

THE MICROBIOLOGY LABORATORY OF *Neisseria gonorrhoeae*

Ayoung Hong

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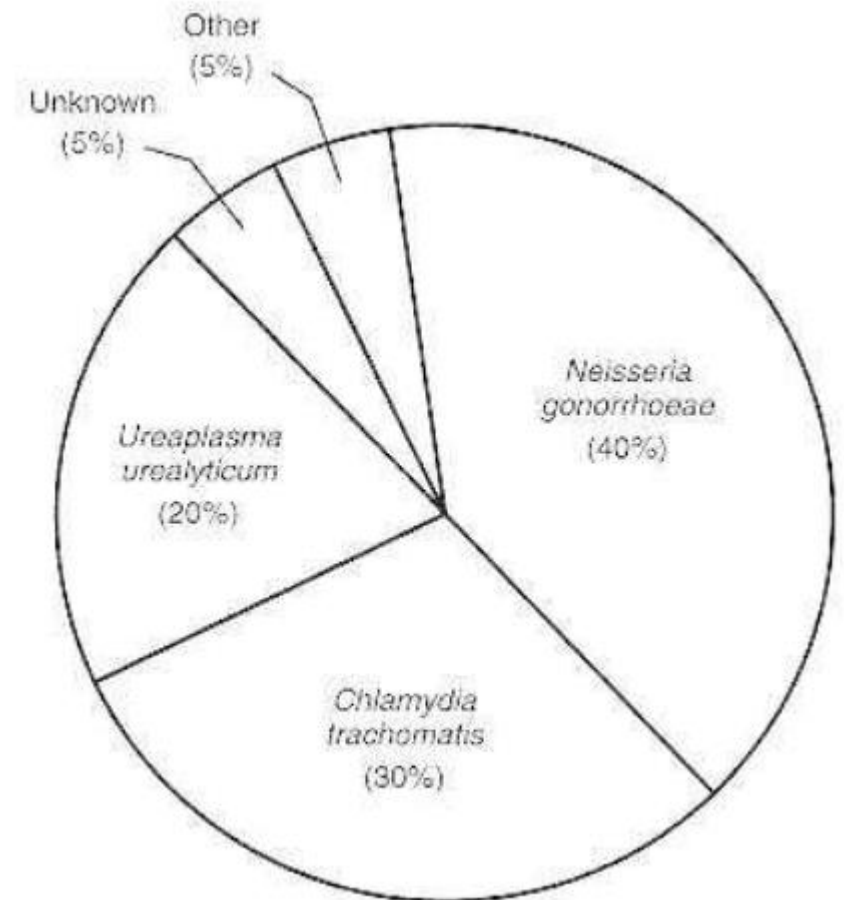


THE MOST COMMON PATHOGENS ASSOCIATED WITH URETHRITIS

https://media.sciencephoto.com/image/co283962/800wm/Co283962-Neisseria_Gonorrhoea,_TEM.jpg

Common Pathogens Causing Urethritis (in STI characterized by urethritis)

- Gonococcal urethritis (1)
: 40% of urethritis cases are caused by ***Neisseria gonorrhoeae***. (2) Naser tested positive for *N. gonorrhoeae*.
- Nongonococcal urethritis (1)
 - ***Chlamydia trachomatis***: 40-60% of nongonococcal urethritis cases (2)
 - Mycoplasma
 - *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Ureaplasma parvum*
 - *Trichomoniasis vaginalis*
- Viral cause
 - Herpes simplex virus (2)



Major causes of urethritis. From (2)

Neisseria gonorrhoeae

- Gonococcal urethritis caused by *N. gonorrhoeae* is a sexually transmitted disease. (1, 2, 3) It is a reportable communicable disease in BC. (4)
- **Epidemiology:** *N. gonorrhoea* is found worldwide. (3) In the United States, gonorrhea is reported as the second most common. (5)
- **Transmission:** Sexual transmission via infected secretion from one mucosal surface to another, by genital, anal, and oral sex (3, 6) It can be transmitted to neonates vertically from infected mother. (3)
- **Pathogenesis:** *N. gonorrhoeae* is a Gram-negative diplococcus. (2, 3) The pathogen colonizes mucosal surfaces. (3) It can adhere via pili and other surface proteins, and invade the mucosal epithelial cells. (2,3) Invasion of epithelial cells causes inflammation, phagocytosis of bacteria, and the influx of polymorphonuclear neutrophils (PMN). (2, 3) Ciliated epithelial cells of mucosal surface are damaged by tumor necrosis factor from phagocytes and gonococcal products (3)
- **Clinical presentations:**
 - In men, mucopurulent urethral discharge secondary to PMN-produced exudate from shedding of urethral epithelial cells. (3, 6) Dysuria (3, 6) and erythema of the urethral meatus have been reported. (3) 10% of the infections are asymptomatic. (3) In women, it is often asymptomatic. (3,6) Other possible symptoms include epididymitis, proctitis, pharyngitis, and conjunctivitis. (3, 6)
 - Symptoms start after 2-8 days of exposure.

