

# **Diagnosis of Sexually Transmitted Infections: Current and Future Landscape**

Salika M. Shakir PhD, D(ABMM)

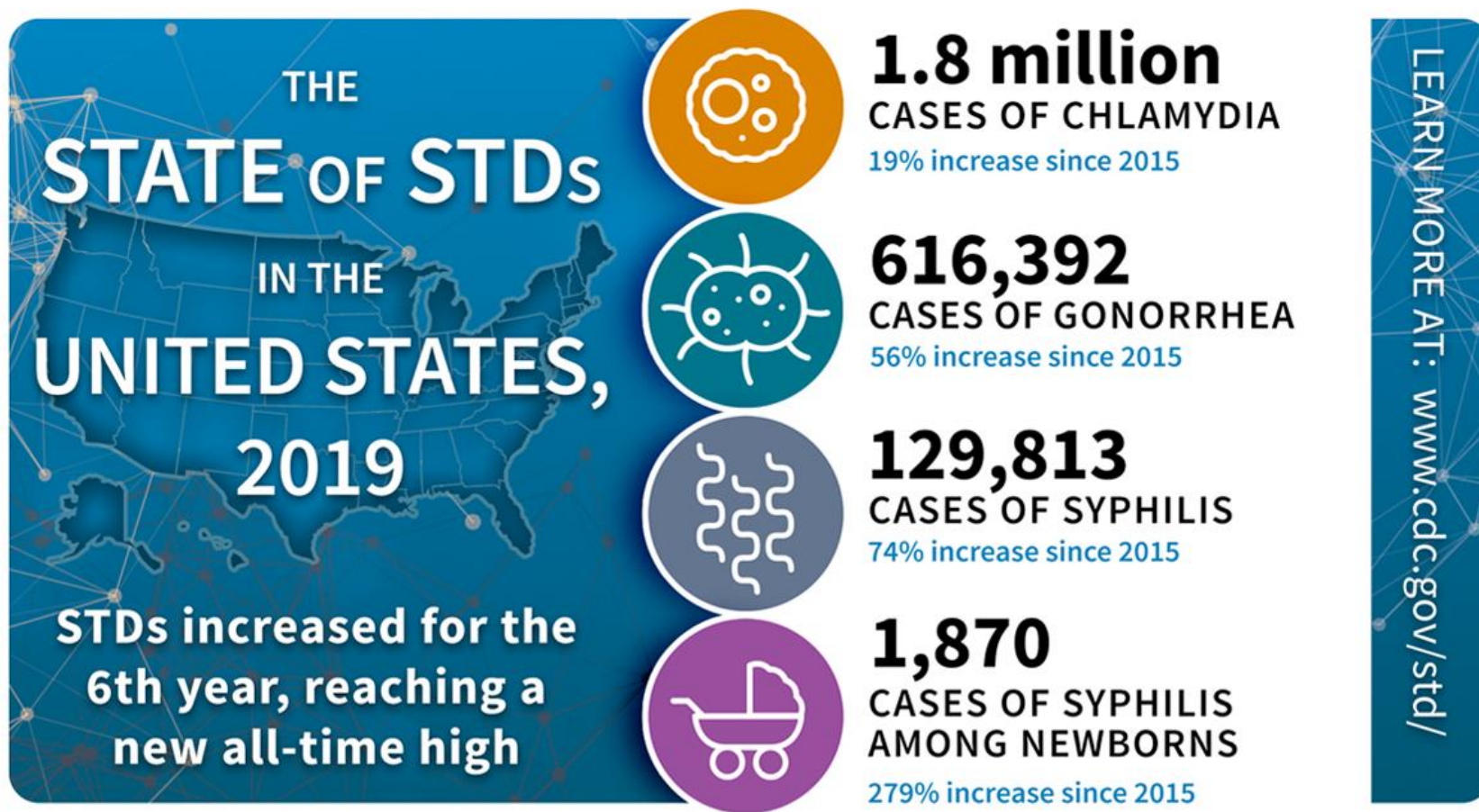
Assistant Professor, University of Utah School of Medicine,  
Department of Pathology

Medical Director, Microbial Amplified Detection Laboratory,  
ARUP Laboratories

# Objectives

1. Recognize the importance of screening and testing for common sexually transmitted infections (STIs)
2. Understand the clinical presentation and organisms associated with STIs
3. Understand the testing methodologies for common STIs

# STIs are on the rise in the US



- Not all STIs are nationally notifiable
- Most STIs are asymptomatic, only those diagnosed are reported
- Influenced by screening coverage and reporting practices

# Why are STI rates increasing?

- COVID-19 pandemic-related disruptions
- Underreported cases
- Lack of funding resources
- Changing sexual habits
- Growing opioid epidemic
- Additional burden with other emerging diseases

# Burden of STIs

## STIs REMAIN COMMON AND COSTLY TO THE NATION'S HEALTH

STIs IN 2018  
(PREVALENCE)



Nearly 68 million infections

ANNUAL NEW STIs  
IN 2018  
(INCIDENCE)



**26 million**

STIs acquired in 2018

DIRECT LIFETIME  
MEDICAL COSTS OF  
2018 NEW STIs



Nearly  
**16 billion**

Total medical costs  
(in 2018 dollars)

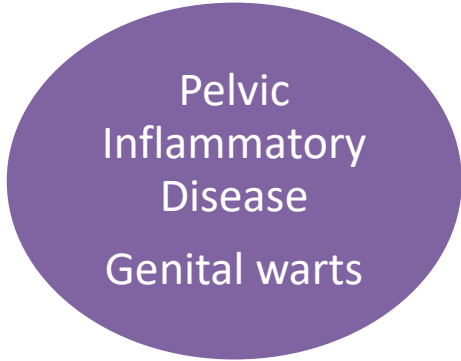
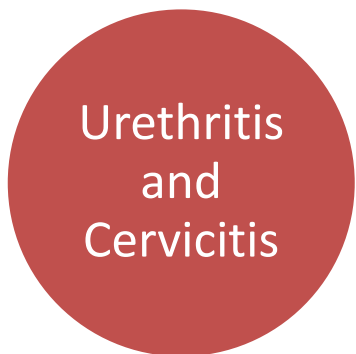
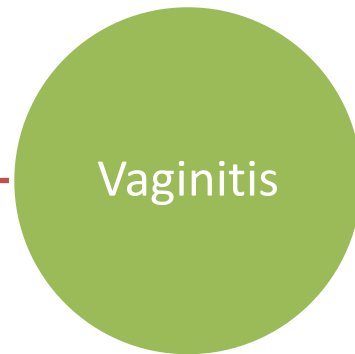


For more information visit [www.cdc.gov/nchstp/newsroom](http://www.cdc.gov/nchstp/newsroom)

# Burden of STIs

- Cervicitis and urethritis
- Pelvic inflammatory disease (PID) in women
- Increase risk for ectopic pregnancies
- Infertility, miscarriage, and preterm birth
- Increase the risk for HIV acquisition among women living with HIV
  - 6% of sexually acquired HIV attributed to chlamydia, gonorrhea (and syphilis)
- Increase the risk for maternal-child transmission of HIV

HSV  
*Treponema pallidum*  
*Haemophilus ducreyi*  
*C. trachomatis* LGV  
*Klebsiella granulomatis*



*Neisseria gonorrhoeae*  
*Chlamydia trachomatis*  
*Trichomonas vaginalis*  
*Mycoplasma genitalium*  
*Ureaplasma urealyticum*

*Neisseria gonorrhoeae*  
*Chlamydia trachomatis*  
*Mycoplasma genitalium*  
HPV

*Trichomonas vaginalis*  
*Gardnerella vaginalis*  
Other Anaerobes  
*Candida* sp.

