UGT Module Lab 2
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Chlamydia

* Gram negative cocci
* It lacks peptidoglycane layer
* 2 Developmental forms :
  1. the elementary body → the infectious form
     ← Incapable of multiplication or reproduction
  2. the reticulate body → the replicated form inside the host E.cells
     ↳ under the expense of ATp of the host cell undergo division and multiplication

Diagnosis

1. Staining
2. Culture → It doesn't grow in routine culture media, it needs cell line culture.
3. Non-culture tests
   - Nucleic Acid Amplification Tests (NAATs)
   - Non-Nucleic Acid Amplification Tests (Non-NAATs)
     - (which include serological method.)

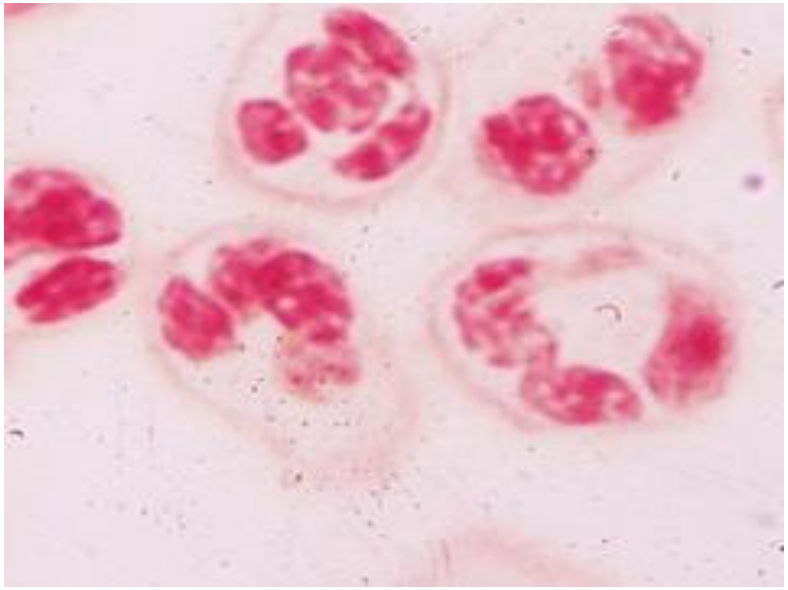
Diagnosis

1- Staining

- Interpretation of results

-Positive leukocyte estrase indicative of urethritis

-Four or more PMNs per 1000X field with no gram negative diplococci indicates NGU



Non-gonococcal urethritis

*Most of it, is caused by chlamydia trachomatis.

*It has PMNs without Neisseria gonorrhea.

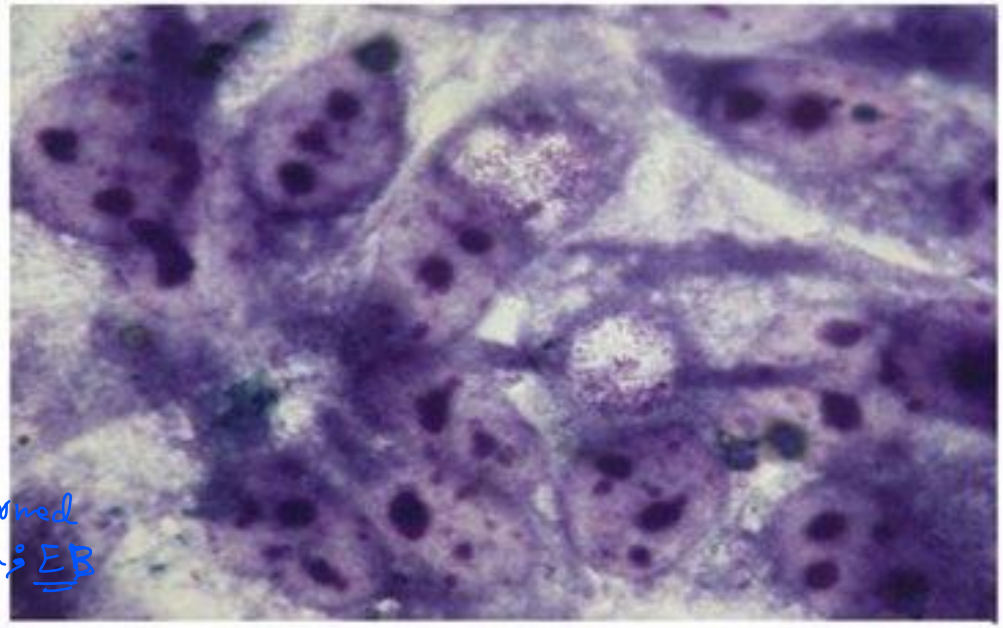
Staining methods

- Gram-negative but Gram stain is not used for identification.
  - ↳ Instead :
- Giemsa stain is often used. EB is purple while RB is blue.

*The dark dots: elementary body inside inclusion body.

↳ Is the place where Chlamydia trachomatis reticulate body undergo division.

↳ After division they transformed into the infectious form: EB



To be seen each cell are two inclusions with elementary bodies. (Giemsa stain)

Staining

:**Giemsa stain:**
Chlamydia inclusion bodies appear as blue-mauve stained mass

*The nucleus is around the rim of the inclusion body.

Diagnosis

2- Culture

- Variable sensitivity (50%-80%) & High specificity
- Not suitable for widespread screening
- The McCoy cell line originally derived from human synovial fluid in 1955, has been later found useful for cultivation of Chlamydia trachomatis.

*The black arrow indicates inclusion body of Chlamydia trachomatis inside Mccoy cell line.

