

UGT Module Lab 2

2023-2024

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Chlamydia

Diagnosis

1. Staining

2. Culture

3. Non-culture tests

➤ Nucleic Acid Amplification Tests (NAATs)

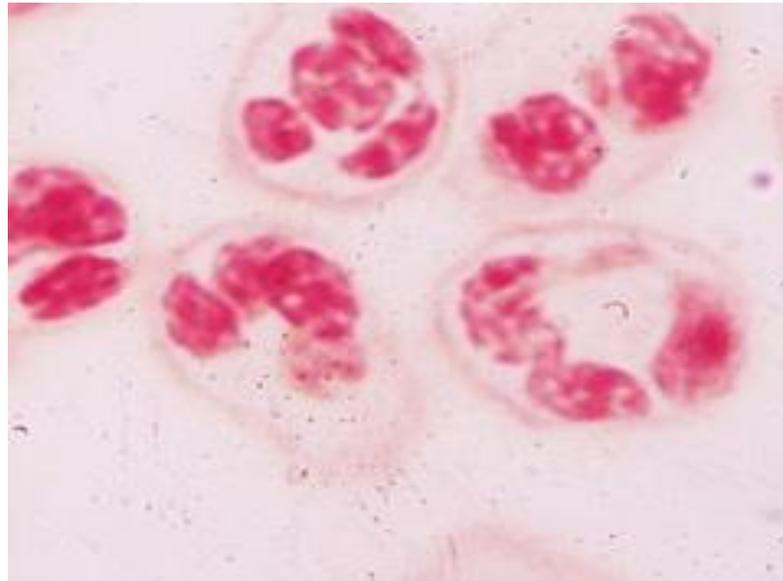
➤ Non-Nucleic Acid Amplification Tests (Non-NAATs)

Diagnosis

1- Staining

➤ Interpretation of results

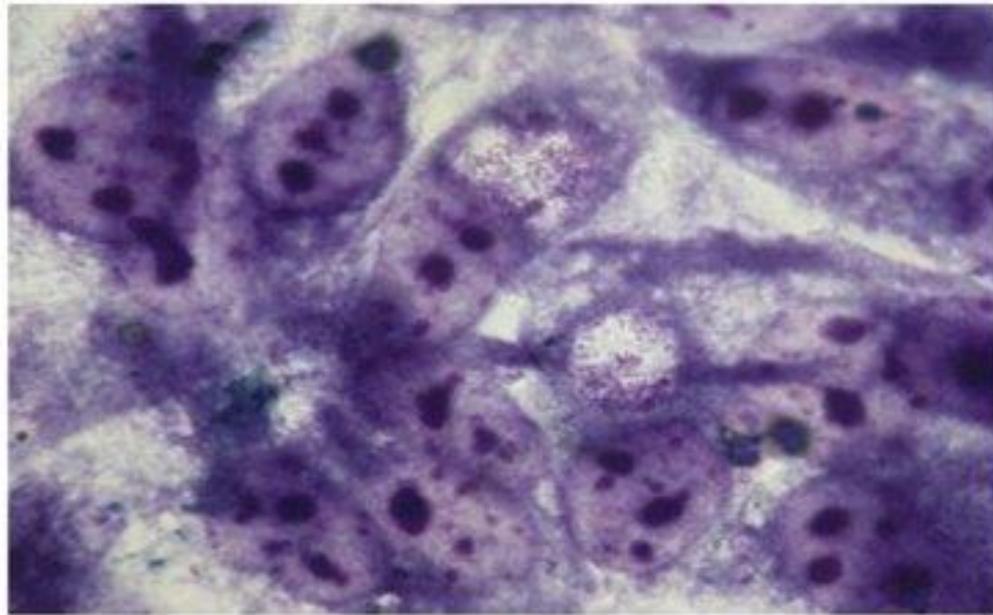
- Positive leukocyte esterase indicative of urethritis
- Four or more PMNs per 1000X field with no gram negative diplococci indicates NGU



