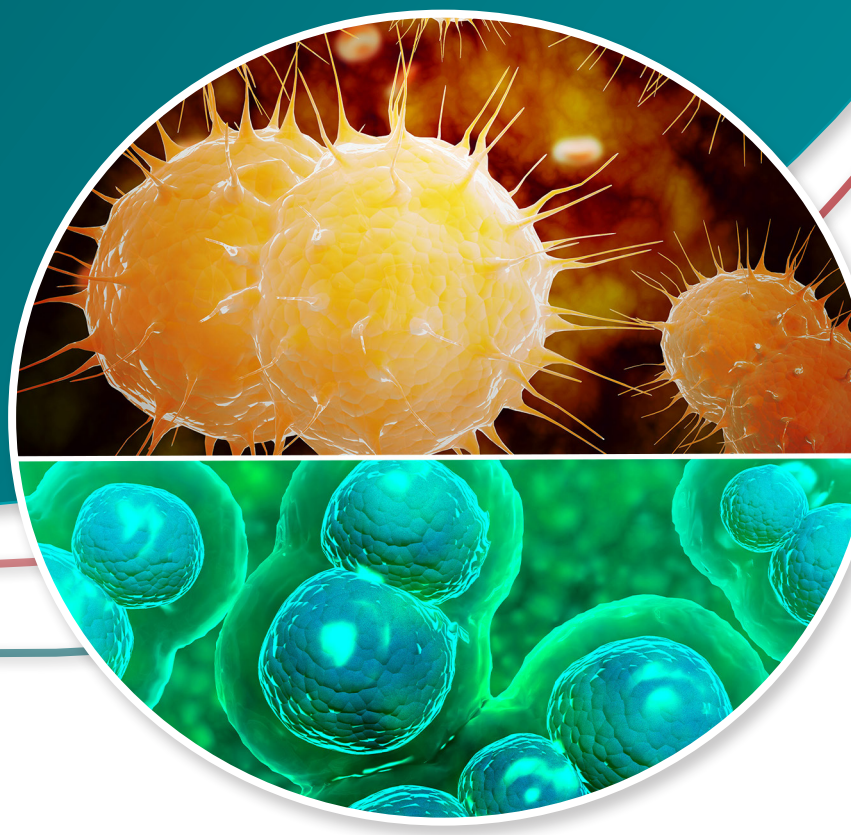
Meeting Summary Report

Laboratory Testing of *Chlamydia trachomatis* and *Neisseria gonorrhoeae:* A Review of the Literature

October 29-30, 2024



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Abbreviations

APHL	Association of Public Health Laboratories
AST	Antimicrobial Susceptibility Testing
BMD	Broth Microdilution
CLIA	Clinical Laboratory Improvement Amendments
CLSI	Clinical & Laboratory Standards Institute
CT	*Chlamydia trachomatis*
DSTDP	Division of Sexually Transmitted Disease Prevention
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	US Food and Drug Administration
GC	*Neisseria gonorrhoeae*
KQ	Key question
LDT	Laboratory-developed test
LGV	Lymphogranuloma venereum
MALDI-TOF	Matrix-assisted laser desorption/ionization time-of-flight
MIC	Minimum inhibitory concentration
MS	Mass spectrometry
MSM	Men who have sex with men
NAAT	Nucleic acid amplification testing
NG	*Neisseria gonorrhoeae*
NGS	Next generation sequencing
PCR	Polymerase chain reaction
POC	Point of care
POCT	Point of care testing
QC	Quality control
SOC	Standard of care
STI	Sexually transmitted infection
SME	Subject matter expert
WGS	Whole genome sequencing

Executive Summary

The United States has faced an epidemic of sexually transmitted infections (STI) as rates of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) surged until recently. The latest published data shows that in 2024 over 2.2 million STIs were reported in the US.[1] GC infections fell below pre-pandemic levels in 2024, with a drop of 10% from 2023, and CT infection rates fell as well with an 8% decline from 2023.[1] Despite the decrease in GC infections, there are still over 500,000 cases per year and antibiotic-resistant GC threatens our ability to treat the infection. Consequently, there is a continued need for improved detection and management strategies. Since the previous publication of recommendations for laboratory-based detection of CT and GC in 2014,[2] advances in diagnostic research, technologies and products have occurred. This happened in parallel with changes in the treatment and prevention landscape, including evolving treatment guidelines, and the advent of doxycycline post-exposure prophylaxis (doxy-PEP).[3,4] In light of these changes, the current state of laboratory diagnostics for GC and CT was reviewed to inform updates to best practice and future directions.

The US Centers for Disease Control and Prevention (CDC) partnered with the Association of Public Health Laboratories (APHL) to examine current capabilities, best practices, and challenges for CT and GC diagnostics in the US. A structured literature review was conducted by external experts, culminating in a two-day consultation, held October 29-30, 2024, in Decatur, GA. The proceedings were guided by key questions (KQ) whose answers and implications are documented in this meeting report for each of five different main topic areas.

Summary points across these topics are outlined here (and further detailed by topic in the body of the report that follows):

CT Resistance, Culture and Identification

Summarized Findings

Culture and Identification

Previously described recommendations[2] accurately inform CT culture and identification as best practice is unchanged.

When to Culture

CT culture could be maintained to monitor antimicrobial susceptibility and support research and surveillance activities.

Antimicrobial Susceptibility Testing

- There is not yet sufficient evidence to support the use of molecular antimicrobial susceptibility testing (AST) to guide therapy.
- AST methods lack standardization for CT.

Serological Testing

The use of serological testing for CT diagnosis is unsupported by the literature.

Future Directions and Research Needs

- In the era of doxy-PEP, broader consideration of CT surveillance may be warranted.

- Demand for CT culture and/or AST may increase in the future.
- Culture-independent sequencing methods, including target amplification/enrichment prior to sequencing, could be used to monitor for diagnostic evasion or antimicrobial resistance.
- Viability assays could help reduce unnecessary antibiotic use; the commercial development of effective viability assays for clinical use is encouraged.

GC Culture and Identification

Summarized Findings

Culture

- GC is a fastidious organism, but effective collection and transport options are available. Methods can be selected based on product capabilities, typical transit times and provider capabilities/practices.
- Patient-collected specimens are acceptable for GC culture.
- Cotton-tipped swabs are acceptable for GC culture collection.
- Cold storage and transport of specimens for GC culture may be advantageous, provided that temperature specifications are met per the manufacturer's package insert.
- Specific specimen collection techniques improve the GC recovery rate from the oropharynx.
- Consideration of GC when working up urine cultures is warranted.
- Previous recommendations[2] adequately describe the media that can be used to culture GC (e.g., selective media such as Thayer-Martin or Martin-Lewis for specimens from non-sterile sites and chocolate agar from sterile sites).

Biochemical Tests

Complete evaluation including colony morphology, Gram stain and appropriate biochemicals are essential when working up a suspected GC isolate.

Matrix Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) Mass Spectrometry (MS)

- MALDI-TOF MS is a reliable method for identification of GC from culture, and secondary confirmation is not necessary. Attention to the US Food and Drug Administration (FDA) authorization status and reference libraries/databases is warranted when implementing MALDI-TOF MS for GC identification from culture.
- To obtain isolates for MALDI, subculture to chocolate agar performs well, but is non-selective. Ensure that a pure isolate is subcultured and worked up.
- Do not use plates after more than 48 hours.

When to Culture

- Culture can be performed when AST is warranted for any reason and for surveillance or epidemiological purposes.
- When culture is indicated, maximize likelihood of success by:
    - performing GC culture in the clinic when possible (bedside culture)
    - Implementing surveillance protocols that target:
        - patients with symptomatic urethritis and Gram-negative intracellular diplococci on urethral Gram stain
        - patients with gonococcal cervicitis\contacts to another person with diagnosed or suspected gonorrhea

- patients seeking treatment following a GC positive NAAT screening test (collect specimens as soon as possible following NAAT result)

Future Directions and Research Needs

- There is limited data on GC culture in pediatric populations and best practice for medico-legal testing.
- Isolation of GC from blood agar could be revisited, given that data from total laboratory automation systems shows increased recovery of GC from urine culture.
- Work to improve recovery rate of GC from extragenital sites is necessary.
- A GC culture-viability assay combination could resolve discrepancy between poor recovery of GC culture from extragenital sites and the more sensitive nucleic acid amplification tests (NAAT).
- Improvement of transport medium that maintains GC viability for at least seven days is sought.
- Reliable genotypic susceptibility testing methods are needed.
- As culture is increasingly outsourced, yet remains necessary for AST, a curated resource that maintains up-to-date information on laboratories with GC culture capacity in the US could be beneficial.

CT and GC Diagnostic Performance

Summarized Findings

Performance of NAATs

- Currently available assays are highly sensitive.
- False positive detections can occur due to many factors including: residual nucleic acid, detection of non-established infection, environmental contamination, cross reactivity with commensal *Neisseria* spp., and others. Communication between the laboratory and the clinician can guide interpretation via review of additional patient data (symptomology, sexual history, population level factors, etc.)
- FDA-cleared sample types now include oropharyngeal and rectal specimens

Self-Collection

- Vaginal swabs self-collected in a clinical setting are now FDA-cleared for use with many different tests.
- Self-collection of rectal and pharyngeal swabs is comparable to clinician-collection.
- There is extensive evidence that self-collection at home can be effective. FDA-authorized options exist for self-collection at home, but options are currently limited and regulatory challenges exist.

Lymphogranuloma venereum (LGV)

- There are no FDA-approved tests to specifically diagnose suspected LGV, but some laboratories offer laboratory developed tests (LDTs).
- Testing rectal sites among men who have sex with men (MSM) may be especially important to LGV detection.

Pooling

There is generally strong agreement between samples pooled across anatomical sites and single-site testing (≥86% across studies). However, these protocols are time consuming and not standardized.

Confirmatory Testing

- There is insufficient data to support routine use of confirmatory testing.

- When performed, confirmation ideally involves testing the same sample for presence of multiple and distinct pathogen targets (orthogonal testing). Use of the same sample may not be possible if multiple assays are used, as required specimen collection devices may differ.
- Assay targets are critical to understand and typically defined in package inserts / instructions for use (IFU).
- Throat/oropharyngeal specimens may require confirmation for GC due to commensal *Neisseria* species and cross reactivity.

Future Directions and Research Needs

- Ongoing surveillance efforts are needed to alert to assay performance limitations and guide assay updates due to emerging diagnostic escape variants.
- Viability assays are needed.
- Test of cure is addressed in CDC's STI Treatment Guidelines;[4] harmonization with future laboratory recommendations may be necessary.
- There remain gaps in research, testing guidance and FDA authorized methodology for neonate and pediatric testing.
- Clearer guidance for NAAT testing and result confirmation in sexual assault is needed. Consistency between the CDC guidelines and laboratory testing recommendations is necessary.
- There is a need for inclusion of LGV in genital ulcer disease panels.
- Pooling strategies across studies are variable; strong future research with well-designed studies conducted across multiple platforms could be especially valuable.
- There is a clear need for research to advance regulatory approval for self-collected specimens and self-testing. Coordination of device manufacturers and regulatory bodies to achieve a universal claim for specimen collection devices would be an ideal goal.
- A compilation of currently FDA-approved tests for GC and CT, assay targets, and approved specimen types in a web-based living document would be beneficial.