# Chlamydia and Gonorrhea screening recommendations (CDC 2021)

## Annual screening of

- Women under 25 yrs of age regardless of risk
- Women (>25 yrs of age) at increased risk
- Men for CT in high prevalence clinical settings
- Men who have sex with men
  - Every 3-6 months with risk factors
- Individuals on HIV PrEP every 3 months
- Collection of urogenital, rectal, pharyngeal specimens



# Retesting and Test of Cure

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Chlamydia and gonorrhea: Retesting men and women 3 months after treatment, or <12 months after initial treatment

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Chlamydia: Test of cure for pregnant individuals at 4 weeks after treatment

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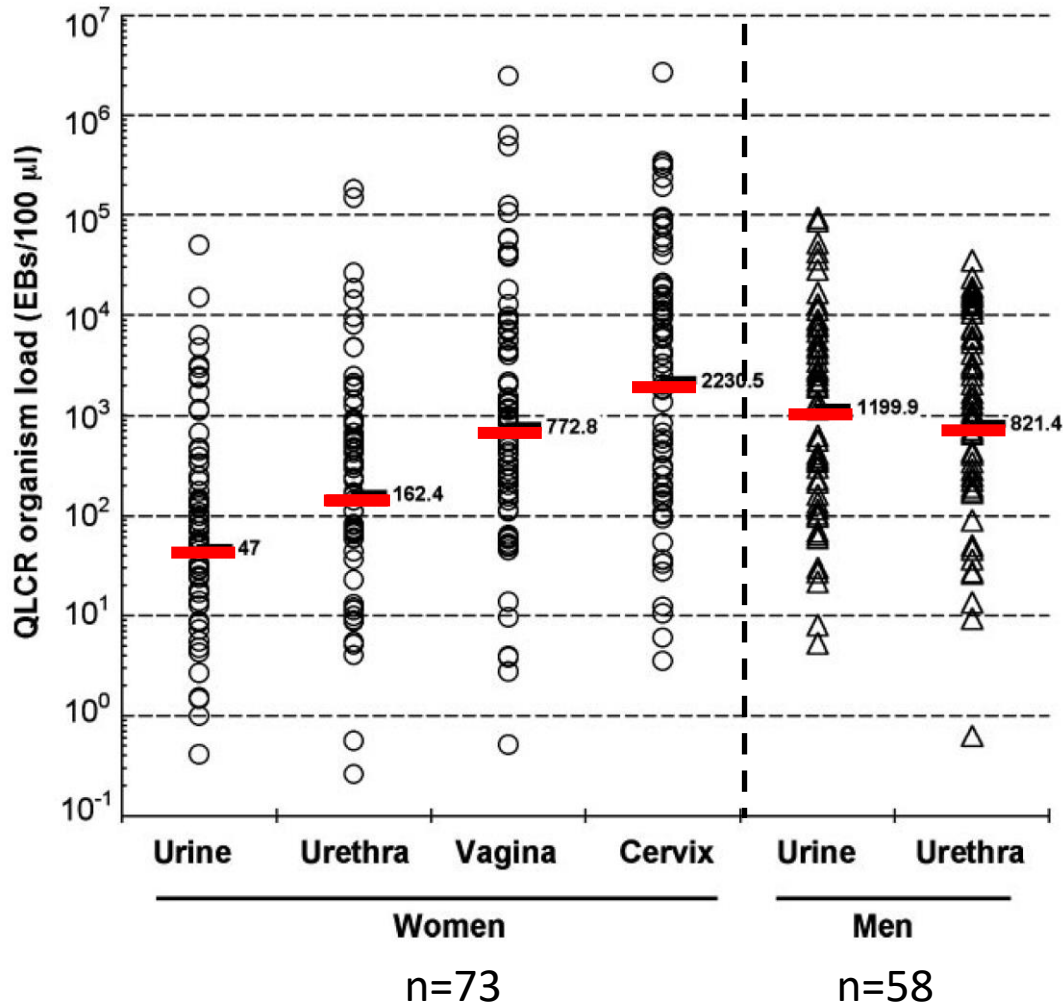
For uncomplicated urogenital or anorectal gonorrhea; no test of cure recommended

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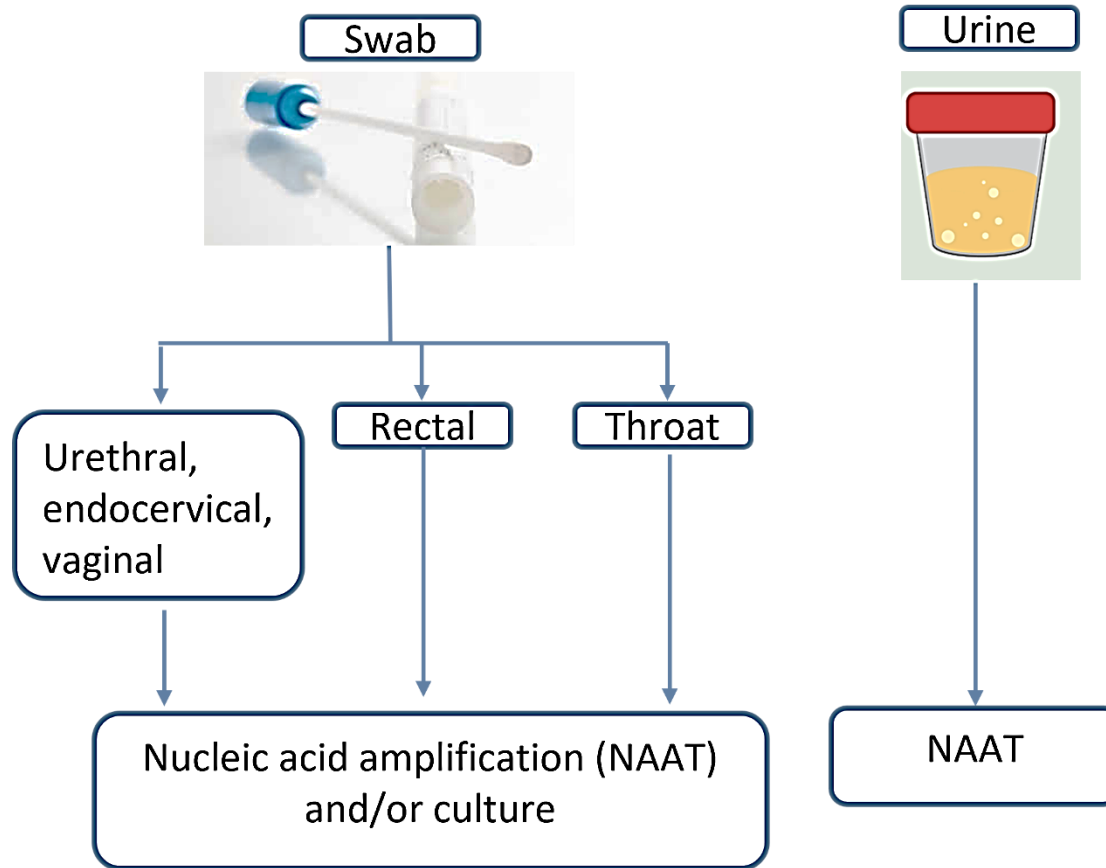
Pharyngeal gonorrhea, a test of cure with either NAAT or culture should be completed 7-14 days after treatment