*Inside inclusion body → reticulate body undergoing divisions

*This is by iodine stain

New Tests:
Nucleic Acid Amplification Tests (NAATs)

- Most sensitive chlamydia tests: amplify nucleic acid sequences specific to C. *trachomatis*
- Do not require viable organisms
- Either swab (vaginal, endocervical, urethral) or urine specimens are FDA-cleared for use
- Can detect GC (Neisseria gonorrhea) and CT (chlamydia T---) in single specimen
- Now widely available

→ This procedure depends on a technique called: Doublex PCR, which can detect gonococcal infection and chlamydia at same time.

Diagnosis

Non-NAATs

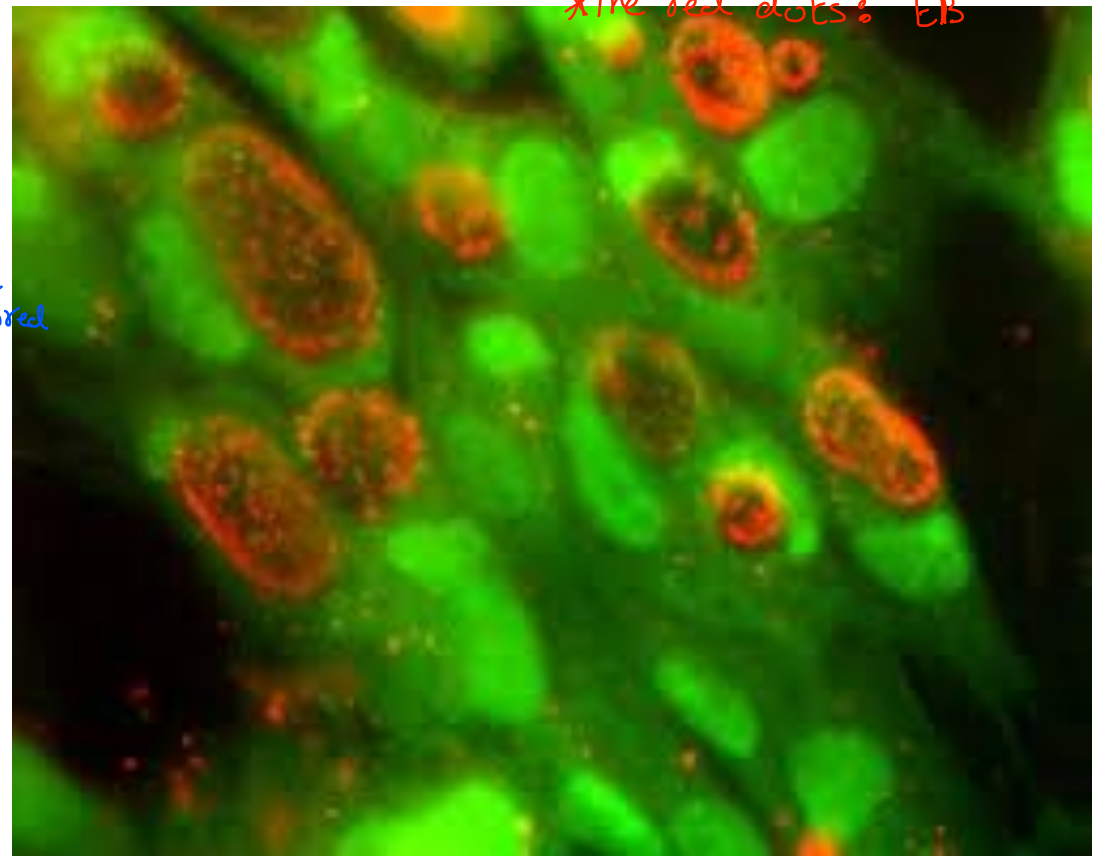
*Depend on serological test.

- Direct fluorescent antibody (DFA)

*This method depends on identification of the antigen specific for chlamydia trichomatis in a clinical sample.

*Ab is labeled with flurescent dye is inculated with samples and monitored under flurescent microscope.

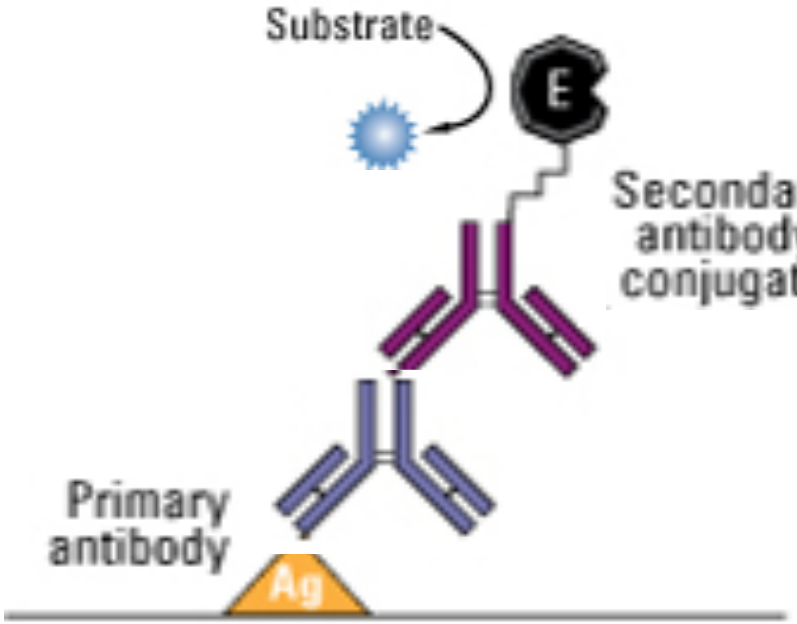
*This picture shows the inclusion body and EB inside.
*The red dots: EB