Non-gonococcal urethritis

Staining methods

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



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

Staining

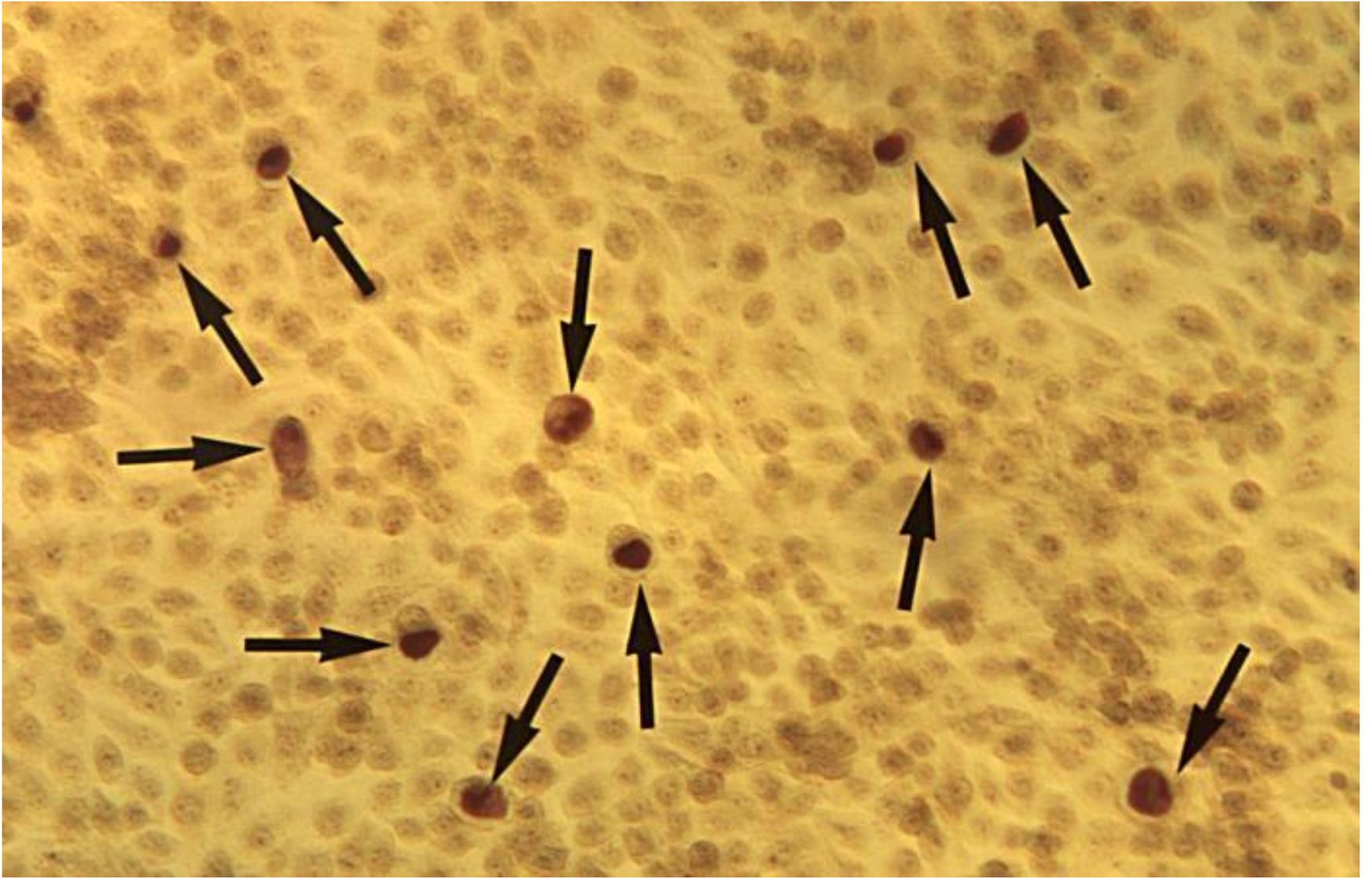
:Giemsa stain:
Chlamydia
inclusion bodies
appear as blue-
mauve stained
mass



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*.



