California Association for Medical Laboratory Technology

Distance Learning Program

MMWR REPORT ON CHLAMYDIA AND NEISSERIA

Course # DL-010

Adapted from Morbidity and Mortality Weekly Report, March 14, 2014 / 63(RR02)
Recommendations for the Laboratory-Based Detection of *Chlamydia trachomatis* and
*Neisseria gonorrhoeae* – 2014

by

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OBJECTIVES:
At the end of this course the participant will be able to
1. discuss the public health effects of Chlamydia trachomatis and Neisseria gonorrhoeae.
2. list the reasons why NAAT testing is recommended.
3. discuss the reasons why NAAT testing is a challenge to the laboratory.
4. outline the culture techniques for identification of Neisseria gonorrhoeae.
5. outline the culture techniques for identification of Chlamydia trachomatis.
6. describe a gram stain of a urethral specimen positive for Neisseria gonorrhoeae.
7. compare the detection of Chlamydia trachomatis and Neisseria gonorrhoeae in women compared to men.
8. discuss the specialized procedures for detection of Chlamydia trachomatis and Neisseria gonorrhoeae in cases of suspected sexual assault.
9. describe Lymphogranuloma venereum infection and laboratory detection.
10. discuss the advantages and disadvantages of pooling specimens for detection of Chlamydia trachomatis and Neisseria gonorrhoeae.

ABSTRACT
This report updates The Centers for Disease and Control’s (CDC’s) 2002 recommendations regarding screening tests to detect Chlamydia trachomatis and Neisseria gonorrhoeae infections and provides new recommendations regarding optimal specimen types, the use of tests to detect rectal and oropharyngeal C. trachomatis and N. gonorrhoeae infections, and circumstances when supplemental testing is indicated. The recommendations in this report are intended for use by clinical laboratory directors, laboratory staff, clinicians, and disease control personnel who must choose among the multiple available tests, establish standard operating procedures for collecting and processing specimens, interpret test results for laboratory reporting, and counsel and treat patients. The performance of nucleic acid amplification tests (NAATs) with respect to overall sensitivity, specificity, and ease of specimen transport is better than that of any of the other tests available for the diagnosis of chlamydial and gonococcal infections. Laboratories should use NAATs to detect chlamydia and gonorrhea except in cases of
child sexual assault involving boys and rectal and oropharyngeal infections in prepubescent girls and when evaluating a potential gonorrhea treatment failure, in which case culture and susceptibility testing might be required. NAATs that have been cleared by the Food and Drug Administration (FDA) for the detection of *C. trachomatis* and *N. gonorrhoeae* infections are recommended as screening or diagnostic tests because they have been evaluated in patients with and without symptoms. Maintaining the capability to culture for both *N. gonorrhoeae* and *C. trachomatis* in laboratories throughout the country is important because data are insufficient to recommend non-culture tests in cases of sexual assault in prepubescent boys and extragenital anatomic site exposure in prepubescent girls. *N. gonorrhoeae* culture is required to evaluate suspected cases of gonorrhea treatment failure and to monitor developing resistance to current treatment regimens. Chlamydia culture also should be maintained in some laboratories to monitor future changes in antibiotic susceptibility and to support surveillance and research activities such as detection of lymphogranuloma venereum (LGV) or rare infections caused by variant or mutated *C. trachomatis*.

**INTRODUCTION**

*Chlamydia trachomatis*

*Chlamydia trachomatis* infection is the most common notifiable disease in the United States with >1.3 million infections reported to CDC in 2010. The majority of persons with *C. trachomatis* infection are not aware of their infection because they do not have symptoms that would prompt them to seek medical care. Consequently, screening is necessary to identify and treat this infection. Direct medical costs of *C. trachomatis* infections were estimated at $516.7 million in 2008 (2012 dollars). The direct costs associated with *C. trachomatis* infections and their sequelae have decreased in recent years as sequelae have been managed in a less-costly manner and ectopic pregnancies have been increasingly managed medically. Also of importance are the tangible costs, including the lost productivity, and the intangible costs, including psychological and emotional injury caused by infertility and ectopic pregnancy. Untreated *C. trachomatis* infections can lead to serious complications. In older observational treatment studies, up to 30% of women with untreated *C. trachomatis* infections developed pelvic inflammatory disease (PID). Of these women, the majority had symptoms that were too mild or nonspecific to cause them to seek medical treatment. Regardless of symptom intensity, the consequences of PID are severe. Left untreated, 20% of those with symptomatic PID might become infertile; 18% will experience debilitating, chronic pelvic pain; and 9% will have a life-threatening tubal pregnancy. The importance of subclinical PID became apparent with observations that most of the women with tubal factor infertility or ectopic pregnancy who had serologic evidence of chlamydial infection apparently had no history of PID. *C. trachomatis* infection during pregnancy might lead to infant conjunctivitis and pneumonia and maternal postpartum endometritis. Among men, urethritis is the most common illness resulting from *C. trachomatis* infection. Complications (e.g., epididymitis) affect a minority of infected men and rarely result in reproductive health sequelae.

*C. trachomatis* infections of the rectum can result from unprotected anal intercourse and are typically asymptomatic but might progress to proctocolitis. Ocular
infections can result in conjunctivitis in neonates and adults. Sexually acquired reactive arthritis also has been reported as a possible consequence.

*Neisseria gonorrhoeae* infections are the second most common notifiable communicable disease in the United States with 309,341 cases being reported to CDC in 2010. Gonococcal infections tend to cause a stronger inflammatory response than *C. trachomatis* but are typically asymptomatic in women until complications such as PID develop. In men, the majority of urethral infections cause urethritis with painful urination and, less commonly, epididymitis or disseminated gonococcal infection.

Subsets of men who have sex with men (MSM) engage in risky sexual behaviors (e.g., having sex with multiple, anonymous partners and unprotected oral and rectal sex), often leading to infections at these extragenital sites. MSM might have a high prevalence of sexually transmitted infections especially at these extragenital sites, which can be a public health problem because of the potential for enhancing HIV transmission. CDC recommends routing laboratory screening of genital and extragenital sites for all sexually active MSM at risk for infection.

