# STI Diagnostics: lab-based diagnostics, POC, and testing trends

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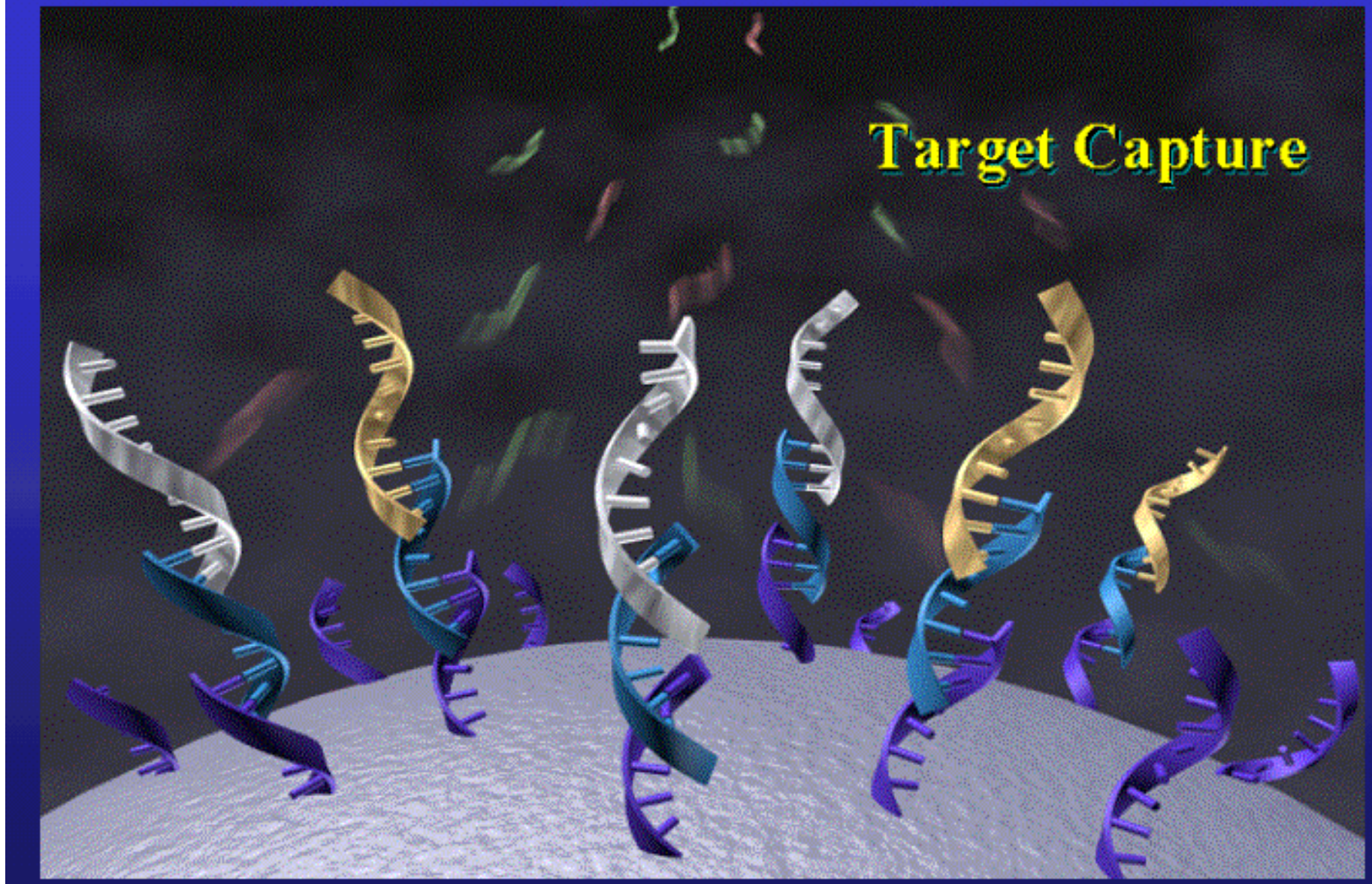
# Learning Objectives

- Review laboratory-based diagnostic testing for STIs
- Discuss point-of-care testing and direct-to-patient testing trends in STI diagnostics
- Introduce planned future diagnostic trends

# Current laboratory-based testing at CUMC

# Hologic Aptima Assays

- Aptima Combo assays – utilize TMA and target capture to detect rRNA for the following pathogens:
  - **CT/GC (combo)**
  - **Trichomonas**



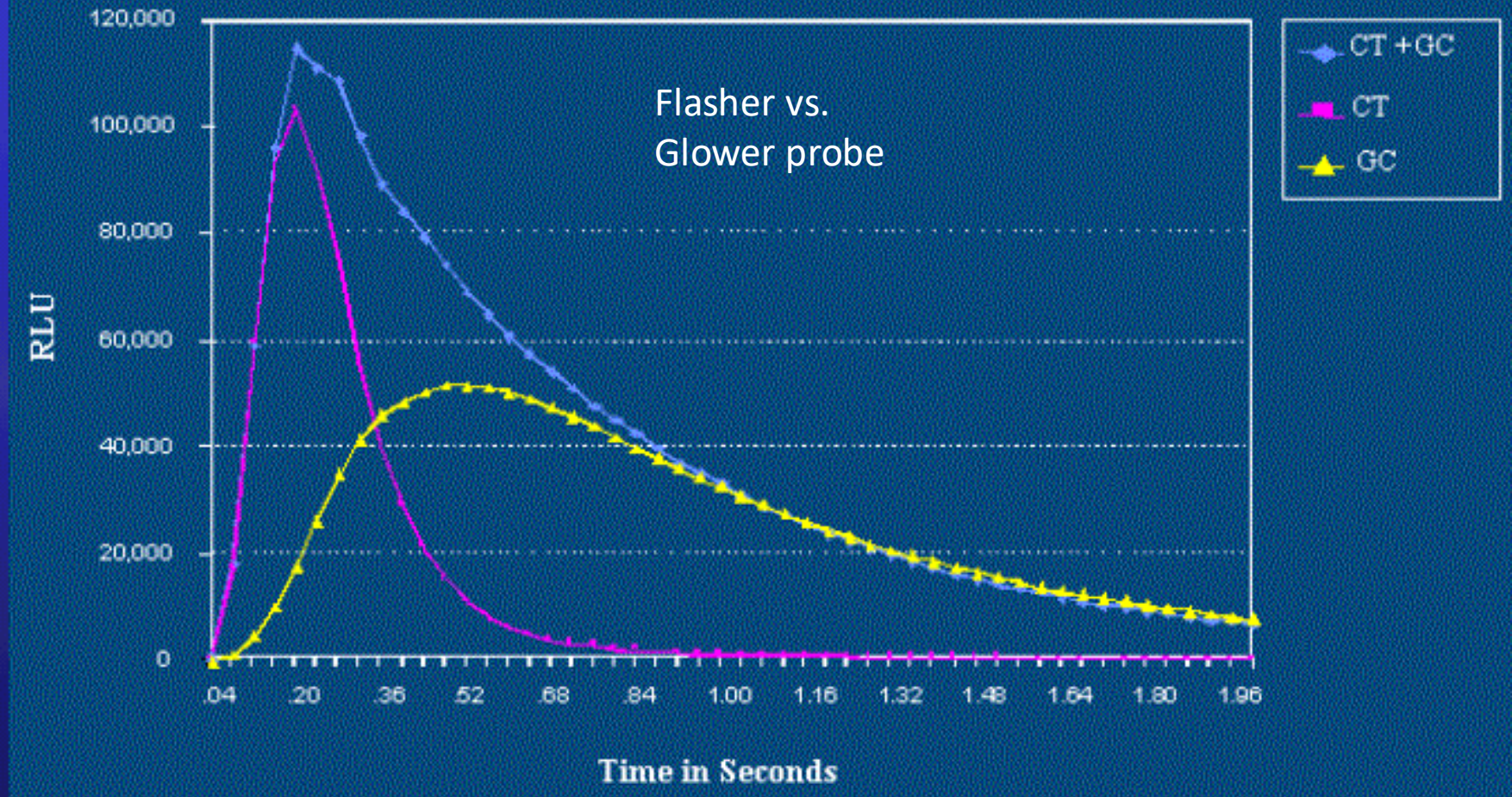
# *Neisseria gonorrhoeae/Chlamydia trachomatis* Assay