CT and GC Point of Care Diagnostic Testing

Summarized Findings

FDA-cleared NAATs for Point of Care Testing (POCT)

- There are currently three FDA-cleared NAAT POCTs for GC and CT (Cepheid GeneXpert® CT/NG, Visby Medical™ Sexual Health test CT, NG, & TV [CLIA-waived], and binx *io* CT/NG [CLIA-waived]).
- Overall, strong diagnostic performance has been demonstrated for these tests.
- Additional technologies will emerge in the near term, and flexible recommendations to address the evolving landscape are needed.
- Broad consideration of the ideal target product profile for a GC/CT POCT is merited.
- Studies conducted internationally and in low-resource areas may not be readily generalizable to other contexts in the US.

POCT Implementation Considerations

- Currently, the most strongly supported use case for CT/GC POCT is testing symptomatic patients in acute care settings (urgent cares and emergency departments).

- For sites seeking to implement POCT, a strong quality assurance program that includes appropriate training, competency assessments, proficiency testing, and quality controls should be in place to ensure accurate results. The PHL in the jurisdiction should be consulted when programs are seeking to establish POCT.

Future Directions and Research Needs

- POCT options continue to be relatively expensive, slower than may be necessary to have the desired impact on practice, and/or have a lot of waste generated from consumables. Continued improvement of these factors is needed.
- Cost effectiveness modeling and further studies to examine the impact of POCT turnaround time are needed.
- There is a lack of metadata provided in many studies to assess the true utility of tests (missing chart info, unknown patient outcomes, lacking information on other testing conducted).
- POCT with FDA-clearance for multiple sample types to enable three-site testing (genital, rectal, oropharyngeal) is needed. There is currently no three-site testing available in a CLIA-waived instrument.
- Approaches to unified data reporting are needed.
- Future research will need to consider the impact of over-the-counter direct-to-consumer tests on the diagnostic market and its relationship to POCT

Characterizing GC Resistance

Summarized Findings

AST Methodologies and Technologies

- Agar dilution remains the gold standard, though laborious and not feasible in all laboratories.
- Gradient and disk diffusion methods are effective for many drugs and less labor intensive than agar dilution.
- Use of broth microdilution is not supported by the literature.
- To optimize the success of culture and AST, consider that:
    - Culture recovery of GC is higher from urogenital sites than from extragenital sites and collection technique matters.
    - Media can alter results; laboratories should defer to the instructions for use (if applicable) and be aware that the most reliable method (in terms of Agar Dilution agreement) is to set up on GC agar over chocolate agar.
- Molecular methods for identifying GC resistance are not yet FDA authorized. However, there is strong evidence for molecular testing of ciprofloxacin susceptibility (*gyrA* gene mutation S91F), with published research displaying excellent correlation with phenotypic AST.

Next Generation Sequencing (NGS)

- There is substantial evidence that sequencing of GC isolates is worthwhile as an ongoing, widely-cast, surveillance effort to help track resistance.

When to Perform

- AST is useful for:
    - Cases of systemic/disseminated GC infection
    - Cases of suspected treatment failure
    - Cases of contact with known or suspected treatment failure
    - Surveillance

Future Directions and Research Needs

- Further characterization and standardization of gradient diffusion strips is needed, including evaluation of appropriate Clinical & Laboratory Standards Institute (CLSI) MIC based breakpoints.
- AST methods for emerging drugs are needed and harmonization with CLSI and/or EUCAST should be considered.
- Continuing research into mass spectrometry (MS) methods for GC AST is needed.
- Research to enable culture-independent sequencing is a priority.
- Advances in the use of NGS approaches for surveillance and development of molecular AST options need to be fostered.
- Molecular ciprofloxacin susceptibility testing (*gyrA* S91F mutation), either commercially as an FDA-authorized assay or as an assay that can otherwise be validated by laboratories under CLIA would be useful.
- Commercial development of collection devices that can be used for both diagnostics and resistance testing is also highly desirable.

Cross-cutting Considerations

Summarized Findings

Themes that arose repeatedly during the consultation included:

- Recommendations for laboratory testing should be harmonized with CDC's clinical guidelines.
- Research focused on understanding the impact of doxy-PEP utilization on diagnostic testing and antimicrobial resistance is needed.
- Recommendations should:
  - Offer clarity on what is FDA-authorized (and what is not).
  - Be actionable and reflect available testing (i.e., FDA-authorized tests) as well as consideration of possible LDT regulatory challenges.
  - Address gaps for additional critical populations including pediatric testing and testing in medicolegal cases (i.e., in instances of sexual assault).
  - Clearly state when practices are not recommended.
- Diagnostic stewardship is important and entails:
  - Consideration of who needs tests, how often, and how to test in differing populations.
  - Guiding laboratorians with information related to diagnostic testing that they may need to communicate to clinicians. What should a lab be prepared to communicate and how can recommendations help?
- Antibiotic stewardship is important and entails:
  - Striking a balance between clinical relevance and test sensitivity, while recognizing the risk of overtreatment when highly sensitive assays detect non-established infections.
  - Integrating viability assays and information on time to clearance of nucleic acid into considerations of appropriate testing (and treatment).
  - Advancing new AST approaches to improve resistance-guided therapy in GC.
- Ensuring proper training and biosafety precautions for laboratorians (and for individuals who may conduct CLIA-waived POCT) including:
  - Proper protective equipment, techniques, and waste disposal.
  - Consideration of the risks of encountering *Neisseria meningitidis*, and importance of the precaution of vaccination.

- Keeping in mind bottlenecks that may occur with supply chain issues or reductions in products for certain diagnostic tests:
    - How can manufacturers, federal agencies, and laboratories work together to strengthen supply chains?
    - Where can flexibility in recommendations allow laboratorians to better manage testing through shortages?
- Identifying ways to bank specimens from, and conduct research to improve diagnostics for, rarer case infections.
    - How can isolates from disseminated infection (blood stream, joints, endocarditis) be collected and studied? Is there a path for making these conditions notifiable and having isolates submitted for banking?
    - How can diagnostic detection of high-mortality infection such as endocarditis be improved?

Summary of Methodology

Review Process

In late 2023, the US Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) began planning an external literature review and consultation on laboratory testing for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC). First, key questions (KQ) were drafted to guide evaluation of the current state of diagnostic testing for these organisms. External experts were then identified and invited to participate in a literature review and consultation (Appendix A). The external expert reviewers were given the opportunity to vet and refine the KQ. For each topic comprising a set of related key questions, two to four external reviewers were selected and CDC expert support from the Division of Sexually Transmitted Disease Prevention (DSTDP) STD Laboratory Reference and Research Branch was assigned (Appendix A).

For each topic area, CDC experts defined associated terms for a comprehensive literature search. They worked with a CDC librarian, providing criteria for conducting the literature searches across multiple databases. Search terms were used to obtain citations for literature published from January 1, 2009, through January 8, 2024 (the day before the search). Resulting citations were imported into the Covidence review management platform, with CDC experts working to ensure access to full-text, electronic manuscripts. CDC experts, APHL staff and APHL contractors performed preliminary title and abstract reviews of the imported data to remove irrelevant references. External reviewers then used pre-designed exclusion and inclusion criteria (Appendix B) to conduct full-text reviews. Following full-text review, external experts extracted data using a uniform template, designed and piloted in Covidence. This data was compiled in tables of evidence (TOE). External reviewers then drafted summary documents, which were provided along with their TOEs to all attendees in advance of the consultation.

Consultation

Consultation participants were identified to bring together a range of perspectives and experiences. Attendees included representatives from public health, commercial and academic laboratories. Staff from the Office of the Director within the National Center for HIV, Viral Hepatitis, STD and Tuberculosis Prevention (NCHHSTP) were invited, as well as from the DSTDP (Office of the Director, STD Laboratory Reference and Research Branch, Behavioral Science and Epidemiology Branch, Clinical Economics and Health Services Research Branch, Program Development and Evaluation Branch, and Surveillance and Data Science Branch). Representatives from the National Institute of Allergy and Infectious Disease (NIAID; Enteric and Sexually Transmitted Infections Branch) also participated (Appendix C).

Attendees listened to KQ presentations by external expert reviewers. Presentations summarized the literature searches, major findings and gaps that need to be addressed. After each presentation, attendees asked clarifying questions and participated in facilitated discussions to provide feedback on the findings. They offered additional considerations or refinements to findings for each KQ based on their understanding of the literature, expert knowledge and their own laboratory experience. The goal of the discussion was not to build consensus, but to identify additional facts and considerations that had not been addressed during the presentations.

Report

Here we summarize the findings from the external experts' literature reviews, combined with the input of individual participants at the convening. For each topic, brief background and summary information is provided followed by a summary of discussion points related to each specific KQ and areas in which further research or diagnostic development should be focused to advance the field and address unmet needs. The findings represent those of the external reviewers, attendees at the meeting and APHL. Recommendations detailed here do not necessarily represent those of DSTDP, CDC or HHS.

Topic 1: CT Resistance, Culture and Identification

Literature review by: Alana Sterkel and Timothy Southern
Presented by: Alana Sterkel

Key Questions

KQ1. Have molecular resistance determinants been defined?

KQ2. Are there molecular methods that can be used to identify potential resistance determinants?

KQ3. Are there molecular methods that can be used for confirming CT viability when treatment failure is suspected?

KQ4. Are there standardized culture-based antimicrobial susceptibility testing (AST) methods for CT?

KQ5. Have culture methods for CT changed since they were described in 2009?

KQ6. What are the various serological tests for CT and what are their performance characteristics and diagnostic value?

KQ7. When should CT culture or resistance-based testing be performed?

Background and Summary

Generally, the CT culture field lags in development behind those of other pathogens, because of the challenges posed by working with intracellular organisms and because the utility of CT culture is low. A review of the literature indicated there has been only modest change to CT culture since previous recommendations (primarily iterative advancements in cell culture models). Progress is being made in identifying relevant molecular mechanisms of antibiotic resistance, but there is not substantial evidence to support the use of molecular AST to guide antibiotic therapy. However, the rapid expansion of next-generation sequencing (NGS) technologies and applications has positioned NGS as a potential growth area for CT characterization and resistance testing.

Reported clinical treatment failure rates for CT infection range from 5-23%.[11,12] However, very few confirmed cases of true resistance have been documented; detecting clinical cases associated with resistance is complicated by re-infections, poor compliance with therapy and inaccurate data on sexual histories. Indeed, consultation participants emphasized that, in the US, there have been no true confirmed cases of CT resistance to date. This critical context framed the discussion of CT-resistance.

Findings

KQ1. Have molecular resistance determinants been defined?

There are a few key genes and mutations recognized by the field as likely associated with CT resistance. There are many more predicted mutations, based largely on data from other organisms like *E. coli*, that remain hypothetical. However, molecular prediction of resistance is not always in alignment with the phenotypic, culture-based AST results for CT.