Chlamydia trachomatis

- **Incidence/Prevalence:** *C. trachomatis* infection is the most common bacterial STI worldwide. (6) (1, 2, 3) It is a reportable communicable disease in BC. (5)
- **Transmission:** sexual transmission via open-skin or mucous membrane by genital, anal, or oral sex. (7) Mother's infected cervix can infect the neonates. (7)
- *C. trachomatis* is a Gram-negative obligate intracellular bacterium. (8)
- **Clinical presentations:**
 - often asymptomatic in both sex. (7) Possible symptoms in women include vaginal discharge, lower abdominal pain, pelvic pain, dysuria, dyspareunia, and postcoital bleeding. (7) Men can present dysuria, urethral discharge, and scrotal pain or swelling. (7)
 - After exposure to the pathogen, symptoms may appear several weeks later. (7)

Relation with *N. gonorrhoeae* infection

- *C. trachomatis* shows high rates of co-infection with *N. gonorrhoeae*. (2, 3) If *N. gonorrhoeae* is treated only in co-infection, post-gonococcal urethritis caused by *C. trachomatis* will be developed. (2). Hence, it is recommended to test and treat both pathogens if chlamydia is not ruled out. (3)

Other bacterial cause in nongonococcal infection

- **Mycoplasma**

: *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum* belong to the Molliculites class and Mycoplasmataceae family. (9) The niche of these bacteria is the human genitourinary tract. (9) It is sexually transmitted. (9) Clinical presentations of these pathogens are similar to *N. gonorrhoeae* and *C. trachomatis*. (9) The pathogens can cause epididymitis, bacterial vaginosis, cervicitis, PID, infertility, and ectopic pregnancy. (9) In a cross-sectional study of 290 men at STD clinic showed that 22.2% of the cases of nongonococcal urethritis are caused by *M. genitalium*. (8)

- ***Trichomoniasis vaginalis***

: *T. vaginalis* is a protozoan of the order Trichomonadidae. (8) The pathogen can infect the vagina, cervix, bladder, and glands in women's genitalia. (8) Symptoms of the infection include vaginal discharge, pruritis, edema, dyspareunia, and dysuria. (8) Vaginal discharge in infection shows excessive, yellow or green, and frothy. (8) In men, *T. vaginalis* can infect the urethra, prostate, and epididymis. (8)



SAMPLES, DIAGNOSTIC TESTING AND TEST RESULT

Recommended Test Samples

Male patients

- Urethral exudate or first-void urine samples can be used for the diagnostic testing
- **First catch urine sample** is the most preferred sample

Female patients

- Cervical or vaginal swabs, or first-void urine samples can be used for diagnostic testing.
- **Vaginal swab** is an optimal sample because the urine sample has low sensitivity. (1)

For Suspected Sexual Assault

- In adult women, the vaginal swab is an optimal sample for NAATs. (1)
- In girls, vaginal swabs or urine samples may be an alternative to culture. (1) Culture is the preferred testing method for the pharyngeal and rectal samples. (1)
- In boys, pharyngeal, rectal, and urethral samples are collected for the cell culture. (1)

Use of Samples

- **First-void urine** of both men and women can be used for the testing of

For the Diagnosis of Urethritis

- Leukocyte esterase test (1): High leukocyte esterase level in urine implies the presence of bacteria in the urinary tract and inflammation. (10)
- Microscopic examination of urine sediment (1): It represents the infection in the genitourinary tract. (11)

For the Diagnosis of *N. gonorrhoeae* caused urethritis

- Nucleic acid amplification test (NAAT): detects the genetic material of *N. gonorrhoeae*. It also can detect *C. trachomatis* at the same time. (1)

- **Urethral exudate** from men, or **vaginal/cervical swab** from women samples can be used to test

For the Diagnosis of *N. Gonorrhoeae* caused urethritis

- Microscopic examination: Gram stain for leukocytes (1) and Gram-negative intracellular diplococci (2). It visualizes the presence of bacteria and allows presumptive diagnosis. (1)
- Culture: direct plant of exudate onto a split plate of Thayer-Marin and chocolate agar. This is to test the antimicrobial susceptibility. (2)
- NAAT (1): detects the genetic material of the pathogen

Summary of Diagnostic Testing of Urethritis

Diagnosis of urethritis (any cause) in men (1)