- Combines target capture, TMA, and DKA
- Cleared for the following specimens on the Panther System:
  - Aptima Transport Vial (Collect as indicated on the vial).
  - Specimen sources include: urine, genital swabs, rectal swabs, and throat swabs

## *Trichomonas vaginalis* Assay

- only female urine, endocervical and vaginal swabs are accepted

# Dual Kinetic Assay (DKA)



# Responding to the Mpox outbreak

The Journal of Molecular Diagnostics, Vol. 25, No. 10, October 2023

the Journal of Molecular Diagnostics
jmdjournal.org

## Evaluation of a Zoonotic *Orthopoxvirus* PCR Assay for the Detection of Mpox Virus Infection

Priya D. Velu,[*†] John Sipley,[*†] Jamie Marino,[*†] Sanjiv Ghanshani,[‡] Georgi Lukose,[*†] Lin Cong,[*†] Liliana Serrano,[*†] Thanh Ly,[†§] Raymond K. Yeh,[†§] Fann Wu,[†§] Mahesh Mansukhani,[†§] Gregory J. Berry,[†§] and Hanna Rennert[*†]

*From the Department of Pathology and Laboratory Medicine,* Weill Cornell Medicine, New York, New York; the NewYork-Presbyterian Hospital,[†] New York, New York; altona Diagnostics,[‡] Hamburg, Germany; and the Department of Pathology and Cell Biology,[§] Columbia University Irving Medical Center, New York, New York*

Accepted for publication June 12, 2023.

Address correspondence to Hanna Rennert, Ph.D., Department of Pathology and Laboratory Medicine, Weill Cornell Medicine, 525 E. 68th St., New York, NY 10065.
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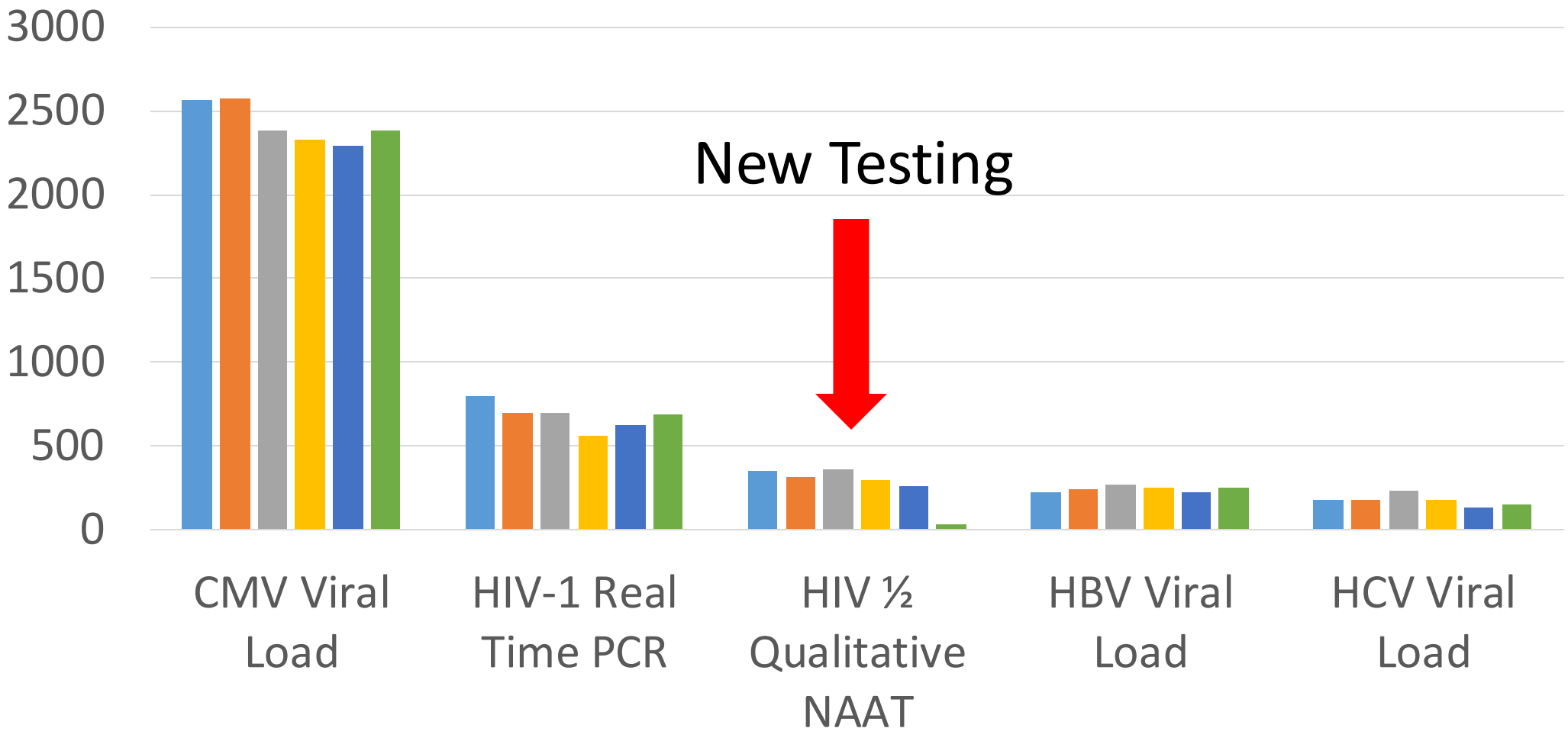
An epidemic caused by an outbreak of mpox (formerly monkeypox) in May 2022 rapidly spread internationally, requiring an urgent response from the clinical diagnostics community. A detailed description of the clinical validation and implementation of a laboratory-developed real-time PCR test for detecting nonvariola *Orthopoxvirus*-specific DNA based on the newly designed RealStar Zoonotic Orthopoxvirus assay is presented. The validation was performed using an accuracy panel ($n = 97$) comprising skin lesion swabs in universal transport media and from mpox virus genomic DNA spiked into pooled mpox virus—negative remnant universal transport media of lesion specimens submitted for routine clinical testing in the NewYork-Presbyterian Hospital clinical laboratory system. Accuracy testing demonstrated excellent assay agreement between expected and observed results and comparable diagnostic performance to three different reference tests. Analytical sensitivity with 95% detection probability was 126 copies/mL, and analytical specificity, clinical sensitivity, and clinical specificity were 100%. In summary, the RealStar Zoonotic Orthopoxvirus assay provides a sensitive and reliable method for routine diagnosis of mpox infections. *(J Mol Diagn 2023, 25: 740—747; https://doi.org/10.1016/j.jmoldx.2023.06.010)*