**TESTING TECHNOLOGIES**

Multiple laboratory test options can be used to detect chlamydia and gonorrhea although some might not be recommended for routine use based on performance. Direct detection of the pathogen using culture or nonculture methods is possible. Of the nonculture tests available, only nucleic acid amplification testing (NAAT) is recommended for routine use whereas other tests (e.g., enzyme immunoassays, nucleic acid probe tests, and genetic transformation tests) are not recommended. Serologic tests that detect a systemic immune response to infection are not recommended because of the lack of precision for the detection of an active infection. Since 2002, improvements in chlamydia and gonorrhea NAAT technologies have enabled significant implementation and expansion of screening programs using less invasive specimen collection. Although these changes have created opportunities for more rapid and accurate chlamydia and gonorrhea diagnosis and a broader understanding of key populations at risk, they also might have created challenges (e.g., increased laboratory costs and physical design constraints requiring unidirectional specimen processing to minimize contamination when laboratories attempt to incorporate new technologies into their existing test repertoire).

The performance of NAATs with respect to overall sensitivity, specificity, and ease of specimen transport is better than that of any of the other tests available. Culture for *C. trachomatis* and *N. gonorrhoeae* was long the reference standard against which all other diagnostic tests were compared. However, better tests have been needed because of difficulties in maintaining the viability of organisms during transport and storage in the diverse settings in which testing is indicated. In addition, the tissue culture methods for *C. trachomatis* isolation are difficult to standardize, technically demanding, expensive, and relatively insensitive. Thus, diagnostic test manufacturers developed nonculture tests. The first nonculture tests for the organisms included enzyme immunoassays (EIAs), which detect specific chlamydial or gonococcal antigens, and direct fluorescent antibody (DFA) tests for *C. trachomatis*, which use fluorescein-conjugated monoclonal antibodies that bind specifically to bacterial antigen in smears. These antigen-specific detection tests
were followed by nucleic acid hybridization tests, which detect \textit{C. trachomatis}-specific or \textit{N. gonorrhoeae}-specific deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) sequences. With the availability of these nonculture tests, some of which could be automated, screening programs for \textit{C. trachomatis} were initiated, and screening programs for \textit{N. gonorrhoeae} began to change from culture to using the more convenient and, for remote settings, more reliable nonculture methods. The primary drawback of these tests, especially for \textit{C. trachomatis}, was that they failed to detect a substantial proportion of infections. This changed with the introduction of NAATs that amplify and detect \textit{C. trachomatis}-specific or \textit{N. gonorrhoeae}-specific DNA or RNA sequences. These tests are approximately 20-35% more sensitive than the earlier nonculture tests.

This report emphasizes the importance of maintaining the capability to culture for both \textit{N. gonorrhoeae} and \textit{C. trachomatis} in laboratories throughout the country because there are insufficient data to recommend nonculture tests in cases of sexual assault in boys and extragenital site exposure in girls. \textit{N. gonorrhoeae} culture is required as a test of cure to evaluate suspected cases of gonorrhea treatment failure and to monitor developing resistance to current treatment regimens. Test of cure should be done when clinically indicated only (i.e., not part of routine care). Chlamydia culture capability also should be maintained in some laboratories to monitor future changes in antibiotic susceptibility and to support surveillance and research activities such as detection of lymphogranuloma venereum (LGV) or rare infections caused by variant or mutated \textit{C. trachomatis} such as the type recently described in Sweden.

**RECOMMENDATIONS**

**Tests to Detect \textit{C. trachomatis} and \textit{N. gonorrhoeae}**

**Isolation and identification of \textit{Chlamydia trachomatis}**

Specimen collection swabs for \textit{C. trachomatis} culture must have a plastic or wire shaft and either rayon, Dacron, or cytobrush tip. Other materials might inhibit isolation. Specimen collection for \textit{C. trachomatis} culture is invasive requiring insertion of a swab 2-3 cm into the male urethra or 1-2 cm into the endocervical canal followed by two or three rotations to collect sufficient columnar or cuboidal epithelial cells. Following the collection, culture samples should be stored in an appropriate transport medium such as sucrose phosphate glutamate buffer or M4 media (Thermal Scientific, Lenexa, Kansas) and transported at \(\leq 4^\circ\text{C}\) to the laboratory within 24 hours of collection to maximize recovery of viable organisms. If transport is delayed >24 hours, the transport medium containing the specimen should be stored at -70\(^\circ\text{C}\). The specimen is inoculated by centrifugation onto a confluent monolayer of McCoy, HeLa 229, or Buffalo green monkey kidney cells that support growth of \textit{C. trachomatis}. Once the specimen has been inoculated, 2\(\mu\text{g/ml}\) of cycloheximide should be added to the growth medium to suppress protein synthesis by the host eukaryotic cell. Inoculated cells are harvested after 48–72 hours of growth; infected cells develop characteristic intracytoplasmic inclusions that contain substantial numbers of \textit{C. trachomatis} elementary and reticulate bodies.

The cell monolayers are reacted with either genus specific or species-specific fluorescein-conjugated monoclonal antibodies to allow specific visualization of the chlamydial inclusions with an epifluorescent microscope. Cell culture detection of \textit{C. trachomatis} is highly specific if a \textit{C. trachomatis} major outer membrane protein (MOMP)-specific stain is used. Monoclonal antibodies directed against the family-
specific lipopolysaccharide (LPS) of Chlamydiaceae cost less but might stain bacteria that share LPS antigens. LPS stains might be suitable for routine use, but a species-specific (MOMP) stain is recommended in situations requiring increased specificity. Less specific inclusion-detection methods using iodine or Giemsa stain are not recommended. Cell culture methods vary among laboratories, resulting in substantial interlaboratory variation in performance. The shell vial method of culture uses a larger inoculum with a reduced risk for cross contamination and therefore provides better accuracy than the 96-well microtiter plate method. In certain laboratories, higher sensitivities are obtained by performing a blind pass in which an inoculated cell monolayer is allowed to incubate for 48–72 hours, after which the monolayer is disrupted and used to inoculate a fresh monolayer that is stained after 48–72 hours of incubation to allow for another cycle of growth.

