CLINICAL AND LABORATORY CHALLENGES IN DIAGNOSIS OF SEXUAL TRANSMITTED INFECTION BY CHLAMYDIA TRACHOMATIS, NEISSERIA GONORRHEA, TRICHOMONAS

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Mrs. A, a 20 year old Female secretary,

- vaginal discharge and irritation for three days.
- The discharge was slight, clear, watery, and non-offensive
- no abnormal vaginal bleeding.
- changed her sexual partner two months previously
- genital thrush, which responded to topical clotrimazole.
- uses a combined contraceptive pill and does not use condoms
- at vaginal examination was that Mrs. A's cervix bled easily when swabbed.
- A high vaginal swab was taken from the posterior fornix, and two swabs were taken from the endocervix and the urethra—a standard cotton swab and a plastic shafted chlamydia swab respectively.
- Mrs. A was prescribed doxycycline (200 mg for seven days) and metronidazole (400 mg three times daily for seven days).

DIFFERENTIAL DIAGNOSIS:

- Vulvovaginitis
- Bacterial vaginosis
- Gonorrhea
- Trichomonas vaginalis
- Mycoplasma infection
- Chlamydia …

Sexually transmitted infections (STIs) remain prevalent and a major burden of morbidity and mortality globally impacting on quality of life, reproductive and child health, and national and individual economies

STIs also facilitate the sexual transmission of human immunodeficiency virus (HIV)

To target the most relevant pathogens, various tools have been developed for the laboratory diagnosis of STIs throughout the years

The choice of the most appropriate diagnostic test :

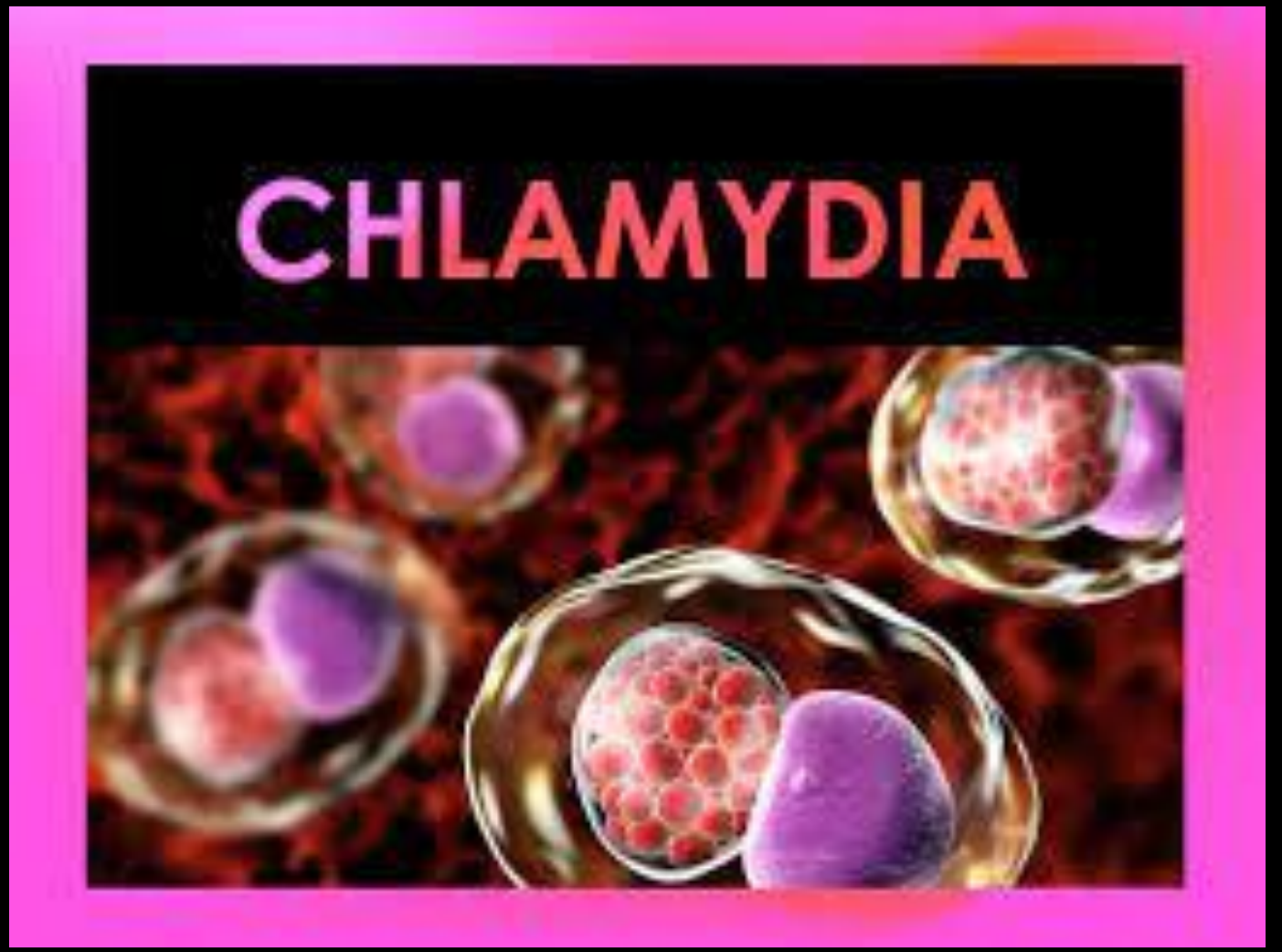
- the performance of the tool
- sensitivity
- specificity
- predictive values
- logistics (technical requirements, cost, throughput)