Primary antimicrobials used for treatment and their associated resistance related mutations have been reviewed: [13,14]

- Azithromycin: *rplD* gene in ribosomal protein L4, 23S rRNA (Peptidyl transferase) point mutations[15–17] and ribosomal protein L22 triple mutant.
- Doxycycline (first line therapy since 2021): Foreign genomic islands can be integrated into the chromosome (6-13.5 kb) encoding for antibiotic efflux pumps (*tetC*), regulatory repressor (*tetR*) and other genes involved in the replication and mobilization of the plasmid. These can be acquired from *C. suis*, which collects resistance more easily.[18] This kind of horizontal gene transfer is considered highly irregular in intracellular organisms and may be extremely rare for CT.
- Fluoroquinolones (note that Levofloxacin is the only fluoroquinolone effective for CT treatment): Point mutations in the *gyrA* gene quinolone resistance determining region (QRDR) (DNA gyrase),[19] and within the *parC* gene (topoisomerase IV) have been identified.[19] There may also be possible roles for drug efflux and permeability in fluoroquinolone resistance. Importantly, spontaneous mutations at the target site of DNA topoisomerase IV can occur through serial passage at subinhibitory concentrations.
- Rifampin: The most common mutations detected are in the *rpoB* gene (point mutations in the mid-portion). Other mutations that may contribute to rifampin resistance impact ribosylation, phosphorylation and glycosylation enzymes, and the activity of efflux pumps.

While ongoing research continues to provide insight into molecular determinants of resistance, there is not yet sufficient evidence to support resistance guided therapy. It must also be reemphasized that there has not been a true confirmed case of resistant CT in the US.

KQ2. Are there molecular methods that can be used to identify potential resistance determinants?

Targeted polymerase chain reaction (PCR) methods exist but are not FDA authorized and have limited ability to detect all mechanisms of resistance. A variety of sequencing and bioinformatics methods have been successfully used to detect resistance mutations, though they have not been standardized. These include culture amplification prior to sequencing,[12,20] PCR amplification prior to sequencing by Sanger,[15,21,22] bait and capture prior to sequencing by Sanger or NGS,[23] and directNGS from a positive specimen.[24]

NGS has significant promise for identifying resistance determinants, but it was discussed that successful sequencing that is representative of the serovars present in infection remains challenging (particularly from rectal swabs). Emphasis should be placed on strategies using amplification/enrichment prior to sequencing.

Ultimately, advances are being made in identifying molecular determinants of resistance, but evidence is insufficient to make a recommendation for the use of molecular AST to support clinical decision making.

KQ3. Are there molecular methods that can be used for confirming CT viability when treatment failure is suspected?

There was a lack of evidence identified in the literature search related to diagnostic laboratory testing that can currently be implemented to address this question. Growth of CT is culture is a definitive way to prove viability, but use of molecular methods is more challenging. Molecular methods are complicated by CT biology. Viability based on detection of mRNA is hard because elementary bodies may be viable but are metabolically inert. Moreover, detection of genomic DNA does not necessarily correlate to detection of infectious elementary bodies and persistence via aberrant reticulate bodies also complicates analysis of CT viability using molecular methods.

The experts discussed recent work from the University of Washington on the use of viability PCR (vPCR) as a method to distinguish viable from non-viable CT; this approach relies on intercalation of propidium monoazide dye into DNA

from dead organisms to prevent their detection in a PCR assay.[25] This study found the methodology to be adaptable to viability assessment in clinical samples and the authors suggested the process could be clinically validated and scaled for use. Furthermore, in discussion amongst participants during the consultation based on expert experience and knowledge from collaborations, it was noted there are assays in development commercially for CT and GC viability, and yet unpublished studies on assessing viability via mRNA that the field. International research is ongoing in this area as well (including in the Netherlands). New information should continue to be monitored.

KQ4. Are there standardized culture-based AST methods for CT?

There are standardized models for CT culture, however there are no standardized methods for culture-based AST. CT antimicrobial susceptibility studies have evaluated isolates derived from diverse clinical specimens such as vaginal, endocervical and anorectal specimens, as well as standard control strains. Increasingly, there is investigation into an expanded range and combination of antimicrobials (not just doxycycline and azithromycin).[22,26–28]

The experts discussed that long standing work by University of Washington researchers Suchland et al[29,30] still accurately informs CT culture and AST testing methodologies.

KQ5. Have culture methods for CT changed since they were described in 2009?

There has been minor evolution in culture models recorded in the literature. There are published reports of new models for CT culture, expanding options beyond long standing use of the McCoy B murine fibroblast and HeLa human epithelial cell lines.[31,32] Use of MCF-7, CACO-2, SiHa and ARPE-19 epithelial cell lines to cultivate clinical isolates of CT has been reported. Differences in the clinical CT isolates themselves appear to impact infectivity and growth rate more profoundly than the differences caused by cell lines.[32] A Study of a HeLa culture model using a low oxygen environment (2% O_2) to understand chlamydial dynamics in the urogenital tract suggested changes in host cell density can significantly impact intracellular growth of CT, and recommended use of RPMI rather than DMEM cell media.[31]

KQ6. What are the various serological tests for CT and what are their performance characteristics and diagnostic value?

Very little data related to serological tests for CT were identified in the literature search (only use of complement fixation testing[33]). No new serological testing approaches were reviewed. Serology is generally problematic given repeat infections (as it does not inform on acute versus past infection), and the approach may be of limited utility. Serological testing is of limited utility and should not be used for diagnosis of urogenital CT infection.

KQ7. When should CT culture or resistance-based testing be performed?

Most CT infections in the US are successfully treated without the need for culture or AST; antimicrobial resistance is currently vanishingly rare. Culture is technically challenging, variable and not well standardized. AST results are not easily compared between laboratories. The greatest strength of these methodologies is as a phenotypic evaluation of resistance. Ultimately, culture is best suited for research or potentially low-volume-clinical testing at specialized laboratories with a standardized protocol.

CT culture should be maintained primarily to monitor antimicrobial susceptibility and support surveillance. Molecular surveillance for mutations that lead to diagnostic evasion is an important research function.

Future Directions and Research Needs

As the absence of true resistance to date in the US has meant that the need for CT culture of clinical samples has been relatively minimal, national capacity for quality CT culture is limited. It was discussed based on expert experience that reference laboratories also face the challenge of dwindling availability of commercial reagents. The diagnostic laboratory community must consider how a potential future increase in need for CT culture or CT AST could be managed.

True antimicrobial resistance is currently rare, but could emerge quickly:

- Resistance to tetracycline in *C. suis* (in pigs) is fairly common
- Implementation of doxy-PEP could lead to a rise in resistance

Current resistance detection methods are insufficient for widespread standardized testing:

- Culture-based AST is slow, complicated, not scalable, not standardized and unreliable.
- Targeted PCR is not comprehensive enough to be useful as the loci for resistance mutations are not well established.
- Sequencing has promise but is still early in development. Standardization is needed.
    - Culture-independent NGS methodologies may become increasingly important absent culture capacity but remain technically challenging.
    - The challenge of obtaining quality sequence directly from samples such as rectal swabs is substantial.
    - Use of enrichment strategies based on PCR amplification before sequencing is likely a valuable methodology to prioritize.
- In the era of doxy-PEP, broader consideration of CT resistance surveillance and what roles public health laboratories may/should play to complement CDC activities is needed.
- Surveillance:
    - Could help improve clinical testing
    - Would expand capacity for AST when needed for treatment failure investigations
    - Could help enhance surveillance for *Lymphogranuloma venereum* (LGV):
        - LGV is spreading, and discerning LGV may be necessary to inform appropriate treatment
        - *OmpA* genotyping alone could misclassify recombinant strains[34]which are strongly linked to differential tissue tropism and disease outcomes (ocular disease, urogenital disease and LGV)
        - Certain MLST strategies could also mis-classify LGV strains produced by recombination[34]

Finally, with increased movement toward antimicrobial stewardship in STI clinical practice, viability assays may become an important tool in reduction of unnecessary antibiotic use; the commercial development of effective viability assays for clinical use is encouraged.

Topic 2: GC Culture and Identification

Literature review by: Sarah Buss and Olusegun Soge, supported by Jennifer Reimche and Matthew Schmerer.
Presented by: Sarah Buss and Olusegun Soge.

Key Questions

KQ1. Have culture methods for GC changed since they were described in 2009?

KQ2. What biochemical tests can be used for the identification of GC?

KQ3. What is the utility of MALDI-TOF for detecting GC?

KQ4. When should culture be performed?

Background and Summary

Culture is still necessary for evaluation of gonorrhea treatment failure and AST. Since 2009, new technologies have been introduced that help optimize culture and identification of GC. Notably, matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has emerged as a reliable method for identification of GC. New products for collection and transport of specimens offer the potential for prolonged viability and improved culture success. When specimens are collected using eSwabs® or Intray™ GC, viability can be maintained for 48 or 72 hours respectively. Continued research also indicated that some long-standing beliefs regarding specimens for GC culture are unfounded. This includes the use of cotton swabs for specimen collection and cold storage or transport of GC culture specimens.

Findings

KQ1. Have culture methods for GC changed since they were described in 2009?

While the foundational methods associated with GC culture have not changed, new specimen collection and transport options have been introduced since 2009. There has been growing interest in self-collected specimens for GC culture, with data to show that patient and clinician collected specimens perform similarly across both genital and extra-genital specimen types (Barbee et al. 2021: results concordant at the pharynx [95%; kappa = 0.85], rectum [99%; kappa = 0.97], urethra/urine [83%; kappa = 0.87], and endocervix/vagina [100%; kappa = 1.0; $P \leq .005$ for all comparisons]).[35] Patient collected specimens are acceptable for GC culture.

Cotton swabs have been shown to perform similarly to polyester swabs for GC culture collection.[36] In a 2021 study by Phillips et al., there was no significant difference in the proportion of FLOQSwab or cotton-tipped swabs that were positive (39.7% [31/78] from cotton-tipped swab versus 38.5% [30/78] from FLOQSwab; P=1.000). Cotton tipped swabs do not kill *Neisseria gonorrhoeae* and are suitable for GC culture collection. However, when specimens are collected for molecular testing it is essential that the laboratory-validated and/or FDA authorized collection device be utilized.

There have been changes in the transport systems available for GC culture. Remel has discontinued production of the JEMBEC system (product#R10320), eliminating one commercial option from consideration. Laboratories are currently using alternatives including modified Thayer-Martin agar (MTM) & CO_2 tabs or InTray® GC when bedside inoculation is utilized. When used according to manufacturer instructions, the Intray® GC helps to extend the viability of GC during transport for up to 72 hours.[37,38] Specimen collection in charcoal-containing Amies has been shown to better preserve GC viability than collection into Amies without charcoal.[39] Flocked swabs in liquid Amies medium are now readily

available and help to extend the viability of GC during transport for 24-48 hours as compared to gel based conventional swab-transport systems.[37,40,41] Chocolate agar slants covered with sterilized paraffin oil may be used to successfully transport swabs for GC culture.[42] GC is a fastidious organism, but effective collection and transport options are available. Laboratories should utilize the method that best suits the needs of their clients, considering the available data, typical transit times and provider practices.

Viability of GC is better when flocked swabs in liquid Amies are stored cold and shipped on ice packs (refrigeration condition) rather than at room temperature. This holds true with other types of collection devices including non-nutritive Amies Agar Gel Medium with charcoal.[21,23,26,27] Laboratories could investigate cold storage and transport of specimens for GC culture but must also ensure that temperature specifications are met per the manufacturer's specifications.