# A recent addition…Mpox testing EUA

- Xpert Mpox* test, authorized for use under FDA Emergency Use Authorization (EUA)
- Lesion swab specimens
- 36 minute run time
- Clade II-specific call out where positive specimens are excluded from select agent program requirements

# HIV-1 and HIV-2 Qualitative NAAT

# NEED OTHER NUMBERS: Total viral load numbers: May-October 2024



# *Neisseria gonorrhoeae* Culture

- Fastidious organism and susceptible to cold
  - In general, poor yield
- Requires special media for isolation (e.g. Thayer-Martin agar) and CO2 incubation
- Collection and transport to lab ASAP (<2 hrs ideal)


**So why do it at all??**

**Antibiotic susceptibility results**

https://microbeonline.com/chocolate-agar-composition-uses-colony-characteristics/

# Point-of-Care Testing for Sexually Transmitted Infections

## A Review of Recent Developments

*Paul C. Adamson, MD, MPH; Michael J. Loeffelholz, PhD; Jeffrey D. Klausner, MD, MPH*

• *Context.*—Sexually transmitted infections (STIs) are among the most common communicable diseases globally and are associated with significant morbidity and mortality worldwide. Point-of-care tests have the potential to revolutionize the prevention and control of STIs by enabling rapid diagnosis and early treatment of infections, thus interrupting transmission and preventing the sequelae of untreated infections. Currently, there are several point-of-care (POC) tests available for the diagnosis of *Treponema pallidum*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* infections, although these tests differ with regard to their performance, turnaround time, and cost.

*Objective.*—To provide an updated review of the POC tests available and under development for the diagnosis of *T pallidum*, *C trachomatis*, *N gonorrhoeae*, and *T vaginalis* infections, to discuss the context for which these tests might be used, and to highlight future directions for test development.

*Data Sources.*—We reviewed the literature pertaining to the recent development and performance evaluations of POC tests for the diagnosis of syphilis, chlamydia, gonorrhea, and trichomonas.

*Conclusions.*—Recently, there has been rapid development of new POC tests for STIs. Although there are inexpensive, rapid, and accurate POC tests available for syphilis, there are few such tests available for the diagnosis of chlamydia, gonorrhea, or trichomonas, and currently none with the ability to detect antimicrobial resistance in *N gonorrhoeae*. Research evaluating implementation strategies for the currently available tests and the development of additional POC tests that are rapid, accurate, and affordable are urgently needed to address the rising number of STIs worldwide.

(*Arch Pathol Lab Med.* 2020;144:1344–1351; doi: 10.5858/arpa.2020-0118-RA)

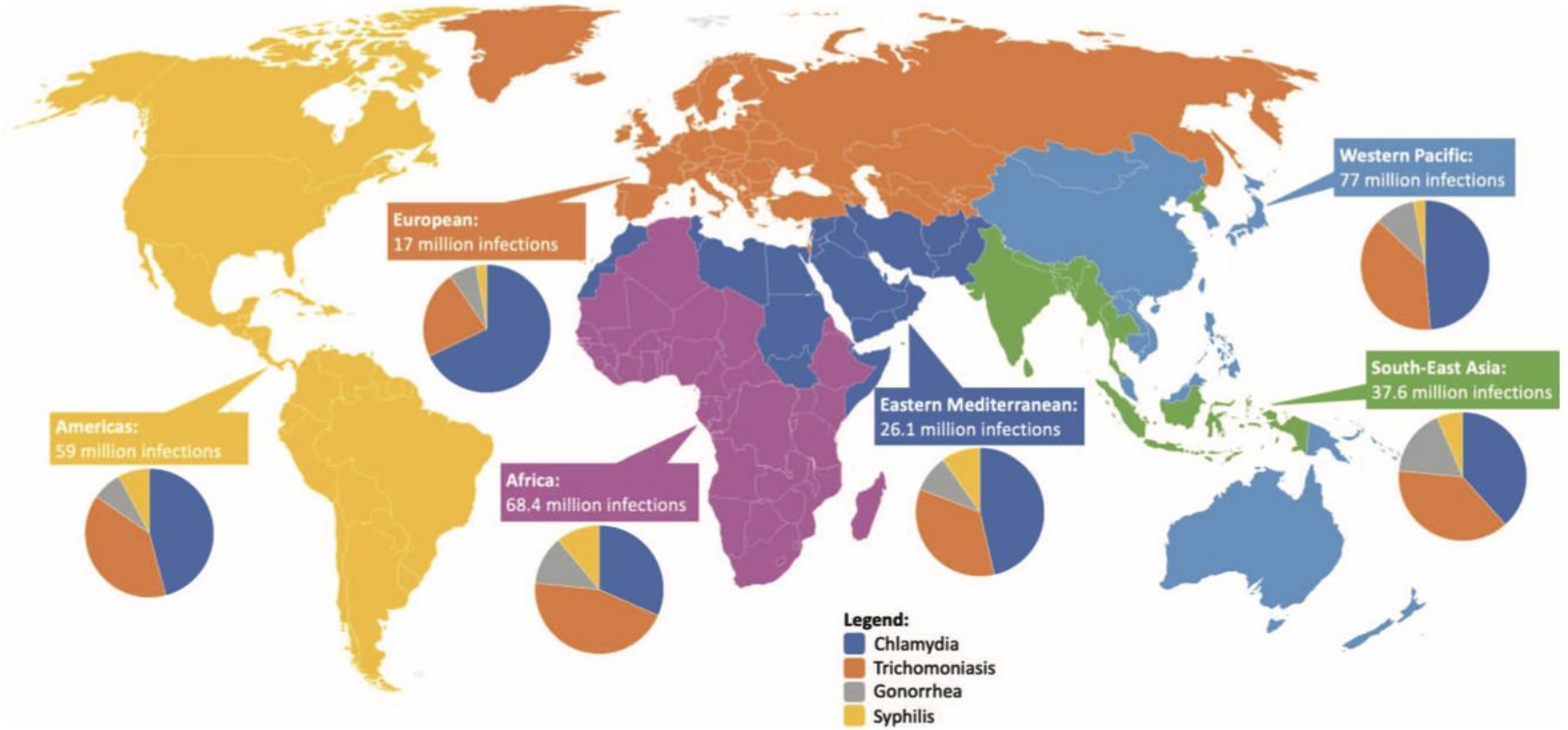



**Figure 1.** *Total number and distribution of new syphilis, gonorrhea, chlamydia, and trichomoniasis infections worldwide in 2016, by World Health Organization Region.*[62]

| ASSURED Criteria Created by the World Health Organization's Special Program for Research and Training in Tropical Diseases to Guide the Development of Point-of-Care Tests | | |
|---|---|---|
| | **Criterion** | **Description** |
| A | Affordable | Tests are affordable to the health system and individuals using the test |
| S | Sensitive | To avoid false negatives |
| S | Specific | To avoid false positives |
| U | User-friendly | Simple to perform, with minimal steps and requiring minimal training |
| R | Rapid and robust | Rapid to enable same-visit treatment<br>Robust to withstand diverse transportation and storage conditions without refrigeration |
| E | Equipment-free | Does not require additional equipment for collection or for processing |
| D | Delivered to end users | Accessible to end users |