Diagnosis

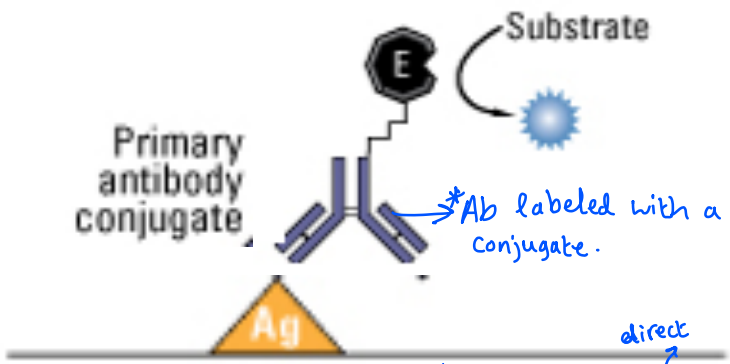
Non-NAATs

- Enzyme immunoassay (EIA)



Indirect Assay

*Depends on the detection of Ab against Chlamydia in a clinical sample.

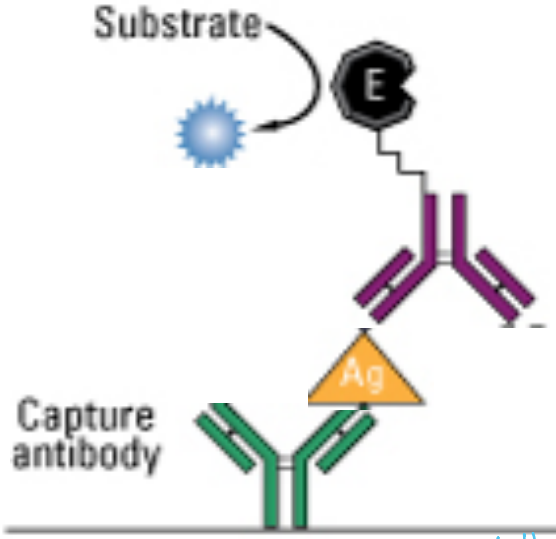


*Ab labeled with a conjugate.

Direct Assay

*It depends on the direct detection of the antigen in a clinical sample

*It gives a colour reaction can be detected.



Capture Assay "Sandwich"

*It depends on detection of Ag first followed by a captured Ab, then by a conjucate Ab with a substrate inside them.

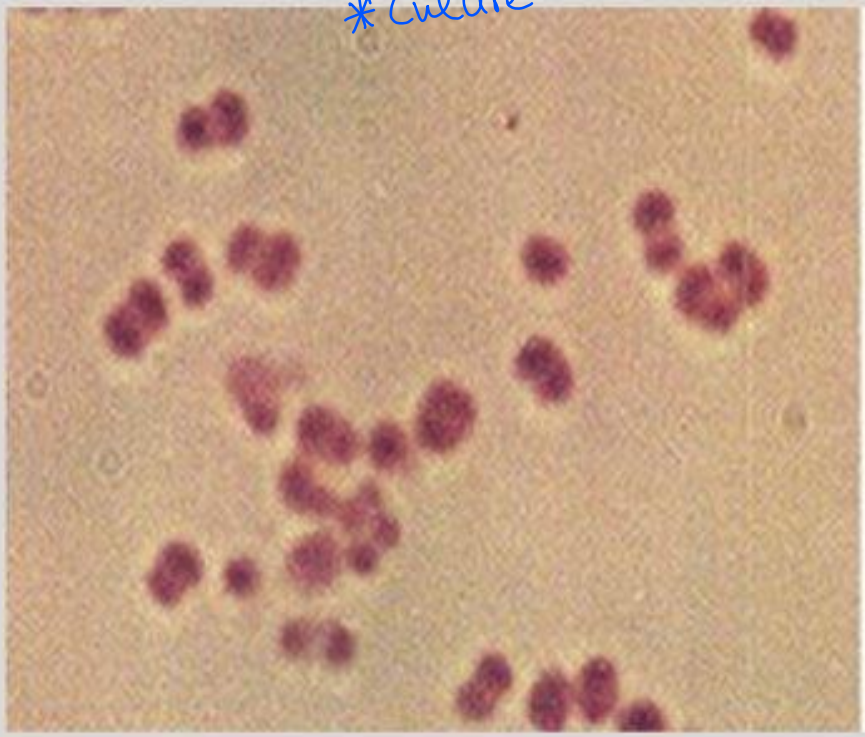
Gonorrhea

Microscopic features:

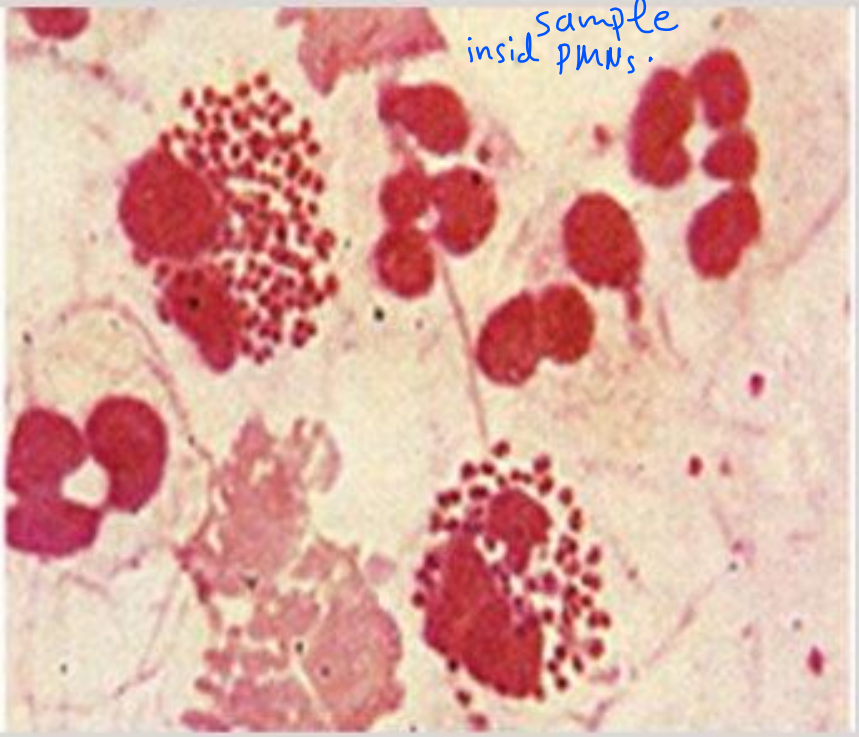
Neisseria gonorrhoeae is a Gram-negative cocci, 0.6 to 1.0 µm in diameter, usually seen in pairs with adjacent flattened sides.

The organism is frequently found as **intracellular** coffee bean-shaped **diplococci** in polymorphonuclear leukocytes of the gonorrhea pustular exudate.

*Culure



*Clinical sample insid PMNs.



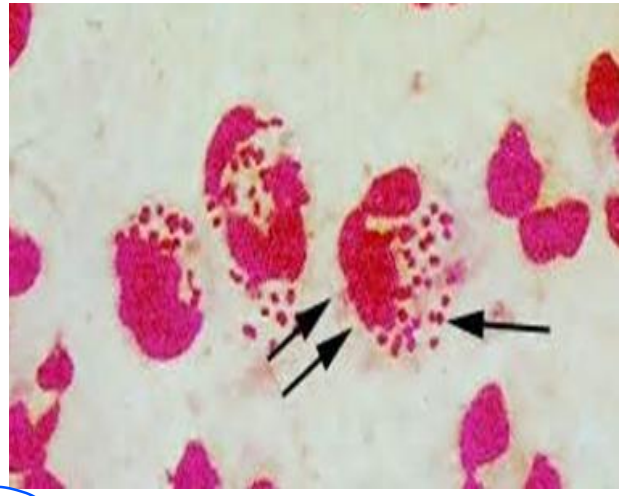
Diagnosis

1- Staining

- 40-96 % (Most of) of Nongonococcal Urethritis (NGU) are due to *C. trachomatis*
- Other 10-20% caused by *Uroplasma urealyticum* and *T. vaginalis*
- Interpretation of results

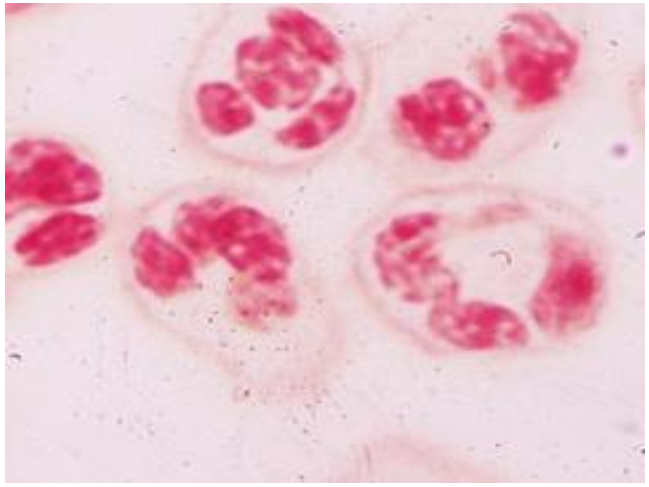
-Positive leukocyte estrase indicative of urethritis.

-PMNs per 1000X field with gram negative diplococci indicates gonococcal infection