GC culture is more successful for symptomatic versus asymptomatic infections. Accordingly, culture from urogenital sites has a higher success rate than culture from extragenital sites which are rarely symptomatic.[9,10,43,44] In terms of urogenital specimens from women, culture recovery is better from endocervical rather than vaginal specimens.[10] Those with infection at multiple sites were more likely to have culture-positive infections from at least one site.[44] When specimens are collected from pharyngeal sites, care should be taken to appropriately collect the specimen. Specific collection techniques should be used to improve GC recovery from the oropharynx:

- The success rate of pharyngeal culture is improved when clinicians reported inducing the gag-reflex during sampling.[45]
- Swabbing a larger surface area, applying more swab pressure and a change in the anatomical sites swabbed to include the oropharynx were all associated with a higher culture success rate.[46]

Data supports the use of urine as a specimen source for GC culture, especially considering advances in laboratory automation:

- Urine was 97% sensitive for GC culture, even when transport was delayed by 4-22 hr.[18]
- Total lab automation (TLA), including the BD Kiestra™ TLA system, may enhance recovery of GC from urine cultures plated onto blood agar.[47]

Laboratories should consider GC when they are working up urine cultures and could consider urine culture in addition to other specimen types as an option for GC recovery.

KQ2. What biochemical tests can be used for the identification of GC?

The biochemical tests used for the identification of GC in culture have not changed (Figure 1), but as technology has advanced the limitations of biochemical tests have become clear. Gram stain of urethral discharge has been historically used in the clinic for the presumptive identification of gonorrhea cases, especially in men with gonococcal urethritis. However, in the laboratory, Gram stain alone should not be used for identification of GC in culture, particularly in the context of increased urethral *N. meningitidis.*[48–51] Complete evaluation including colony morphology, Gram stain and appropriate biochemicals are essential when working up a suspected GC isolate. API-NH, Rapid-NH, Gram stain, oxidase, superoxol and Phadebact Monoclonal GC kit can all be used to identify GC, with varying levels of success.[52,53] Laboratories should review data on the performance of these methods and determine the best and most practical option for their facility. It was discussed that these assays have limitations, and it is important to know that horizontal gene transfer between GC and commensal *Neisseria* spp., including *Neisseria meningitidis,* have led to challenges. For example, mis-identification of GC as *N. meningitidis* by the API-NH and the Gonochek-II was recently attributed to a homologus recombination event involving the *ggt* gene.[49]

Figure 1. Relevant biochemical test results for Gram-negative, oxidase-positive *Neisseria* and

related species, modified from [2]

Species	Acid From					Superoxol	Reduction of nitrate	Polysaccharide from sucrose
	Glucose	Maltose	Lactose	Sucrose	Fructose			
N. gonorrhoeae	+	-	-	-	-	+	-	-
N. meningitidis	+	+	-	-	-	+	-	-
N. cinerea	-	-	-	-	-	+	-	-
N. elongata	-	-	-	-	-	-	-	-
N. flava	+	+	-	-	+	+	-	-
N. flavescens	-	-	-	-	-	+	-	+
N. lactamica	+	+	+	-	-	+	-	-
N. mucosa	+	+	-	+	+	+	+	+
N. perflava	+	+	-	+	+	+	-	+
N. polysaccharea	+	+	-	-	-	+	-	-
N. sicca	+	+	-	+	+	+	-	+
N. subflava	+	+	-	-	-	+	-	-
Moraxella catarrhalis	-	-	-	-	-	+	+	-
Kingella denitrificans	+	-	-	-	-	-	+	-

KQ3. What is the utility of MALDI-TOF for detecting GC?

MALDI-TOF MS is a reliable method for the identification of GC[54–58] in culture, although the identification of *N. meningitidis* via MALDI-TOF MS can be a challenge. Secondary confirmation is not necessary when MALDI TOF MS is used for GC identification.[54,59,60] However, when pharyngeal specimens are cultured, care should be taken when subculturing for antimicrobial susceptibility testing as commensal *Neisseria* spp. are likely to be contained on the same plate as GC.[61] The specificity of MALDI-TOF MS for species differentiation is dependent upon the number of isolates contained in the reference library.[56] There are both FDA cleared and Research Use Only databases for MALDI-TOF MS analysis. Attention to the FDA authorization status and reference libraries/databases are warranted when implementing MALDI-TOF MS for GC identification.

Based on expert experience, it was discussed that isolation methods for MALDI-TOF MS are important. Specifically, users should know that subculture to chocolate agar to obtain isolates for MALDI-TOF MS performs well, but subculture is non-selective. Plates should not continue to be used after more than 48 hours.

KQ4. When should culture be performed?

The CDC's guidelines[4] should be utilized for guidance on when to perform culture. In general SMEs felt that culture could be performed when test of cure is indicated (as described in CDC Guidelines) and should be performed when AST is warranted for any reason including:

- in cases of suspected or documented treatment failure (in patient and contacts of patient)
- in cases of disseminated gonorrhea infection,

SMEs also believed in use of culture for surveillance or epidemiological purposes:

- to monitor trends in antimicrobial resistance
- for strain typing purposes.

SMEs believed that limited national capacity may negatively impact GC culture and felt that a curated database of

laboratories with GC culture capability would be beneficial. The importance of culture in cases of disseminated infection was stressed, given the lack of FDA-cleared molecular tests for uncommon specimen types. Routine orders for culture from sterile sites typically include chocolate agar that would support growth of GC, however when disseminated GC is suspected, a specific order for GC culture may be beneficial.

SMEs have observed that practical issues, including difficulties with isolation of pure cultures from female patients, and the known lack of sensitivity associated with oropharyngeal cultures may drive dissatisfaction with the method and further reduce use of culture. Therefore, when culture is indicated, likelihood of success should be maximized by:[10,41,43,45,46,55,62,63]

- Performing GC culture in the clinic when possible (bedside culture)
- Collecting specimens from all potential sites of exposure (genital and extra-genital)
- Following best practice in terms of specimen collection
- Implementing surveillance protocols that target:
    - Patients with symptomatic urethritis and Gram-negative intracellular diplococci on urethral Gram stain
    - Patients with gonococcal cervicitis or contacts to another person with diagnosed or suspected gonorrhea
    - Patients seeking treatment following a GC nucleic acid amplification test (NAAT) positive screening test (collect specimens as soon as possible following NAAT result)

Future Directions and Research Needs

There continues to be limited data on GC culture in pediatric populations and on best practice for medico-legal testing. The 2014 Recommendations for the Laboratory-Based Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* and the 2021 CDC STI Treatment Guidelines are not currently aligned with respect pediatric testing for GC (specific to sexual assault or abuse) at extragenital sites in prepubescent girls or at all in prepubescent boys. To mitigate confusion, these documents should be aligned in future revisions.

Isolation of *Neisseria gonorrhoeae* from blood agar should be revisited. The persistent assumption that GC does not grow on blood agar may need to be reevaluated in the face of new evidence from total lab automation that has shown significant increase in GC recovery from blood agar.[47,64] More data in the impact of automation on GC recovery rate is needed. It may be particularly interesting to obtain GC isolates from TLA culture growth on blood agar for molecular surveillance purposes.

GC is a fastidious organism and despite advances, the recovery rate is still poor, especially from extra-genital sources. Regardless, GC culture is beneficial for cases of disseminated infection, AST and surveillance work. Future efforts should focus on:

- Work to improve recovery rate of GC from extragenital sites
- GC culture-viability assay combination to resolve discrepancy between poor recovery of GC culture from extragenital sites and the more sensitive NAAT
- Improvement of transport medium that maintains GC viability for at least seven days
- Improved media formulations that prevent overgrowth of commensal flora
- Development of reliable genotypic susceptibility testing methods

Efforts to expand selective media options for GC would be of particular interest, especially as material and reagent shortages (e.g., defined supplements like BD BBL™ IsoVitaleX™ Enrichment) have continued to plague manufacturers and consumers can be reliant upon a limited number of products.

Topic 3: CT and GC Diagnostic Performance

Literature review by: Megan Crumpler, Susan Realegeno, Salika Shakir and Barbara Van Der Pol; supported by Julio Ayala, Bill Davis, Shelby Hutton and Amanda Smith.

Presented by: Megan Crumpler.

Key Questions

KQ1. Has the performance of NAATs changed since 2009? Have new tests or specimen types been FDA approved and how do their performance characteristics compare to previous NAATs?

KQ2. What types of self-collected specimens do laboratories accept? Do the performance characteristics of tests vary when specimens are self-collected in a clinical or non- clinical settings?

KQ3. What are the testing recommendations when treatment failure is suspected, and do they vary across anatomic sites?

KQ4. What methods can be used to diagnose suspected LGV? What are the performance characteristics of those methods?

KQ5. How does pooling of multiple specimens or specimens across anatomical sites affect the performance characteristics of available assays?

KQ6. When should confirmatory testing be performed and does this vary across anatomic sites?

Background and Summary

The sensitivity and specificity of modern NAAT assays for detection of CT and GC are generally very high, and routine use of these tests is still advisable.[5,65–78] Since the previous recommendations[2] were published, new platforms, assays and sample types have been FDA-cleared for diagnostic testing, but some gaps remain for particular populations. Published evidence supporting the efficacy and equivalency of self-collection outside of clinical settings exists, but regulatory barriers remain to testing these specimens in US laboratories, although some progress in this area has been made.

Recommendations

KQ1. Has the performance of NAATs changed since 2009? Have new tests or specimen types been FDA approved and how do their performance characteristics compare to previous NAATs?

Inclusivity of NAATs has been improved by adding variant probes, and currently available assays have achieved sufficient sensitivity levels. SMEs discussed that the clinically relevant sensitivity threshold is likely at or above the current limits of detection for commercially available assays. Analytical specificity of new assays is generally high, though specificity may vary by testing platform and specimen type. Regardless, false positive or clinically irrelevant detections can still occur due to many factors including residual nucleic acid, non-established infection suppressed by doxy-PEP, environmental contamination and others. Laboratorians should be aware of the performance characteristics of these tests and implement all federal and state mandated guidelines that surround quality assurance and control for molecular testing.

New FDA-cleared sample types since previous recommendations include oropharyngeal and rectal swabs[5,68]

- The Hologic Aptima Combo 2® Assay and the Cepheid Xpert® CT/NG were FDA-cleared in 2019 for throat and rectal testing; others have followed including Abbott Alinity m STI and Roche Cobas® CT/NG (5800/6800/8800).
- For pharyngeal sources, NAAT is more sensitive than culture. However, specificity varies among the NAAT methods (TMA >95% compared to PCR 70-90%.)[79,80]
- Pharyngeal specimens may require supplemental testing; false positives can occur with PCR-based assays. Positive predictive values fall below 90% and confirmation rates were 80% as compared to other sites.[81,82]
- Rectal specimens achieve comparable performance to urogenital specimens across multiple platforms, without the need for confirmatory testing.[81,83,84]
- For rectal sources, NAAT testing is more sensitive than culture, with culture detecting roughly 66.7% of GC and 36.1% of CT infections as compared to NAAT.[79,80]
- Specificity of NAAT is comparable to culture for rectal swabs at approximately 98.3-98.8% for GC and 95.6-98.5% for CT depending on the NAAT method.[79,80]

Self-collected urine and vaginal swabs:

- Self-collection of vaginal swabs in a clinical setting is comparable to endocervical testing by NAAT[85,86]
- Diagnostic yield of urine specimen is high in men, in women newer studies demonstrate self-collected vaginal specimens are superior to urine for NAAT.[75,87,88]

Recently approved NAAT assays and platforms also include options focused on use at or near the point-of-care (POC): Cepheid GeneXpert® CT/NG, Visby™ medical sexual health test (CT, NG, & TV) and binx *io* CT/NG. Overall, strong diagnostic performance has been demonstrated for these tests; these also could have a role in laboratory settings for confirmatory testing (more information provided below in POCT topic section).

