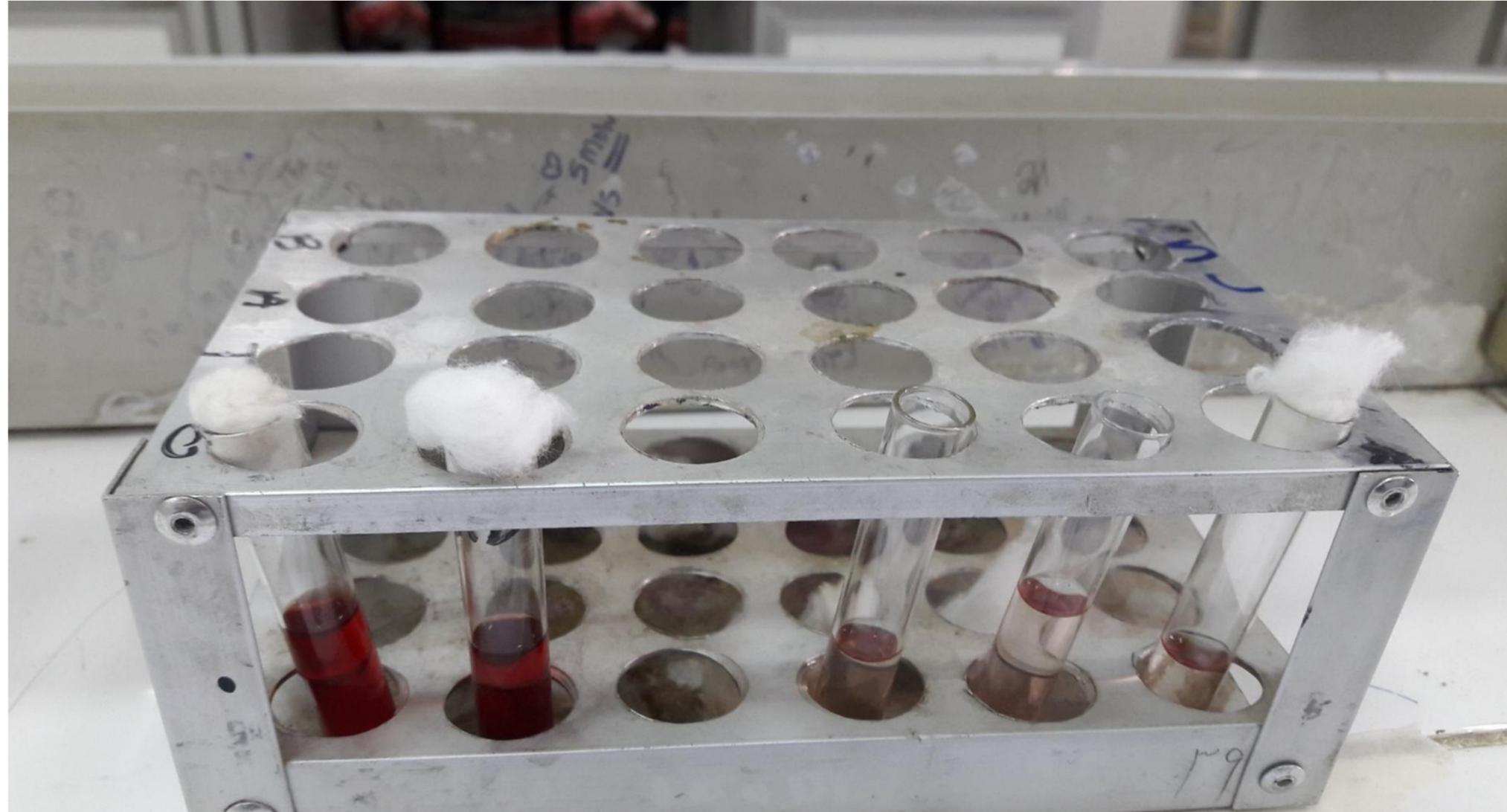
**- Hemolysis → Negative**



**Wassermann test (Mild positive)**



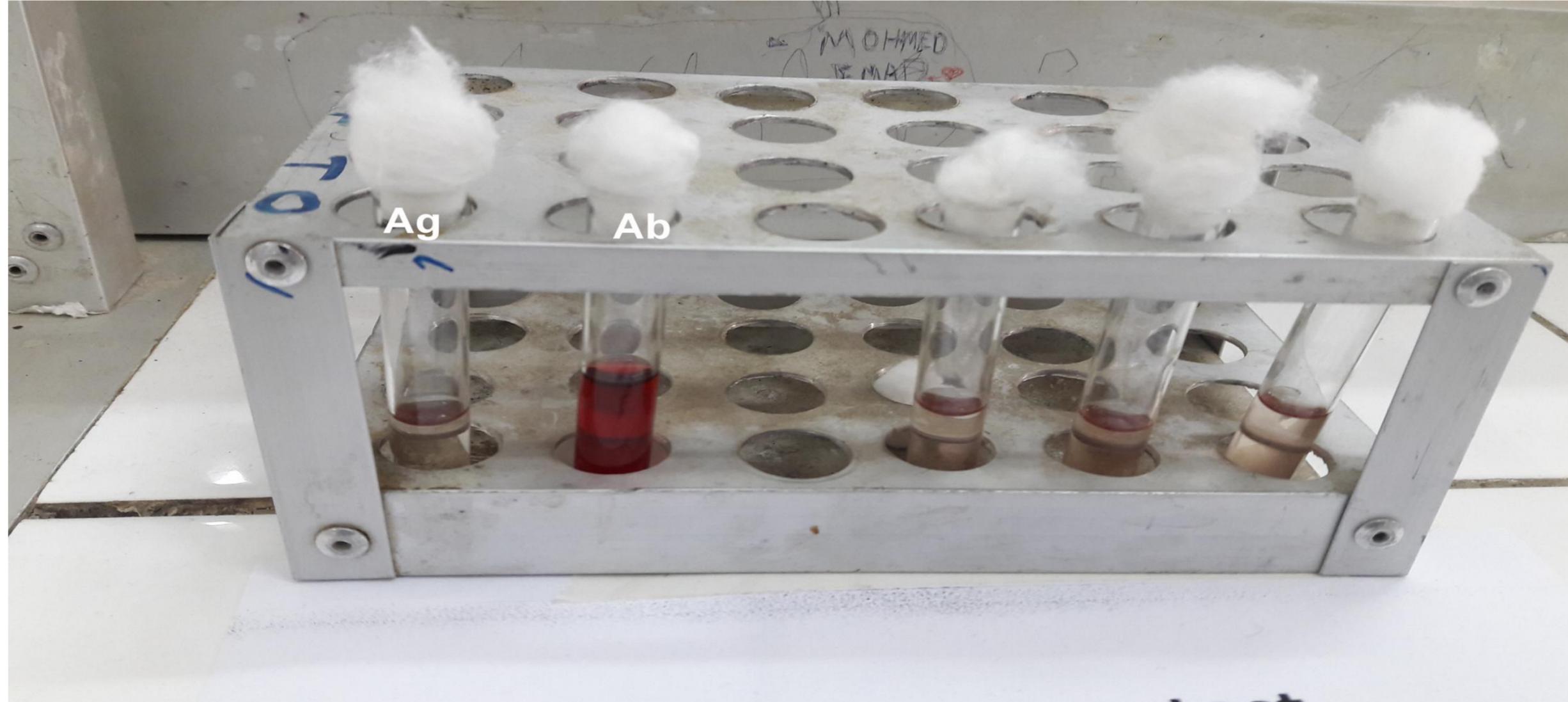
**Wassermann test (Moderate positive)**



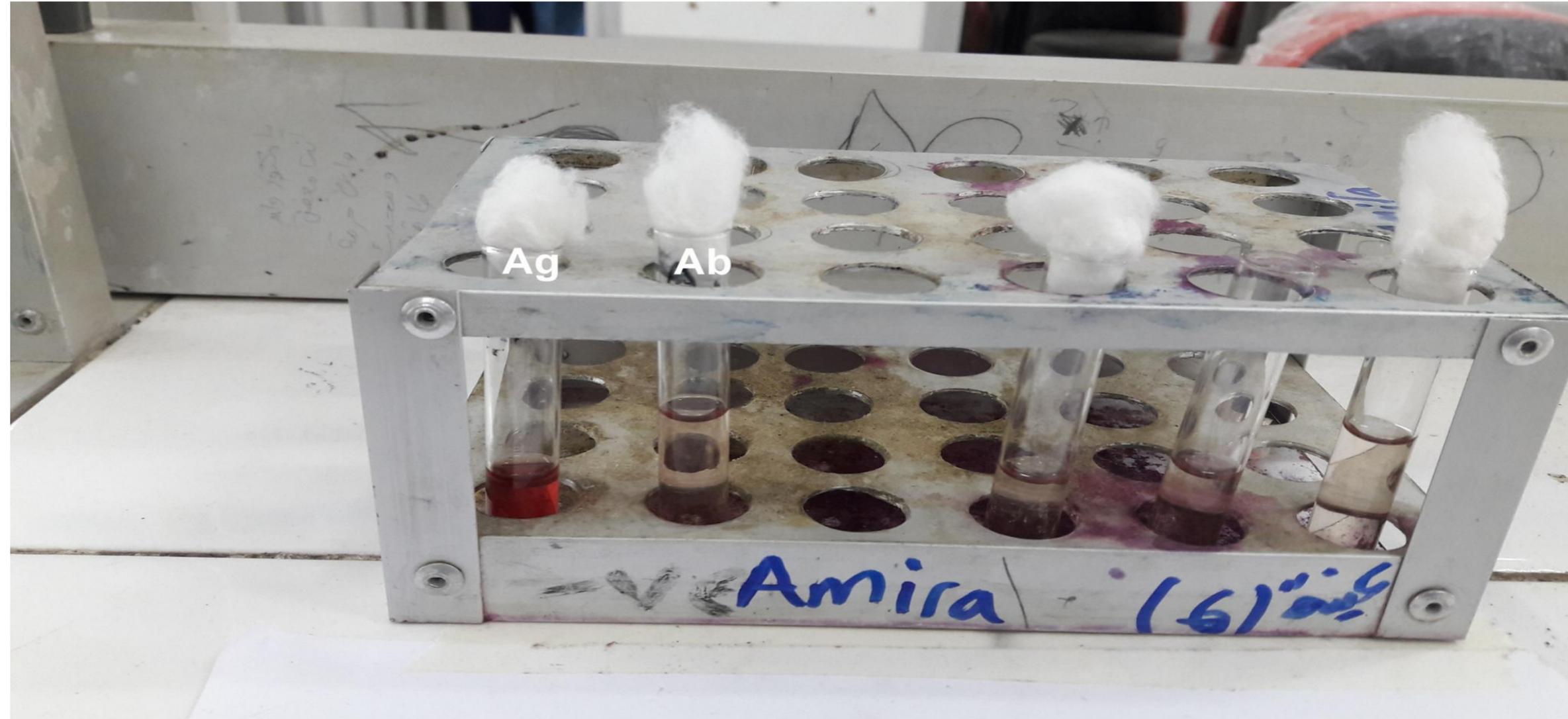
**Wassermann test (Strong positive)**



**Wassermann test (Negative)**



**Wassermann test**  
**(Ag anticomplementary)**



**Wassermann test**  
**(Ab anticomplementary)**



## Family *Chlamydiaceae*

One genus *Chlamydia*

- ✓ *C. trachomatis*,
- ✓ *C. pneumoniae* (TWAR)
- ✓ *C. psittaci*



## Family *Chlamydiaceae* - *C. trachomatis*

- ✓ 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.
- ✓ It is caused by *Chlamydia trachomatis*



## Family *Chlamydiaceae* laboratory diagnosis:

### 1. Specimens:

- ✓ Scrapping from the urogenital tract.
- ✓ Urethral or Cervical exudates.