It reveals gram -ve diplococci inside PMNs

Gonorrheal urethritis



non-gonococcal urethritis

Diagnosis

2. Culture

In men

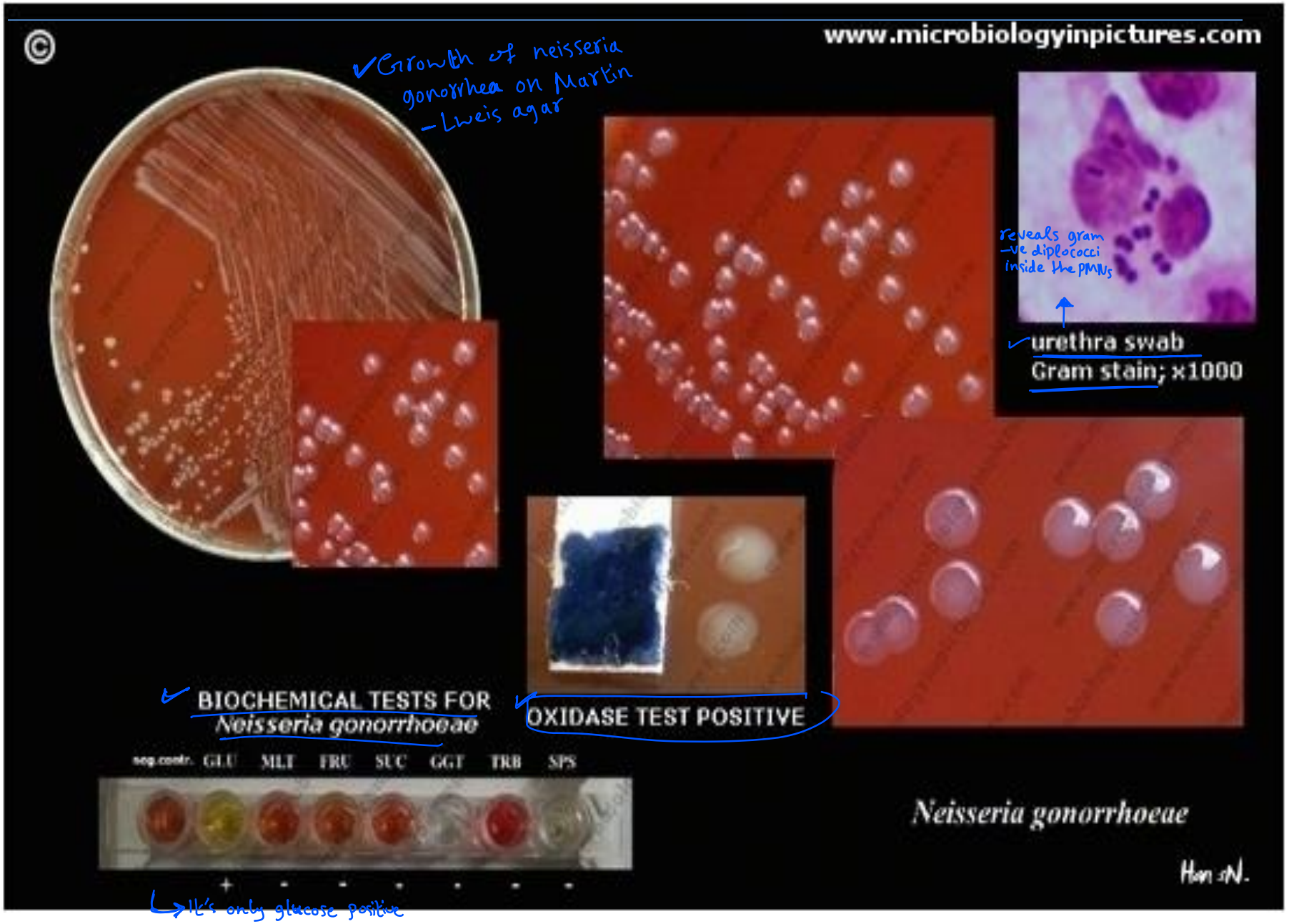
- the best specimen is urethral **exudates** or **urethral scrapings** (obtained with a loop or special swab).

In women

- **Cervical, urethral, or vaginal swabs**
- Swabs may be streaked directly onto culture medium or transmitted to the laboratory in a suitable transport medium if the delay is not more than 4 hours.
- The most common medium is **Martin–Lewis agar**, an enriched selective chocolate agar.
  - ↳ supplemented with antibiotics that selectively inhance the growth of neisseria gonorrhea.

Oxidase test positive

Diagnosis


©
www.microbiologyinpictures.com
Growth of neisseria gonorrhea on Martin-Lweis agar
reveals gram -ve diplococci inside the PMNs
urethra swab
Gram stain; ×1000
BIOCHEMICAL TESTS FOR
Neisseria gonorrhoeae
OXIDASE TEST POSITIVE
neg.contr. GLU MLT FRU SUC GGT TRB SPS
+ - - - - - -
It's only glucose positive
Neisseria gonorrhoeae
Hans N.

Diagnosis

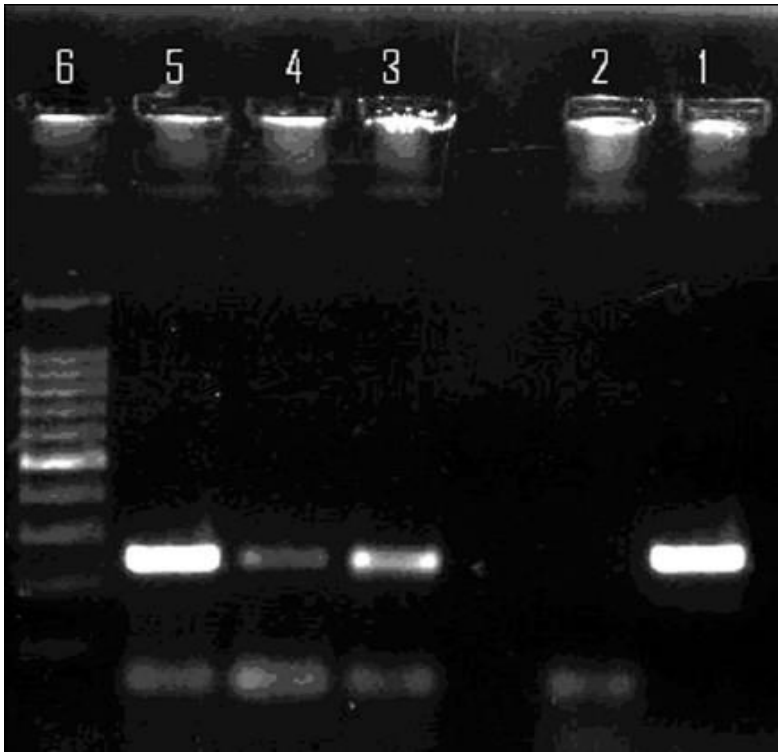
(non cultural methode)

3. Direct detection

DNA amplification methods that detecting gonococci in clinical specimens without culture