Compilation of currently approved tests for GC and CT and their approved specimen types in a web-based living document would be beneficial.

KQ2. What types of self-collected specimens do laboratories accept? Do the performance characteristics of tests vary when specimens are self-collected in a clinical or non- clinical settings?

FDA-cleared tests:

- Self-collected vaginal swabs are acceptable when collected in a clinical setting; this sample type is FDA-cleared for use with many different testing kits / platforms. Refer to manufacturer provided package inserts for most accurate information.
- LetsGetChecked Simple 2 test is the first FDA-authorized CT and GC test for at-home sample collection (2023, via De Novo pathway[89]) but can only be used by the Let's Get Checked Laboratory, so is not applicable in any other setting. Additional tests have been recently FDA-authorized for home-collection that are more widely applicable (e.g., simpli-COLLECT STI Test, Abbott Molecular and Visby Medical Sexual Health Test) and additional authorizations are expected in the near future.

Data for non-FDA-cleared tests:

- Self-collection for rectal and pharyngeal is comparable to clinician-collected swabs.[5–8] There is no commercial FDA-cleared assay available for self collection of rectal and pharyngeal specimens.
- Self-collected penile meatal swabs are not FDA-authorized with any commercially available test, but have comparable sensitivity to urine or clinician collected urethral swab (>90%).[90–92]
- Home vaginal self-collection has been studied extensively in the US and internationally.

- Recent meta-analysis of at-home specimen self-collection for GC and CT identified high concordance between specimens collected at home and in clinical settings and higher uptake of testing with home collection.[93]
- LetsGetChecked, I Want The Kit (IWTK and Indigi-IWTK, John Hopkins) and other published studies have pursued home self-collection[94–96]
- In general, there is extensive evidence that self-collection of specimens at home can be effective, but there remain regulatory challenges for laboratory testing of self-collected specimen. Laboratories may not validate specimen collection devices as laboratory-developed tests (LDTs) and FDA-authorized options for self-collection in a non-clinical setting (i.e., at home) are limited.
- Challenges for ensuring quality testing of at-home self-collection include ensuring stability of samples, understanding invalid results, and potential need for internal control (such as beta globin) to assess for sample adequacy.[97]

KQ3. What are the testing recommendations when treatment failure is suspected, and do they vary across anatomic sites?

During discussions surrounding this key question, the SME felt the focus should be on time to negativity as opposed to test of cure. The rationale was that there are subtle differences between a test of cure, a retest, a viability assay and the time to negativity of a molecular test. The SME also discussed that the distinct differences between GC and CT should be made clear when addressing this question and test of cure.

Data addressing time to negativity of NAAT testing results was reviewed in the literature search and discussed during the consultation. The identified studies have significant limitations when they are compared to each other. They are not standardized and are variable in the questions asked, treatments included, co-infection status, methodology, subsequent sexual exposure and other variables.

GC	CT
• Majority of infected persons will be NAAT negative by day 14 post treatment and almost all will be negative after 30 days.[98–100] • Rectal and oropharyngeal specimens are more likely to be positive by NAAT after 7 days compared to urine.[98] Median clearance times have been reported from 2-6 days. • Median time to clearance is similar between assays that detect RNA vs DNA. [101] • No association between clearance and symptoms or bacterial load.[99] • Positive “blips” may occur after patient has tested negative with both RNA and DNA assays.[98,101]	• Majority of infected persons will be negative by 14 days, even more by 21 days and almost all will be negative after 30 days including both pregnant and nonpregnant females.[99,100,102] • Median time to negative CT NAAT 6-14 days for assays that detect either RNA or DNA.[99,100,102,103]there are limited data about the optimal time to perform TOC using nucleic acid amplification tests (NAATs) • No association between clearance and symptoms or bacterial load.[99] • Positivity at day 21 was associated with concurrent STI.[103] • Positive “blips” are associated with HIV-positivity, chlamydia infection in the preceding six months, and absence of sign or symptoms.[100]

Moving forward, recommendations must clearly address the challenge of differentiating time to nucleic acid clearance (negativity) from test of cure. Information reviewed on the duration of nucleic acid after treatment may be helpful to inform test of cure; test of cure should be directly addressed in clinical guidelines and any information related to test of cure or time to clearance included in laboratory testing recommendations should be harmonized with clinical guidelines.

KQ4. What methods can be used to diagnose suspected LGV? What are the performance characteristics of those methods?

Testing rectal specimens from men who have sex with men (MSM) is especially important to LGV detection.[104] FDA-cleared CT NAATs detect but do not differentiate serovars L1-L3 that cause LGV. Collection devices used with common CT testing platforms may be appropriate specimens for confirmation of LGV (*i.e.,* Aptima and Roche collection devices), but there are no FDA-approved tests to specifically diagnose suspected LGV. Some public health and commercial laboratories offer LDTs and diagnostics used internationally with rectal samples have been described:[105]

- Allplex Genital Ulcer Assay kit in the Seegene RT-PCR instrument (multiplex panel containing LGV + 6 other pathogens): PPA, NPA and OPA, were 88.9%, 100% and 97.26%, respectively.
- CERTEST BIOTECH VIASURE: PPA, NPA and OPA were 91.6%, 100% and 97.9%, respectively.

LDTs for LGV diagnosis:

- Two-step assay using RT-PCR – first detect CT, then detect LGV using *pmpH* gene target with high PPV and NPV[106]
- BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies) was used to sequence purified ompA amplicons. Performance not assessed, just ability to differentiate between serovars.[107]

The *pmpH, ompA* and *pgp3* are potential gene targets; the multi-copy gene target *pgp3* is more sensitive than a single copy gene such as *pmpH*.[108]

KQ5. How does pooling of multiple specimens or specimens across anatomical sites affect the performance characteristics of available assays?

There is generally strong agreement between samples pooled across anatomical sites versus single-site testing (≥86% across studies, see Table 1). Compared to taking a single site sample, pooled specimens have been shown to improve identification of infection.[8] Depending on the economic considerations of different testing programs and the prevalence of CT or GC in target populations, there may be value in pooling, although many SMEs felt that the practice is difficult to implement.

Pooling strategies across studies are variable. Some studies included gargle specimens; these are not FDA-cleared and data to suggest equivalency to oropharyngeal swabbing are limited. It should also be noted that in general specimen pooling results in off-label use of FDA authorized CT and GC tests. This further complicates implementation as laboratories must understand if and how they might be able to conduct studies to validate pooled testing as an LDT.

Table 1. Pooled Testing of GC and CT.

	Pooled testing (combined rectal, gargle or pharynx/oropharyngeal and urine)	
	GC	CT
Agreement	High agreement between pooled and single-site testing (kappa 0.943[109]; kappa 0.895[110]).	Relatively high agreement between pooled and single-site testing (kappa 0.945[109]; kappa 0.860[110]).
Sensitivity	90-98% sensitivity for pooled[8,109–113] • Some studies report lower sensitivity in pooled samples compared to standard of care (SOC),[114]rectal, and urethral/first-void urine samples while others report no significant differences in sensitivity compared to individual testing.[112,113] • Sensitivity may vary between anatomic sites [114]rectal, and urethral/first-void urine samples and be lower for pooled pharynx/pharyngeal samples.[110,111] • Pooled sensitivity was higher compared to collecting a single site sample (VVS or FCU).[8]	86.2%-96% sensitivity for pooled[8,109–114] • Sensitivity in pooled was not significantly different from SOC.[113,114] • Individual sample testing sensitivity was significantly higher than pooled (99.6 vs 96%), but still >95% for one study.[8,112] • Pooling urogenital and extragenital in asymptomatic MSM reduced analytical sensitivity by 10% in one study.[112] • Sensitivity varies per anatomic site.[114]
Specificity	>98% in pooled specimens[8,109,111]	>99% in pooled specimens[8,109,111]
NPV/PPV	• NPV >96%[8,113,114] • PPV >99%[8]	• NPV >96%; not significantly different from SOC.[8,113,114]
Missed Infections	• Tend to be single site[112] • 1 missed NG infection for every 43 people tested by pooled vs SOC[114]	• Tend to be single site[8] • 1 missed CT infection for every 153 people tested by pooled vs SOC[114]
Patient Status	Symptom status did not impact individual vs pooled results.[8]	• Symptom status did not impact individual vs pooled results.[8] • Slightly lower sensitivity in pooled samples for MSM vs non-MSM population.[111]

KQ6. When should confirmatory testing be performed and does this vary across anatomic sites?

Generally, there is insufficient data to support broad recommendation of confirmatory testing. Current FDA-cleared NAATs have high sensitivity and specificity. Some recently developed NAATs integrate a second target; users should review package inserts to ensure they are familiar with the targets included in tests. It would also be beneficial for the target(s) to be included in a web-based document detailing available FDA-cleared NAATs.

Throat/oropharyngeal specimens are more likely to require confirmation for GC due to commensal *Neisseria* species and cross reactivity. This may vary by assay. Pathogen prevalence is directly correlated with the PPV of any assay and some studies suggest confirmatory testing can increase PPV in low GC prevalence settings.[115,116] However, there is a lack of consensus and detailed guidance available to explicitly define and determine what constitutes a low prevalence setting. During discussions, SMEs expressed that it is important to be explicit about who should be tested, especially using a throat/oropharyngeal specimen. SME felt that testing the right patients was one of the most important factors involved in the testing process.

Ideally, confirmatory testing, when performed, should be done on the same sample on an assay that has different targets (orthogonal testing). Laboratories would need to consider:

- Compatibility of collection media with different tests/instruments (or lack thereof)
- Regulatory requirements associated with validation of alternative collection media

It was discussed, for example, that Hologic offers Aptima tests with alternative targets that can be used for confirmatory testing for CT or GC individually, which are FDA-cleared. In some cases, however, a new sample must be collected for this testing when initial collection device was incompatible (e.g., when Aptima collection devices are not used initially).

Finally, recommendations put forth for NAAT and any confirmation testing necessary in cases of sexual assault should be considered and in line with CDC Guidelines.

Future Directions and Research Needs

Current performance of FDA-cleared CT/GC NAATs in US populations is strong, though ongoing surveillance efforts are needed to continue to alert to assay performance limitations due to emerging variants and provide data to guide assay updates.[117] Indeed, laboratorians should be aware that the high sensitivity of these tests can on occasion lead to false positive or clinically irrelevant detections; the harms inflicted by this need to be minimized (both for an individual and at the level of contributing to antibiotic resistance). In addition, the clinical significance of detecting nucleic acid in individuals regularly prescribed doxy-PEP may require investigation. Laboratorians should be prepared to discuss the sensitivity of these tests and the results obtained in specific cases with clinicians, whose diagnostic expertise and review of additional patient data (symptomology, sexual history, population level factors, etc.) must be used to guide interpretation. In addition, continued research and development of viability assays should be sought to provide another tool to this end.

