The prevalence of sexually transmitted infections (STIs) is increasing in men who have sex with men (MSM). High-risk sexual behavior among MSM has become more common following the introduction of highly active anti-retroviral therapy (HAART) and — as a consequence — recreational drug and alcohol use. As a result, the incidence of STIs, specifically gonorrhea (GC), chlamydia (CT), and syphilis, is rising in MSM in North America as well as in Europe.

Although high-risk sexual practices are commonly recognized in MSM, the frequency of oral and anal sex is also increasing among young heterosexual adults. This increase in high-risk behavior could lead to an increase in the prevalence of STIs in this patient group. The rise in STIs related to high-risk sexual behavior raises concern about increases in HIV transmission, as there is evidence linking both ulcerative and non-ulcerative STIs with transmission and acquisition of HIV. For example, HIV-positive patients with urethral infection are known to have increased HIV-1 RNA levels in semen. This increase in seminal HIV viral load is highly relevant as seminal HIV-RNA levels are thought to correlate with HIV transmissibility.

It is unknown whether STIs at extra-genital sites lead to increases in HIV viral shedding similar to that seen in urethritis. It has been established, however, that gonococcal proctitis is an independent risk factor for HIV acquisition. This increase is presumably related to breakdown of the mucosal barrier...
secondary to rectal inflammation; and, therefore, one could infer that a similar risk would be associated with other causes of proctitis, including chlamydia.

Gonorrhea and chlamydia commonly cause rectal and pharyngeal infections in MSM. Several studies report that chlamydia is found more frequently than gonorrhea in the rectum; in contrast, gonorrhea is more commonly isolated from the oropharynx than chlamydia.

There are currently 10 studies in the literature evaluating the utility of NAATs in the diagnosis of chlamydia and gonorrhea in rectal and pharyngeal specimens.

Looking at the prevalence of GC and CT infections of the rectum, Klausner, et al, retrospectively reviewed the etiology in 101 cases of clinical proctitis in MSM and found that gonorrhea and chlamydia were the most frequently recovered pathogens (30% and 19%, respectively). Secondly, Kent, et al, used self-collected rectal swabs as a means to screen for gonococcal and chlamydial infection in MSM seeking HIV testing. Out of 174 collected rectal specimens, they found a prevalence of rectal chlamydia of 5.3%, while the prevalence of rectal gonorrhea was 2.9%. Similarly, Lister, et al, screened MSM for rectal infections in male-only saunas in Seattle; they detected chlamydia in 5.9% and gonorrhea in 2.2% in 507 patients screened.

Rectal gonococcal infection is frequently seen in HIV-positive patients. Specifically, Kim, et al, studied 564 MSM and found an overall prevalence of rectal gonorrhea of 7.1%. In a subgroup analysis, prevalence of rectal gonorrhea in HIV-positive MSM was 15.2%, and was found 3.5 times more frequently in HIV-positive than HIV-negative MSM.

In sexually transmitted pharyngeal infection, chlamydia is less frequently isolated than gonorrhea. For example, using polymerase chain reaction (PCR) to test pharyngeal specimens of 521 MSM, Lister, et al, detected chlamydia in only 0.6% and gonorrhea in 2.5%. Prevalence of gonococcal pharyngeal infection ranges between 1% to 6% in men and women attending sexually transmitted-disease (STD) clinics. In addition, of MSM diagnosed with gonorrhea at any site, 10% to 25% have only pharyngeal infection.

Notably, many patients with pharyngeal and rectal gonococcal infections do not have simultaneous genital infections. Additionally, it is important to note that gonorrhea and chlamydia often cause co-infection — not only in the urethra but also in the rectum and possibly the pharynx. Treatment for either organism generally requires a single dose of an antimicrobial (typically an intramuscular or oral cephalosporin for gonorrhea and an oral tetracycline or azalide for chlamydia); however, there is evidence suggesting that pharyngeal gonococcal infections are more difficult to treat and, therefore, may require a longer course of treatment.