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# Choice of Specimen



# Choice of Specimen



# Testing of Children

## Diagnostic testing

- Mostly same considerations as for adults
- No FDA-cleared NAAT assays available
- Laboratory-validated tests for off-label use
- CDC recommends retesting or obtaining new specimen

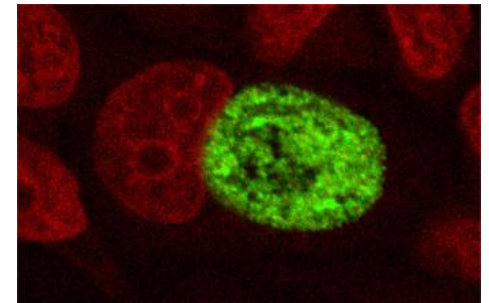
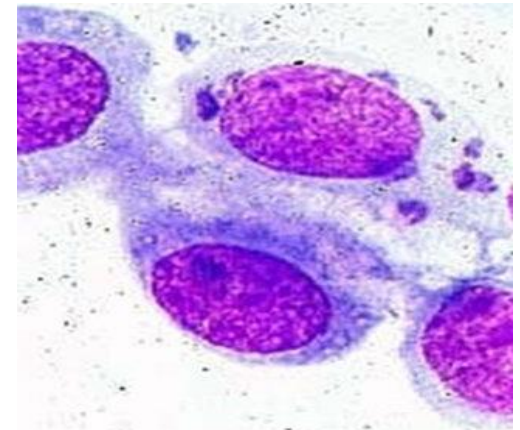
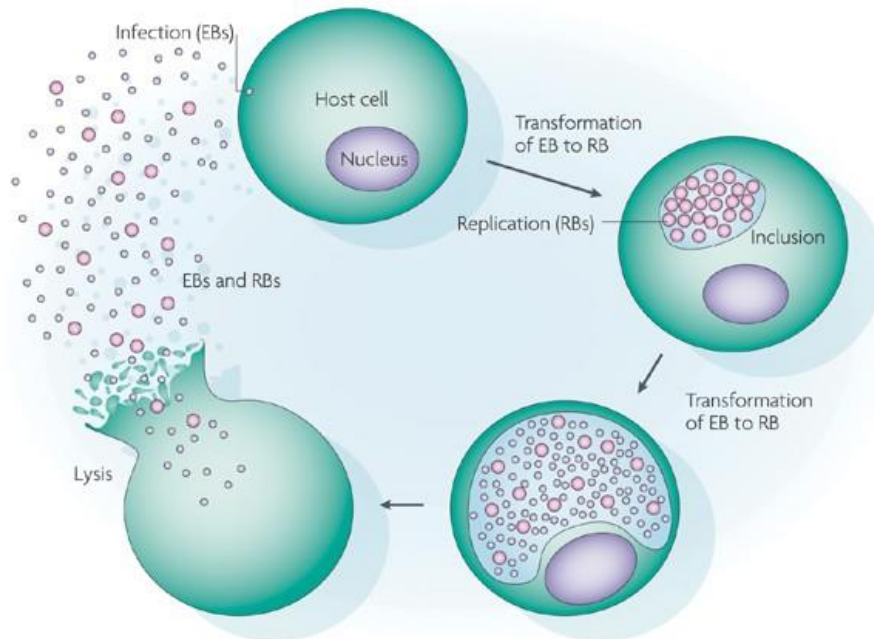
## Forensic testing for STIs

- After neonatal period: CT, NG, TV almost 100% indicative of sexual contact
- Chain of custody preferred
- Testing depends on state (culture vs. NAAT)

# Specific Pathogens

# *Chlamydia trachomatis*

- Obligate intracellular pathogen
- Elementary body: extracellular, infectious
- Reticulate body: intracellular, replicating
- 1 Mb genome, 7.5 kb plasmid



# *Chlamydia trachomatis*

- 15 serovars
  - **A, B1, B2, C**: Trachoma
  - **D-K**: STD (urethritis, cervicitis, salpingitis), conjunctivitis, pneumonia (neonates)
  - **L1, L2, L3**: Lymphogranuloma venereum
- Infects the oropharynx, rectum, eye, and urogenital tract
- More than 70% of urogenital and 90% of rectal and pharyngeal infections are asymptomatic



# Vertical Transmission

- Prenatal screening and treatment of mothers
- Conjunctivitis 5-12 days after birth
  - Conjunctival swab from everted eyelid
  - Culture, nucleic acid amplification
  - Also test for *N. gonorrhoeae*
- Sub-acute, afebrile pneumonia at 1-3 months
  - Nasopharyngeal sample
  - Culture
- Re-testing (erythromycin 80% efficacious)