Given the high sensitivity and specificity of commercially available CT/GC NAATs, emphasis should now be on making tests easier, faster, approved for use with specimens from more anatomical sites and more affordable to the laboratories and patients that need them.There remain gaps in research and testing guidance for pediatric patients. FDA-authorized tests for pediatric patients and clearer guidance for NAAT testing and confirmation in sexual assault are also needed.

Inclusion of LGV in genital ulcer disease (GUD) evaluation panels makes sense clinically and may make it more feasible for commercial vendors to obtain FDA authorization of an LGV assay, which is needed. Distinguishing LGV impacts the duration of treatment that is prescribed by clinicians. Without FDA-cleared testing available, there are limited options for LGV diagnostic testing in some public health laboratories and reference laboratories; however, this is reliant on continued ability to validate these assays as LDTs.

Finally, there is a clear need for research that could help advance regulatory approval for self-collected specimens and testing. Innovations in regulatory science to enable researchers and manufacturers to bring together multi-site data to effectively support submission for claims related to self-testing and sample collection devices are needed. Pathways to claims that can be applied across multiple diagnostic testing platforms are needed. Coordination of device manufacturers and regulatory bodies to achieve a universal claim for specimen collection devices is an ideal goal.

Topic 4: CT and GC Point of Care (POC) Diagnostic Testing

Literature review by: Yukari Manabe, Anthony Tran and Barbara Van Der Pol; supported by Amanda Smith.
Presented by: Yukari Manabe.

Key Questions

KQ1. What POC or near-POC NAATs for CT/GC are currently FDA-cleared? Which are CLIA-waived?

KQ2. What are the acceptable specimen types and the performance characteristics?

KQ3. When should POC testing (POCT) be used?

Background and Summary

Previously published recommendations for laboratory testing of CT and GC did not yet address POCT.[2] Until 2019, the most compact and rapid tests were immunoassay lateral-flow devices, rarely achieving clinical sensitivity over 90%.[118] Recently, NAAT technologies that can be used at the POC have been FDA-authorized, representing a significant advance. Studies of currently approved POC NAATs generally demonstrate strong performance, but current assays still present limitations (e.g., expense, longer than desired turn-around times, limited number of approved specimen types). Data are rapidly emerging for new tests and additional manufacturers are known to be in the process of seeking regulatory approvals for tests on POC platforms.

Findings

KQ1. What POC or near-POC NAATs for CT/GC are currently FDA-cleared? Which are CLIA-waived?

At the time of this consultation (October 2024), there were three FDA-cleared POC NAATs for GC and CT and two are CLIA waived:

- Cepheid GeneXpert® CT/NG
- Visby medical™ Sexual Health test (CT, NG, & TV) (CLIA-waived)
- Binx *io* CT/NG (CLIA-waived)

Additional technologies will be emerging in the near term and any recommendations put forth related to POCT should allow for flexibility to address the evolving landscape of POCT, rather than focus too narrowly on the characteristics of only what is presently commercially available.

KQ2. What are the acceptable specimen types and the performance characteristics?

Overall, strong diagnostic performance has been demonstrated for all of these tests (Cepheid,[119–12] Visby,[118] Binx[124,125]).

Table 2 provides introductory information on the three FDA-cleared POC NAAT for GC and CT; the information captured here comes from studies conducted to support clearance and reported in the instructions for use (IFUs) for these products. These data provide an indication of the analytical capabilities of these POCTs, but may not necessarily reflect clinical performance under varying conditions. Studies described and referenced in the next section provide additional insight into the conditions and performance of these tests in clinical trials and implementation studies in various settings. Table 2 also includes the accepted specimen types for each POCT.

Table 2. Currently FDA-Cleared NAAT POCTs for GC and CT

Assay	Accepted Specimens	Sensitivity/PPA*	Specificity/NPA*	LOD*
Cepheid GeneXpert CT/NG IFU[110,126–142] **Turnaround Time: 90 min** **CLIA-waived: No**	• Female and male urine • Patient-collected (in a clinical setting) vaginal swabs • Clinician-collected endocervical swabs • Clinician-collected female and male pharyngeal and rectal swabs	Female VS CT Symptomatic: 100 (95.4-100) Asymptomatic: 99.2 (95.5-100) Female ES CT Symptomatic: 96.2 (89.3-99.2) Asymptomatic: 95.9 (90.7-98.7) Female Urine CT Symptomatic: 98.8 (93.6-100) Asymptomatic: 97.6 (93.2-99.5) Male Urine CT Symptomatic: 97.6 (93.0-99.5) Asymptomatic: 100.0 (95.1-100) Pharyngeal CT Symptomatic: 100.0% (70.1-100.0) Asymptomatic: 95.0% (83.5-98.6) Rectal CT Symptomatic: 81.5% (63.3-91.8) Asymptomatic: 86.6% (81.3-90.7) Female VS GC Symptomatic: 100 (87.2-100) Asymptomatic:100 (86.3-100) Female ES GC Symptomatic: 100 (87.2-100) Asymptomatic: 100 (86.3-100) Female Urine GC Symptomatic: 96.6 (82.2-99.9) Asymptomatic: 92.0 (74.0-99.0) Male Urine GC Symptomatic: 99.1 (94.9-100) Asymptomatic: 92.3 (64.0-99.8) Pharyngeal GC Symptomatic: 92.9% (81.0-97.5) Asymptomatic: 95.1% (90.7-97.5) Rectal GC Symptomatic: 97.4% (86.8-99.6) Asymptomatic: 89.8% (84.2-93.5)	Female VS CT Symptomatic: 98.4 (97.5-99.0) Asymptomatic: 99.5 (99.2-99.8) Female ES CT Symptomatic: 99.6 (99.0-99.9) Asymptomatic: 99.5 (99.2-99.8) Female Urine CT Symptomatic: 99.7 (99.2-99.9) Asymptomatic: 99.9 (99.7-100) Male Urine CT Symptomatic: 99.7 (98.8-100) Asymptomatic: 99.8 (99.6-99.9) Pharyngeal CT Symptomatic: 100.0% (98.7-100.0) Asymptomatic: 99.6% (99.3-99.8 Rectal CT Symptomatic: 99.4% (96.6-99.9) Asymptomatic: 99.4% (98.9-99.6) Female VS GC Symptomatic: 99.8 (99.4-100) Asymptomatic: >99.9 (99.8-100) Female ES GC Symptomatic: 99.9 (99.6-100) Asymptomatic: 100 (99.8-100) Female Urine GC Symptomatic: 100 (99.7-100) Asymptomatic: >99.9 (99.8-100) Male Urine GC Symptomatic: 100 (99.4-100) Asymptomatic: 99.9 (99.7-100) Pharyngeal GC Symptomatic: 98.9% (96.7-99.6) Asymptomatic: 98.8% (98.2-99.2) Rectal GC Symptomatic: 99.4% (96.6-99.9) Asymptomatic: 99.4% (98.9-99.6)	Vaginal Swabs CT serovar D = 84 EB/mL CT serovar H = 161 EB/mL GC= 1.5-1.6 CFU/mL Urine CT serovar D = 75 EB/mL CT serovar H = 134 EB/mL GC= 1.2-2.7 CFU/mL Pharyngeal CT serovar D = 161 EB/mL CT serovar H = 225 EB/mL GC= 6.4-7.1 CFU/mL Rectal CT serovar D = 88 EB/mL CT serovar H = 161 EB/mL GC= 4.9-5.3 CFU/mL
Visby medical sexual health test (CT, NG, & TV) IFU[143] **Turnaround Time: <30 min** **CLIA-waived:Yes**	Patient-collected (in any setting) vaginal swabs	CT Symptomatic: 97.9% (92.8-99.4%) Asymptomatic: 96.4% (87.9-99.0%) NG Symptomatic: 100% (86.7-100%) Asymptomatic: 95.0% (76.4-99.1%)	CT Symptomatic: 96.8% (95.4-97.8%) Asymptomatic: 98.8% (97.7-99.3%) NG Symptomatic: 99.1% (98.3-99.6%) Asymptomatic: 99.0% (98.1-99.5%)	CT serovar H = 16.0 EB/ML CT Serovar D= 5.9 EB/ML GC= 5.7-6.2 CFU/mL

Assay	Accepted Specimens	Sensitivity/PPA*	Specificity/NPA*	LOD*
Binx *io* CT/NG IFU[144–146] **Turnaround Time: <30 min** **CLIA-waived:Yes**	Clinician and patient-collected (in a clinical setting) vaginal swabs Male Urine	Female VS CT Symptomatic: 95.2% (86.7-98.3%) Asymptomatic: 97.0% (89.8-99.2%) Female VS GC Symptomatic: 100% (88.3-100%) Asymptomatic: 100% (80.6-100%) Male Urine CT Symptomatic: 91.7% (81.9-96.4%) Asymptomatic: 93.3% (89.8-99.2%) Male Urine GC Symptomatic: 98.4% (91.4-99.7%) Asymptomatic: 91.7% (64.6-98.5%)	Female VS CT Symptomatic: 98.9% (97.9-99.5%) Asymptomatic: 99.2% (98.2-99.7%) Female VS GC Symptomatic: 99.9% (99.3-100%) Asymptomatic: 100% (89.2-100%) Male Urine CT Symptomatic: 99.6% (97.8-99.9%) Asymptomatic: 99.1% (97.9-99.6%) Male Urine GC Symptomatic: 100% (98.5-100%) Asymptomatic: 100% (98.6-100%)	Vaginal Swabs CT serovar E = 407.4 GE/mL CT serovar F = 755.5 GE/mL GC= 2.1-2.5 CFU/mL Urine CT serovar E = 485.3 GE/mL CT serovar F = 769.3 GE/mL GC= 1.1-2.5 CFU/mL
*Assay performance & limit of detection are referenced from the instructions for use CT: *Chlamydia trachomatis*; NG or GC = *Neisseria gonorrhoeae*; TV: *Trichomonas vaginalis*; VS: Vaginal swab				

KQ3. When should POCT be used?

Testing symptomatic patients in acute care settings (urgent cares and emergency departments) is currently the most strongly supported use case for CT/GC POCT.[147,148] Same-visit results can guide treatment and prevent the challenge of loss to follow-up. Rule out of STI in an acute care setting can also reduce antibiotic overuse.

A study of implementation of the Xpert POCT at a high-volume urgent care showed improved appropriateness of treatment (prevention of under and over treatment) versus a retrospective review of data relying upon traditional urogenital GC/CT laboratory-based testing and treatment.[148] Similarly, in a small randomized controlled trial of Xpert CT/NG use in an emergency department, test patients with negative results (8 of 37, 21.6%) were less likely to receive empirical antibiotic treatment than control patients (11 of 20, 55%).[149]

A small study showed that use of the Visby STI Panel in an outpatient clinic also would have prevented both overtreatment (in 33/33 overtreated patients) and undertreatment (in 13 out of 15 undertreated patients). Clinicians valued the rapid return of results and patients were comfortable waiting up to 30 minutes.[150] A paper by Hsieh et al., noted by expert reviewers as in preparation, will further detail the use of Visby in an emergency department, enabling faster throughput of patients.