→ * specific sequence of nucleic acid

Patients



Syphilis

* Treponema pallidum

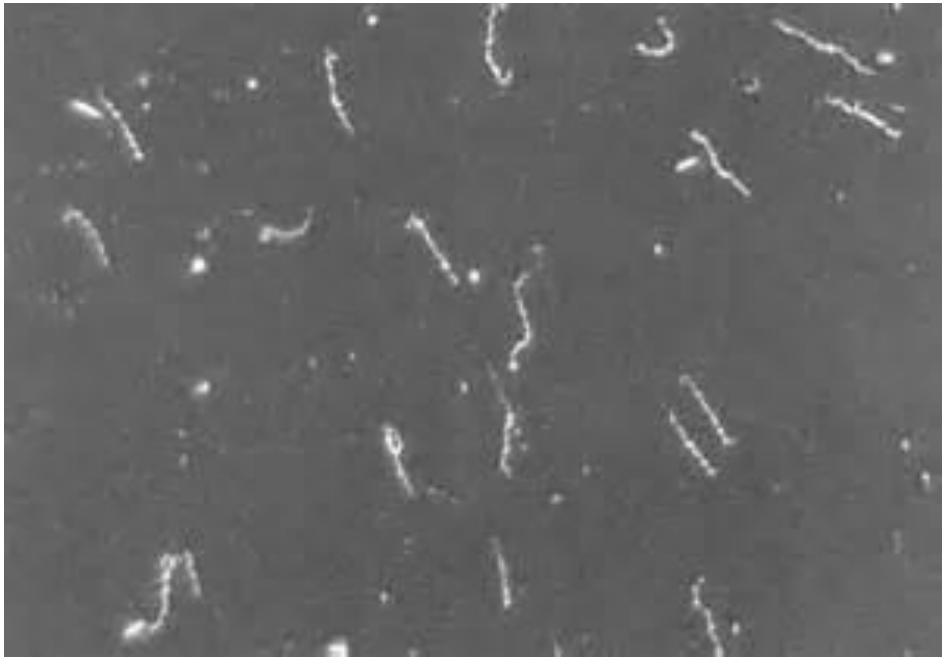
Syphilis

Methods of laboratory diagnosis of syphilis:

1. **Treponemal tests (Direct detection of spirochetes):**

- Darkfield microscopy - Specimen obtained from lesion is evaluated using darkfield microscopy for characteristic corkscrew morphology.

Treponema pallidum

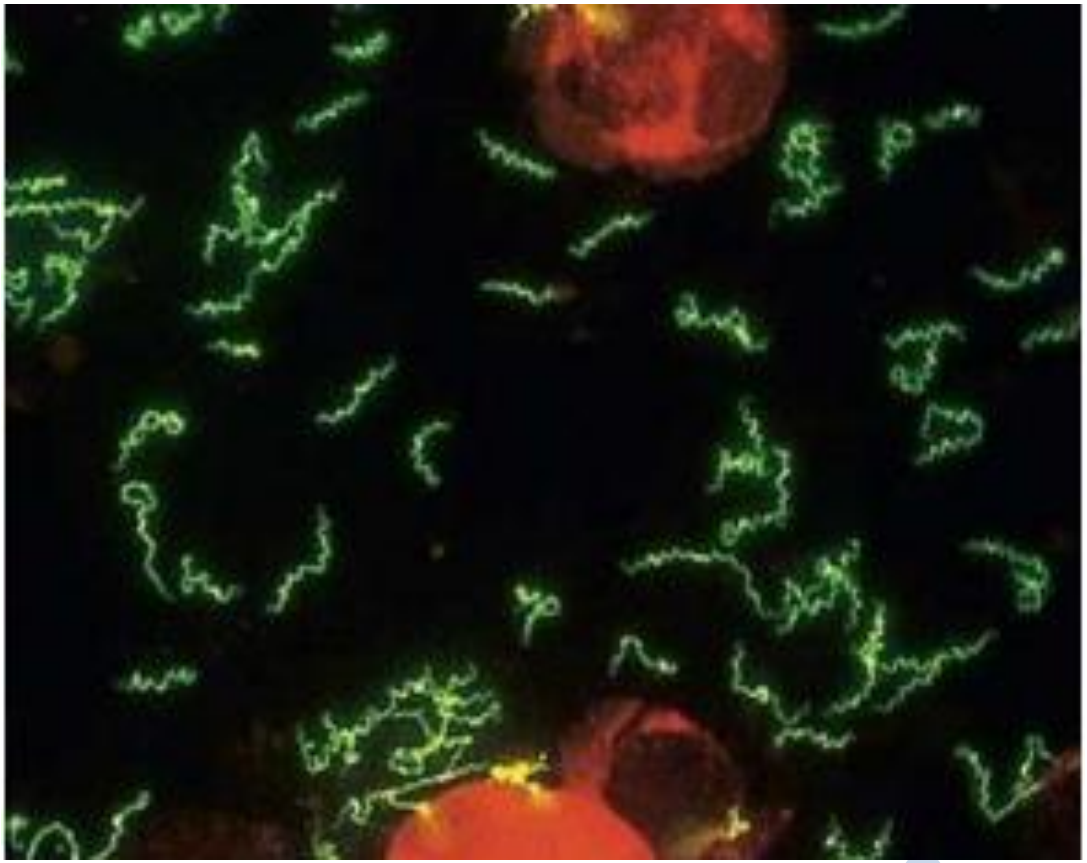


- Specific fluorescent Antibody Testing: direct or indirect methods

Results of direct fluorescence tests

*Depends on Ab labeled with a fluorescent dye that's directed against specific Ag in the treponema pallidum.

*Viewed under fluorescent microscope.