Despite the technical difficulties, cell culture, when performed by an experienced analyst, was the most sensitive diagnostic test for chlamydial infection until the introduction of NAATs. The relatively low sensitivity, extended testing turnaround time, difficulties in standardization, labor intensity, technical complexity, stringent specimen collection and transport requirements, and relatively high cost are disadvantages of cell culture isolation of *C. trachomatis*. Recommended procedures for *C. trachomatis* isolation and culture detection using a species specific stain must be followed when using this test in cases of suspected child sexual assault in boys and extragenital infections in girls.

**Isolation and identification of *N. gonorrhoeae***

Because of its high specificity (>99%) and sensitivity (>95%), a Gram stain of a male urethral specimen that demonstrates polymorphonuclear leukocytes with intracellular Gram-negative diplococcic can be considered diagnostic for infection with *N. gonorrhoeae* in symptomatic men. However, because of lower sensitivity, a negative Gram stain should not be considered sufficient for ruling out infection in asymptomatic men. In addition, Gram stains of endocervical specimens, pharyngeal, or rectal specimens also are not sufficient to detect infection and therefore are not recommended. Specific testing for *N. gonorrhoeae* is recommended because of the increased utility and availability of highly sensitive and specific testing methods and because a specific diagnosis might enhance partner notification.

If multiple specimens are being collected from an anatomic site, *N. gonorrhoeae* culture specimens should be obtained first; this sequence maximizes the load collected, which increases the likelihood of a successful culture. Specimens collected for gonorrhea culture should be obtained by using swabs with plastic or wire shafts and rayon, Dacron, or calcium alginate tips. Other swab material such as wood shafts and cotton tips might be inhibitory or toxic to the organism and should be avoided. Although collection of epithelial cells is less important for culture detection of *N. gonorrhoeae*, swabs should be inserted 2–3 cm in the male urethra or 1–2 cm into the endocervical canal followed by two or three rotations. In cases of urethritis, collection of the exudates is sufficient for *N. gonorrhoeae* culture.

Several nonnutritive swab transport systems are available, and some studies suggest that these transport systems might maintain gonococcal viability for up to 48 hours in ambient temperatures. However, environmental conditions might vary by
location and season, which could affect the viability of gonorrhea in these transport systems; thus, additional local validation of transport conditions might be needed.

Culture medium transport systems are preferred because there are some advantages over swab transport systems (e.g., extended shelf life and better recovery because cultivated isolates are being transported rather than a clinical specimen). Culture medium is inoculated with the swab specimen and then placed immediately into a CO₂-enriched atmosphere for transportation to the laboratory. Because *N. gonorrhoeae* has demanding nutritional and environmental growth requirements, optimal recovery rates are achieved when specimens are inoculated directly and when the growth medium is incubated in an increased CO₂ environment as soon as possible.

Specimens from normally nonsterile sites are streaked on a selective (e.g., Thayer-Martin or Martin-Lewis) medium and specimens from sterile sites are streaked on nonselective (e.g., chocolate agar) medium. Culture for *N. gonorrhoeae* isolation include a base medium supplemented with chocalatized (heated) equine or bovine blood to support the growth of the gonococcus. Commercially prepared chocolate agar containing synthetic hemin and growth factors for *N. gonorrhoeae* are available from various vendors. Selective media differ from routine culture media in that they contain antimicrobial agents (i.e., vancomycin, colistin, and nystatin or another antifungal agent) that inhibit the growth of other bacteria and fungi. Using selective media might improve isolation if the anatomic source of the specimen normally contains other bacterial species, although some strains of *N. gonorrhoeae* have been demonstrated to be inhibited on selective media. Inoculated media are incubated at 35°C–36.5°C in an atmosphere supplemented with 5% CO₂ and examined at 24 and 48 hours postcollection. Supplemental CO₂ can be supplied by a CO₂ incubator, candle-extinction jar using unscented candles (e.g., votive candles), or CO₂-generating tablets.

Isolates recovered from a genital specimen on selective medium that are Gram-negative diplococcus- and oxidase-positive might be presumptively identified as *N. gonorrhoeae*. A presumptive identification indicates only that a Gram-negative, oxidase-positive diplococcus (e.g., any *Neisseria* species or *Branhamella catarrhalis*) might be isolated from such specimens. Certain coccobacilli, including *Kingella denitrificans*, might appear to be Gram-negative diplococci in Gram-stained smears. A confirmed laboratory diagnosis of *N. gonorrhoeae* requires additional biochemical tests (Table 1). A presumptive test result is sufficient to initiate antimicrobial therapy, but additional tests must be performed to confirm the identity of an isolate as *N. gonorrhoeae*.

Culture for *N. gonorrhoeae* is inexpensive to perform from genital sites and is specific and sensitive if the specimen is collected and transported properly to the laboratory. However, it is less than ideal for routine diagnostics because of stringent collection and transport requirements, and confirmation might take several days from the time of specimen collection. The primary advantage of isolating *N. gonorrhoeae* by culture is the ability to characterize the isolate further by antimicrobial susceptibility testing and genetic analysis if necessary. Cephalosporins are the sole class of antibiotics recommended for the treatment of *N. gonorrhoeae* infections in CDC’s 2010 STD treatment guidelines (available at http://www.cdc.gov/std/treatment/2010/default.htm), and the availability of gonococcal culture capacity at the local level is an important consideration if a patient fails therapy.

**Antibiotic susceptibility testing**
Gonorrhea treatment is complicated by the ability of *N. gonorrhoeae* to develop resistance to antimicrobial therapies. Genetic mutations and/or acquisition of genetic material from closely related bacteria species might result in antibiotic-resistant *N. gonorrhoeae*. Plasmid mediated resistance to penicillin can be conferred by extrachromosomal genes encoding for ß-lactamase that destroys penicillin. Resistance to tetracycline also might occur when the organism acquires an extrachromosomal gene from streptococcus, the tetM gene, which allows for ribosomal protein synthesis that is normally impaired by tetracycline. Testing for these plasmid genes provides limited information because genetic changes in the chromosome also might confer resistance to penicillin and tetracycline in addition to spectinomycin and fluoroquinolones. Penicillin-, tetracycline-, and fluoroquinolone-resistant *N. gonorrhoeae* isolates now are disseminated widely throughout the United States and globally. These antimicrobial agents are no longer recommended regimens for *N. gonorrhoeae* treatment, and thus susceptibility testing is not needed to make recommendations for clinical management. Laboratory capacity for *N. gonorrhoeae* culture and antibiotic susceptibility testing is critical to monitor for emerging resistance. Updated information regarding *N. gonorrhoeae* antibiotic susceptibility testing is available from CDC at http://www.cdc.gov/std/gisp.