OUR APPROACH IN INFECTIOUS OR GYNECOLOGY CLINIC

- History
- Physical exam
- Consultation
- Laboratory work up if possible
- Symptomatic treatment according to probable diagnosis

WHAT IS SUPPOSED TO BE DONE?

- History
- Physical exam
- Consultation
- Laboratory work up
- Treatment according to definite diagnosis


CHLAMYDIA

- obligate intracellular bacteria
- commonest treatable sexually transmitted disease
- most common in sexually active women aged under 20
- Serological studies suggest that chlamydial infection may account for a large proportion of cases of tubal infertility and ectopic pregnancy
- 60-80% of genital chlamydia infections in women may be asymptomatic
- In one randomised trial, screening high risk women and treating those found to be infected reduced the incidence of pelvic inflammatory disease by about half in 12 months

CHLAMYDIA SYMPTOMS

MEN

penile discharge

burning/painful urination

testicular swelling

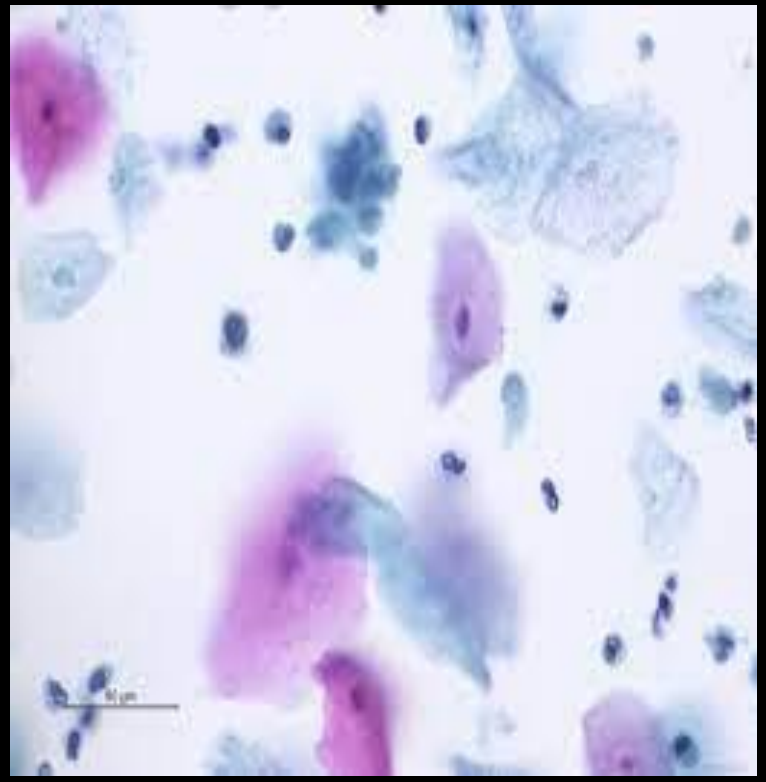
WOMEN

vaginal discharge

burning/painful urination

bleeding between periods

- Many women and most men with trichomoniasis have no symptoms, at least not at first.
- An often foul-smelling vaginal discharge — which might be white, gray, yellow or green
- Genital redness, burning and itching
- Pain with urination or sexual intercourse
- rarely causes symptoms in men
- Irritation inside the penis
- Burning with urination or after ejaculation
- Discharge from the penis



- Pelvic Inflammatory Disease (PID)
- Chronic Pelvic Pain
- Female Infertility
- Ectopic Pregnancy
- Male Infertility
- Pregnancy Complications
- Rectal Scarring and Fissures
- Cervical Cancer Risk
- Human Immunodeficiency Virus (HIV) Risk
- Lymphogranuloma Venereum
- Newborn Complications
- Eye infections
- Pneumonia

microorganisms

Testing for chlamydia is indicated in:

- patients with urogenital
- anorectal
- ocular symptoms
- patients with STI other than chlamydia
- sexual contacts of persons with STI
- persons destined for chlamydia screening

Indirect methods

- depend on detection of antibodies against *C. trachomatis*
- evaluation of chronic/invasive infection (PID, LGV) and post infectious complications, like sexually acquired reactive arthritis (SARA)
- pathogens have crossed the epithelial and may no longer be detectable in swabs.
- serology is inappropriate to diagnose acute infections of the lower genital and anal tract, as the antibody response becomes detectable only after weeks to months and is often less pronounced.

direct pathogen detection

localized infections were examined by assays for

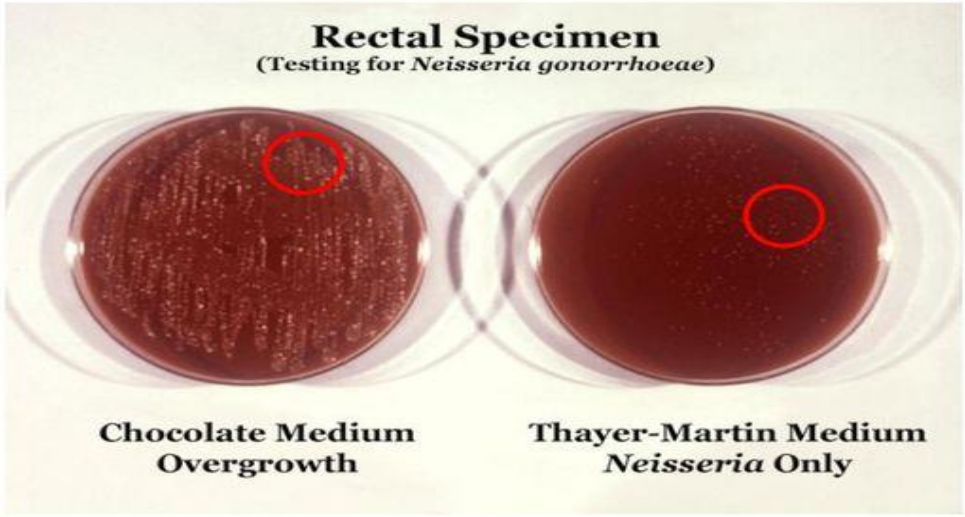
- culture
- antigen tests (EIA)
- direct fluorescent antibody (DFA)
- immune chromatographic RDTs)
- nucleic acid hybridization
- amplification tests

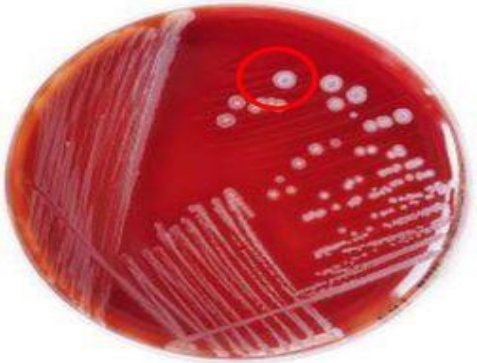
ISOLATION OF *C. TRACHOMATIS* IN CELL CULTURE

- Swabs from different anatomical sites (endocervix, urethra, anal canal, conjunctivae)
- must be collected using special devices and transport media
- centrifuged onto confluent cell monolayer and analyzed for the development of characteristic intra cytoplasmatic inclusions after 48–72 h by staining with Giemsa, iodine, or fluorescence labelled antibodies to chlamydial antigens (LPS or MOMP)

Expected Results

Examples of Positive Cultures for Gonorrhea and Chlamydia:





Chlamydia Trachomatis

- culture depends on vital organisms the detection rate is at best 60%–80%, even when performed in laboratories with experienced technicians

Sensitivity of culture may be impaired by:

- inadequate specimen collection
- storage
- transport
- toxic substances in clinical specimens
- overgrowth of cell cultures by commensal microbes
- Additional disadvantages are represented :
- the extended turn-around time
- labor intensity
- difficulties in standardization.

THUS:

cell culture is rarely used nowadays in diagnostic laboratories, but the methodology is still needed,:
at least in some reference laboratories

- to monitor antibiotic susceptibility
- changes of virulence
- when a test with the highest specificity is required as in case of suspected sexual assault.

NUCLEIC ACIDS AMPLIFICATION TESTS (NAATS)

- the most sensitive tests to detect chlamydia.

- high specificity comparable to culture, but in contrast to culture, do not depend on viable pathogens, facilitating specimen transport.
- the test of choice for chlamydia and have replaced culture as the diagnostic gold standard
- Antigen tests (EIA, DFA, RDTs) are no longer recommended for chlamydia testing due to insufficient diagnostic accuracy

- NAATs are based on polymerase chain reaction (PCR) and use fluorescence labelled probes to detect amplification products in real time, thereby significantly reducing the test duration
- Recombination primarily reflects adaptation to changing environmental conditions but may also result in development of new variants with increased virulence
- Recombination is of relevance for laboratory diagnostics, especially when using tests based on nucleic acid detection.