Gonococcal and chlamydial infections are frequently asymptomatic. One study found that of 56 MSM who tested positive for either gonococcal or chlamydial urethritis, proctitis, or pharyngitis, only 23.2% reported symptoms at the relevant site. When symptomatic, gonococcal or chlamydial pharyngitis presents with a sore throat and can be associated with fever and enlarged submandibular lymph nodes. Gonococcal or chlamydial pharyngeal infection, however, is most frequently asymptomatic. For example, in a study in which 200 MSM in San Francisco underwent screening for pharyngeal gonorrhea, all patients who tested positive by culture or a NAAT denied symptoms. Similarly, in a Seattle-based study, 24 of 666 men screened for gonococcal pharyngeal infection tested positive, of whom only four (16%) were symptomatic. While proctitis may present with rectal pain, itching, rectal discharge, or bleeding, Kent, et al, found that approximately 85% of rectal gonococcal and chlamydial infections were asymptomatic.

For all patients with these infections, the paucity of symptoms in extra-genital sites is problematic as asymptomatic patients frequently do not seek medical care, leading to untreated reservoirs of infection in the community. For this reason, current guidelines from the Centers for Disease Control and Prevention (CDC) recommend at least annual screening for urethral, pharyngeal, and rectal sexually transmitted diseases in MSM, regardless of symptoms.

In addition, the CDC guidelines recommend more frequent screening (three- to six-month intervals) in patients at highest risk of acquiring infection (i.e., those with multiple sex partners or those who use or whose partners use illicit drugs). Despite these recommendations, asymptomatic screening is not regularly per-
formed outside of STD clinics, largely due to physician unawareness and a perceived low prevalence of infection in asymptomatic patients. Because of the association between STDs and HIV, however, it is critical that the CDC screening recommendations be implemented.

**Diagnosis of pharyngeal and rectal chlamydial and gonococcal infections**

For decades, bacterial culture was the standard diagnostic modality for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Culture of *Neisseria gonorrhoeae*, a Gram-negative diplococcus, requires plating on selective media and incubation at 36 °C in a CO₂-enriched atmosphere. This process is followed by colony identification by morphology, oxidase-positivity, and confirmation using various carbohydrate utilization or chemical tests. Other confirmation tests like direct fluorescent antibody or Gonostat are available. *Chlamydia trachomatis* is difficult to grow in culture as it is an obligate intracellular bacterium. Culture technique requires specific methods of specimen collection, transport, storage, and the use of a sensitive cell line. Importantly, because of the equipment and technique required, chlamydia culture is also relatively expensive.

Although it is the gold standard for the diagnosis of chlamydial and gonococcal infections, culture has several disadvantages. Specifically, gonococcal cultures can be falsely negative in the setting of a low bacterial load. On the other hand, false-positive gonococcal cultures may occur when cultures are taken from sites such as the pharynx, which is commonly colonized with other non-gonococcal Neisseriae species (e.g. *Neisseria meningitidis*). The use of the biochemical tests on suspected gonococcal colonies should obviate this disadvantage; however, their use adds additional laboratory cost and may impact turnaround time. Secondly, because chlamydia is an intracellular bacterium, its specific culture requirements are not available in all laboratories, and the culture system has modest sensitivity.

Despite the imperfections of culture, however, it remains important to maintain capacity for performance of gonorrhea culture and antimicrobial-susceptibility testing in order to monitor changing resistance patterns. This is especially true for gonorrhea, given the increase in antibiotic resistance, particularly due to the fluoroquinolones. In addition, bacterial culture facilitates isolate subtyping. Lastly, in the context of forensic microbiology, gonococcal and chlamydial cultures may be the method of choice for definitively establishing the diagnosis of these infections.

Because of the challenges of culture, non-culture-based tests for gonorrhea and chlamydia have been developed. The first non-culture-based tests were enzyme immunoassays (EIA) and direct fluorescent antigen tests (DFA). Subsequently, nucleic acid hybridization probes, which detect DNA or RNA sequences, were developed. These non-culture-based tests were developed...
for use in specimens from the urogenital tract, and with the exception of DFA, they have not been evaluated for use in the detection of gonococcal or chlamydial infection in extragenital sites.