Assessing *N. gonorrhoeae* isolates for antibiotic susceptibility requires viable isolates because accurate genetic markers of antibiotic resistance to recommended therapies have not been documented. Agar plate dilution testing that provides minimum inhibitory concentration values of tested antibiotics is the preferred method for testing the susceptibility of *N. gonorrhoeae* but might be too difficult to perform in laboratories with limited capacity and low testing volumes. Disk diffusion and E-test are simpler methods for determining susceptibilities of gonococcal isolates, although cefixime E-test strips are not FDA-cleared for use in the United States. Isolates that appear to be less susceptible than the current Clinical and Laboratory Standards Institute (CLSI) interpretive criteria for susceptible organisms (available at http://www.cdc.gov/std/gonorrhea/arg/criteria.htm) should be submitted to CDC for reference testing using the agar plate dilution method because there are no CLSI interpretive criteria for resistance to CDC-recommended therapeutic agents. Procedures for agar dilution and disk diffusion testing are available at http://www.cdc.gov/std/gonorrhea/lab/testing.htm. Clinicians who diagnose *N. gonorrhoeae* infection in a patient with suspected treatment failure should contact their local or state public health laboratory or local clinical laboratory for guidance on submitting specimens for culture and susceptibility testing. Local and state public health laboratory directors are encouraged to maintain culture and antimicrobial susceptibility testing capabilities for *N. gonorrhoeae* or identify public health or private laboratories in their area with such capacity if they do not perform such testing.

No standard procedures exist to assess in vitro susceptibility of *C. trachomatis* to antibiotics. Further research is required to determine the relationship between in vitro data and outcome of treatment.

**Nucleic acid amplification tests (NAATs)**

As of May 2013, five manufacturers in the United States had commercially available and FDA-cleared NAAT assay platforms for the detection of *C. trachomatis* and *N. gonorrhoeae*. NAAT assays are recommended for detection of urogenital
infections caused by \textit{C. trachomatis} and \textit{N. gonorrhoeae} infections in women and men with and without symptoms. These tests have been shown to be cost-effective in preventing sequelae due to these infections. A list of FDA-cleared specimen types and transport and storage requirements is provided (Table 2). These include Abbott RealTime m2000 CT/NG (Abbott Molecular Inc., Des Plaines, Illinois), Amplior and cobas CT/NG test (Roche Molecular Diagnostics, Branchburg, New Jersey); Aptima (Hologic/Gen-Probe, San Diego, California); BD ProbeTec ET and Qx (Becton Dickinson, Sparks, Maryland), and Xpert CT/NG Assay (Cepheid, Sunnyvale, California).

NAATs are designed to amplify and detect nucleic acid sequences that are specific for the organism being detected. Similar to other nonculture tests, NAATs do not require viable organisms. The increased sensitivity of NAATs is attributable to their theoretic ability to produce a positive signal from as little as a single copy of the target DNA or RNA. This high sensitivity has allowed the use of less invasively collected specimens such as first catch urines and vaginal swabs to detect shed organisms. Use of such specimens greatly facilitates screening.

Commercial tests differ in their amplification methods and their target nucleic acid sequences (Table 2). The two Roche tests and the Abbott RealTime CT/NG use polymerase chain reaction (PCR) and both Becton Dickinson tests use strand displacement amplification (SDA) to amplify \textit{C. trachomatis} DNA sequences in the cryptic plasmid that is found in >99% of strains of \textit{C. trachomatis}. The Hologic/Gen-Probe Aptima Combo 2 assay for \textit{C. trachomatis} uses transcription-mediated amplification (TMA) to detect a specific 23S ribosomal RNA target. The Roche cobas CT/NG test, Abbott, Becton Dickinson, and Hologic/Gen-Probe tests detect the new variant of \textit{C. trachomatis} (nvCT) strain. These nucleic acid amplification methods also are used to detect \textit{N. gonorrhoeae}, and each manufacturer has marketed a duplex assay that allows for simultaneous detection of both organisms. The nucleic acid primers used by commercial NAATs for \textit{C. trachomatis} are not known to detect DNA from other bacteria found in humans. However, the primers employed by the Becton Dickinson \textit{N. gonorrhoeae} NAATs might detect nongonococcal \textit{Neisseria} species (Table 3). Most commercial NAATs have been cleared by FDA to detect \textit{C. trachomatis} and \textit{N. gonorrhoeae} in vaginal and endocervical swabs from women, urethral swabs from men, and first catch urine from both men and women (Table 2).

Because NAATs are so sensitive, efforts are warranted to prevent contamination of specimens in the clinic or spread of environmental amplicon in the laboratory. Laboratories should

- follow standard molecular method techniques and
- clean workspaces and equipment frequently,
- include multiple negative controls in each run, and
- monitor the rate of indeterminate and positive results, as a change in monthly trends might indicate a need to investigate the accuracy of the results.

Environmental monitoring might be required as recommended by the manufacturer. If environmental amplicons are found, robust cleaning of the laboratory is needed until negative results are obtained. Steps to prevent cross-contamination include proper testing of laboratory workflow design and strict adherence to testing and quality assurance protocols.

\textbf{Detection of Genitourinary \textit{C. trachomatis} and \textit{N. gonorrhoeae} Infections in Women}
Screening programs have been demonstrated to reduce both the prevalence of *C. trachomatis* infection and rates of PID in women. Sexually active women aged ≤25 years and women aged >25 years with risk factors (e.g., those who have a new sex partner or multiple partners) should be screened annually for chlamydial infections.