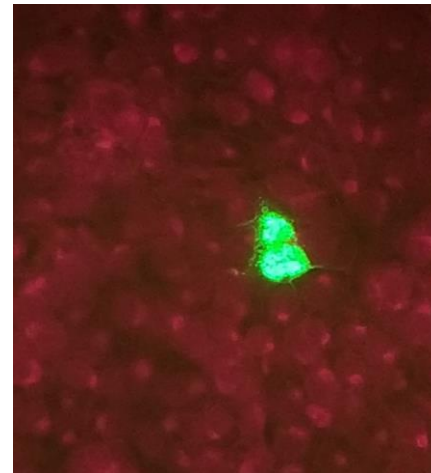
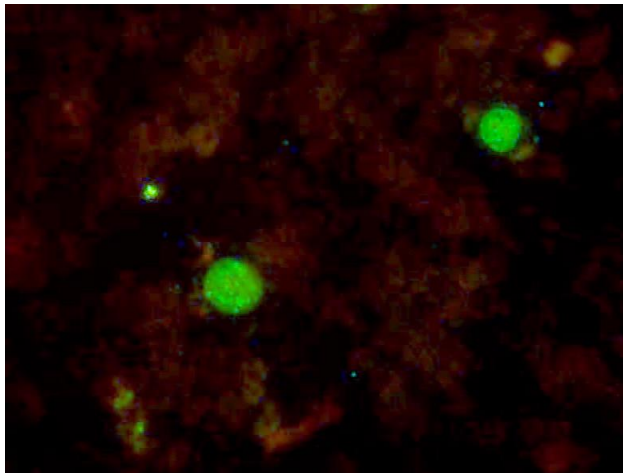
# Testing Modalities

- **Nucleic acid amplification tests (NAAT)**
  - Highest sensitivity, high specificity
  - Less invasive sample acquisition (vaginal swabs, urine)
  - Many commercial FDA cleared-NAATs
  - CDC recommendation to test rectal and pharyngeal site in MSM and women
  - Cannot discriminate between non-LGV and LGV strains

# Testing Modalities (cont'd)

- **Culture**

- Gold Standard
- McCoy, BGM, Hep-2, Hela (48-72h)
- Very high specificity, low sensitivity
- Difficult to standardize, maintain viability, technically demanding
- For sites not validated for NAAT testing





## Testing Modalities cont'd

- **Serology**
  - Abs to chlamydial endotoxin by complement fixation titers  $>1:64$
  - Inguinal , but not rectal LGV
  - Not recommended for detection of active infection or diagnosis of uncomplicated urogenital infections

# ***C. Trachomatis* Test Performance**

<b>Method</b>	<b>Sensitivity</b>	<b>Specificity</b>
Cell culture	40-80%	> 99%
Serology	60-70%	95-99%
DFA	50-70%	95-99%
<b>NAAT (TMA, PCR)</b>	<b>&gt; 95%</b>	<b>&gt; 99%</b>

# ***C. trachomatis* treatment**

- Uncomplicated genitourinary chlamydia infection
  - Doxycycline (100 mg twice daily for seven days)
    - Highly efficacious in all sites
  - Alternative
    - Azithromycin 1 g orally in a single dose or  
Levofloxacin 500 mg orally once daily for 7 days
      - Lower efficacy in urethritis, poor efficacy in  
rectum

# *Chlamydia trachomatis* L serovars

- Lymphogranuloma venerum (LGV)
- Serovars L1, L2, or L3
- **Primary:** genital papule
- **Secondary**
  - Inguinal syndrome: lymphadenitis **w/ bubo formation**
  - Anogenitoretal syndrome: hemorrhagic proctitis
- **Tertiary**
  - Genital ulcers, fistulas, rectal strictures
- Accurate diagnosis is important!
  - Prolonged therapy with Doxycycline 100 mg PO BID for 21 days



# LGV Epidemiology

- E/W Africa, India, SE Asia, South America, Caribbean
- Mostly sporadic: North America, Europe, Australia
- Increased incidence of LGV proctitis in MSM since 2003
  - Outbreak of LGV proctocolitis among HIV positive MSM due to the **L2b strain**
  - Not routinely screened
  - Reportable only in 24 states
  - National prevalence is not known

# LGV Laboratory Testing

- NAAT
  - Detection of *C. trachomatis* by lab-developed test to detect polymorphic membrane protein H (*pmpH*) gene
  - Genotyping for L1, L2, or L3
  - Some public health laboratories
- Serology
  - Complement fixation
  - Cross-reactivity, other serotypes
  - LGV generally >1:64 (often >1:1024)
  - Urethritis, cervicitis, conjunctivitis usually ≤1:16

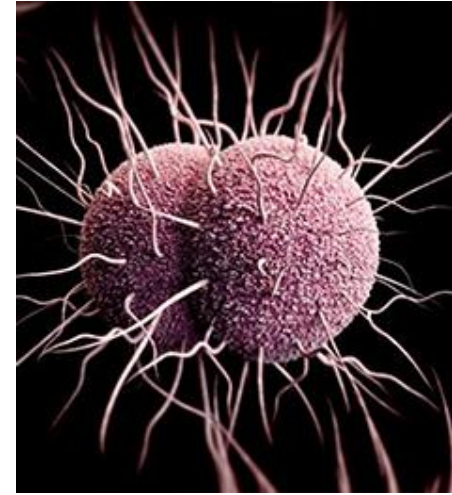


# *Neisseria gonorrhoeae*



# *Neisseria gonorrhoeae*

- Gram-negative diplococci
- Environmentally labile, fastidious, obligate pathogen
- Often asymptomatic in women
  - >50% can have asymptomatic urogenital infections
- Sequelae as with *C. trachomatis*
  - Urethritis in men, cervicitis in women
- Rectal and pharyngeal infections in MSM
- Ophthalmia neonatorum



# Disseminated Gonococcal Infection

- 0.5%-3% of gonococcal infection
- More common in women than in men
- Overt signs of genital disease may be absent
- Dissemination to the skin and joints
- Host risk factors
  - complement deficiencies (C5-C8)
  - Menstruation
  - pregnancy
  - history of pelvic surgery, and intrauterine devices (IUD)