- In populations with low prevalence of chlamydia, the predictive value of positive results is low, even when using tests with high specificity

- NAATs : more sensitive than culture to detect CT infections in victims of sexual assault

- when used for evaluation of sexual abuse, positive results must be confirmed with another NAAT using another target region.

RAPID DIAGNOSTIC TESTS (RDTS)

- NAATs were performed in a central laboratory and require transportation of specimens and transmission of test results to the clinicians.
- NAAT-based diagnostics requires a second visit of patients, potentially leading to delayed treatment or no treatment at all if patients do not re-appear again, which may contribute to the high incidence of infection.
- they allow near-patient (point-of-care) testing and provide results in a few minutes, so that patients may receive antibiotic therapy immediately when they test positive

Compared to culture and PCR these antigen-based RDTs are significantly less sensitive and less specific.

The low sensitivity of RDTs may relate to low bacterial load in asymptomatic patients, but even when testing endocervical swabs of symptomatic patients RDTs were only 22.7%–37.7% sensitive

Based on the combined data from 4 studies, the sensitivity for first void urine and vaginal swabs was 77% and 80%, respectively, each with a specificity of 99% .

the antigen-based RDTs were not recommended for CT testing of both asymptomatic (screening) and symptomatic patients

SEROLOGY

- Testing for chlamydia antibodies is not useful to diagnose local epithelial infections of the lower genital tract, because antibodies are detectable with
  - delay of several weeks
  - antibody titers may be low
  - many serologic test are not able to differentiate antibodies against different chlamydia species
- serology may be helpful in the diagnosis of chronic and invasive infections (PID, LGV, SARA)
- As persistent CT infections and complications of ascending infections are usually associated with a positive antibody response, negative serology most likely rules out the involvement of chlamydia

- The micro immune fluorescence (MIF) test was long considered the reference method of chlamydia antibody testing.
- Serum IgM titer of 1:32 or greater is considered diagnostic of infection
- testing for IgG is not useful because they may represent passively transferred maternal antibody
- Current guidelines do not recommend serologic testing to diagnose infant pneumonia

enzyme immunoassays (EIA) and immunoblots or line assays are currently used more frequently to detect chlamydia antibodies

- Chlamydial LPS is generally considered a genus-specific antigen, but cross-reactivity with antibodies against LPS of other Gram-negative bacteria has been observed


Gonorrhea

- consequential worldwide morbidity both in resource-abundant and resource-limited nations
- diagnosis and treatment require costly expenditures
- Impact on young adult populations
- An ancient disease with biblical references (Hebrew Bible; Leviticus 15:1-3), gonorrhea has many slang references, including “the clap,” which likely derived from the name of the ancient Parisian red-light district Les Clapier

Symptoms of Gonorrhea.

- Some men and women have no symptoms at all.
- However some men have signs or symptoms two to five days after infection. Can take as long as 30 days for symptoms to appear.
- Most women mistake gonorrhea for a bladder infection.
- Complications include:
  - Pelvic Inflammatory Disease (PID)
  - Epididymitis
  - Infertility
- Women:
  - Painful or burning when urinating.
  - Increased vaginal discharge.
  - Bleeding between periods.

- obligate pathogen *N. gonorrhoeae*
- infects only humans in nature
- most commonly manifests as urethritis in men and cervicitis in women

in women :

- Increased vaginal discharge
- Painful urination
- Vaginal bleeding between periods, such as after vaginal intercourse
- Abdominal or pelvic pain
- fever/septicemia, tenosynovitis, arthritis, and vasculitis

- In men:
- Painful urination
- Pus-like discharge from the tip of the penis
- Pain or swelling in one testicle