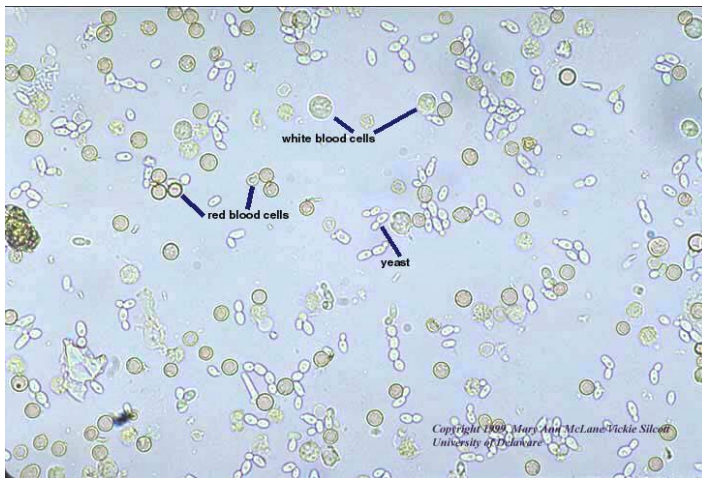
- Gram stain of urethral exudate: ≥ 2 white blood cells (WBC) per oil immersion field
- Clinical presentation: Mucopurulent discharge on exam
- Leucocyte esterase: positive on first-void urine
- Microscopic examination of first-void urine: ≥ 10 WBC per high power field

Specific testing for *N. gonorrhoeae* as the cause of urethritis (1)

- Gram stain of exudate/swab
 - Presence of gram-negative diplococci
 - Only presumptive diagnosis
- Culture of urethral swab
 - It used to be gold standard for diagnosis.
 - Culture and antimicrobial susceptibility testing for the suspected resistant infection
- Nucleic acid amplification test (NAAT) of urethral swab and first-void urine
 - Recommended testing by CDC and IUSTI/WHO for *N. gonorrhoeae* infection in men

Microscopy & Expected Result

Diagnostic testing of any cause of urethritis Urine Sediment Test



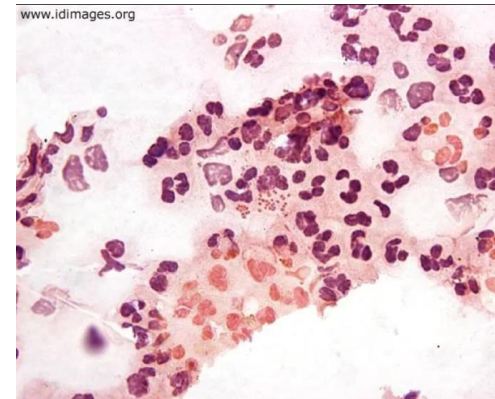
Urine sediment testing result showing WBC, RBC and yeast

From <https://www.medical-labs.net/red-and-white-blood-cells-and-yeast-in-urine-sediment-191/>

- First-void urine sediment ≥ 10 WBC per high power field (1)

Diagnostic testing of *N. Gonorrhoeae* specific testing

Gram stain of a urethral sample

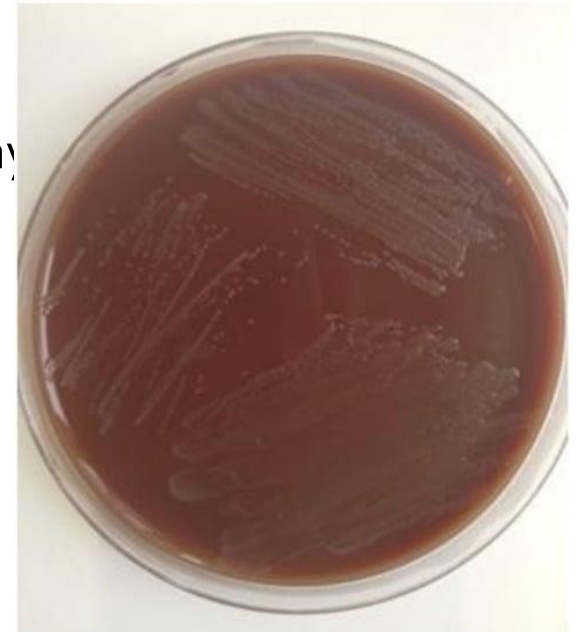


Gram stain of a urethral swab showing *N. gonorrhoeae* (1)

- Co-presence of PMN leukocyte and gram-negative diplococci \rightarrow sensitivity $> 95\%$, specificity $> 99\%$ (1)
- Visualization of intracellular diplococci within PMN cell (1)
- Not sufficient to rule out gonorrhea infection in asymptomatic men (1)

Culture

- Usually culture of urethral swabs only, because the culture from conjunctiva, rectum, and oropharynx are difficult to cultivate. (12) Culture from vaginal swab and urine also does not successfully cultivated. (12)
- *N. gonorrhoeae* is a fastidious pathogen. (1, 12) They are susceptible to dry conditions. (12) The swab sample should be cultured immediately after. (12)
- **Incubation condition** of culture plates: 35-37°C, humidity of 70-80%, pH 6.75-7.5, 4-6% CO₂ enriched (12)
- **The Expected result of plate:** After 18-24 hours (up to 48 hours), small, shiny gray
- **After cultivation:** other detection methods are needed to distinguish between other *Neisseria* species. (12)
 - Biochemical test: enzyme production, metabolic reactions (12)
 - Immunological test
 - Spectrometric test
 - Molecular test