Syphilis

Methods of laboratory diagnosis of syphilis:

2. Nontreponemal tests Indirect detection of spirochetes: (Depends on)

A. Venereal Disease Research Laboratory (VDRL)

B. Rapid plasma reagin (RPR)

Principle

→ During:

Treponemal infection

↓

→ there will be:

Desrruction of cells

↓

→ which will:

Release of lipid materials from the damaged host cells *called lipoidal material cells as well as lipoprotein-like materials released from the treponemes*

↓

→ This material will produce immune response with a:

Production of antibody against this lipoidal materials

↓

VDRL and RPR

↓

This antibody called reagin Ab

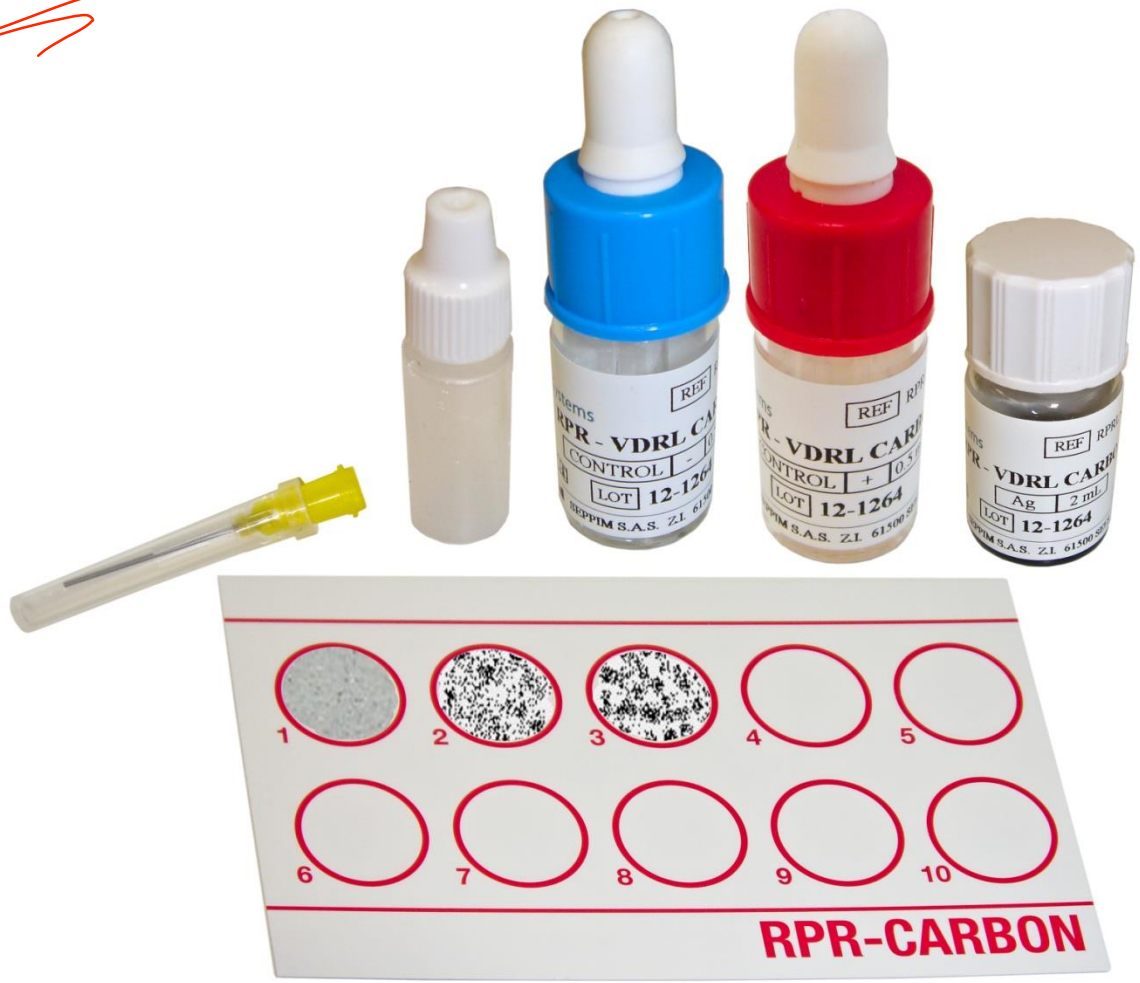
↓

In VDRL: The basis of the test is that the reagin antibody produced by a patient with syphilis reacts with a lipoid reagent extracted from the ox heart (cardiolipin antigen). The agglutination is seen under microscope.

↓

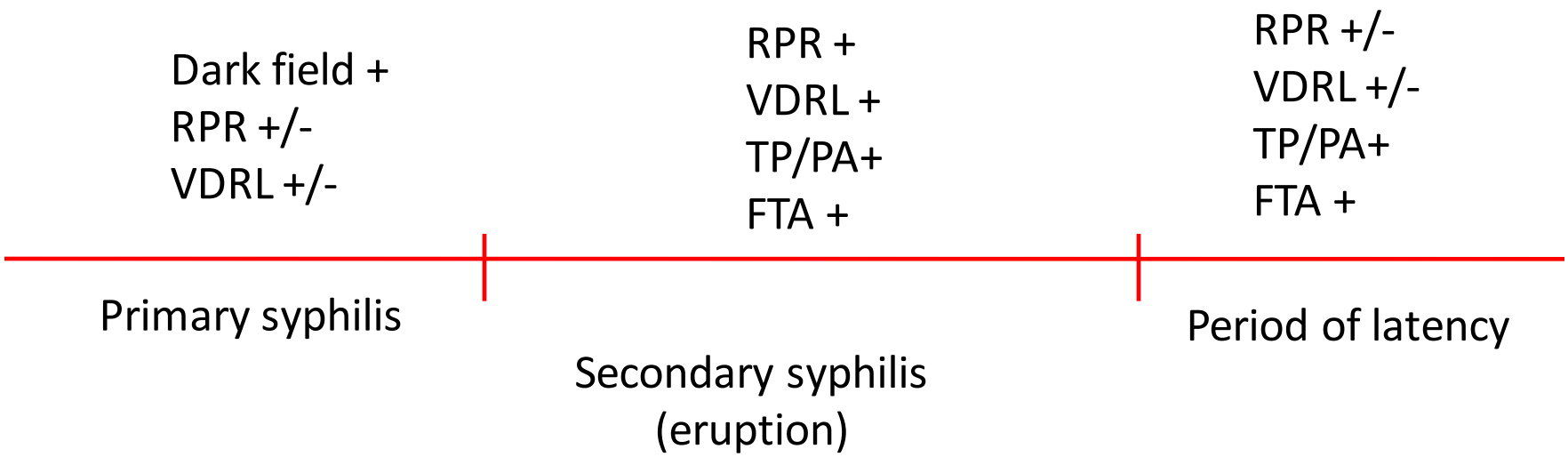
In the RPR test: the same as VDRL , but in that test, the antibody is bounded to several other molecules, including a carbon particle to allow visualization of the reaction without the need of a microscope.