POCTs for Sexually Transmitted Infections—Adamson et al

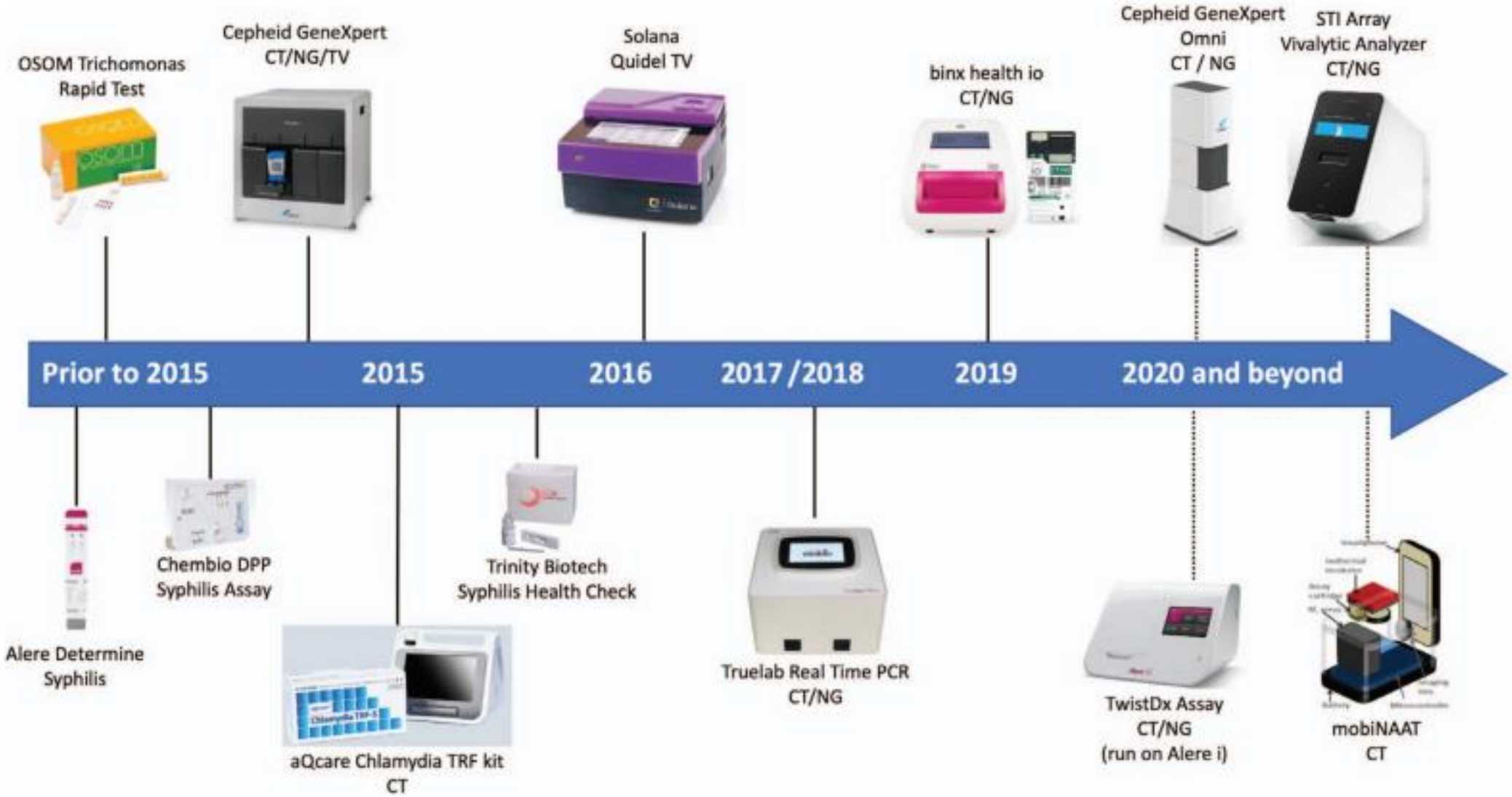



**Figure 3.** *Point-of-care tests, available and under development, for the diagnosis of syphilis, chlamydia, gonorrhea, and trichomoniasis. Dotted lines indicate tests that are not yet commercially available. Figure adapted from prior report and used with author's permission.*[42] *Abbreviations: CT,* Chlamydia trachomatis*; NG,* Neisseria gonorrhoeae*; PCR, polymerase chain reaction; TV,* Trichomonas vaginalis.

# Developments in “Near Patient” Testing

FDA NEWS RELEASE

## FDA Allows for First Point-of-Care Chlamydia and Gonorrhea Test to be Used in More Near-Patient Care Settings

Share | Tweet | Linkedin | Email | Print

**For Immediate Release:** March 30, 2021

English

Today, the U.S. Food and Drug Administration announced it is allowing the use of the Binx Health IO CT/NG Assay at point-of-care settings, such as in physician offices, community-based clinics, urgent care settings, outpatient health care facilities and other patient care settings, operating under a CLIA Certificate of Waiver, Certificate of Compliance or Certificate of Accreditation. This action is the result of the FDA granting a waiver under the Clinical Laboratory Improvement Amendments of 1988 ("CLIA") for the Binx Health IO CT/NG Assay.

“The ability to diagnose at a point-of-care setting will help with more quickly and appropriately treating sexually-transmitted infections, which is a major milestone in helping patients,” said Tim Stenzel, M.D., Ph.D., director of the Office of In Vitro Diagnostics and Radiological Health in the FDA’s Center for Devices and Radiological Health. “More convenient testing with quicker results can help patients get access to the most appropriate treatment. According to the CDC, one in five Americans are diagnosed

## CLIA Waiver by Application Approval Determination

### Decision Summary

**A. Document Number**

CW200003

**B. Parent Document Number**

K200748

**C. CLIA Waiver Type:**

Dual 510(k) and CLIA Waiver by Application (Dual Submission)

**D. Applicant**

Visby Medical

**E. Proprietary and Established Names**

Visby Medical Sexual Health Click Test

**F. Measurand (analyte)**

*Chlamydia trachomatis* DNA,
*Neisseria gonorrhoeae* DNA, *and*
*Trichomonas vaginalis* DNA

**G. Sample Type(s)**

Female Vaginal Swabs (self-collected in healthcare settings)

**H. Type of Test**

Qualitative, Polymerase Chain Reaction (PCR)

*Original research article*

INTERNATIONAL JOURNAL OF STD & AIDS

International Journal of STD & AIDS
2016, Vol. 27(14) 1275–1282

DOI: 10.1177/0956462415615775
std.sagepub.com
SAGE

# Multiplex PCR testing for nine different sexually transmitted infections

**John D Kriesel[1], Amiteshwar S Bhatia[1], Cammie Barrus[2], Mike Vaughn[3], Jordan Gardner[4] and Robert J Crisp[3]**

## Abstract

Current sexually transmitted infection (STI) testing is not optimal due to delays in reporting or missed diagnoses due to a lack of comprehensive testing. The FilmArray® (BioFire Diagnostics, LLC, Salt Lake City, Utah) is a user-friendly, fully automated, multiplex PCR system that is being developed for rapid point-of-care use. A research-use-only STI panel including multiple PCR primer sets for each organism was designed to detect *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *Trichomonas vaginalis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, *Haemophilus ducreyi*, and herpes simplex virus (HSV) types 1 and 2. Standard clinical testing included Gram stain, nucleic acid amplification, wet mount examination, herpes simplex virus culture, and syphilis IgG. Standard clinical tests were not available for all the organisms tested by the FilmArray STI panel. Two hundred and ninety-five clinical specimens from 190 subjects were directly compared to standard testing. Urine ($n = 146$), urethral/cervical swabs (31), oral swabs (60), rectal swabs (43), and ulcer swabs (15) were tested. Among the tested samples, FilmArray detected *C. trachomatis* in 39 (13%), *N. gonorrhoeae* in 20 (7%), *T. vaginalis* in nine (3%), HSV 1 in five (2%), HSV 2 in five (2%), *U. urealyticum* in 36 (12%), *M. genitalium* in eight (3%), and *T. pallidum* in 11 (4%). Concordance between the FilmArray STI panel and standard nucleic acid amplification testing for *C. trachomatis* was 98% and for *N. gonorrhoeae* was 97%. Multiplex PCR STI testing has the potential to improve public health by providing rapid, sensitive, and reliable results within the clinic or nearby laboratory.