A very large multisite study of Binx *io* compared against commonly utilized NAATs in the US demonstrated the utility of a rapid molecular POCT (~30 min) to provide timely results to patients who were unwilling to wait over 90 min for a test result.[124,125]

Patients are generally willing to have specimens tested using a POCT and wait at the testing site to receive their results (95.95%, n = 1633), but tolerance for wait time varies:[61]

- 22.4% were willing to wait up to two hours
- 41.6% up to one hour
- 30.8% up to 30 minutes

While the capabilities of these POCT show promise for improving testing and subsequent care in some settings, discussion of the use of POCT by experts at the consultation placed significant emphasis on the need for quality assurance and continued trainings efforts. For sites seeking to implement POCT, a strong quality assurance program that includes appropriate training, competency assessments, proficiency testing and quality controls should be in place to ensure accurate results and compliance with federal, state or manufacturer specified requirements. Specifically, molecular best practices should also be included in training, as CLIA-waived options now include sensitive NAAT testing that may be performed by individuals with limited molecular laboratory knowledge. Issues of biosafety and waste management should also be thoroughly considered and integrated in POCT planning. The public health laboratory in the jurisdiction should be consulted when programs are seeking to establish POCT.

Future Directions and Research Needs

Overall, while there has been significant progress in the ability to bring testing for GC and CT to the POC, it was discussed that available options continue to be relatively expensive (even as compared to reimbursement rates), slower than may be necessary to have the desired impact on practice, and/or have a lot of waste generated from consumables. Moreover, there are significant limitations associated with acceptable specimen types, especially when it comes to CLIA waived POCT. Continued improvement of these factors is necessary and should be sought.

Further evaluation of economic viability of testing would help to guide commercial development—can the greater expense of POCT still be cost-saving in settings where faster turnaround time (TAT) enables greater throughput of patients (specifically emergency departments)? Cost effectiveness modeling is needed. Further information is needed to understand these potential challenges:

- POCT may be expensive for asymptomatic screening (may not be reimbursed or be reimbursed at a deficit).
- Reimbursement may also be a challenge in the context of STI screening for HIV pre-exposure prophylaxis (PrEP).
- Costs of instrument and/or test purchase must be considered along with costs of maintenance/service of equipment.
- The combination of analytes associated with commercially available POCT may not align with recommendations for use in target populations, potentially increasing expenses with unnecessary testing or creating additional billing and reimbursement challenges (*e.g.*, inclusion of *Trichomonas vaginalis* in approved POCT, and inclusion of *Mycoplasma genitalium* in future POCT currently in the pipeline).

Studies to continue to address the impacts of POCT TAT and over-the-counter (OTC) testing are also needed. How critical is same day versus same visit resulting? It will be important to determine whether patients are treated faster when they receive results faster, even if they have left the clinical setting before receiving those results. It was discussed that new data may be emerging on this soon from US public health departments using POCT or establishing express clinic models and should be examined as available. Equally important will be an evaluation of the appetite for and impact of OTC testing, as those options become more widely available.

Many studies of POCT for GC and CT were conducted internationally and in low-resource settings with differing recommendations for testing and differing population risks that may not be readily generalizable to other contexts in the US. There is a lack of the necessary context on participants provided in many studies to assess the true utility of tests (missing chart info, unknown patient outcomes, lacking information on other testing conducted).

While more expensive to develop, seek approval and commercialize, POCT with clearance for multiple sample types to enable three-site testing (genital, rectal, oropharyngeal) is needed. There is currently no three-site testing available on a CLIA-waived instrument. Although it is understood this is not always immediately possible in initial commercialization

of new POCTs, there should be emphasis on developing and seeking approval of POCT with a greater range of sample types.

Finally, it is also important to consider how information is transferred from POCT. Approaches to unifying reporting are needed to ensure the necessary information can be captured at both the state and federal level. Ensuring that reporting systems differentiate POCT from laboratory diagnostics will also aid future analysis and identification of any data trends on difference with POCT testing. Reporting challenges related to OTC testing also merit further investigation, as clinics and laboratories will have to navigate how to make best use of results generated with OTC tests in the future, whether by reflex to additional confirmatory testing measures and / or by treatment.

Topic 5: Characterizing GC Resistance

Literature review by: Kara Mitchell and Mark Pandori; supported by Julio Ayala and Matthew Schmerer.
Presented by: Mark Pandori.

Key Questions

KQ1. What methods are available for resistance guided therapy and what is their performance?

KQ2. Has next generation sequencing been used for characterizing resistance?

KQ3. What options are available for culture-based AST and what needs to be considered when selecting a culture-based AST method?

KQ4. When should susceptibility testing for GC be performed?

Background and Summary

There is a lack of forward advancement in methods to phenomenologically assess GC resistance. Gradient and disk diffusion methods are still the only alternatives to agar dilution and broth microdilution (BMD). Gradient and disk diffusion are acceptable for determining resistance, but their use would benefit from standardization and further study of breakpoints. Strong evidence exists for molecular prediction of ciprofloxacin resistance; the case for molecular markers of azithromycin and cephalosporin resistance is less clear.

There has been some exploration of the application of nanopore sequencing, NGS and MALDI-TOF MS to resistance prediction in GC, but data is limited and mostly derived from retrospective data sets. However, NGS approaches have tremendous value for tracking and characterizing emerging resistance. Clinical use of NGS could be beneficial but is not yet practical.

Findings

KQ1. What methods are available for resistance guided therapy and what is their performance?

Different methods that have been considered for resistance guided therapy were reviewed and discussed:

- Agar Dilution: Remains the gold standard, though laborious and not feasible in all laboratories.
- Broth Microdilution (BMD): Limited data was reviewed for BMD. Minimum inhibitory concentrations (MICs) are higher with BMD compared to agar dilution[151] but more similar to gradient diffusion.[152] BMD is not recommended by the Clinical & Laboratory Standards Institute (CLSI) and generally over decades of experience it has been found that GC is not well suited to AST in broth culture. There was agreement that use of BMD is not supported by the literature.
- Gradient Diffusion (e.g., ETEST) and Disk Diffusion: These methods have proven to be effective with interpretations available for many drugs and they are more feasible for many laboratories than agar dilution. Gradient diffusion strips are generally preferred for the ability to quantitate an MIC. Data supporting the use of gradient and disc diffusion predated the scope of this literature review for many drugs, including those not represented below.[153] Since 2009 new work has shown:
    - Ceftriaxone – many ETEST based gradient diffusion studies achieve a high level of agreement with agar dilution (twofold MIC agreement, ranging from 88.7-98%)[92–95]

- Cefixime – generally high agreement in ETEST studies, with some inter-lab variability (74.3-96% agreement)[154,155]
- Azithromycin – high level of agreement (twofold MIC, 90.7-95%)[154–156]
- Gentamicin – gradient strip studies (ETEST) have shown agreement with agar dilution[157] and two studies showed comparability between disk diffusion and agar dilution.[158,159] However there are no CLSI defined zone diameter or MIC breakpoints, which negatively impacts usability and the lack of gentamicin resistant isolates for evaluation is a challenge.
- In limited data from singular studies, lower twofold agreement of ETEST with agar dilution was seen for penicillin (65%) and tetracycline (38-76%).

Existing studies of gradient diffusion methods are not always harmonized by media type.[154–157,160–167] However, many US studies have used GC agar. Overall, experts agreed that these studies are challenging and not always ideal, but there is strong data for agreement between gradient strips and agar dilution for the antibiotics used to treat GC.[155]

- Molecular - currently no molecular methods are FDA-authorized for resistance prediction in GC. However, there is strong evidence for molecular testing of ciprofloxacin resistance (*gyrA* gene mutation S91F), with published research displaying excellent correlation with phenotypic AST.
  - This mutation is the basis of SpeeDX ResistancePlus® GC commercial kit.[168]
  - LDTs based on molecular detection of this mutation have been implemented in clinical labs to guide patient treatment and care.[169–173]

PCR-based assessment of cephalosporin resistance would be immensely beneficial, but is stymied by many factors including the vast number of *penA* alleles and other mutations at play, large spectrum of corresponding phenotypes, and ongoing evolution of this resistance genomically.[174–178]

- Mass Spectrometry (MS) – current research to determine potential approaches via MS is early stage; MS targets that correlate with GC resistance are not yet defined.[179]
- NGS – (addressed below under KQ2)

KQ2. Has next generation sequencing been used for characterizing resistance?

NGS methods are currently used to look at retrospective data sets; none are being used clinically or even side by side in real time. Metagenomic sequencing from specimens using Nanopore/long-read technology, in real time, could be tremendously powerful for GC diagnostic AST and rapid strain typing for identification of transmission networks, but the current technology is far too slow and expensive to do this.[105–107]

There is substantial evidence that sequencing of GC isolates is a worthwhile endeavor as an ongoing, widely-cast, surveillance effort to help track resistance.[160,180–182]

- It could allow resistance prediction and provide for real-time detection of novel mutations associated with resistance, if paired with AST. In these instances, strong epidemiological response networks could work to facilitate further analysis when resistant cases arise.
- It would simultaneously provide information on what strains are dominant and what strains are succeeding in geographically distinct regions.
- There may also be a role in resolving individual cases if phenotypic results are confusing or do not match the clinical profile.

KQ3. What options are available for culture-based AST and what needs to be considered when selecting a culture-based AST method?

Disk diffusion and gradient diffusion strips are both available to a broad range of laboratories. Gradient diffusion strips are generally preferred because of their ability to generate MICs. There are multiple brands of gradient diffusion tests and not all achieve the same performance for each antibiotic. The regulatory status of strips can also vary even within a brand – for example, bioMérieux ETEST strips have been FDA-cleared for some drugs, while others currently are research use only (*e.g.*, cefixime, azithromycin). Laboratories should identify AST diffusion products with proven performance and ideally choose FDA-authorized products.

- Currently CLSI M 100 and European Committee on Antimicrobial Susceptibility Testing (EUCAST) have break points for disk diffusion and MIC based agar dilution for most, but not all, drugs. Several only have S and NS criteria. The FDA's Antibacterial Susceptibility Test Interpretive Criteria database provides additional guidance on FDA's recognized standards.
- CLSI M100 and EUCAST lack specific break points for gradient diffusion, however studies have been performed applying the agar dilution and disk diffusion breakpoints to gradient diffusion strips.
- CLSI, FDA or manufacturer guidance should be followed when available for diffusion-based AST; future updates or guidance specifically for gradient diffusion would be tremendously beneficial.
- Additional studies may need to be done for some drugs given the limited number of resistant strains available at this time (*e.g.*, gentamicin, ceftriaxone).

To optimize the success of culture-based AST, it should be kept in mind that media types can alter results. Laboratories should be made aware that the most reliable method (in terms of agar dilution agreement) is to set up on GC agar instead of chocolate agar.[167]

KQ4. When should susceptibility testing for GC be performed?

Susceptibility testing should be performed for:

- Cases of systemic / disseminated GC infection
- Cases of suspected treatment failure
- Cases of contact with known or suspected treatment failure
- Surveillance purposes

It is important that there is a clear understanding of how treatment failure is defined when guidelines are established. Considerations for susceptibility testing should be consistent with CDC's clinical guidelines.