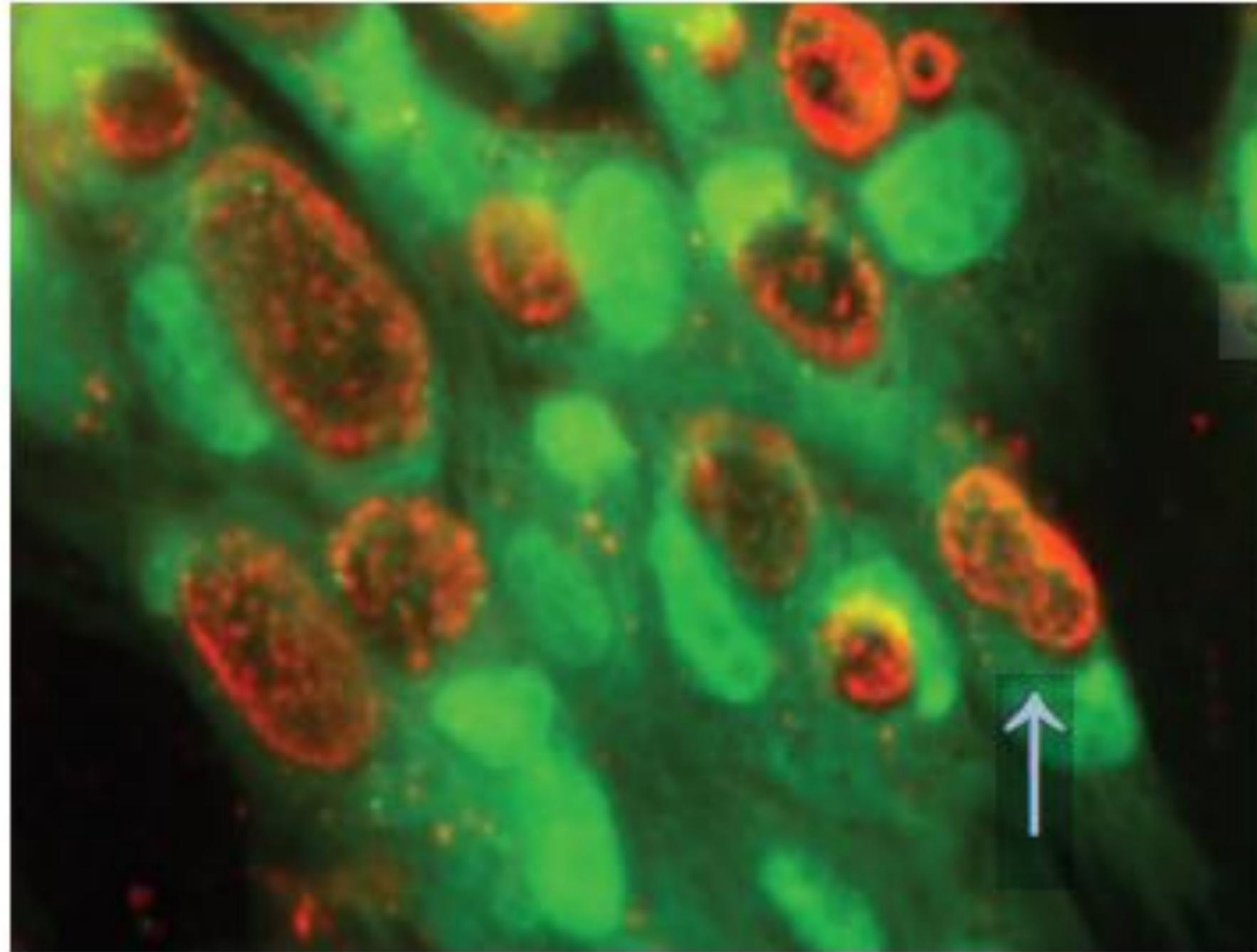
### 2. Microscopic examination:

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



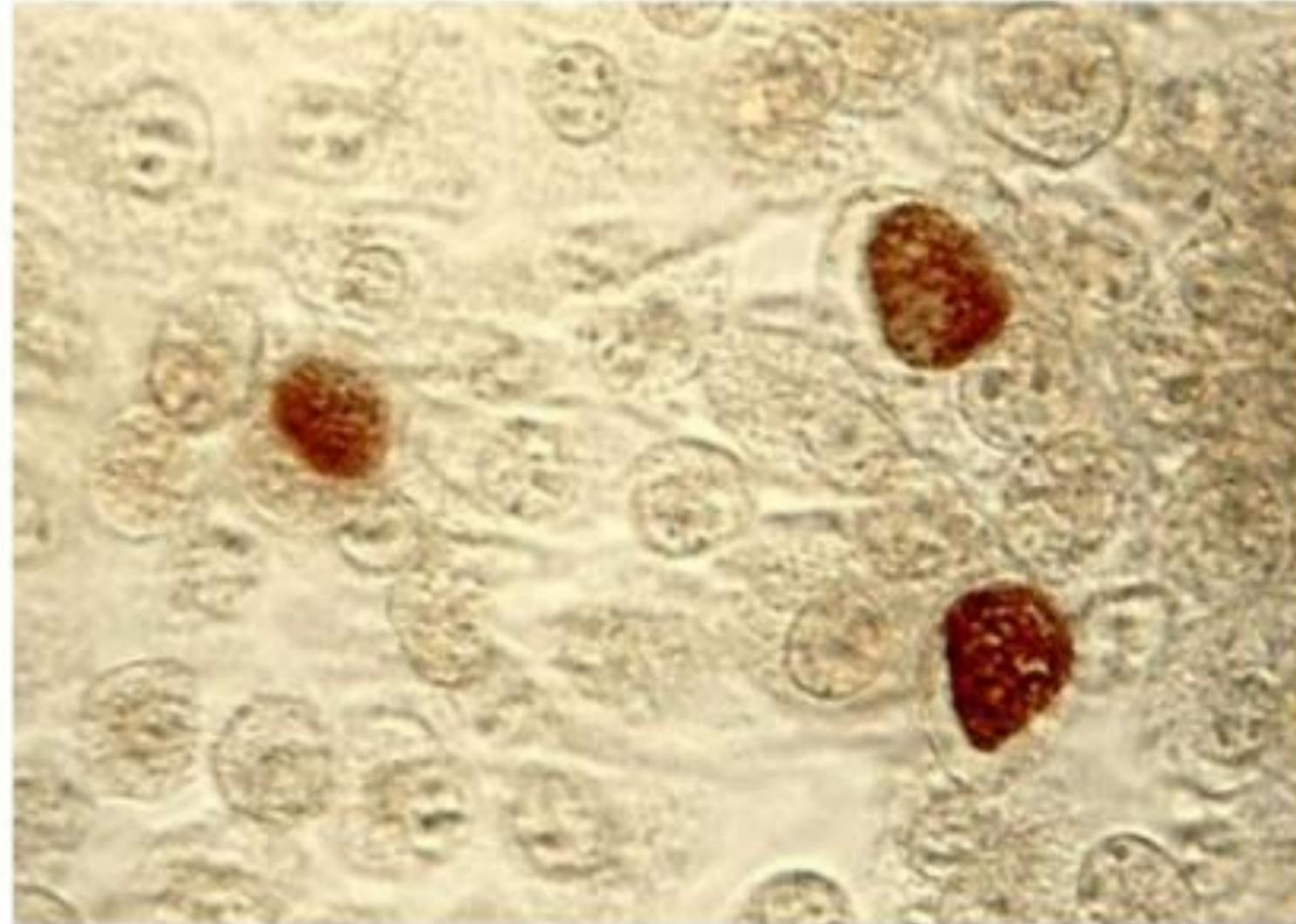
### 3.Culture:

- 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 ( $\times 1000$ ). 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.

c) Frei test:

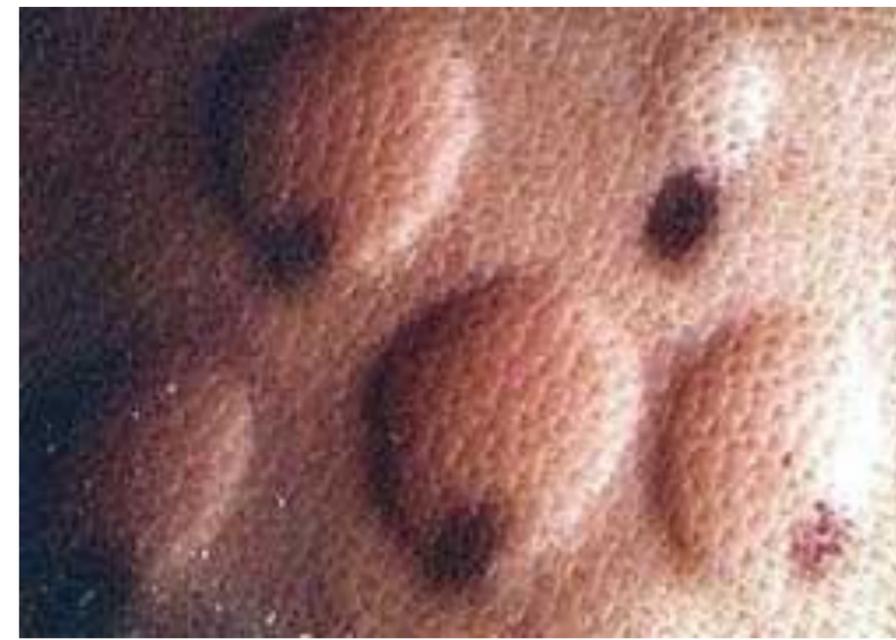
- ✓ ID, killed *chlamydia* from genitourinary infections, detects present or past infection of LGV, ID injection of killed organism
- ✓ non sensitive, non specific
- ✓ Delayed cell mediated reaction



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# Frei test





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- 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
- Practical Microbiology and Immunology 2018-2019: Textbook by staff members of medical Microbiology and Immunology Department; volume II and volume III; Neisseria, Treponema, Chlamydia



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Thank you  
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