# Disseminated Gonococcal Infection



**Arthritis-dermatitis syndrome** – fever, flu-like symptoms and flitting polyarthralgia or tenosynovitis

Pustular rash present on the extremities – especially the palms and soles

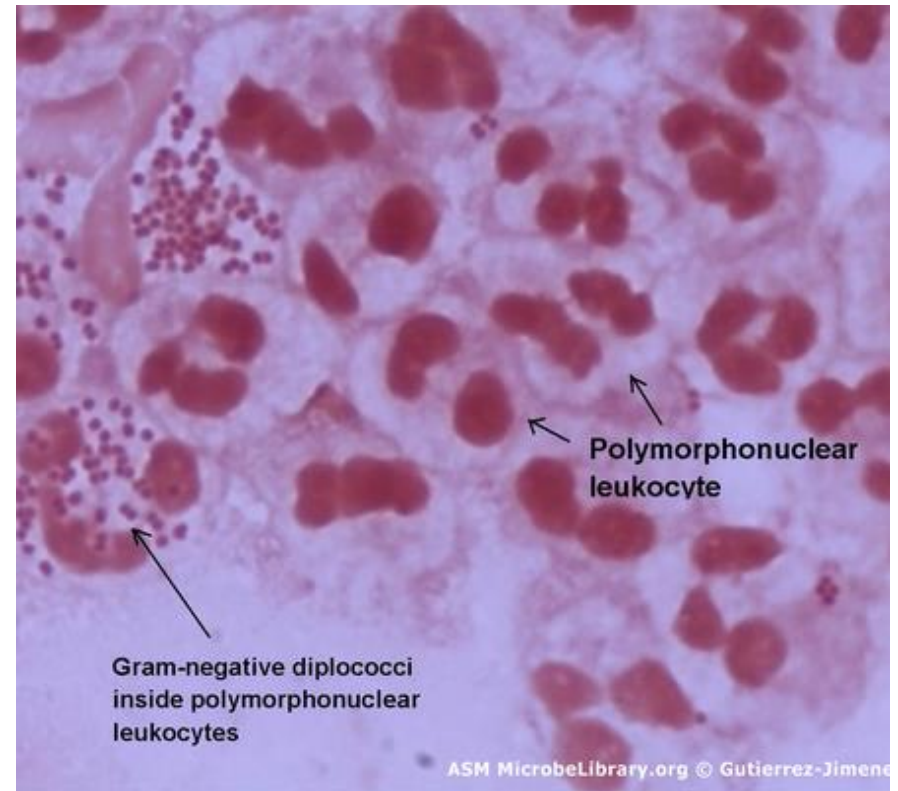
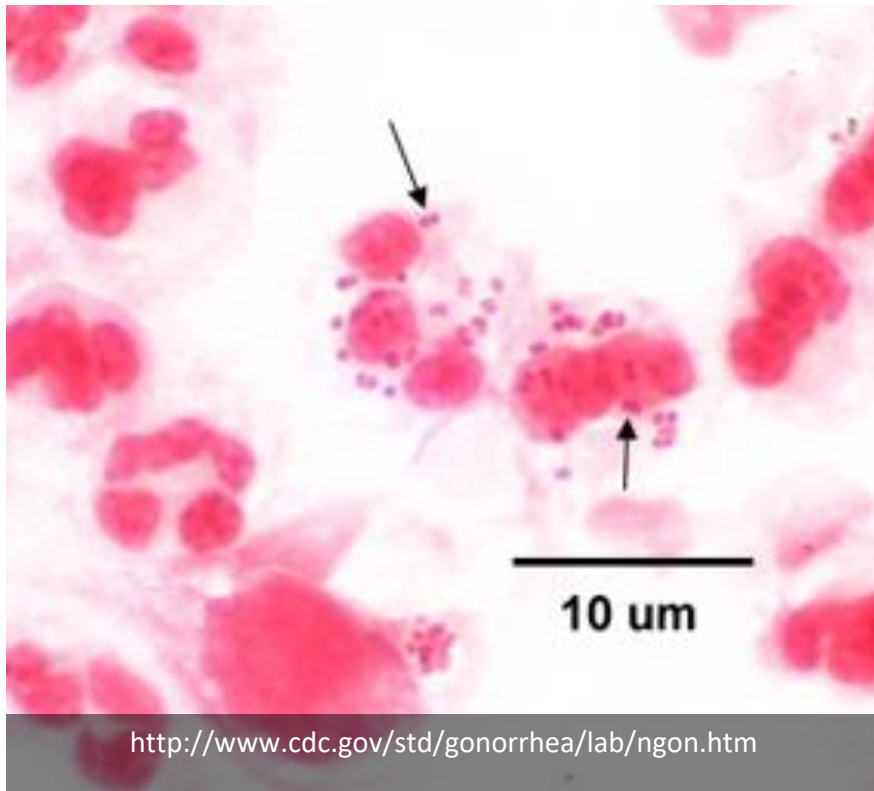
Isolated from the blood, but not usually from the skin lesions



**Purulent Arthritis** – most common in the knees, ankles, and wrists

~50% isolated from synovial fluid culture

No FDA cleared NAAT testing available

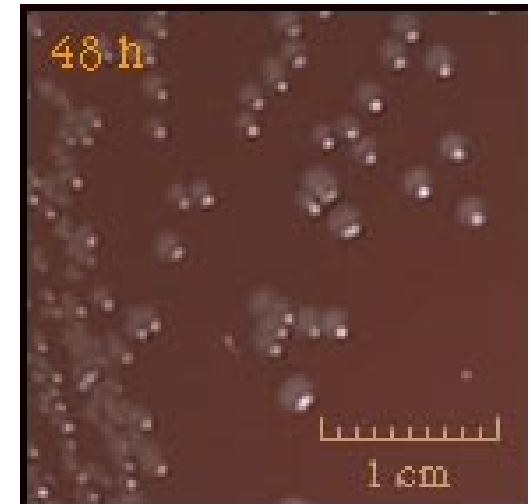


## Testing Modalities

- **Gram stain**
  - Gram-negative Diplococcus
  - Urethral swab in symptomatic male
  - Sensitivity 90-95%

# • Culture

- Modified Thayer-Martin or Martin-Lewis
- Additional biochemical tests for confirmation
  - **Catalase positive**
  - **Oxidase positive**
  - Acid only from glucose
- MALDI-TOF
- Required for susceptibility testing
- Endocervical and urethral samples



# Testing Modalities cont'd

- **NAATs**
  - **Recommended test method**
  - Vaginal swab, endocervical swab (women), first-catch urine, or urethral swab (men)
  - CDC recommends NAATs for rectal and pharyngeal (MSM)
  - FDA-cleared tests in combination with CT

# *N. gonorrhoeae* Test Performance

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<b>Method</b>	<b>Sensitivity</b>	<b>Specificity</b>
Culture	50%-80%	> 95%
Gram stain	50-70% (females) 90-95% (males)	95-99%
NAAT (PCR, TMA)	90-100%	92-100%






# *N. gonorrhoeae* Treatment Recommendation

- **CDC 2021 recommended treatment for uncomplicated gonorrhea**
  - **Ceftriaxone (500 mg IM single dose)**
- **Alternative treatment (if cephalosporin allergy)**
  - Gentamicin (240 mg single IM) plus azithromycin (2 g single oral)
  - Cefixime (800 mg single oral dose)
- **Azithromycin or doxycycline (no longer recommended)**
  - Burden of resistant isolates in MSM
  - More likely elevated ceftriaxone, azithromycin MICs
  - Ciprofloxacin, penicillin, tetracycline resistance

*CDC: MMWR, March 14, 2014, Vol. 63, No. 2*  
*CDC: Sexually Transmitted Disease Surveillance, 2018*  
*CDC STI 2021 guidelines*

**DRUG-RESISTANT  
*NEISSERIA GONORRHOEAE***

THREAT LEVEL **URGENT**

 <b>550,000</b> Estimated drug-resistant infections each year	 <b>1.14M</b> Total new infections each year	 <b>\$133.4M</b> Annual discounted lifetime direct medical costs
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*Neisseria gonorrhoeae* causes gonorrhea, a sexually transmitted disease (STD) that can result in life-threatening ectopic pregnancy and infertility, and can increase the risk of getting and giving HIV.