New Tests:

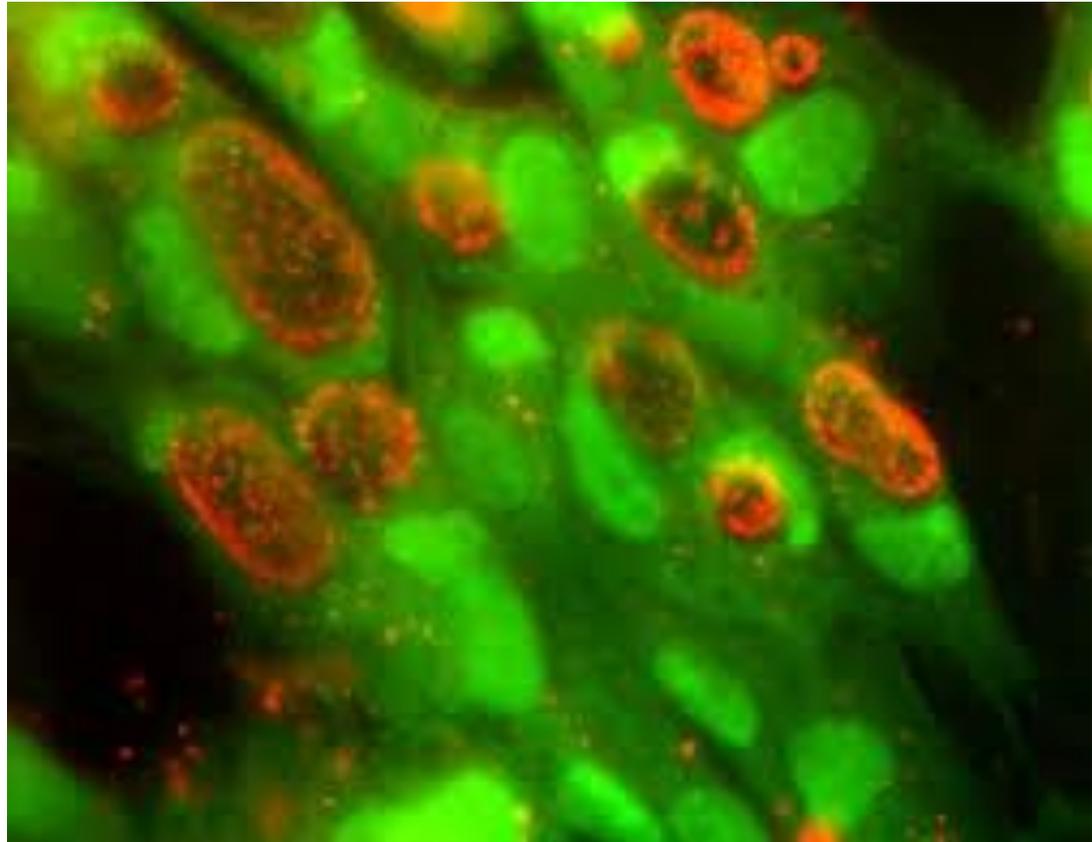
Nucleic Acid Amplification Tests (NAATs)

- 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 and CT in single specimen
- Now widely available