## Keywords

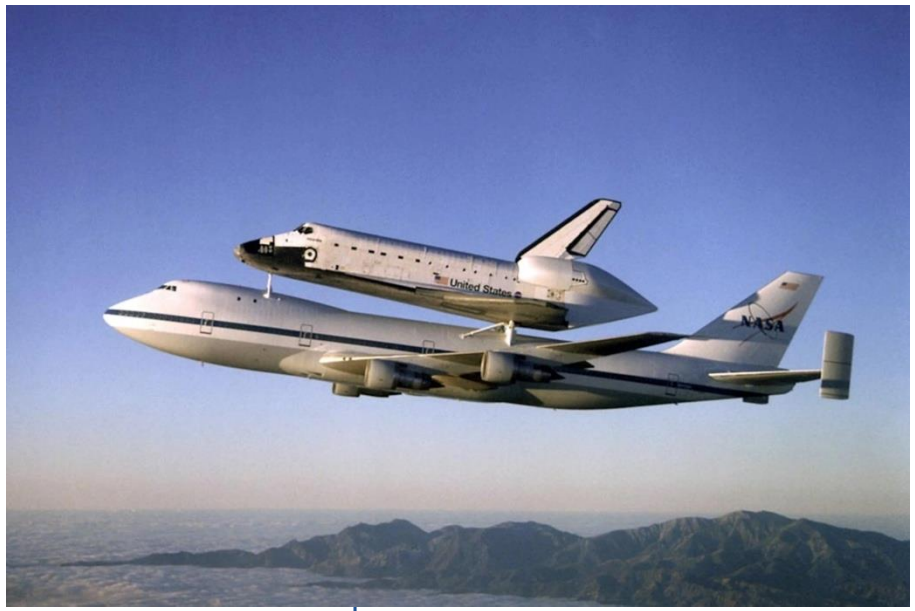
FilmArray, sexually transmitted diseases, sexually transmitted infections, STI, diagnostic test performance, multiplex PCR

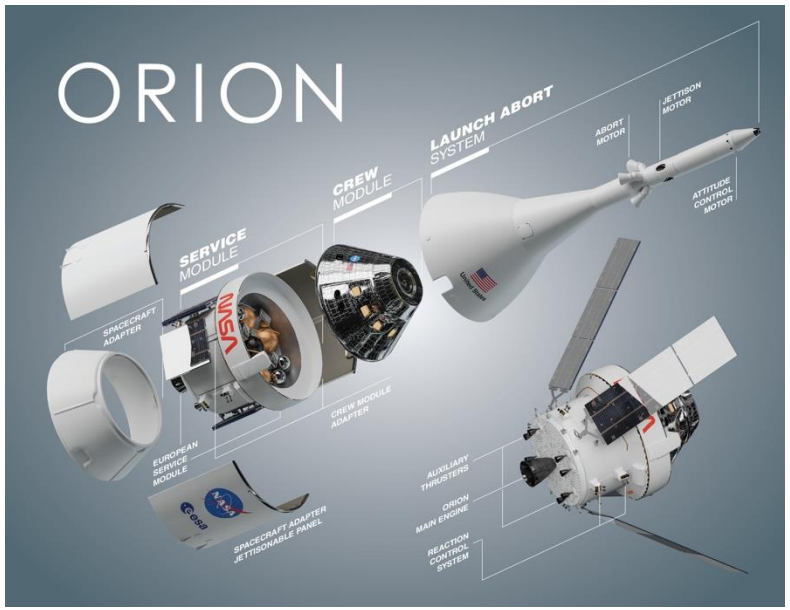
Date received: 24 June 2015; accepted: 14 October 2015

**Table 4.** Results for specimens (by type) tested by the FilmArray STI panel. Two hundred and ninety-five specimens from 190 subjects were selected for testing on the FilmArray device. The table header shows the total number of samples tested for each specimen type. The table body shows the number of tested specimens that were positive by the FilmArray.

| Organism | Urine $n=146$ | Urethral/ | Ulcer swab $n=15$ | Total $n=295$ |
|---|---|---|---|---|
| *Chlamydia trachomatis* | 23 | 5 | 0 | 39 |
| *Neisseria gonorrhoeae* | 9 | 1 | 0 | 20 |
| *Treponema pallidum* | 2 | 0 | 5 | 11 |
| *Trichomonas vaginalis* | 5 | 3 | 0 | 9 |
| HSV1 or HSV2 | 5 | 1 | 1 | 10 |
| *Mycoplasma genitalium* | 5 | 1 | 0 | 8 |
| *Ureaplasma urealyticum* | 9 | 9 | 0 | 36 |
| *Haemophilus ducreyi* | 0 | 0 | 0 | 0 |
| Total | 58 | 20 | 6 | 133 |

CANCELLED

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https://www.nationalgeographic.com/science/article/space-shuttle-program
https://www.nasa.gov/feature/meet-nasa-s-orion-spacecraft/

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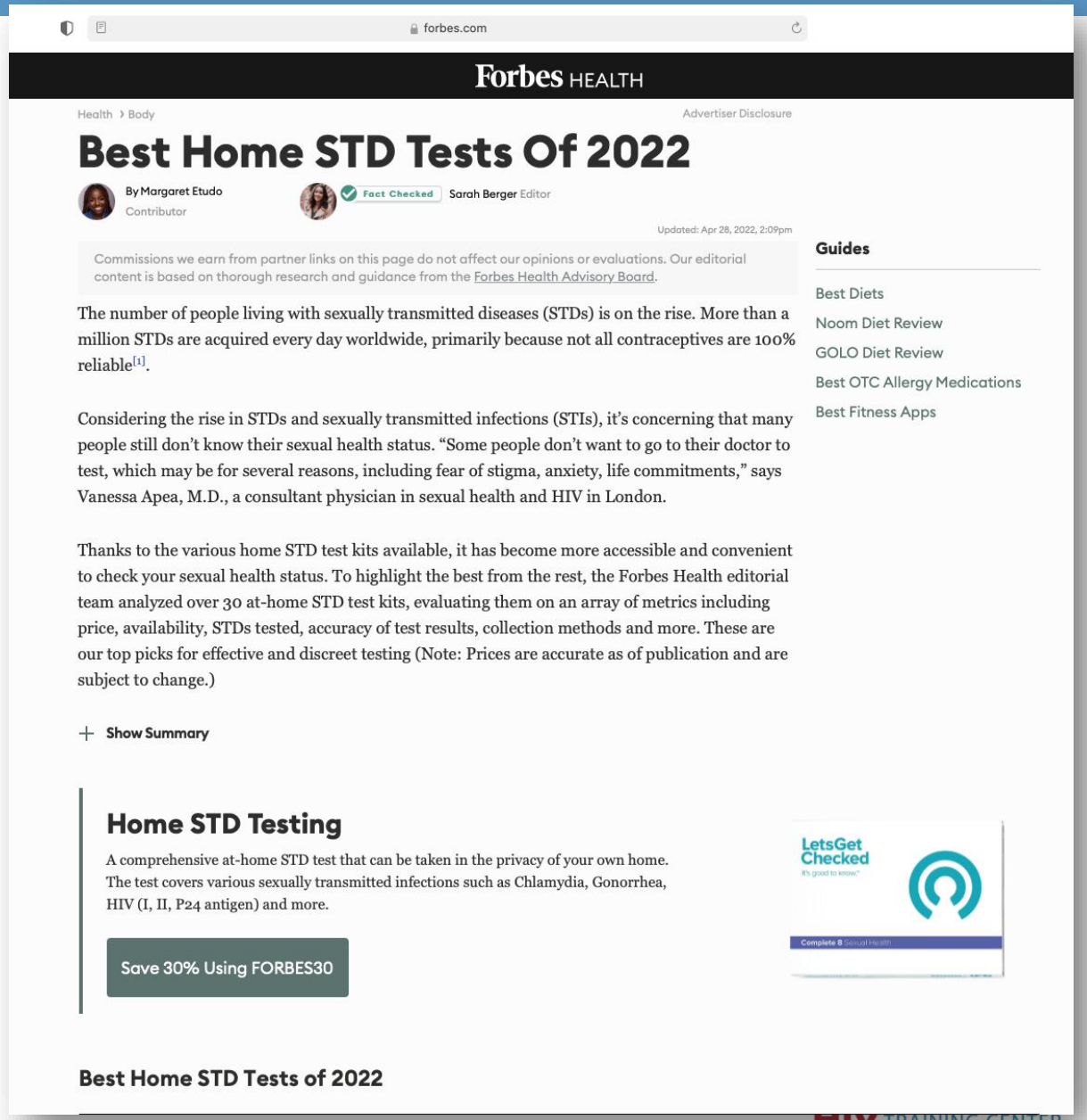
# Alternate Future of STI testing?