FORBES > INNOVATION > HEALTHCARE

## New Multi-Drug Resistant Gonorrhea Detected In U.S., 2 Cases In Massachusetts



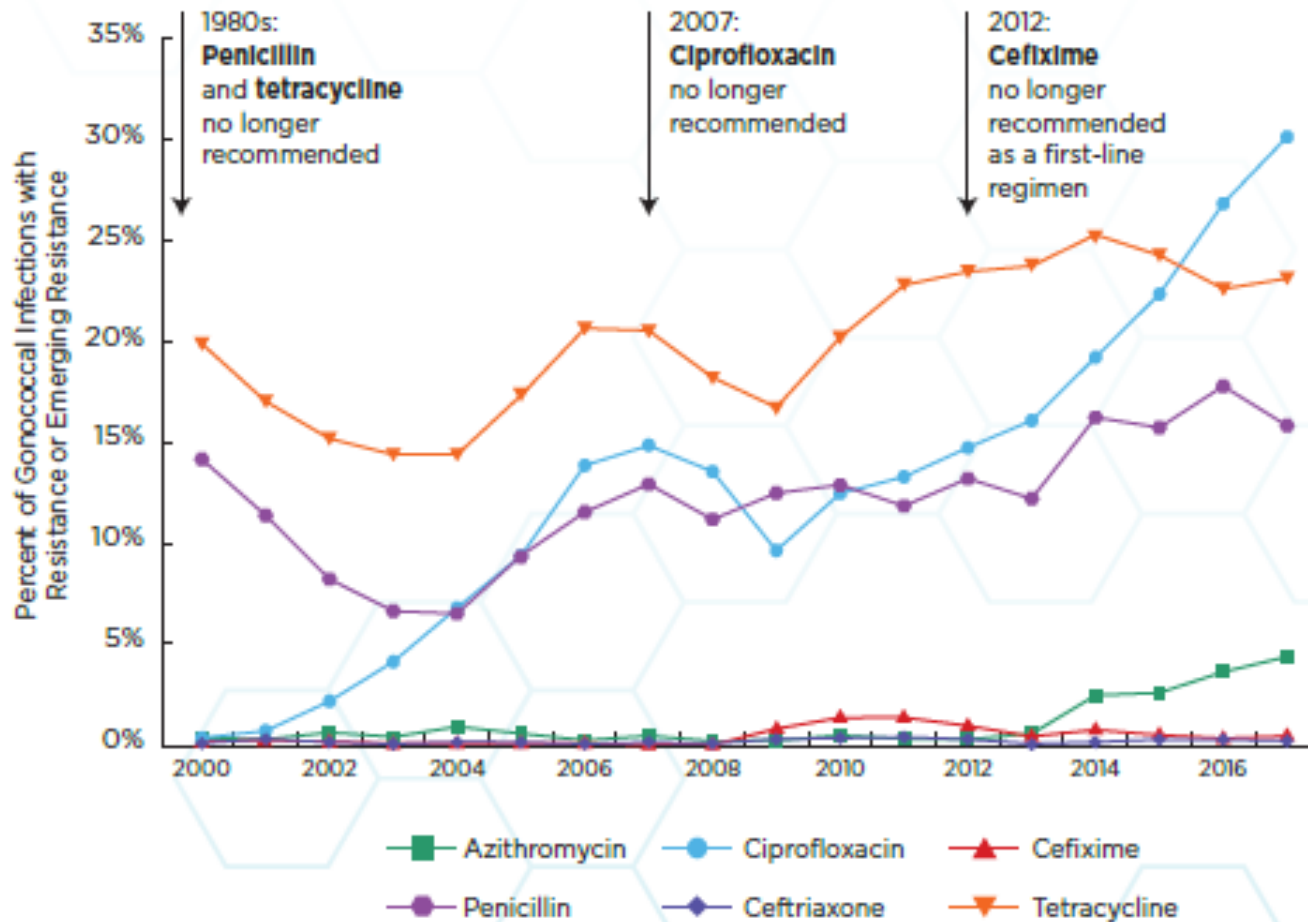
HEALTH

## New 'Superbug' Strain of Gonorrhea Is Outsmarting Most Antibiotics

Two cases in Massachusetts involve a novel strain more impervious to existing antibiotics than other strains in the U.S.

# Antimicrobial resistance in *N. gonorrhoeae*

Gonorrhea rapidly develops resistance to antibiotics—ceftriaxone is the last recommended treatment.



# Antimicrobial Susceptibility Testing

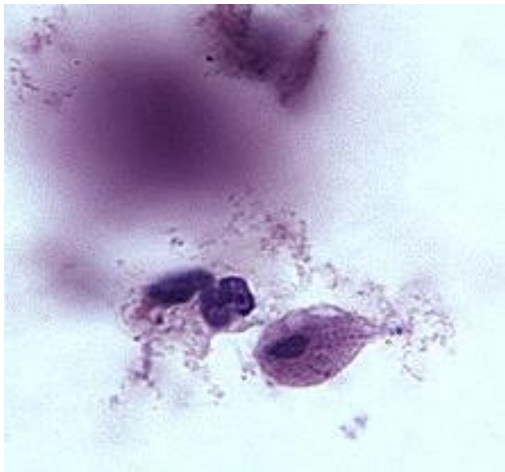
- Requires culture for antimicrobial susceptibility testing
- Agar dilution (gold standard), Disk diffusion, gradient strip method
- CLSI breakpoints established for:
  - penicillin, cephalosporin agents, azithromycin, tetracycline, ciprofloxacin, and spectinomycin
- No molecular tests for prediction of antimicrobial resistance determinants validated for clinical use