Diagnosis

Non-NAATs

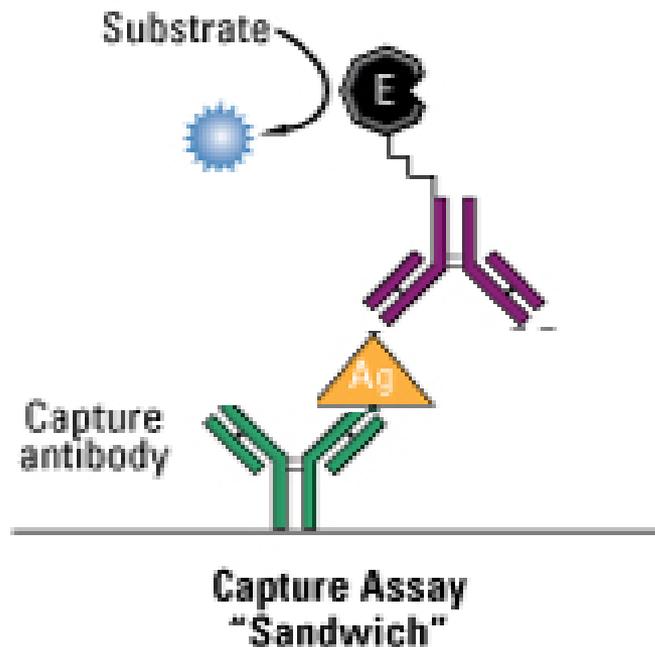
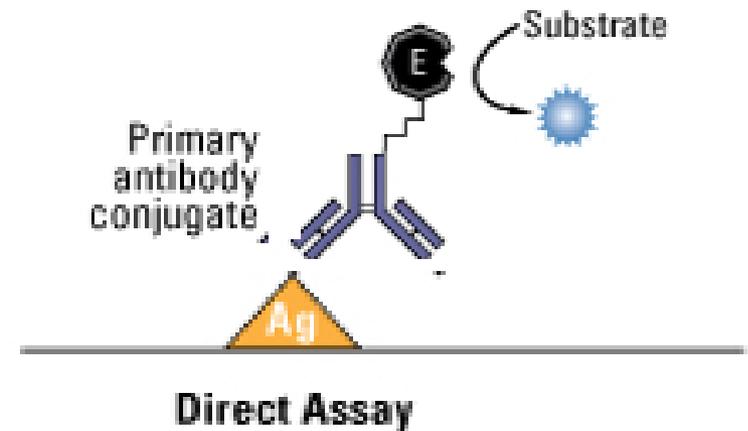
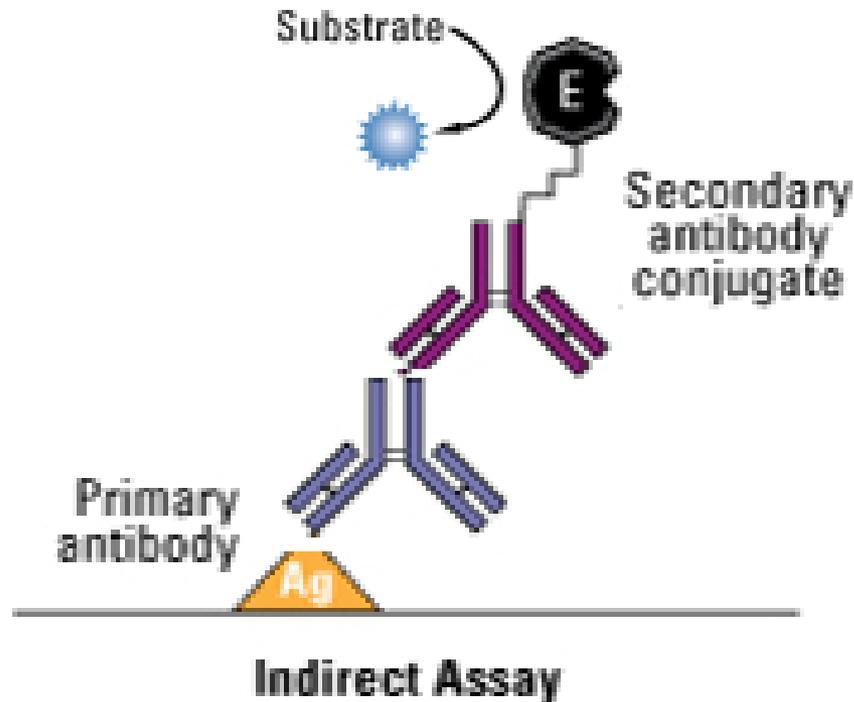
- Direct fluorescent antibody (DFA)



Diagnosis

Non-NAATs

- Enzyme immunoassay (EIA)