- Does “Home Testing” mean POC, or something else??
- Convenient (and discreet) vs. Rapid



# What's Missing?

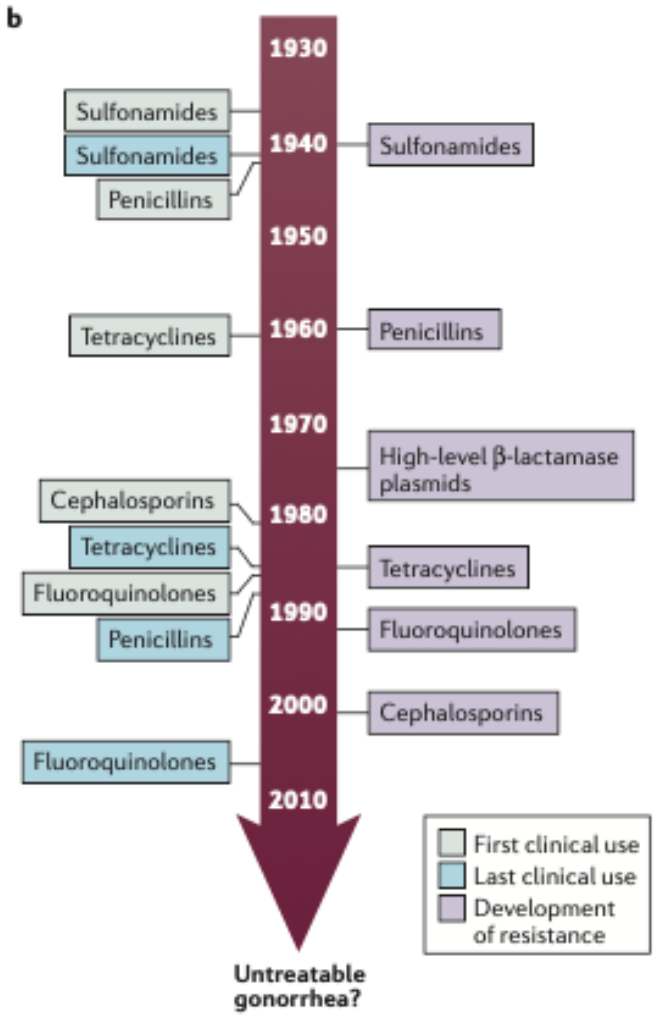
These assays all give an ID, but where's my AST result??
More importantly…why do I care?!?

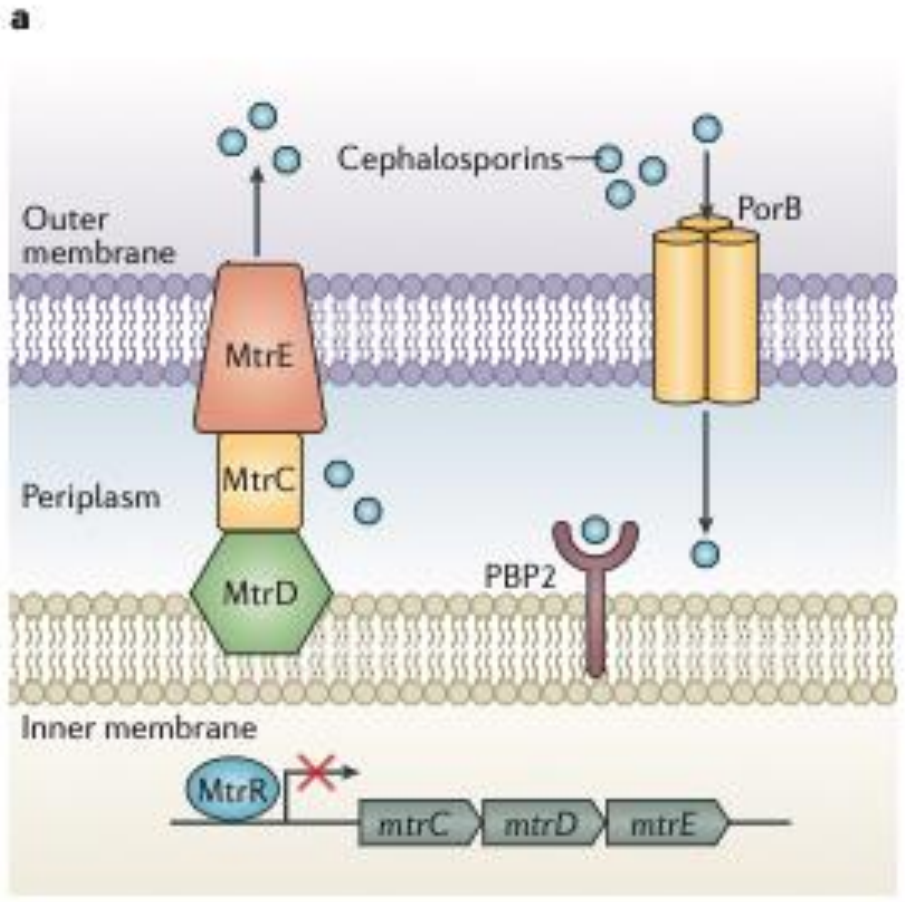
# Multi-drug resistant *Neisseria gonorrhoea*

- Antimicrobial resistance in gonorrhoea has increased rapidly in recent years and has reduced the options for treatment.
- Eighty-two million new cases of gonorrhoea occurred in 2020.
- Most gonorrhea cases in 2020 were in the WHO African Region and the Western Pacific Region.
- Most people affected are aged 15–49 years.

https://www.who.int/news-room/fact-sheets/detail/multi-drug-resistant-gonorrhoea

# Antibiotic resistance in *Neisseria gonorrhoeae*

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Quillin, S., Seifert, H. *Neisseria gonorrhoeae* host adaptation and pathogenesis. *Nat Rev Microbiol* **16**, 226–240 (2018). https://doi.org/10.1038/nrmicro.2017.169

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OPEN ACCESS

SHORT REPORT

# Low gonorrhoea antimicrobial resistance and culture positivity rates in general practice: a pilot study

Maartje Visser ,[1] Mireille van Westreenen,[2,3] Jan van Bergen,[1,4,5] Birgit H B van Benthem[1]

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/sextrans-2019-054006).