# *Trichomonas vaginalis*



# *Trichomonas vaginalis*

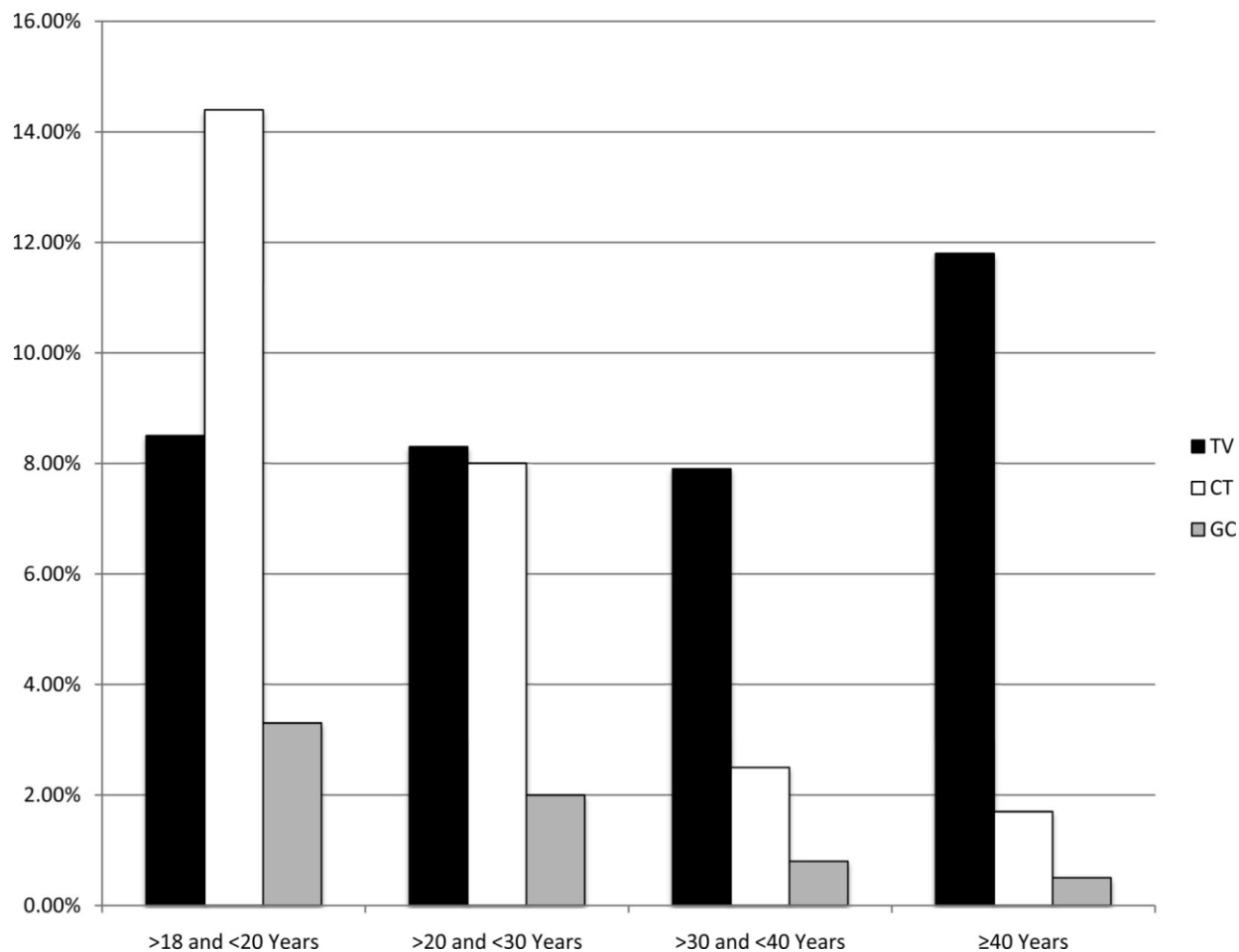
- Most common curable STI in young women
- WHO estimates 1.3 million new infections
- Vaginitis, urethritis, and cervicitis, PID
- Preterm delivery, low birth weight
- Increase HIV transmission in HIV endemic areas
- Men: often asymptomatic



<https://www.cdc.gov/dpdx/trichomoniasis/index.htm>

J Clin Microbiol. 2016 Sep;54(9):2278-83

# *Trichomonas vaginalis* Prevalence



# *Trichomonas vaginalis*

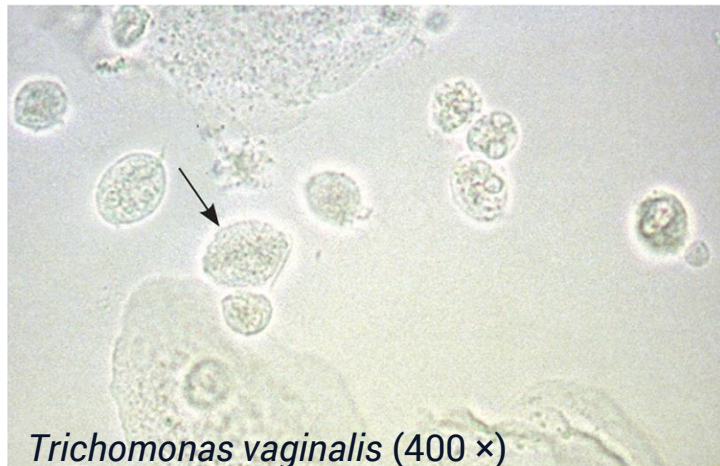
- Indications for testing
  - Women seeking care for vaginal discharge
  - High risk of infection (new/multiple partners, history of STIs, commercial sex worker, IUD)
- Serious adverse outcomes occur primarily among pregnant women and individuals at high risk for sexually transmitted diseases
- Oral and rectal testing not recommended
- Retesting <3 months after initial treatment because of high rate of reinfection



# *T. vaginalis* Diagnosis

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- Wet mount (vaginal, urethral discharge, urine, prostatic secretions)
- Trophozoite: 8-15  $\mu\text{m}$ , single nucleus, 4 anterior flagella
- Miss more than 50% of trichomoniasis



# *T. vaginalis* Diagnosis

- Culture more sensitive than wet mount, not widely available
- Gold standard before NAATs
- Inoculation within 1 hr of collection
- Sensitivity 70%-85%, lower in men
- Specificity of 100%



InPouch<sup>®</sup> kit or Diamond's medium, TAT 5-7 days, requires daily sampling for microscopic examination

# *T. vaginalis* Diagnosis

- **Nucleic acid testing**
  - PCR or transcription-mediated amplification
  - DNA probe (BD Affirm VPIII)
- CDC recommends NAAT in symptomatic or high-risk women
- Higher sensitivity than saline microscopy (95% to 100% vs. 51% to 65%)
- Can be performed on endocervical, vaginal, or urine specimens, or on liquid-based Pap test samples

# ***T. vaginalis* Treatment**

- Metronidazole is drug of choice
  - Women: 500 mg 2 times/day for 7 days
  - Men: 2 g orally in a single dose
- Alternative regimen
  - Tinidazole 2 g orally in a single dose

# *Mycoplasma genitalium*

- Considered the “new kid on the block”
- Mollicutes class, smallest replicating bacteria
- Lack a cell wall,  $\beta$ -lactam antibiotics are ineffective
- **No current US recommendations for *M. genitalium* screening in any population**
- Testing indicated for patients with persistent urethritis or cervicitis, or severe pelvic inflammatory disease
- Two-step, resistance guided therapy >90% cure rate
- CDC’s watch list for increasing macrolide resistance