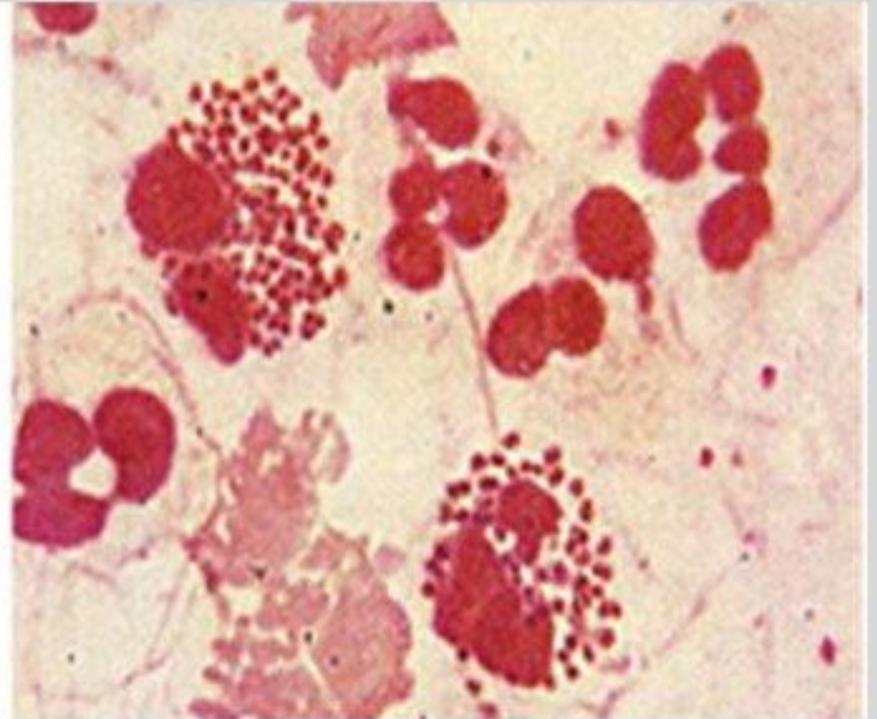
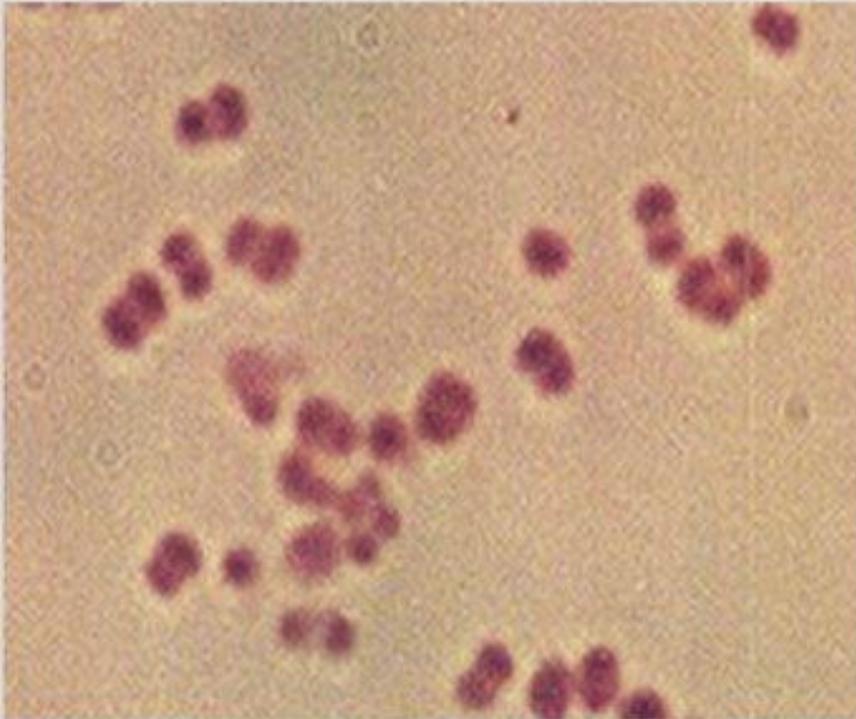


Gonorrhoea

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.

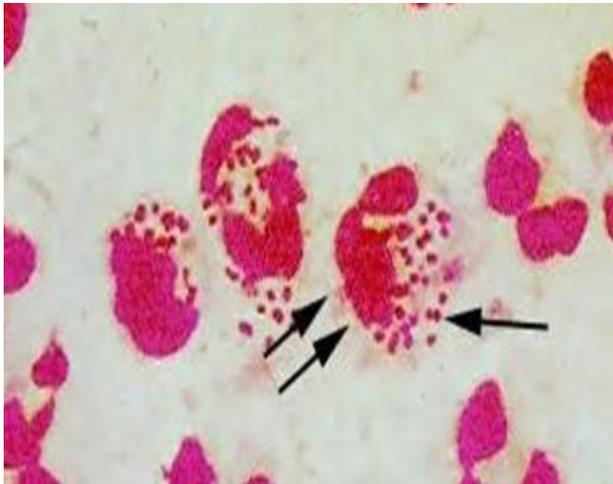
The organism is frequently found as **intracellular** coffee bean-shaped **diplococci** in **polymorphonuclear leukocytes** of the gonorrhea pustular exudate.