The prevalence of gonorrhea varies widely among communities and populations; health-care providers should consider local gonorrhea epidemiology when making screening decisions. Although widespread screening in not recommended, targeted screening of young women (i.e., those aged ≤25 years) at increased risk for infection is a primary component of gonorrhea control in the United States because gonococcal infections among women are frequently asymptomatic. For sexually active women, including those who are pregnant, the U.S. Preventive Services Task Force (USPSTF) recommends that clinicians provide gonorrhea screening only to those at increased risk for infection (e.g., women with previous gonorrhea infection, other STDs, new or multiple sex partners, and inconsistent condom use; those who engage in commercial sex work and drug use; women in certain demographic groups; and those living in communities with a high prevalence of disease). UPSTF does not recommend screening for gonorrhea in women who are at low risk for infection.

For female screening, a specimen obtained with a vaginal swab is the preferred specimen type. Vaginal swab specimens are as sensitive as cervical swab specimens, and there is no difference in specificity. Self-collected vaginal swabs are equivalent in sensitivity and specificity to those collected by a clinician. Cervical samples are acceptable when pelvic examinations are done, but vaginal swab specimens are an appropriate sample type, even when a full pelvic exam is being performed. Cervical specimens collected into a liquid cytology medium for Pap screening are acceptable for NAATs that have been cleared by FDA for such specimen types. However, following Pap screening, there should be a clinical indication for reflex additional testing of liquid cytology specimens for chlamydia and gonorrhea since these specimen types are more widely used in older populations at low risk for infection. First catch urine from women, while acceptable for screening, might detect up to 10% fewer infections when compared with vaginal and endocervical swab samples (Box 2).

**Detection of Genitourinary *C. trachomatis* and *N. gonorrhoeae* Infections in Men**

*C. trachomatis* and *N. gonorrhoeae* control efforts in men differ substantially from those recommended for women. Although chlamydia prevalence data have provided a basis for setting age guidelines for routine annual screening and behavioral guidelines for targeted screening in women, no such consensus has been reached regarding control program definitions in men who have sex with women. There are no recommendations to screen heterosexual men in clinical settings with a high prevalence of *C. trachomatis* (e.g., STD clinics, adolescent clinics, and detention and correctional facilities) and among persons entering the Armed Forces or the National Job Training Program.

The prevalence of *N. gonorrhoeae* varies widely among communities and populations; health-care providers should consider the local gonorrhea epidemiology when making screening recommendations. There is insufficient evidence for or against routine screening for gonorrhea in sexually active heterosexual men at increased risk for infection. However, it is not recommended to screen for gonorrhea infections in men at low risk for infection.
Overwhelming evidence described the performance of male first catch urine samples as equivalent to, and in some situations superior to, urethral swabs. Use of urine samples is highly acceptable and might improve the likelihood of the initiation of routine screening in men (Box 3).

**Detection of Extragenital C. trachomatis and N. gonorrhoeae Infections in Men and Women**

Infections with *C. trachomatis* and *N. gonorrhoeae* are common in extragenital sites in certain populations such as MSM. Because extragenital infections are common in MSM, and most infections are asymptomatic, routine annual screening of extragenital sites in MSM is recommended. No recommendations exist regarding routine extragenital screening in women because studies have focused on genitourinary screening, but rectal and oropharyngeal infections are not uncommon.

A 2003 study that assessed NAATs for diagnosing *C. trachomatis* and *N. gonorrhoeae* infections in multiple anatomic sites in MSMs used Becton Dickinson’s ProbeTec NAAT, which had been validated previously for such use. Among 6,434 MSM attending an STD or a gay men’s clinic, the study found that the prevalence by site for *C. trachomatis* was 7.9% for the rectum, 5.2% urethral, and 1.4% pharyngeal; and prevalence by site for *N. gonorrhoeae* was 6.9% for the rectum, 6% urethral, and 9.2% pharyngeal. The great majority (84%) of the gonococcal and chlamydial rectal infections were asymptomatic. More than half (53%) of *C. trachomatis* and 64% of *N. gonorrhoeae* infections were at nonurethral sites and would have been missed if the traditional approach to screening of men by testing only urethral specimens had been used.

The scope of the problem of extragenital infection in MSM is not known at the national level. In 2007, CDC coordinated an evaluation of MSM attending several community-based organizations and public or STD clinics and found that of approximately 30,000 tests performed, 353 (5.4%) MSM were positive for rectal infection with *N. gonorrhoeae*, and 468 (8.9%) were positive for rectal *C. trachomatis*. Pharyngeal *N. gonorrhoeae* tests were positive for 759 MSM (5.3%) and 54 (1.6%) were positive for *C. trachomatis*.

In the United Kingdom, some studies on screening MSMs have been performed using NAATs, and in one study of 3,076 MSM attending an STD clinic, there was an 8.2% prevalence of infection with *C. trachomatis* in the rectum and 5.4% in the urethra. The majority (69%) of the men with *C. trachomatis* were asymptomatic, stressing the need for screening.

A study that compared culture to two NAATs (Hologic/Gen-Probe’s Aptima Combo2 [AC2]) and Becton Dickinson’s ProbeTec) for the detection of *C. trachomatis* and *N. gonorrhoeae* in pharyngeal and rectal specimens collected from 1,110 MSM being seen in an STD clinic confirmed all NAAT positive results when either the original test or a test using alternate primers was positive. The number of infections detected was more than doubled when a more sensitive NAAT was used as compared with the use of standard culture. Other researchers also have demonstrated the superiority of NAATs as compared with culture for diagnosing *C. trachomatis* and *N. gonorrhoeae* in rectal and oropharyngeal sites.

Although commercially available NAATs are recommended for testing genital tract specimens, they have not been cleared by FDA for the detection of *C. trachomatis* and *N. gonorrhoeae* infections of the rectum and oropharynx. Results from commercially
available NAATs can be used for patient management if the laboratory has established specifications for the performance characteristics according to CLIA regulations. If a moderate complexity test such as the GeneXpert is modified in any manner, the test defaults to high complexity and the laboratory must meet all high complexity CLIA requirements, including those for personnel. Certain NAATs that have been demonstrated to detect commensal Neisseria species in urogenital specimens might have comparable low specificity when testing oropharyngeal specimens for N. gonorrhoeae. Thus, a N. gonorrhoeae NAAT that does not react with nongonococcal commensal Neisseria species is recommended when testing oropharyngeal specimens (Table 3).