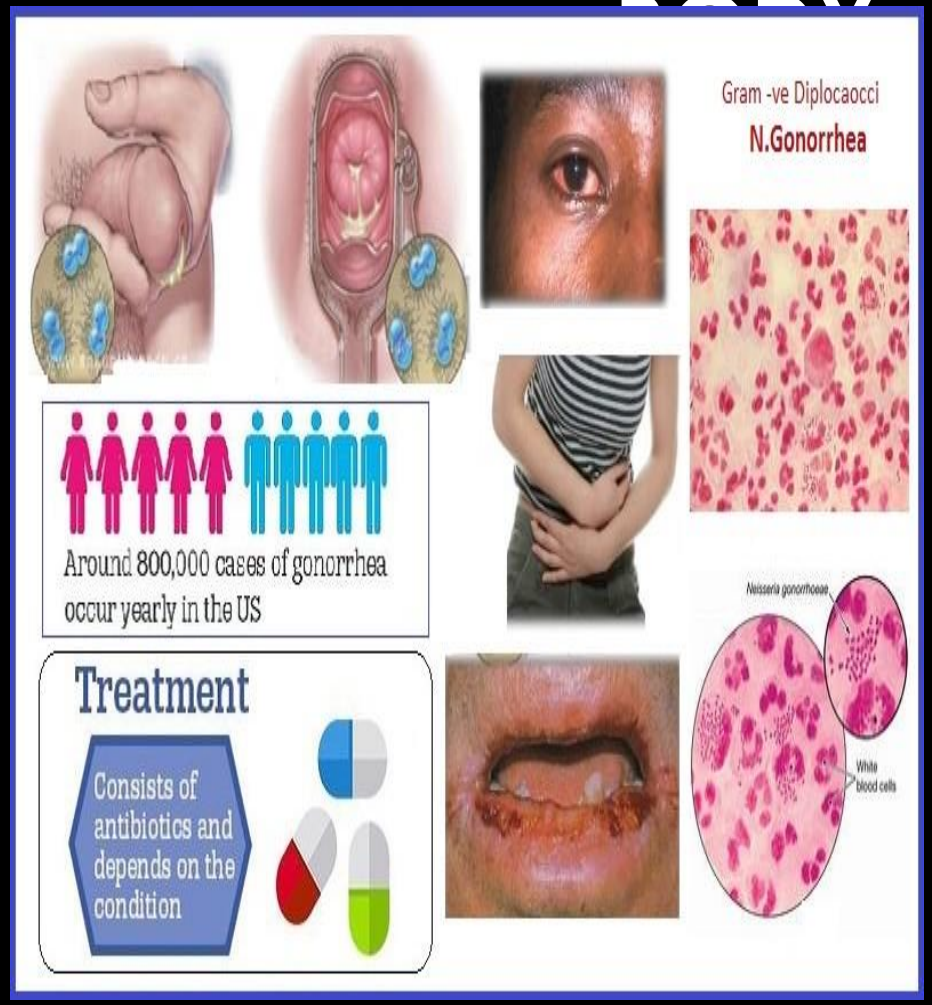
GONORRHEA AT OTHER SITES IN THE BODY

- **Rectum.** Signs and symptoms include anal itching, pus-like discharge from the rectum, spots of bright red blood on toilet tissue and having to strain during bowel movements.

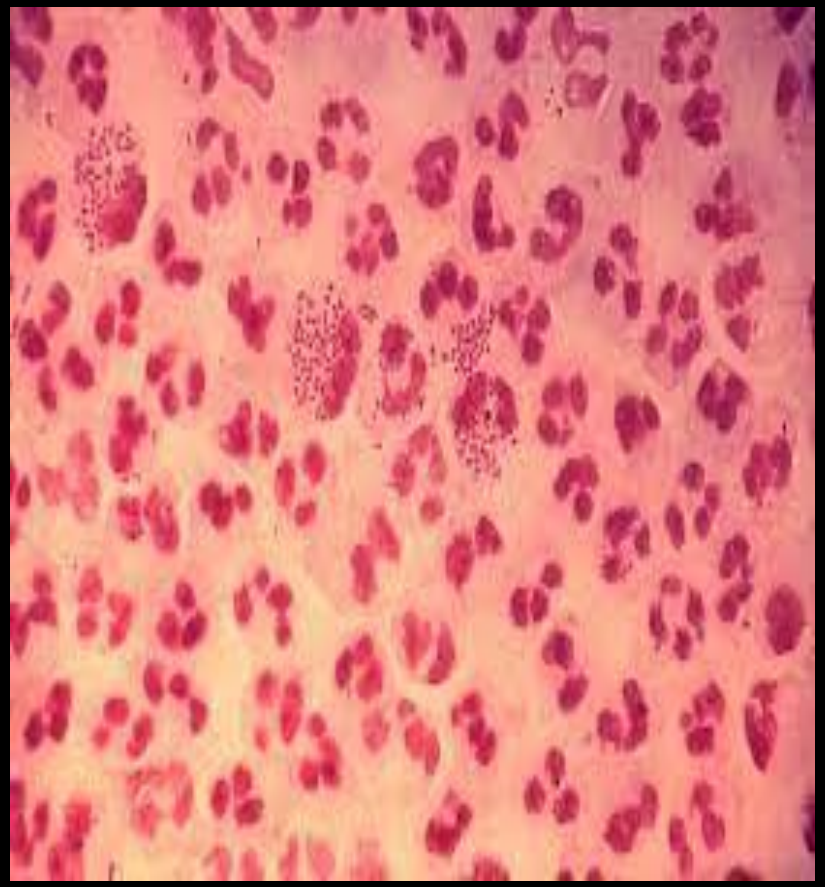
- **Eyes.** Gonorrhea that affects your eyes can cause eye pain, sensitivity to light, and pus-like discharge from one or both eyes.