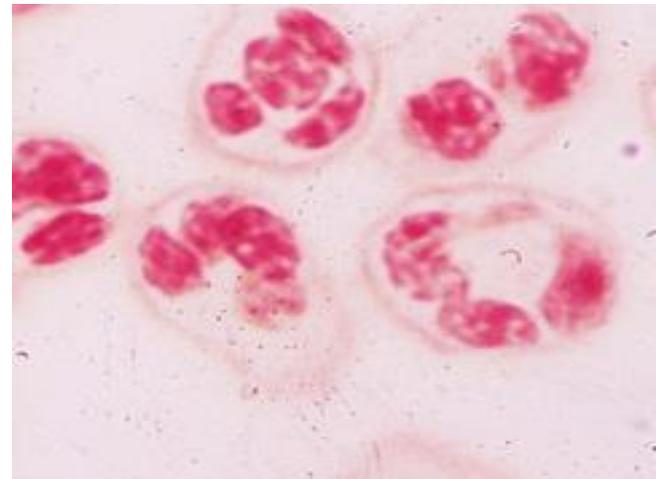
Diagnosis

1- Staining

- 40-96 % of Nongonococcal Urethritis (NGU) are due to *C. trachomatis*
- Other 10-20% caused by *Ureaplasma urealyticum* and *T. vaginalis*
- Interpretation of results
 - Positive leukocyte esterase indicative of urethritis.
 - PMNs per 1000X field with gram negative diplococci indicates gonococcal infection



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

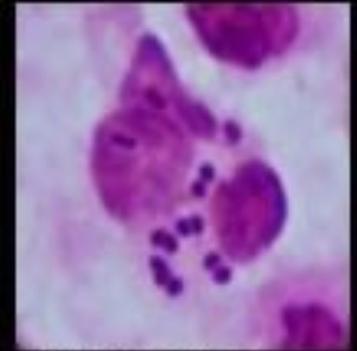
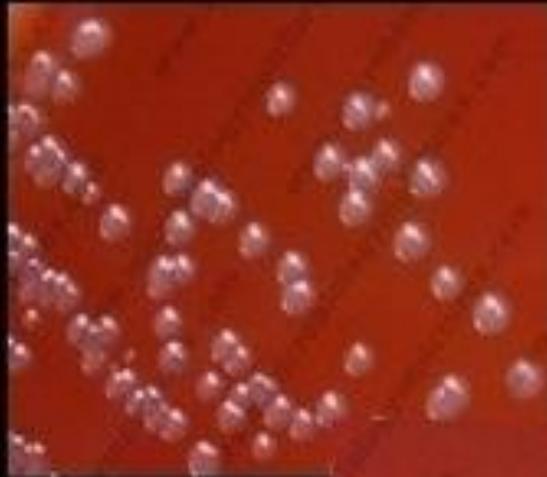
- **Cervical, urethral, or vaginal swabs**
- Swabs may be streaked directly onto culture medium or transmitted to the laboratory in a suitable transport medium if the delay is not more than 4 hours.
- The most common medium is **Martin–Lewis agar**, an enriched selective chocolate agar.

Oxidase test positive

Diagnosis

©

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urethra swab
Gram stain; x1000



OXIDASE TEST POSITIVE



BIOCHEMICAL TESTS FOR
Neisseria gonorrhoeae

neg. contr. GLU MLT FRU SUC GGT TRB SPS



+ - - - - - - -

Neisseria gonorrhoeae

Hem AN.

Diagnosis

3. Direct detection

DNA amplification methods that detecting gonococci in clinical specimens without culture