Detection of Genitourinary and Extragenital C. trachomatis and N. gonorrhoeae Infections in Cases of Sexual Assault

Detailed information about evaluation and treatment of suspected victims of sexual assault can be obtained from the 2010 STD treatment guidelines. General recommendations pertaining only to C. trachomatis and N. gonorrhoeae testing are presented here. Examination of victims is required for two purposes: 1) to determine if an infection is present so that it can be successfully treated and 2) to acquire evidence for potential use in a legal investigation. Testing to satisfy the first purpose requires a method that is highly sensitive, whereas satisfying the second purpose requires a method that is highly specific. Although NAATs meet these criteria, acceptance of any test results is determined by local legal authorities. Local legal requirements and guidance also should be sought for maintaining and documenting a chain of custody for specimens and results that might be used in a legal investigation and for which test results are accepted as evidence.

NAATs for C. trachomatis and N. gonorrhoeae are preferred for the diagnostic evaluation of adult sexual assault victims, from any sites of penetration or attempted penetration. Data on use of NAATs for detection of N. gonorrhoeae in children are limited. Consultation with an expert is necessary before use of NAATs for this indication in children to minimize the possibility of positive reactions with nongonococcal Neisseria species and other commensals. NAATS can be used as alternative to culture with vaginal specimens or urine specimens from girls. Culture remains the preferred method for urethral specimens from boys and extragenital specimens (pharynx and rectum) in boys and girls.

Using highly specific tests is critical with prepubescent children for whom the diagnosis of a sexually transmitted infection might lead to initiation of an investigation for child abuse. Specimen collection for culture for N. gonorrhoeae includes the pharynx and rectum in boys and girls, the vagina in girls, and the urethra in boys. Cervical specimens are not recommended for prepubertal girls. For boys with a urethral discharge, a meatal specimen discharge is an adequate substitute for an intra-urethral swab specimen. Standard culture procedures must be followed. Gram stains are inadequate to evaluate prepubertal children for N. gonorrhoeae and should not be used to diagnose or exclude N. gonorrhoeae. Specimens from the vagina, urethra, pharynx, or rectum should be streaked onto selective media for isolation of N. gonorrhoeae, and all presumptive isolates of N. gonorrhoeae should be identified definitively by at least two tests that involve different principles (e.g., biochemical, enzyme substrate, or serologic). Isolates should be preserved to enable additional or repeated testing.
Cultures for *C. trachomatis* can be collected from the rectum in both boys and girls and from the vagina in girls. The likelihood of recovering *C. trachomatis* from the urethra of prepubertal boys is too low to justify the trauma involved in obtaining an intraurethral specimen. However, a meatal specimen should be obtained if urethral discharge is present. Pharyngeal specimens for *C. trachomatis* are not recommended for children of either sex because the yield is low, perinatally acquired infection might persist beyond infancy, and culture systems in some laboratories use antibody stains that do not distinguish between *C. trachomatis* and *C. pneumoniae*. All specimens must be retained for additional testing, if necessary, regardless of a positive or negative test result.

Only standard culture systems for the isolation of *C. trachomatis* should be used. The isolation of *C. trachomatis* should be confirmed by microscopic identification of inclusions by staining with fluorescein-conjugated monoclonal antibody MOMP specific for *C. trachomatis*; stains using monoclonal antibodies directed against LPS should not be used. EIAs are not acceptable confirmatory methods. Isolates should be preserved. Nonculture tests for *C. trachomatis* such as EIAs and DFA are not sufficiently specific for use in circumstances involving possible child abuse or sexual assault. NAATs can be used for detection of *C. trachomatis* in vaginal specimens or urine from girls. No data exist on the use of nucleic acid amplification tests in boys and extragenital specimens (rectum) in boys and girls. Culture remains the preferred method for extragenital sites in these circumstances.

**Detection of Lymphogranuloma venereum Infections**

Lymphogranuloma venereum (LGV) is a long-term sexually transmitted infection of the lymph system caused by three different types of *C. trachomatis* (L1, L2, and L3 serovars). The infection is not caused by the same bacteria that cause genital chlamydia (D-K serovars).

Serologic testing for LGV is not widely available in the United States. The chlamydial complement fixation test (CFT), which measures antibody against group-specific lipopolysaccharide antigen, has been used as an aid in the diagnosis of LGV. A CFT titer ≥1:64 typically can be measured in the serum of patients with bubonic LGV. The micro-immunofluorescence (MIF) test was initially developed for serotyping strains of *C. trachomatis* isolated from the eye and genital tract but was soon adapted to measure antibody responses in patients with chlamydial infections. Although the original MIF method was complicated, involving the titration of sera against numerous antigens, it was found to have many advantages when compared with CFT. The MIF test can be used to detect type-specific antibody and different immunoglobulin classes. The MIF test is more sensitive than the CFT with a larger proportion of patients developing an antibody response and at higher titer. Patients with LGV tend to have broadly cross-reactive MIF titers that are often greater than 1:256. Microtiter plate format enzyme immunoassays have been developed but comparative performance data are lacking. Serologic test interpretation for LGV is not standardized, tests have not been validated for clinical proctitis presentations, and *C. trachomatis* serovar-specific serologic tests are not widely available. More detailed information concerning the diagnosis and treatment of LGV has not been published.

Genital and lymph node specimens (i.e., lesion swab or bubo aspirate) can be tested for *C. trachomatis* by culture, direct immunofluorescence, or nucleic acid detection. Commercially available NAATs for *C. trachomatis* detect both LGV and non-
LGV *C. trachomatis* but cannot distinguish between them. Additional molecular procedures (e.g., PCR-based genotyping) can be used to differentiate LGV from non-LGV *C. trachomatis*, but these are not widely available.

For patients presenting with proctitis, *C. trachomatis* NAAT testing of a rectal specimen is recommended. While a positive result is not definitive diagnosis of LGV, the result might aid in a presumptive clinical diagnosis of LGV proctitis.