- **Throat.** Signs and symptoms of a throat infection might include a sore throat and swollen lymph nodes in the neck.

- **Joints.** If one or more joints become infected by bacteria (septic arthritis), the affected joints might be warm, red, swollen and extremely painful, especially during movement.

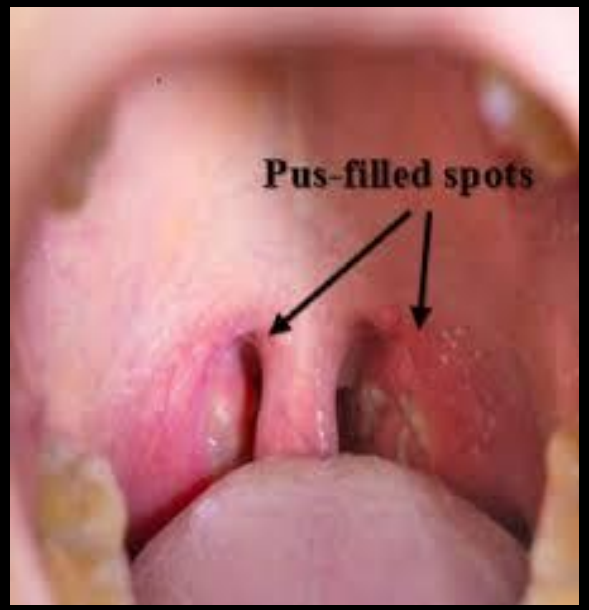


- Light microscopy of Gram-negative or methylene blue-stained smears of *N. gonorrhoeae* urethral or cervical mucus samples will demonstrate neutrophils with intracellular diplococci.
- Light microscopy has high sensitivity and specificity for symptomatic males with urethral discharge;
- light microscopy has a lower sensitivity for the diagnosis of cervical, pharyngeal, and rectal gonorrhea



- ascending gonococcal infection
- upper reproductive tract involvement such as salpingitis
- pelvic inflammatory disease
- increase the risk of ectopic pregnancy
- adverse pregnancy outcomes such as low birth weight newborns
- transmission to newborns resulting in oropharyngeal or conjunctival infections.

❑ females, more than 50%, will not manifest symptoms of their gonococcal cervix infections, most males, more than 90%, will manifest urogenital gonorrhea symptomatically.


Pus-filled spots


GALANT
0943 108 138


Infection that spreads to the joints and other areas of your body
The bacterium that causes gonorrhea can spread through the bloodstream and infect other parts of your body, including your joints. Fever, rash, skin sores, joint pain, swelling and stiffness are possible results.

DIAGNOSIS:

- by direct detection of the gonococcal pathogen in urogenital, anorectal, pharyngeal, or conjunctival swab specimens or first-catch urine.
- Populations that engage in anogenital sexual intercourse and/ or insertive oral sex will require screening for gonorrhea from the anus and pharynx in addition to urogenital screening.
- while these point-of-care assays have few false-positive results, i.e., they demonstrate high levels of specificity, their sensitivity profiles range widely, with false-negative rates varying from 13% to greater than 90%.
- Repeat laboratory screening after patient and partner treatment for gonococcal infection enhances eradication efforts.
- NAATs are generally more than 95% sensitive and specific in urethral and cervical swabs and first catch urine of males

- genital or extragenital samples by light microscopy of stained smears, culture, or nucleic acid amplification tests (NAATs)
- routine utilization of NAATs to screen at-risk asymptomatic patients have demonstrated that pharyngeal and rectal gonococcal infections are not uncommon manifestations
- NAATs : 95% sensitive and specific in urethral and cervical swabs and first catch urine of males

TREATMENT

empiric therapy : initial clinical visit based on historical factors :

- sexual intercourse with a person with an STI
- clinical exam suspicious for an STI, such as penile drip or abnormal vaginal discharge

infections in males and females most commonly consists of dual therapy :
single intramuscular or intravenous dose of 500 mg of ceftriaxone in conjunction with doxycycline 100 mg orally twice a day for 7 days.