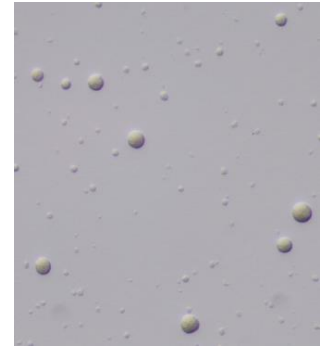


# *M. genitalium* Prevalence

- Asymptomatic mostly
- Co-infections with other STIs are common
- In men
  - 30%-40% of NGU cases
  - Reported among 1%-26% of MSM
  - Higher prevalence is reported among men with rectal symptoms
- In women
  - Detected among 10%-30% of women with cervicitis
  - High prevalence of 12%-18% in pregnant women
  - Increase the risk of PID, endometritis, and infertility

# *M. genitalium* Diagnosis

- Culture can take several weeks, extremely slow growing
- Nucleic acid amplification tests (NAAT) are recommended
- Preferred specimens are a first-void urine sample in males and a vaginal swab in females
- Two FDA-cleared NAAT available
  - Hologic Aptima Mycoplasma genitalium Assay
  - Abbott Alinity m STI assay for CT, MG, NG and TV
  - Roche cobas TV/MG
- Resistance marker detection RUO or lab-developed



Spherical *M. genitalium* colonies (G37) growing on SP4 agar after 10 days of incubation

# Drug resistance in *M. genitalium*

- Resistance to azithromycin is increasing
- Mutations in 23S rRNA marker for macrolide resistance (A2058G/C/T and 2059G/C)
- High correlation with treatment failure 44%-90% in the US
- Quinolone resistance is much lower (0%-15% in US)
- Several CE-marked assays available
  - ResistancePlus MG Flexible, AllplexMG

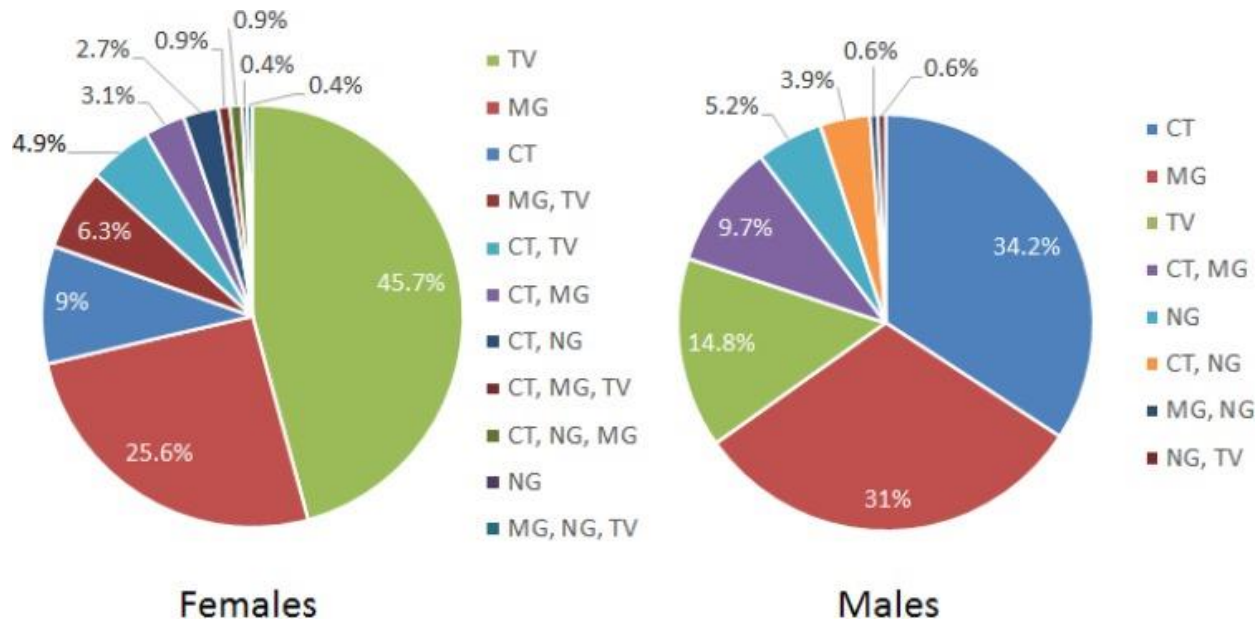


# Current and future molecular diagnostic options



# Molecular diagnosis of CT, NG, TV, MG

- More than 25 FDA-cleared NAATs for CT/NG ,
  - Transcription-mediated amplification
  - Polymerase chain reaction
- Some include TV and MG



# Molecular diagnosis of CT, NG, TV, MG

- New generation molecular assays
  - High throughput, increased efficiency
  - Expanded sample types
  - Expanded test menus with multiple targets
  - 1 sample for most assays



- Novel diagnostics
  - Rapid, <1 hr
  - Point-of-care



# FDA-cleared Point-of-care STI tests

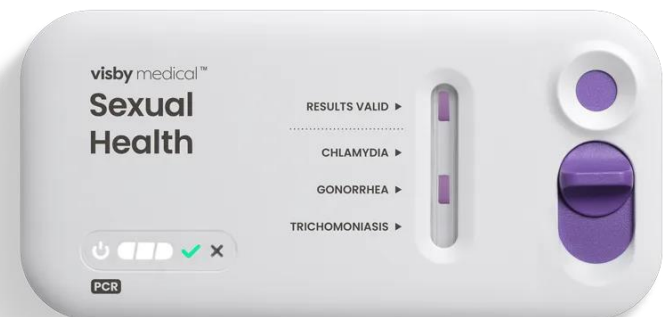
## Binx Health io STI

- CLIA- waived POC
- CT, NG
- Vaginal swab, male urine
- Rapid, 30 minutes
- CT sens 96.1%, spec 99.1%
- NG sens 100%, spec 99.9%



## Visby STI panel

- CLIA- waived POC
- Instrument-free platform
- CT, NG, and TV
- Self- and clinician collected vaginal swab
- Rapid, 30 minutes



# Self-collection is the way

- Five commercially available FDA cleared NAAT assays for self-collected vaginal swabs and first-catch urine
- Self-collected urogenital specimens provided accurate results
- Rectal and throat self-collection not FDA-cleared but studies show equivalent or better detection rates for CT/NG
- Self-collected rectal swabs are a reasonable alternative to clinician-collected rectal swabs in low resource settings (CDC 2021)
- Improved patient satisfaction overall

# Summary

- Sharp rise in STI's are prompting calls for new prevention, testing and treatment methods
- For CT and NG: screening recommendations well defined, less for other STIs
- Many infections in non-genital sites
- Antimicrobial resistance of *N. gonorrhoeae* is progressing
- Highly sensitive NAATs are recommended for *T. vaginalis* diagnosis
- *M. genitalium* is increasing in prevalence with concern for macrolide resistance
- POC tests and self-collection is the way of the future

Thank you!

Questions?

Salika M. Shakir, PhD, D(ABMM)

salika.shakir@aruplab.com



*ARUP is a nonprofit enterprise of the University of Utah and its Department of Pathology.*