Nucleic acid amplification tests (NAATs) have been available since 1993 and are the newest generation of the non-culture-based tests. The NAATs detect and amplify specific bacterial DNA or RNA sequences specific for the targeted organism, and each NAAT uses a slightly different method of amplification (see Table 1). The currently available NAATs include PCR (COBAS Roche Amplicor), strand displacement amplification (SDA) (BD ProbeTec), and transcription-mediated amplification (TMA) (Gen-Probe Aptima). The NAATs offer many advantages compared to bacterial culture (see Table 2).

Of particular importance is the improved sensitivity of NAATs in comparison to culture of these two organisms. This superior sensitivity has revolutionized the ability to diagnose gonococcal and chlamydial infections. In fact, because of their high sensitivity and specificity, NAATs have essentially replaced bacterial culture for the diagnosis of gonococcal and chlamydial urogenital infections in both men and women. Importantly, their development has improved our understanding of the epidemiology of those STIs. Additional key advantages of NAATs include use of non-invasively obtained specimens (e.g., urine), ease of provider collection of specimens for testing, and feasibility of patient self-collected specimens (e.g., vaginal swabs). Recent research demonstrated the reliability of NAATs using self-collected rectal specimens for screening in non-clinical settings.17,18

<table>
<thead>
<tr>
<th>Culture</th>
<th>NAATs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Advantages</td>
<td>• Nearly perfect specificity&lt;br&gt;• Ability to retain the isolate to perform antimicrobial susceptibility testing and isolate subtyping&lt;br&gt;• Do not require viable organisms for detection&lt;br&gt;• A single NAAT can detect GC and CT simultaneously&lt;br&gt;• High sensitivity, as nucleic acid can be amplified from a single organism&lt;br&gt;• Rapid processing (result is available often in 4 to 5 hours)&lt;br&gt;• Can be performed on non-invasively performed specimens (urine, self-collected vaginal swabs, self-collected rectal swabs)</td>
</tr>
<tr>
<td>Disadvantages</td>
<td>• Technique is labor-intensive, difficult to standardize and expensive&lt;br&gt;• Long turn-around time (24 to 72 hours)&lt;br&gt;• Relatively poor sensitivity (particularly for CT, as it is an obligate intracellular bacterium)&lt;br&gt;• Decreased sensitivity if organism viability is compromised or if specimen transport, storage conditions are inadequate&lt;br&gt;• Decreased sensitivity in the setting of a low bacterial load&lt;br&gt;• Specificity may be decreased due to sample contamination if strict quality-control measures are not implemented&lt;br&gt;• Decreased specificity due to cross-reactivity with genes from related species (particularly for GC)&lt;br&gt;• Decreased sensitivity due to amplification inhibitors seen with specific NAATs (e.g., PCR, SDA)&lt;br&gt;• DNA-based NAATs have decreased sensitivity due to low numbers of target DNA in each bacterium&lt;br&gt;• Certain NAATs have difficulties with reproducibility (e.g., SDA)</td>
</tr>
</tbody>
</table>

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Despite their advantages, there are several limitations of NAATs for diagnosing gonococcal and chlamydial infections. Test systems using PCR and SDA can be inhibited due to amplification inhibitors resulting in false-negative test results. Of note, inhibitors are less frequently associated with male urethral or female endocervical specimens compared with urine specimens. Examples of urine inhibitors include hemoglobin, glucose, nitrites, beta-human chorionic gonadotropin, and crystals. Laboratory methods such as dilution, heat treatment or freeze-thawing of samples have been found to reduce inhibition. In addition, in order to identify inhibition, NAAT manufacturers have included internal controls in the test kits. Notably, one advantage of the TMA is its unique target capture step that may essentially eliminate amplification inhibition, thereby improving the sensitivity of that NAAT.

In the diagnosis of lymphogranuloma venereum (LGV), an STI caused by the Chlamydia trachomatis subtypes L1-L3, a limitation of commercially available NAATs for chlamydia detection is their inability to distinguish between those Chlamydia trachomatis subtypes (LGV (L1-L3) vs. non-LGV (A-K). From an epidemiological standpoint and because the treatment for LGV differs from treatment given for other chlamydial infections, there is a need for further advances in molecular-diagnostic testing to enable differentiation between those subtypes.