For complicated gonococcal infections :

- pelvic inflammatory disease (PID),
- epididymitis
- Proctitis

dual therapy with a single intramuscular or intravenous dose of 500 mg of ceftriaxone is paired with oral doxycycline 100 mg BID for seven days, rather than a single 1 g dose of azithromycin, because of doxycycline's effectiveness against *C. trachomatis* and documented efficacy in treating epididymitis and proctitis

- Current diagnostic tests for NG include Gram staining of urethral discharge smears and bacterial culture
- Smears have limited sensitivity in populations other than men with urethral discharge, whereas culture takes several days and cannot be performed at the point of care (POC).
- Nucleic acid amplification tests for NG and CT are available
- To enable effective etiological case management, a rapid and easy-to-use POC diagnostic test that can provide accurate results that immediately inform treatment is required.

- Current methods to detect AMR in patients with gonorrhea rely on slow bacterial culture techniques

- A POC AMR test :
  to inform the selection of antibiotics for treatment
  prescribe appropriate antibiotics
  reduce the development of resistance

Trichomonas vaginalis

REVIEW
2021 CDC UPDATE: TREATMENT AND COMPLICATIONS OF SEXUALLY TRANSMITTED INFECTIONS (STIS)

- a protozoa transmitted through unprotected oral, vaginal, or anal sex
- It is the third most common cause of vaginitis and often associated with other infections.
- yellow-green, malodorous, frothy vaginal discharge with an elevated pH > 4.5.
- Females may also have urethritis and irritation of the vulva along with a “strawberry cervix” due to punctate hemorrhages and tiny ulcerations of the cervix.

Trichomoniasis Symptoms

- Grey or yellowish vaginal discharge
- Vaginal bleeding
- Genital burning
- Genital swelling
- Frequent urge to urinate
- Pain during sex

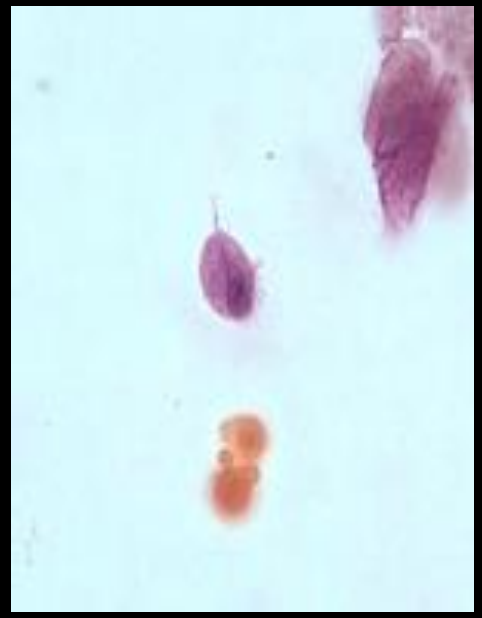
- Discharge from urethra
- Burning during urination or after ejaculation
- Urge to urinate frequently

All symptomatic individuals—and especially those with high-risk behaviors—should be tested for T. vaginalis.

In contrast to a wet mount, NAAT has very high sensitivity and high specificity for female vaginal, endocervical, or urine specimens.

- TMA (transcription mediated amplification) assays : acceptable testing modalities for male urine or urethral swabs
- expensive and can take several days to result , microscopic evaluation through wet mount preparations is the most common method for diagnosis despite requiring immediate evaluation (less than 1h) of the specimen for optimal results

- motile organisms with flagella can be seen moving in the preparation when viewed with a microscope.
- only 40% - 60% sensitive but is typically the most common testing method used due to convenience and low cost.
- vaginal pH is usually more than 4.5 in the presence of trichomoniasis, but this is not a specific finding
- The whiff test is done by adding drops of KOH to a sample of vaginal discharge. This generates a fishy smell.



Nucleic acid amplification tests (NAATs) are gaining favor when testing for *Trichomonas vaginalis*

the gold standard when testing for gonorrhea and chlamydia.

greater than 90% sensitivity and specificity

Before NAATs and other point-of-care : the gold standard was a culture when testing for *Trichomonas vaginalis* with a negative wet prep and a symptomatic patient.