Patients



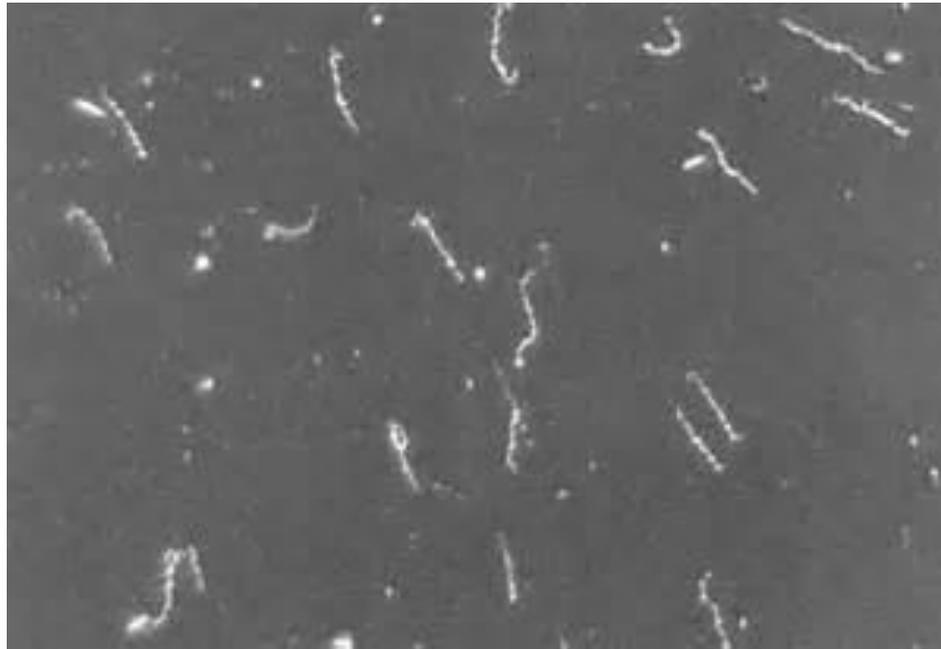
Syphilis

Syphilis

Methods of laboratory diagnosis of syphilis:

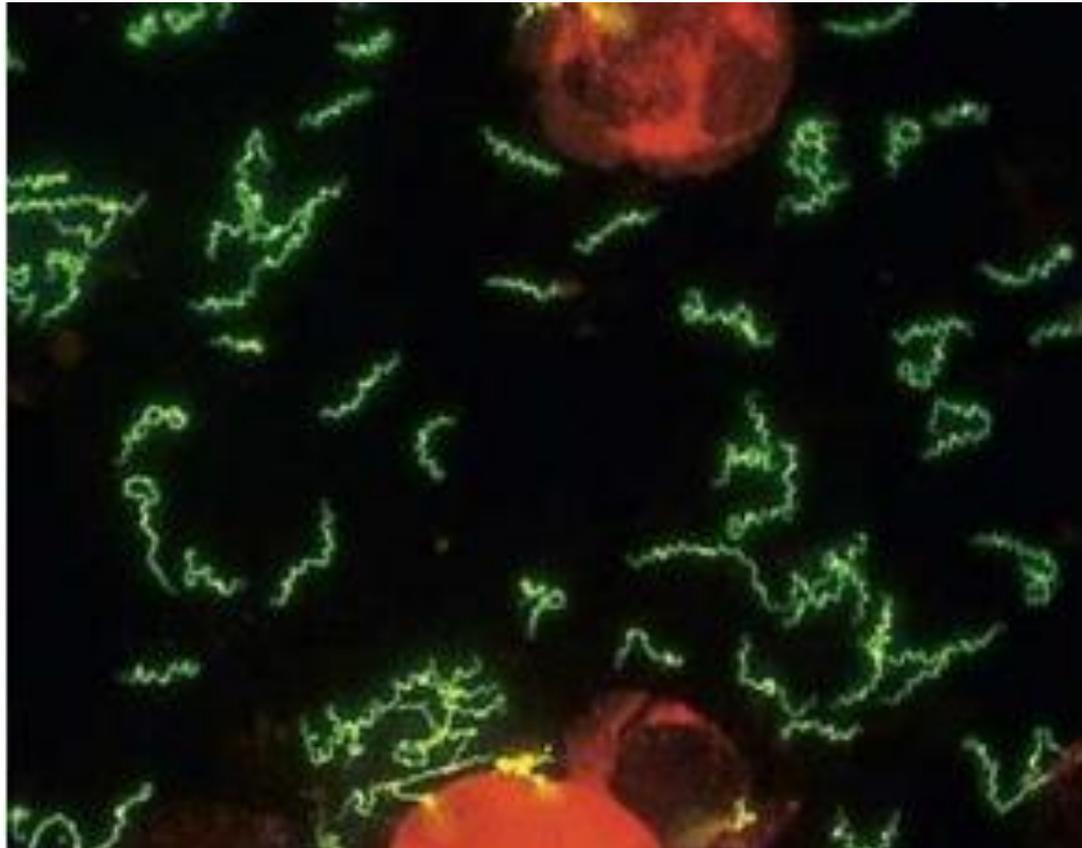
1. Treponemal tests (Direct detection of spirochetes):