In general, NAAT processing is less labor intensive than performing culture, although care must be taken to avoid sample contamination as this can lead to false-positive test results. Of note, NAAT processing is more expensive than culture, and it is this reason that is most commonly cited by medical providers when explaining why less sensitive screening tests like culture or non-amplified tests may be preferred over NAATs. When evaluating the cost-effectiveness of using NAATs to screen asymptomatic young women as a means to prevent pelvic inflammatory disease, however, Shafer, et al, found that the use of a NAAT to test urine specimens was not only less expensive but also increased the number of women screened when compared to the use of cervical swab specimens collected through pelvic examination.

NAATs are FDA-cleared for use with male urethral and urine as well as female endocervical and urine specimens. Most recently, TMA was FDA-cleared for use with self-collected vaginal-swab specimens. Current research to evaluate the performance NAATs in rectal and pharyngeal specimens

There are currently 10 studies in the literature evaluating the utility of NAATs in the diagnosis of chlamydia and gonorrhea in rectal and pharyngeal specimens.
tion studies for those organisms are difficult, as frequently there is no optimal gold standard for comparison due to the lower sensitivity of culture. Therefore, those studies used an expanded reference standard that includes a positive culture result, a second confirmatory NAAT or DFA, or a confirmatory NAAT using a different primer target.

It is the difference in sensitivity which is the most striking between the NAATs and GC and CT culture (see Table 3). Generally, NAAT sensitivity is at least as good as culture, and frequently, it is superior. Specifically, for rectal gonococcal infection, TMA had the highest sensitivity, followed by SDA. PCR, which essentially has equivalent sensitivity to culture, had the lowest sensitivity of the NAATs for rectal GC. For rectal chlamydia, TMA again had the highest sensitivity, followed by PCR and, lastly, SDA. Finally, for pharyngeal gonococcal infection, TMA had the highest sensitivity, followed by SDA. PCR had the lowest sensitivity for GC detection in the pharynx. Conclusions regarding the sensitivity of NAATs for pharyngeal CT cannot be made as the prevalence across the studies was quite low (only 19 total subjects tested positive out of 694 subjects screened in the four available studies).

As with bacterial culture, the specificity of the NAATs for GC and CT detection in extra-genital sites varies between studies. Secondly, the reference standards were not standardized across studies. A third limitation is that individual studies had small sample sizes ranging from 20 to 491 total patients screened and a low frequency of infection ranging from one to 47 infected patients per study.

Overall, it appears that culture is suboptimal for the detection of gonorrhea or chlamydia infection in the rectum and pharynx due to its low and variable sensitivity. Despite the limitations of available studies, NAATs appear to have great potential for use in testing non-genital specimens. Due to its consistently high sensitivity in both the pharynx and rectum, TMA appears especially promising for the detection of gonorrhea and chlamydia in the rectum and for gonorrhea in the pharynx. In contrast, PCR appears to have lower sensitivity than other NAATs for the detection of gonorrhea and chlamydia in extra-genital sites. Because they are not yet FDA-cleared for extra-genital screening, CLIA verification for NAATs at local laboratories is required prior to their use for gonorrhea and chlamydia screening in the pharynx and rectum.

### Table 3. Mean sensitivity and specificity of culture and NAATs for GC/CT detection in the rectum and pharynx GC rectum.

<table>
<thead>
<tr>
<th>Diagnostic test</th>
<th>Study</th>
<th>Number positive tests / Total subjects screened</th>
<th>Combined number of positive tests</th>
<th>Mean sensitivity</th>
<th>Mean specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>Young et al. Klausner et al.</td>
<td>9/205 MSM</td>
<td>18/322</td>
<td>92.2%</td>
<td>100%</td>
</tr>
<tr>
<td>PCR</td>
<td>Cook et al. Klausner et al.</td>
<td>3/10 W and M</td>
<td>7/27</td>
<td>75.0%</td>
<td>93.3%</td>
</tr>
<tr>
<td>SDA</td>
<td>Klausner et al.</td>
<td>12/205 MSM</td>
<td>42/395</td>
<td>73.3%</td>
<td>99.1%</td>
</tr>
<tr>
<td>TMA</td>
<td>Klausner et al.</td>
<td>12/205 MSM</td>
<td>6/220</td>
<td>45.5%</td>
<td>99.5%</td>
</tr>
</tbody>
</table>