TREATMENT

TABLE 3

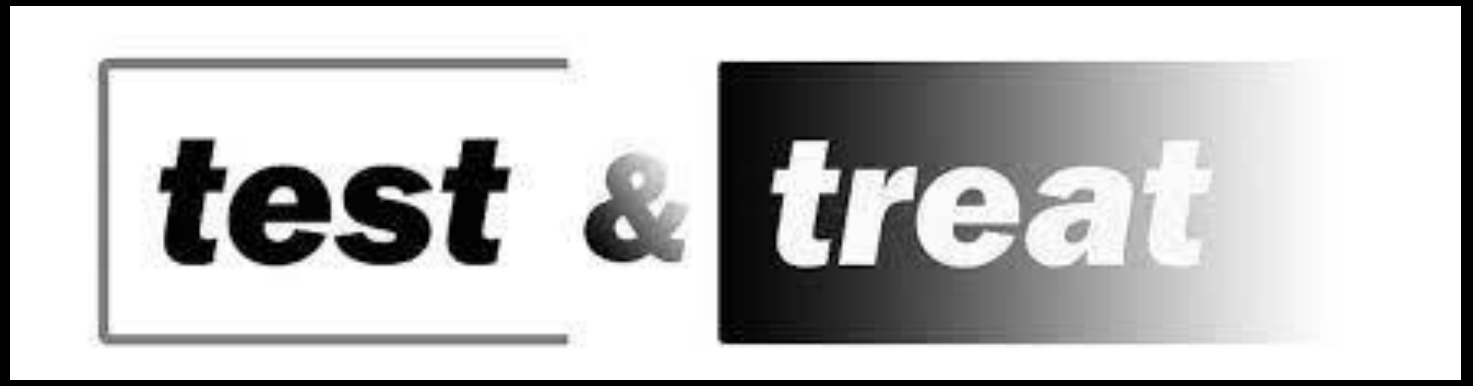
CDC-Recommended Regimens for Treatment of Chlamydial Infection in Pregnant Women

Recommended
Erythromycin base 500 mg orally four times per day for seven days
Amoxicillin 500 mg orally three times per day for seven days
Alternatives
Erythromycin base 250 mg orally four times per day for 14 days
Erythromycin ethylsuccinate 800 mg orally four times per day for seven days
Erythromycin ethylsuccinate 400 mg four times per day for 14 days
Azithromycin (Zithromax) 1 g orally in a single dose

CDC = Centers for Disease Control and Prevention.

Information from reference 2.

What is done in our clinics?
What should be done?



- **Primary prevention** (including behavioral counselling; promoting sexual health, safer sex, condom use; and reducing the partner change rate) to reduce transmission efficiency

- case management (appropriate diagnostic, clinical, and partner notification services)

- opportunistic/targeted testing in key populations(e.g., those visiting certain venues such as STI clinics or housed in jails or congregating in schools); and organized community based testing/screening (e.g., young women)

- Test-and-treat strategies aim to reduce the duration of infectiousness, thereby also reducing subsequent complications and future transmission

CURRENT AND FUTURE TRENDS IN THE LABORATORY DIAGNOSIS OF SEXUALLY TRANSMITTED INFECTIONS

In 2016, the WHO launched its global strategy for tackling STIs .

One of the main cornerstones includes improved surveillance through the development and implementation of better diagnostic algorithms and tests.

Treatment based on this syndrome is simple, inexpensive and cost-effective

the syndromic approach has

- poor predictive value
- should not be used for screening
- it can lead to either overtreatment or undertreatment.

The use of the most sensitive and specific tests is often impractical in resource-poor or remote areas due to their high cost and technical requirements.

considering the clinical presentation and the severe complications associated with some STIs if left untreated "ideal" diagnostic test should be quick so that the patient is treated on the spot ted,

POINT OF CARE TESTING (POC)

POCT should be:

- purchased over the counter for home use
- simple to use and interpret
- Take around 20 minutes to run and release the result.
- the turn around time should coincide with the time spent for the patient–client interaction

- Most HCPs indicate that the accuracy of the test should be the same as that of a laboratory-based NAAT

- Point-of-care (POC) tests represent an answer to the problem of needing to reach a diagnosis quickly outside of a standard laboratory.

- a POC test can be performed at the patient's hospital bedside or own house, the physician's office, or in the field

- a POC test should meet the WHO affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free and deliverable to end-users (ASSURED) criteria

SOME CHALLENGES REMAIN FOR STI DIAGNOSTICS

- NAATs powerful tool, but further improvements are needed to make them less technologically demanding so that they may be more affordable in resource poor settings
- Rapid testing for the presence of antimicrobial resistance remains an important issue for STIs
- the extensive use of next generation sequencing, metagenomics, and culture omics on clinical samples could provide assistance, not only in the characterization and
  detection of AMR mechanisms, but also in developing strategies for previously unculturable organisms.

- research to improve the development of microfluidic technologies and their definitive establishment in diagnostic platforms

- Whole-genome sequencing can be used to track the origin and evolution of hospital outbreaks and allows for the high-resolution typing of microorganisms

- Untargeted metagenomic sequencing from a clinical sample can, in principle, identify any microorganism present without previous knowledge of its genome.

OVERALL

- novel technological solutions should be focused on improving the sensitivity, specificity, and cost of current POC tests.
- Cheap and user-friendly tests for STIs could be routinely used on a much larger scale, resulting in a significant reduction in long-term morbidity and also in costs for the healthcare system
- Due to the hidden nature of STIs, ensuring the extensive and rapid screening of at-risk people and their partners is pivotal to successfully controlling these infections.


Enjoy
the
little
things
#lablife