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## ABSTRACT

**Objective** In the Netherlands, the Gonococcal Resistance to Antimicrobials Surveillance (GRAS) programme is carried out at Centres for Sexual Health (CSH), which provide care for sexual high-risk populations. However, half of gonorrhoea infections are diagnosed in general practice (GP). We performed a pilot study to explore expanding GRAS to GPs using laboratory-based surveillance. Additionally, antimicrobial resistance patterns of GP and CSH patients were compared.

**Methods** Three laboratories from different regions were included, which all perform gonorrhoea diagnostics for GPs and used [illegible] surveillance. Additional

**Results** During the study period, 484 samples were put in culture. 16.5% of cultures were positive (n=80).

in CSH GRAS data (first half of 2018) were 19.2% for azithromycin, 31.5% for ciprofloxacin, 1.9% for cefotaxime and 0.0% for ceftriaxone.

**Conclusions** Culture positivity rates for GP patients were low, probably due to long transportation times and awaiting PCR test results before attempting culture. Positivity rates might be improved by making changes in sampling and/or transportation methods, but that would require involvement of GPs and patients instead of keeping the surveillance lab based. Resistance levels appeared to be lower at GPs than at the CSH, indicating that resistance might emerge first in more high-risk populations. It is important to consider all potentially relevant patient populations when establishing a gonococcal antimicrobial resistance surveillance programme. However, based on the findings from this study the current GRAS programme will not be extended to GPs.

Netherlands,[2] but has been seen in other countries, including the UK.[3]

To monitor gonorrhoea antimicrobial resistance in the Netherlands, the Gonococcal Resistance to Antimicrobials Surveillance (GRAS) programme was established in 2006. GRAS is a sentinel surveillance system including 18 out of 24 Centres for Sexual Health (CSH). However, more than half of gonorrhoea diagnoses in the Netherlands are carried out in general practice (GP) (in 2016: 6092 CSH diagnoses vs approximately 9000 GP diagnoses).[2] Thus, many patients are not included in GRAS. Currently, it is unknown to what extent

but it is known that, for example, extragenital testing is less often performed by GPs.[4] Culture is also not routinely performed, but is necessary to determine antimicrobial susceptibility. Because it requires more effort from GPs and their patients to collect additional samples for culturing, we first wanted to explore implementation of a laboratory-based surveillance that requires no additional sample collection. Therefore, the primary goal of this pilot study was to explore the feasibility of a laboratory-based GP surveillance of gonococcal antimicrobial resistance. Additionally, we aim to describe antimicrobial resistance patterns of patients with gonorrhoea in GP, and compare these to patterns of CSH patients.

METHODS

Published in final edited form as:
*Sex Transm Dis*. 2018 May ; 45(5): 312–315. doi:10.1097/OLQ.0000000000000755.

# Molecular characterization of markers associated with antimicrobial resistance in *Neisseria gonorrhoeae* identified from residual clinical samples

**Johan H. Melendez, MS, PhD**[1,2,*], **Justin Hardick, MS**[1], **Mathilda Barnes, MS**[1], **Perry Barnes, MSPM**[1], **Christopher D. Geddes, PhD**[2], and **Charlotte A. Gaydos, MS, DrPH**[1]

[1]Johns Hopkins Medical Institutions, Baltimore, Maryland [2]Institute of Fluorescence, Department of Chemistry and Biochemistry, University of Maryland Baltimore County, Baltimore, Maryland

## Abstract

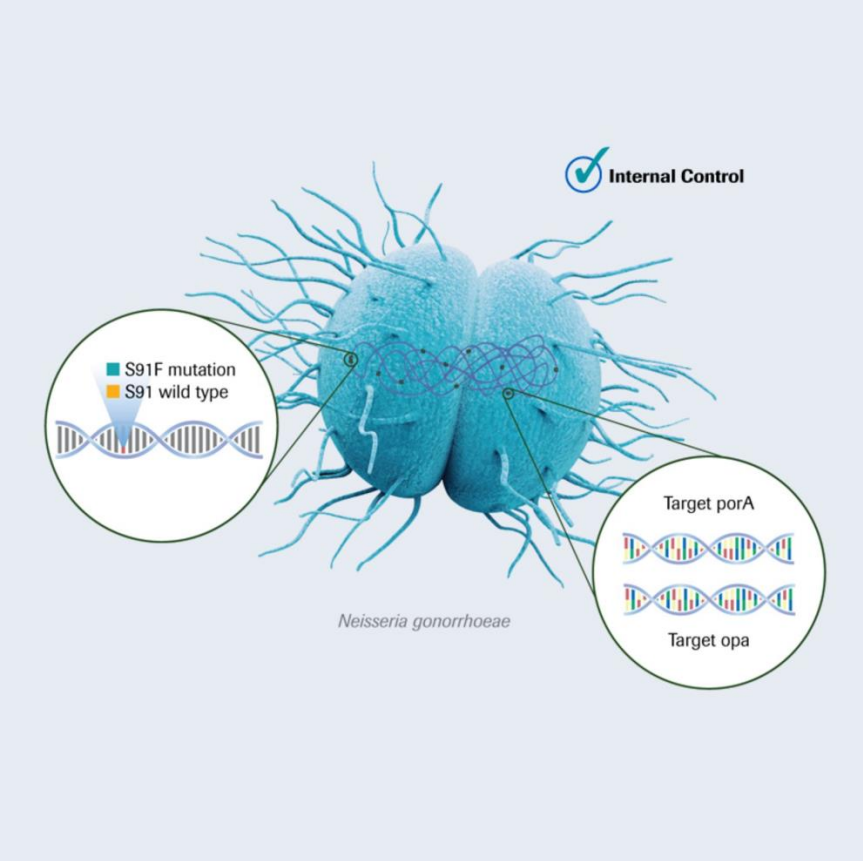
**Background—**The emergence and spread of antimicrobial-resistant (AMR) *Neisseria gonorrhoeae* (NG) is a major public health concern. In the era of nucleic acid amplifications tests (NAATs), rapid and accurate molecular approaches are needed to help increase surveillance, guide antimicrobial stewardship, and prevent outbreaks.