CT rectum

<table>
<thead>
<tr>
<th>Diagnostic Test</th>
<th>Study</th>
<th>Number positive tests / Total subjects screened</th>
<th>Combined number of positive tests</th>
<th>Mean sensitivity</th>
<th>Mean specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>Winkowski et al. Cook et al. Klausner et al.</td>
<td>13/20 W</td>
<td>21/271</td>
<td>76.6%</td>
<td>100%</td>
</tr>
<tr>
<td>PCR</td>
<td>Winkowski et al. Cook et al. Klausner et al. Lister et al.</td>
<td>3/10 W and M</td>
<td>7/33</td>
<td>77.7%</td>
<td>95.8%</td>
</tr>
<tr>
<td>SDA</td>
<td>Klausner et al.</td>
<td>12/205 MSM</td>
<td>42/350</td>
<td>73.3%</td>
<td>99.1%</td>
</tr>
<tr>
<td>TMA</td>
<td>Klausner et al.</td>
<td>12/205 MSM</td>
<td>24/270</td>
<td>88.9%</td>
<td>99.5%</td>
</tr>
</tbody>
</table>

GC pharynx

<table>
<thead>
<tr>
<th>Diagnostic test</th>
<th>Study</th>
<th>Number positive tests / Total subjects screened</th>
<th>Combined number of positive tests</th>
<th>Mean sensitivity</th>
<th>Mean specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>Stary et al. Pie-Picado-Shair et al. Young et al. Klausner et al.</td>
<td>2/4 W</td>
<td>28/325</td>
<td>41.7%</td>
<td>100%</td>
</tr>
<tr>
<td>PCR</td>
<td>Leslie et al. Klausner et al.</td>
<td>7/124 M and W</td>
<td>19/320</td>
<td>84.3%</td>
<td>88.8%</td>
</tr>
<tr>
<td>SDA</td>
<td>Klausner et al.</td>
<td>15/205 MSM</td>
<td>19/205</td>
<td>90.5%</td>
<td>99.5%</td>
</tr>
<tr>
<td>TMA</td>
<td>Klausner et al.</td>
<td>15/205 MSM</td>
<td>19/205</td>
<td>90.5%</td>
<td>99.5%</td>
</tr>
</tbody>
</table>

Legend:

NAAT = nucleic acid amplification test, PCR = polymerase chain reaction, SDA = strand displacement amplification, TMA = transcription mediated amplification, M = men, W = women, MSM = men who have sex with men.
dation” process, aimed at documenting that a test, which has already been verified, is repeatedly yielding the expected results as the test is performed over a period of time. Validation is an integral part of the laboratory’s ongoing quality assurance program.42

Since commercial NAATs are not FDA-cleared for rectal and pharyngeal specimens, individual labs may verify NAATs for non-genital sites; however, using culture as a comparison can be challenging — and if new-patient samples are used, it may require consideration by a local investigational review board. That said, the use of clinical-diagnostic specimens without patient identifiers is not human research and is exempt from human-subjects considerations as defined in the Code of Federal Regulations, Title 45, Part 46. The low sensitivity of culture in the detection of gonorrhea and chlamydia creates a problem for verification studies because the commercial NAAT may outperform the gold standard, culture. A method called latent class analysis requires using three or more conditionally independent tests to define true positives.43 The performance in sensitivity and specificity of any three out of four positive or any two out of three comparators appears to be similar.44

For instance, the San Francisco Department of Public Health Laboratory, in collaboration with colleagues at the University of California San Francisco, conducted a latent class analysis on the performance of three NAATs and culture for gonorrhea and chlamydia at non-genital sites.45 In that study, the sensitivity and specificity of culture, SDA, TMA, and PCR were compared for gonorrhea and chlamydia in the pharynx and rectum. The SDA and TMA methods were found to be more sensitive than culture, while PCR was less sensitive for pharyngeal chlamydia and rectal gonorrhea. In that study, a true positive was defined by a positive culture or two or more positive NAATs.