**ADDITIONAL CONSIDERATIONS**

**Supplemental testing of NAAT-positive specimens**

In 2002, CDC recommended that consideration be given to performing an additional test routinely after a positive NAAT screening test for *C. trachomatis* and *N. gonorrhoeae*. This approach was advised to improve the positive predictive value (PPV) of a NAAT screening test. This was particularly important when the test was used in a population with a low prevalence of infection. However, studies since 2002 addressing the utility of routine repeat testing of positive specimens demonstrated >90% concurrence with the initial test for either *C. trachomatis* or *N. gonorrhoeae*. Therefore, routine additional testing following a positive NAAT screening test for *C. trachomatis* is no longer recommended by CDC unless otherwise indicated in the product insert.

Some NAATs might detect nongonococcal *Neisseria* species (Table 3). When these NAATs are used, consideration should be given to retest these specimens with an alternate target assay if the anatomic site from which the specimen was collected is typically colonized with these commensal organisms, e.g., oropharyngeal specimens. As with any diagnostic test, if there is a clinical or laboratory reason to question a test result, a repeat test should be considered.

**Test interpretation**

The laboratory should interpret and report results according to the manufacturer’s package insert instructions. In the event of discordant results from multiple tests, the report should indicate the results of both the initial and any additional tests. An interpretation of “inconclusive,” “equivocal,” or “indeterminate” would be appropriate. A new specimen should be requested for testing in these situations. All test results should be interpreted by clinicians within the context of the patient-specific information to determine appropriate patient management.

**Test of cure**

Culture is the only method that can be used to properly assess the efficacy of antibiotic therapy because commercial NAATs are not FDA-cleared for use as a test of cure. Residual nucleic acid from bacteria rendered noninfective by antibiotics might still give a positive *C. trachomatis* NAAT up to 3 weeks after therapy. Detection of *N. gonorrhoeae* nucleic acid has been observed for up to 2 weeks following therapy although the vast majority of patients who were treated effectively for gonorrhea had a negative NAAT 1 week after treatment. However, data from these studies were derived from older NAATs and should be repeated with current NAATs.

**Pooling of specimens**

The superior performance characteristics of NAATs for detection of *C. trachomatis* and *N. gonorrhoeae* have led some researchers to pool urine specimens in an attempt to reduce the higher material costs associated with their use. Samples of individual specimens are first combined into a pool, which is then tested by a NAAT. If
the pool is negative, all specimens forming the pool are reported as negative. If the pool
is positive, a second aliquot of each specimen that contributed to the pool is tested
individually. The potential cost-savings with pooling increases with decreasing
prevalence of infection, because more specimens can be included in a pool without
increasing the probability of a pool testing positive. Available evidence indicates that
pooled aliquots from up to 10 urine specimens can be a cost-effective alternative to
testing individual specimens without any loss of sensitivity or specificity. Savings from
reduced reagent costs have ranged from 40% to 60%. However, the increased complexity
of the pooling protocol might require more personnel time to deconstruct positive pools
for individual specimen testing. The use of pooled specimens for testing is not cleared by
FDA and, therefore, the CLIA requirements applicable to modifying a test procedure
must be met before implementation and reporting results intended to guide patient care.
Laboratories must be aware that the process of pooling specimens requires extensive
handling of samples which increases the potential for cross-contamination. Studies for
pooling clinical specimens other than urine are required before extending this
recommendation.

TESTS NOT RECOMMENDED FOR ROUTINE USE

Direct fluorescent antibody (DFA) tests
This assay should not be used for routine testing of genital tract specimens.
Rather, DFA tests are the only FDA-cleared tests for ocular \textit{C. trachomatis} infections.
Depending on the commercial product used, the antigen that is detected by the antibody
in the \textit{C. trachomatis} DFA procedure is either the MOMP or LPS molecule. No DFA
tests exist for the direct detection of \textit{N. gonorrhoeae} in clinical specimens.

Nucleic acid hybridization/probe tests
Two nucleic acid hybridization assays are FDA-cleared to detect \textit{C. trachomatis}
and \textit{N. gonorrhoeae}: the Hologic/Gen-Probe PACE 2 and the Digene Hybrid Capture II
assays. The Hybrid Capture assay is not widely available and the PACE 2C test was
discontinued December 31, 2012.

Nucleic acid genetic transformation tests
The Gonostat test (Sierra Diagnostics, Incorporated, Sonora, California) uses a
gonococcal mutant that grows when genetically altered by DNA extracted from a swab
specimen containing \textit{N. gonorrhoeae}. \textit{N. meningitides} causes false-positive results. The
test has received limited evaluation in published studies, which included an evaluation of
its use with mailed specimens. Amplified and hybridization tests that detect \textit{N.
gonorrhoeae} nucleic acid have better performance characteristics than the Gonostat test.
The gonorrhea nucleic acid genetic transformation test might have some utility in settings
that lack the stringency for gonorrhea culture. However, it is not recommended as an
alternative test to \textit{N. gonorrhoeae} NAATs. A genetic transformation test is not available
for detection of \textit{C. trachomatis} infection.

Enzyme immunoassay (EIA) tests
A substantial number of EIA tests have been marketed for detecting \textit{C.
trachomatis} infection. The performance and cost characteristics of EIA tests for \textit{N.
gonorrhoeae} infection have not made them competitive with other available tests. None
of the EIAs are as sensitive or specific as NAATs, and their use is discouraged.

Serology tests
Serology has little, if any, value in testing for uncomplicated genital \textit{C. trachomatis} infection. It should not be used for screening because previous chlamydial infection might or might not elicit a systemic antibody response. A serologic screening or diagnostic assay is not available for \textit{N. gonorrhoeae}.

CONCLUSION

Technological evolution in clinical laboratory diagnostics has advanced considerable by allowing for the direct molecular detection of a pathogen in a clinical specimen rather than relying on isolation and cultivation. This approach has decreased the time required to identify a pathogen because the laboratory is no longer limited by the growth kinetics of the organism. Therefore, patients can be evaluated and, if infected, can be treated promptly, thereby diminishing progression to disease and disrupting transmission. As with all changes in laboratory technology, a synthesis of scientific evidence is required for an informed decision regarding the implementation of a new or improved test platform. Previous CDC recommendations now specify that vaginal swabs are the preferred specimen for screening women and include the use of rectal and oropharyngeal specimens among populations at risk for extragenital tract infections. FDA clearance is important for widespread use of a test, and it is important that clearance be obtained for NAAT use with rectal and oropharyngeal specimens, and with vaginal swabs collected in other than clinical settings.