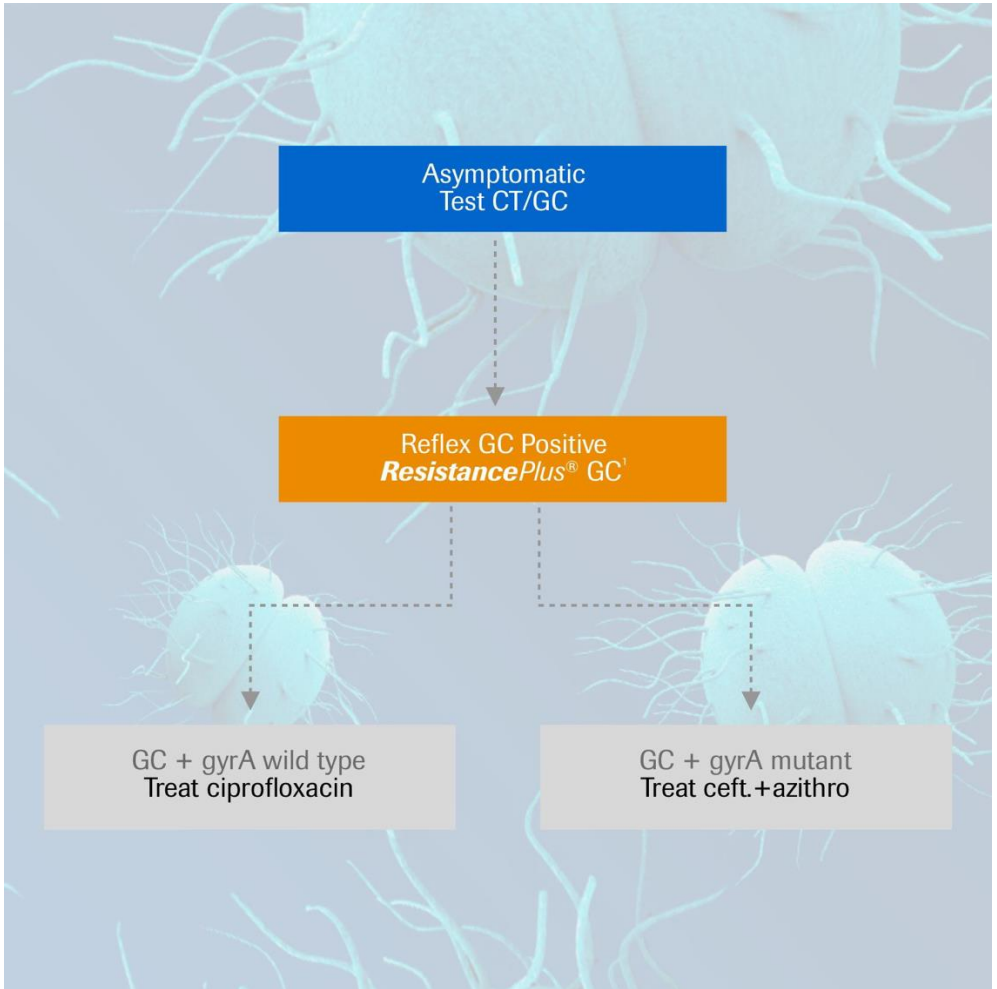
**Methods—**Residual urethral swabs, collected prospectively in the Baltimore City Health Department during a six-month period, were analyzed by real-time PCR assays for NG DNA and AMR determinants to fluoroquinolones, penicillin, and extended-spectrum cephalosporins (ESCs).

**Results—**NG DNA was detected in 34.8% (73/210) of samples, including 67.3% (68/101) of the swabs which had been previously identified as NG-positive by culture. Markers associated with decreased susceptibility to fluoroquinolones were detected in 22.4% of the PCR NG-positive samples. The rate of penicillinase-producing *Neisseria gonorrhoeae* (PPNG) was very low (1.6%) and no markers associated with decreased susceptibility to ESCs were detected in this cohort of men using the AMR assays herein described.

**Conclusions—**Detection of molecular markers associated with AMR in NG can be performed directly from residual clinical samples, even though the recovery rate of adequate DNA for molecular testing from these samples can be sub-optimal. A high number of samples with mutations associated with decreased susceptibility to fluoroquinolones were identified.

# *N. gonorrhoeae* resistance testing




**Registration status: CE-IVD, not yet available in the U.S.**

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https://diagnostics.roche.com/global/en/products/params/resistanceplus-gc.html

# Antibiotic Resistant *Mycoplasma genitalium*

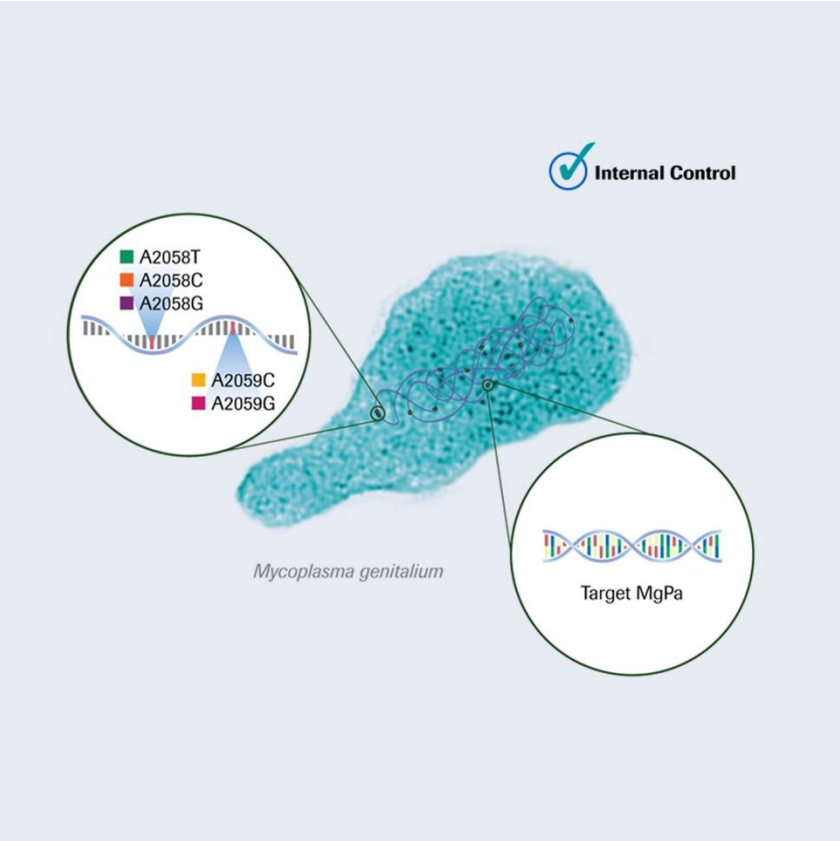
- *M. genitalium* cases are on the rise in both men and women

- Resistance to azithromycin rapidly increasing

- Molecular markers for macrolide resistance strongly correlate with treatment failure
  - Studies have shown prevalence ranging from 44% to 90% in the United States, Canada, Western Europe, and Australia

https://www.cdc.gov/std/treatment-guidelines/mycoplasmagenitalium.htm

# *Mycoplasma genitalium* resistance testing




- detection of 5 mutations in the 23S rRNA gene (A2058G, A2059G, A2058T, A2058C, and A2059C) associated with resistance to azithromycin

**Registration status: CE-IVD, not yet available in the U.S.**

https://diagnostics.roche.com/global/en/products/params/resistanceplus-mg.html

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# *Mycoplasma genitalium* resistance testing


- Detection of 4 mutations in the 23S rRNA gene (A2058G, A2059G, A2058T, A2058C) associated with resistance to azithromycin

***ResistancePlus*® MG FleXible is validated on a wide range of sample types including rectal, male and female urine, and common collection swab kits including Xpert® CT/NGVaginal/Endocervical Specimen Collection kit and Xpert® CT/NG Urine Specimen Collection Kit.****

**CE-IVD *in Vitro* Diagnostic Medical Device. Not available in the U.S.**

https://www.cepheid.com/en/tests/Sexual-Health/SpeeDx-RPMG-FleX

# What is in the pipeline?

# Diagnostic Summary- STI testing

- Molecular techniques have largely replacing traditional culture-based ID
  - Dramatically increased sensitivity, turnaround time
- Trend toward near patient testing will continue
- There is a critical need for continued development and implementation of molecular-based antimicrobial resistance testing to combat antibiotic resistance.

# Acknowledgments

Clinical Microbiology Service at CUMC

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- Dr. Daniel Green
- Aisha Diallo and Thanh Ly

# Thank you!