RPR test



Syphilis stages and possible test results

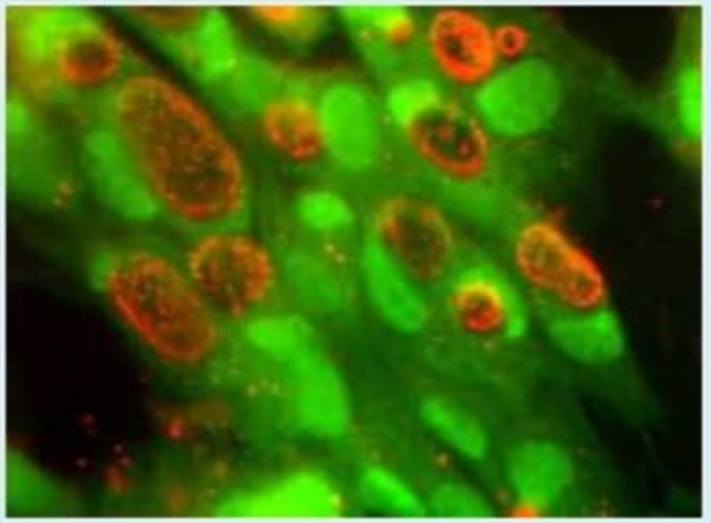
*The 2 approaches, ① Laboratory diagnosis depends mainly on non treponemal test → If it's +ve → it must be confirmed by the treponemal test by the dark microscope or by the fluorescent Ab.



fluorescent treponemal antibody-absorbed (FTA-ABS)

T pallidum particle agglutination (TP-PA)

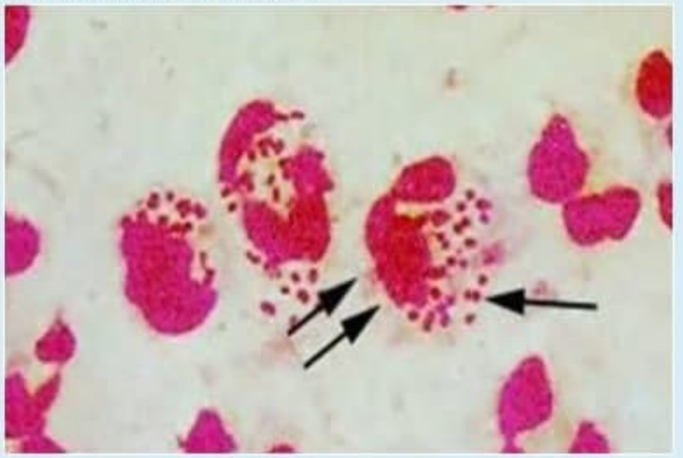
This image indicates?



Select one:

- a. Gonorrhea.
- b. Chlamydia infection.
- c. Syphilis.
- d. Non-gonococcal urethritis.
- e. Artifact.

This staining pattern indicates?



Select one:

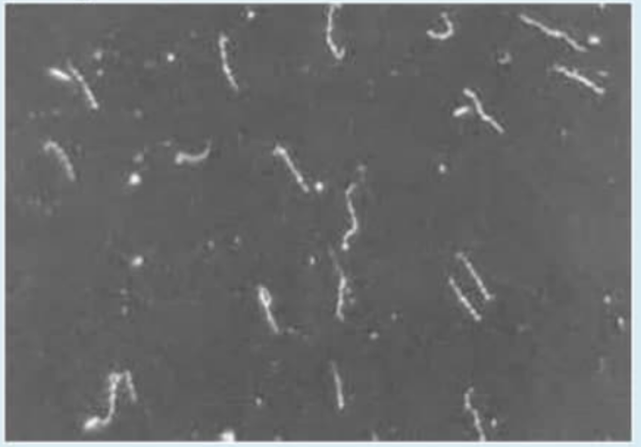
- a. Group A streptococci.
- b. N. gonorrhoeae.
- c. T. pallidum.
- d. Group B streptococci.
- e. Candida.

This test is used to diagnose?

Select one:

- a. Group A streptococci.
- b. N. gonorrhoeae.
- c. T.pallidum.
- d. Group B streptococci.
- e. Candida.

This image shows?



Select one:

- a. Group A streptococci.
- b. N. gonorrhoeae.
- c. T. pallidum.
- d. Group B streptococci.
- e. Candida.

Gonorrhea

Diagnosis:

1- Staining.

2- Culture.

3- PCR.