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



- Specific fluorescent Antibody Testing: direct or indirect methods

Results of direct fluorescence tests



Syphilis

Methods of laboratory diagnosis of syphilis:

2. Nontreponemal tests Indirect detection of spirochetes:

- A. Venereal Disease Research Laboratory (VDRL)
- B. Rapid plasma reagin (RPR)

Principle

Treponemal infection



Desruction of cells



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



Production of antibody
against this lipoidal materials



VDRL and RPR



This antibody called reagin Ab

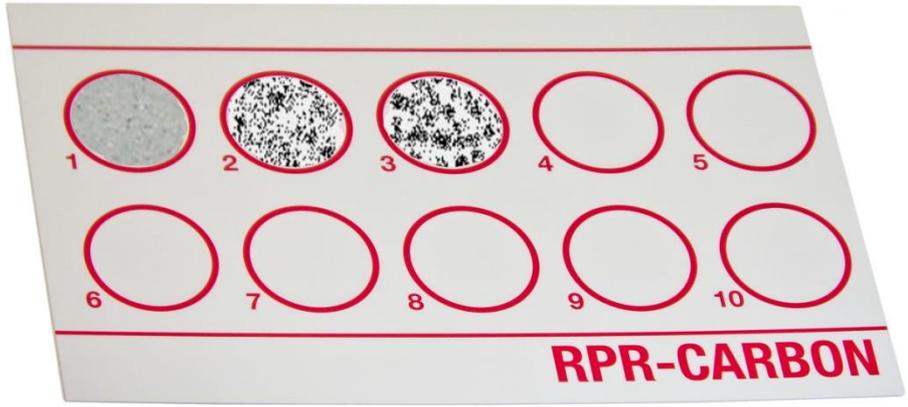


In VDRL: The basis of the test is that the reagin antibody produced by a patient with syphilis reacts with a lipoid reagent extracted from the ox heart (cardiolipin antigen).

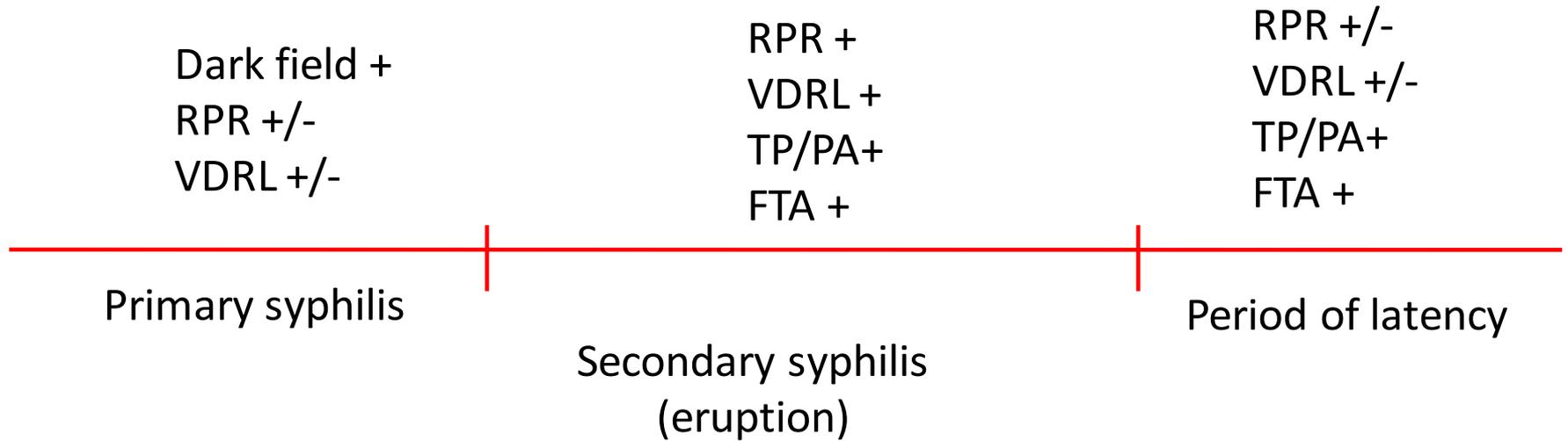
The agglutination is seen under microscope.



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.



Syphilis stages and possible test results



fluorescent treponemal antibody-absorbed (FTA-ABS)

T pallidum particle agglutination (TP-PA)