To avoid the complexity and expense of a verification study of this scope, an alternative approach is to work with a reference laboratory that has previously verified the test by either having the reference laboratory 1) verify a suitable number of test samples, or 2) provide a panel of samples previously characterized by the reference lab to be tested at the lab undertaking verification. The reference lab aliquots and stores samples with a known result and the testing lab may then run the samples in its operating NAAT system. The existing NAAT system needs to have been established in accordance with CLIA regulations. The reference samples can only be used for verification of the same NAAT system that the reference lab has used unless duplicate samples are collected in separate transportation media and then tested by both methods.

The number of samples suggested for a verification of an FDA-cleared indication by the American Society of Microbiology is 20 positives and 50 negatives and by the National Committee for Clinical Laboratory Standards is 50 positives and 100 negatives.46 The availability of samples may be limited and, therefore, dictate the protocol; however, as the number of positives becomes less, the confidence limits of the sensitivity estimate will become broader. As such, use of fewer than 10 samples would not be recommended. Since specificity has important implications on positive predictive value, it is also important that the NAAT test ultimately will be employed in a population setting with sufficient disease prevalence to avoid excessive false-positives.

Individual labs must develop their own protocol for verification and documentation of the study must be recorded and maintained anticipating CLIA-inspector requests of the local laboratory’s verification process. The protocol design typically involves a reference panel of at least 20 positive and 20 negative specimens, obtained from a laboratory that has completed verification. The samples are run on a CLIA-approved NAAT system in the verifying laboratory. Positives and negatives, along with controls, are tested by at least two microbiologists and by a single microbiologist on different days, to demonstrate consistent results from day to day and among different operators. Target sensitivity and specificity should be equivalent to that demonstrated in the reference laboratory from which the samples are obtained.

A review of screening tests to detect gonorrhea and chlamydia, with a brief discussion of NAAT test verification, has been published by the CDC and is available at www.cdc.gov/std/labguidelines/rr5115.pdf.41 Further information on local laboratory verification of GC/CT NAATs may be obtained at www.stdtraining.org/gcnaat.

Conclusion
The significant proportion of high-risk patients found to have asymptomatic gonococcal and chlamydial infections has led to the realization that a largely unrecognized reservoir of asymptomatic infection exists, particularly among MSM. Because of the association of STIs and HIV transmission, the diagnosis and treatment of asymptomatic STIs is a crucial strategy in the prevention of HIV transmission and acquisition. Therefore, consistent with CDC guidelines, patients practicing high-risk sexual behaviors should be routinely and periodically screened for STIs, independent of symptoms.

Given the low sensitivity of gonococcal and chlamydial culture, non-culture-based testing should be expanded to facilitate the accurate and prompt diagnosis of gonorrhea and chlamydia. The development of molecular-diagnostic methods has greatly advanced the ability to diagnose STIs. Similar to their performance in urogenital infections, NAATs show great promise for the detection of chlamydia and gonorrhea using non-genital site specimens. Many experts expect that following formal evaluation of extra-genital specimen testing, use of NAATs will become the standard of care for the diagnosis of gonococcal and chlamydial infections in the pharynx and rectum. Many are urging NAAT manufacturers to request FDA clearance for the use of these tests for the testing of clinical specimens from non-genital sites to improve medical-care and –screening practices. Prior to FDA clearance, public-health officials, healthcare providers, and local laboratories should work together to perform local verification studies to facilitate the use of these molecular assays for that purpose.

There is continued excitement about advances in molecular technology and the potential uses of molecular methods to improve the ability to detect STIs. Future directions for gonorrhea and chlamydia diagnostic testing include the use of real-time PCR and self-collected oral washings (e.g., mouthwash)
to detect gonococcal oropharyngeal infections. Ultimately, more widespread STI screening offers multiple benefits, such as improvement in sexual health, reduced sequelae of genital and extra-genital infections, and interruption of HIV transmission among those at risk for STIs. □

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