Future Directions and Research Needs

Characterizing rapidly evolving resistance in GC is critically important. Advances in research and diagnostic tools are especially crucial and implementation of surveillance strategies and consideration of their impact on treatment needs to be forward-thinking. The experts discussed that recent conversations at meetings in the field have emphasized the need to start surveillance studies for new antimicrobials (e.g:, zolifloxacin and gepotidacin) now and consider their use before ceftriaxone use is no longer an option. AST methods for new drugs are needed and harmonization between FDA, CLSI and EUCAST should be considered.

In the near term, it is important to emphasize maintaining capacity for culture-based methods. Further characterization and standardization of gradient diffusion methods is needed, including evaluation of appropriate breakpoints. Guidance is needed for the use of gradient diffusion with CLSI's MIC based breakpoints. Continuing research into MALDI-TOF

MS methods for GC AST, should also be supported. While still requiring a culture isolate, MALDI-TOF MS may offer other advantages in workflow and reduced reagent costs. However, MALDI-TOF MS has been shown to poorly predict azithromycin susceptibility in GC.[18]

Considering the challenges of GC culture and understanding the relatively limited capacity for culture in the US, SMEs expressed that research to enable culture-independent sequencing and molecular AST should be prioritized. Looking further, use of NGS with machine learning methodologies may eventually enable successful prediction of phenotypes and merits exploration. Given that a vast majority of GC diagnostic testing is currently conducted with NAAT, and in most cases culture will not be performed, molecular testing may be the most practical AST method to pursue. A path forward for molecular ciprofloxacin testing (*gyrA* S91F mutation) either commercially as an FDA-cleared assay or as an assay that can be validated as an LDT should be pursued. There is opportunity to implement reflex molecular testing of *gyrA* and base treatment off of resutls.[170,18] It is known there is regional variability in resistance to drugs geographically and in certain patient populations; and this approach could extend the effective use of ciprofloxacin against susceptible infections. This is a situation in which commercial development of collection devices that can be used for both diagnostics and resistance testing is also highly desirable.

Cross-cutting Considerations

A number of themes, ideas or concerns arose repeatedly during the consultation, spanning multiple topic areas. These included:

- Harmonizing recommendations for laboratory testing with CDC's clinical guideline
- Ensuring research examines the impact of doxy-PEP implementation on diagnostic testing and AST
- Ensuring recommendations offer clarity on what is FDA cleared (and what is not)
- Ensuring recommendations can be enacted by laboratories (testing that is available, FDA authorized tests, consideration of possible regulatory challenges of LDTs)
- Addressing gaps for additional critical populations including neonate testing, pediatric testing
- Considering clear statements of practices that are not recommended
- Practicing diagnostic stewardship:
    - Consideration of who needs tests, how often and how tests are performed in differing populations.
    - Guiding laboratorians with information related to diagnostic testing that they may need to be able to communicate to clinicians. What should a lab be prepared to communicate and how can these guidelines help?
- Practicing antibiotic stewardship:
    - Ensuring issues of clinical relevance and possible overtreatment are considered
    - Integrating viability assays and information on time to clearance of nucleic acid into considerations of appropriate testing and treatment.
    - Advancing new AST approaches to improve resistance-guided therapy in GC
- Ensuring proper training and biosafety precautions for laboratorians and for individuals who may conduct CLIA-waived POCT:
    - Proper protective equipment, technique and waste disposal
    - Consideration of the risks of encountering *Neisseria meningitidis,* and importance of the precaution of vaccination
- Keeping in mind bottlenecks that may occur with supply chain issues or reductions in products for certain diagnostic tests:
    - How can manufacturers, federal agencies and laboratories work together to strengthen supply chains?
    - Where can flexibility in recommendations allow laboratorians to better manage testing through shortages?
- Identifying ways to bank specimens from and conduct research to improve diagnostics for rarer case infections.
    - How can isolates from disseminated infection (blood stream, joints, endocarditis) be collected and studied? Is there a path for making these conditions notifiable and having isolates submitted for banking?
    - How can diagnostic detection of high mortality infection such as endocarditis be improved?

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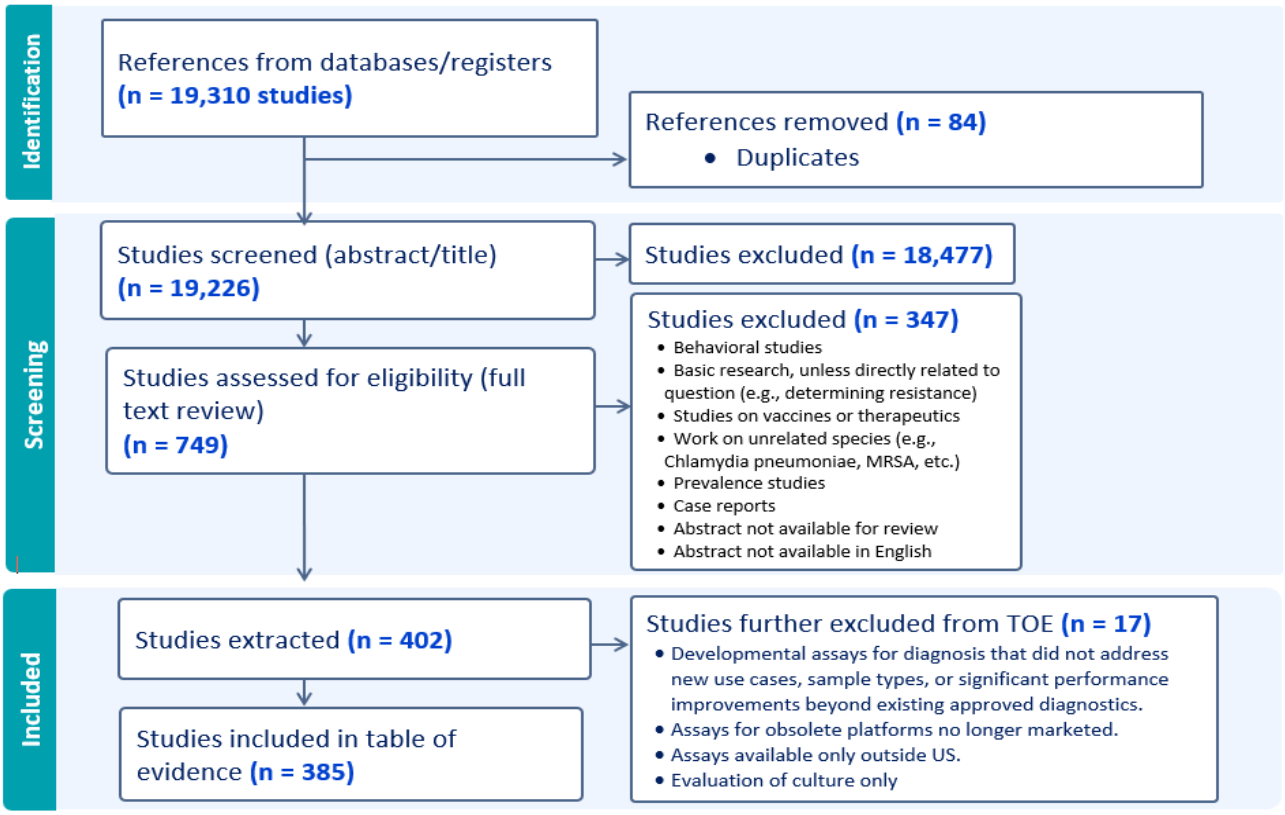
Appendix A. External Review Summary

Chlamydia trachomatis	
Characterizing Resistance	
KQ1. Have molecular resistance determinates been defined? KQ2. Are there molecular methods that can be used to identify potential resistance determinants? KQ3. Are there molecular methods that can be used for confirming CT viability when treatment failure is suspected? KQ4. Are there standardized culture-based antimicrobial susceptibility testing (AST) methods for CT?	**External Expert Reviewers:** Timothy Southern, MS, PhD, D(ABMM) Alana Sterkel, PhD, D(ABMM), SM(ASCP)CM **CDC Expert Support:** Travis Loya, PhD
Culture & Identification	
KQ1. Have culture methods for CT changed since they were described in 2009? KQ2. What are the various serological tests for CT and what are their performance characteristics and diagnostic value? KQ3. When should CT culture or resistance-based testing be performed?	**External Expert Reviewers:** Timothy Southern, MS, PhD, D(ABMM) Alana Sterkel, PhD, D(ABMM), SM(ASCP)CM **CDC Expert Support:** Travis Loya, PhD
Neisseria gonorrhoeae	
Characterizing Resistance	
KQ1. What methods are available for resistance guided therapy and what is their performance? KQ2. Has next generation sequencing been used for characterizing resistance? KQ3. What options are available for culture-based antimicrobial susceptibility testing (AST) and what needs to be considered when selecting a culture-based AST method? KQ4. When should susceptibility testing for GC be performed?	**External Expert Reviewers:** Kara Mitchell, PhD Mark Pandori, PhD, HCLD(ABB) **CDC Expert Support:** Julio Ayala, PhD Matthew Schmerer, PhD
Culture & Identification	
KQ1. Have culture methods for GC changed since they were described in 2009? KQ2. What biochemical tests can be used for the identification of N. gonorrhoeae? KQ3. What is the utility of MALDI-TOF for detecting GC? KQ4. When should culture be performed?	**External Expert Reviewers**: Sarah Buss, PhD, D(ABMM) Olusegun Soge, PhD, MSc **CDC Expert Support:** Jennifer Reimche, PhD Matthew Schmerer, PhD

Chlamydia trachomatis* and *Neisseria gonorrhoeae	
Diagnostic Performance	
KQ1. Has the performance of NAATs changed since 2009? Have new tests or specimen types been FDA approved and how do their performance characteristics compare to previous NAATs? KQ2. What types of self-collected specimens do laboratories accept? Do the performance characteristics of tests vary when specimens are self-collected in a clinical or non- clinical settings? KQ3. What are the testing recommendations for performing a test of cure, and do they vary across anatomic sites? KQ4. What methods can be used to diagnose suspected LGV? What are the performance characteristics of those methods? KQ5. How does pooling of multiple specimens or specimens across anatomical sites affect the performance characteristics of available assays? KQ6. When should confirmatory testing be performed and does this vary across anatomic sites?	**External Expert Reviewers:** Megan Crumpler, PhD, HCLD Susan Realegeno, PhD, D(ABMM), M(ASCP)CM Salika Shakir, PhD, D(ABMM) Barbara Van Der Pol, PhD, MPH **CDC Expert Support:** Julio Ayala, PhD Bill Davis, PhD Shelby Hutton, MS Amanda Smith, PhD
Point of Care Tests	
KQ1. What POC or near-POC NAATs for CT/GC are currently FDA-cleared? Which are CLIA-waived? KQ2. What are the acceptable specimen types and the performance characteristics? KQ3. When should POCT be used?	**External Expert Reviewers:** Yuka Manabe, MD, FIDSA, FRCP Tony Tran DrPH, MPH, D(ABMM) Barbara Van Der Pol, PhD, MPH **CDC Expert Support:** Amanda Smith PhD

Appendix B. Prisma Diagram of Review Process



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Association of Public Health Laboratories

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