Culture of *N. gonorrhoeae* from a male urethral swab (12)

Culture and Sensitivity

- Culture and sensitivity (C&S) test used to be the gold standard test for gonorrhea. (1) Not preferred anymore because sensitivity of C&S test is lower than sensitivity of NAAT (1)
- Currently, C&S testing is performed after a positive NAAT for the test of cure. (1)
 - CDC recommends the C&S test, when NAAT is positive even after 7 days of antibiotic treatment. (1)
 - IUSTI/WHO recommends C&S test for all patients with positive NAAT. (1)
- Susceptibility testing is done by disc diffusion assay and minimum inhibitory concentration (MIC) gradient testing. Test results reveal the pathogen's susceptibility to the antimicrobial and help decide on antimicrobials for the treatment. (12)
- Antibiotics tested for susceptibility include ceftriaxone, azithromycin, and ciprofloxacin. (12)
- Azithromycin-resistant *N. gonorrhoeae* is increasing, and susceptibility testing is necessary for patients with persistent infection. (1, 12)



Disc diffusion assay (12)



MIC gradient strip test method (12)

Nucleic Acid Amplification Tests (NAATs)

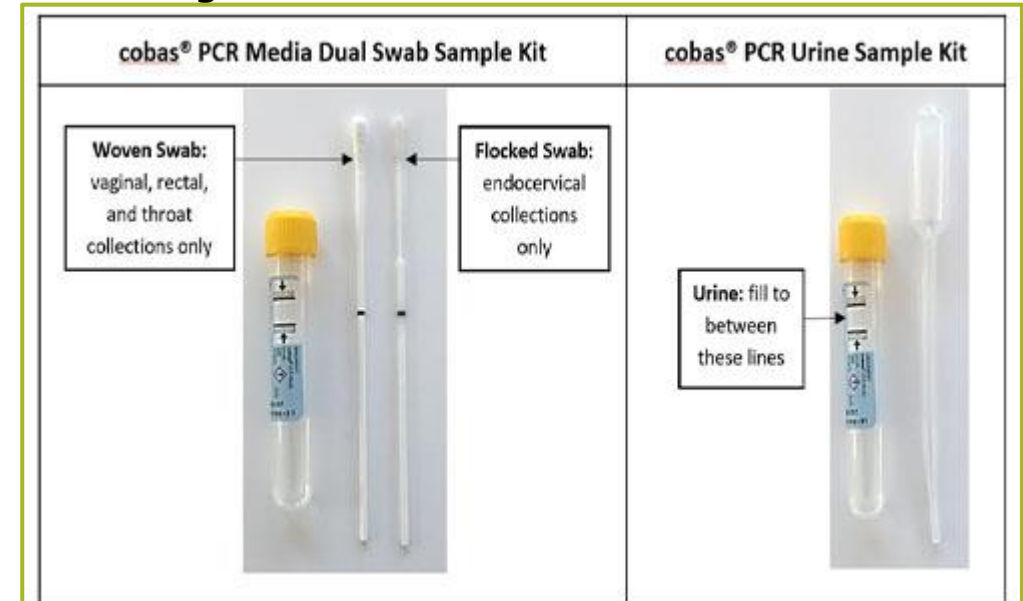
- **Types:** Polymerase chain reaction (PCR) or isothermal transcription-mediated amplification
- NAAT detects the gene of *N. gonorrhoeae*: Opa gene, two different targets in the DR g region, 16S-rRNA, OpcA gene, pillin-gene inverting protein homologue, two distinct chromosomal targets. (12)
The expected result of NAAT is positive for the *N. gonorrhoeae* gene.

- **Advantage**

- Highest sensitivity (>95%) and specificity (>99%) in swabs and first-catch urine (12)
- Sensitive for extragenital gonorrheal infection as well. (1, 12)
NAATs are recommended testing for the diagnosis of rectal or pharyngeal gonococcal infection. (12)
- NAATs can detect chlamydial infection at the same time. (1)
Considering that chlamydial co-infection is common and it can cause post-gonococcal urethritis, *C. trachomatis* should be tested along with *N. gonorrhoea* (1)

- **Disadvantage**

- Lacks antimicrobial resistance information → needs C&S test for the test of cure



PCR NAAT for *N. gonorrhoeae* testing.
From <https://www.publichealthontario.ca/en/laboratory-services/test-information-index/chlamydia-trachomatis-naat-swabs>

MIC gradient strip test method (12)

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