Future revision to these recommendations will be influenced by the development and marketing of new laboratory tests, or indications for use of existing tests for chlamydia and gonorrhea. Improvements in molecular tests that continue to decrease detection time and decrease the test complexity might facilitate the use of NAATs in non-traditional laboratory settings such as physician offices, health fairs, school clinics, or other outreach venues. Shifting chlamydia and gonorrhea diagnostics from laboratories might require new recommendations on test application or reporting positive cases of reportable diseases. Periodic updated to these recommendations will be available on the CDC Division of STD Prevention website (http://www.cdc.gov/std).
Table 1. Characteristics of gram-negative, oxidase-positive *Neisseria* and related species of human origin

<table>
<thead>
<tr>
<th>Species</th>
<th>Acid from</th>
<th>Reduction of nitrate</th>
<th>Polysaccharide from sucrose</th>
<th>Tributyrin hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
<td>Maltose</td>
<td>Lactose</td>
<td>Sucrose</td>
</tr>
<tr>
<td><em>N. gonorrhoeae</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>N. meningitidis</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>N. lactamica</em></td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>N. cinerea</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>N. polysaccharea</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>N. subflava</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>V</td>
</tr>
<tr>
<td><em>N. sicca</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>N. mucosa</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>N. flavescens</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>N. elongata</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Branhamella catarrhalis</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>Kingella denitrificans</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Abbreviations:** + = strains typically positive but genetic mutants might be negative; - = negative; V = variable.

* Certain strains grow on selective media for the isolation of *N. gonorrhoeae*.

† Includes biovars sublava, flava, and perflava. *N. sublava* biovar perflava strains produce acid from sucrose and fructose and produce polysaccharide from sucrose; *N. subflava* biovar flava strains produce acid from fructose; *N. subflava* biovar flava and *N. subflava* biovar subflava do not produce polysaccharide.

§ Rod or cocccobacillus.
<table>
<thead>
<tr>
<th>FDA-cleared NAAT</th>
<th>FDA-cleared specimen types</th>
<th>Specimen transport and storage conditions</th>
</tr>
</thead>
</table>
| **Abbott RealTime CT/NG (Abbott Molecular Inc., Des Plaines, IL)** | Asymptomatic women: clinician-collected vaginal swab, patient-collected vaginal swab in a clinical setting, and urine.  
Asymptomatic men: urine.  
Symptomatic women: endocervical swab, clinician-collected vaginal swab, patient-collected vaginal swab in a clinical setting, and urine.  
Symptomatic men: urethral swab and urine. | 14 days at 2°–30°C  
90 days at -10°C or lower  
Thaw frozen specimens at 2°–30°C  
Specimens must not undergo more than four freeze/thaw cycles |
| **Aptima COMBO 2 assay**                            | Asymptomatic women: endocervical swab, clinician-collected vaginal swab, patient-collected vaginal swab in a clinical setting, gynecologic specimens collected in PreservCyt solution and urine.  
Asymptomatic men: urethral swab and urine.  
Symptomatic women: endocervical swab, clinician-collected vaginal swab, patient-collected vaginal swab in a clinical setting, gynecologic specimens collected in PreservCyt solution and urine.  
Symptomatic men: urethral swab and urine. | 24 hours at 2°–30°C (urine specimen in primary cup)  
30 days at 2°–30°C (urine specimen in Aptima urine transport tube)  
60 days at 2°–30°C (swab in Aptima swab transport tube)  
12 months at -20° to -70°C (urine specimen and swab specimens in respective Aptima transport tubes) |
| **BDProbeTec ET CT/GC Amplified DNA assay** (Becton Dickinson and Company, Sparks, MD) | Asymptomatic women: endocervical swab and urine.  
Asymptomatic men: urethral swab and urine.  
Symptomatic women: endocervical swab and urine.  
Symptomatic men: urethral swab and urine. | 30 hours at 2°–30°C (urine specimen in primary cup)  
7 days at 2°–8°C urine specimen in primary cup  
30 days at 2°–30°C (urine specimen in urine processing tube)  
60 days at -20°C or lower (neat urine specimen or urine in urine processing tube)  
6 days at 2°–27°C (swab specimens)  
30 days at 2°–8°C (swab specimens) |
| **BD ProbeTec QX CT Amplified DNA assay** (Becton Dickinson and Company, Sparks, MD) | Asymptomatic women: endocervical swab, patient-collected vaginal swab in a clinical setting, gynecologic specimens collected in BDSurePath or PreservCyt solution and urine.  
Asymptomatic men: urethral swab and urine.  
Symptomatic women: endocervical swab, patient-collected vaginal swab in a clinical setting, gynecologic specimens collected in BD SurePath or PreservCyt solution and urine.  
Symptomatic men: urethral swab and urine. | 30 hours at 2°–30°C (urine specimen in primary cup)  
7 days at 2°–8°C (urine specimen in primary cup)  
30 days at 2°–30° (urine specimen in urine processing tube)  
180 days at -20°C or lower (neat urine specimen or urine in urine processing tube)  
30 days at 2°C–30°C or lower (endocervical and urethral swab specimens)  
180 days at -20°C or lower (endocervical and urethral swab specimens)  
14 days at 2°–30°C (dry vaginal swab specimens)  
30 days at 2°–30°C (expressed vaginal swab specimens)  
180 days at -20°C or lower (dry or expressed vaginal swab specimens) |
<table>
<thead>
<tr>
<th>Test</th>
<th>Collection Types</th>
<th>Storage Conditions</th>
</tr>
</thead>
</table>
| **Xpert CT/NG assay**       | Asymptomatic women: endocervical swab, patient-collected vaginal swab in a clinical setting, and urine.  
Asymptomatic men: urine.  
Symptomatic women: endocervical swab, patient-collected vaginal swab in a clinical setting, and urine.  
Symptomatic men: urine. | 24 hours at room temperature (female urine specimen in primary cup)  
3 days at room temperature (male urine specimen in primary cup)  
8 days at 4°C (female and male urine specimen in primary cup)  
3 days at 15°–30°C (female urine specimen in Xpert CT/NG Urine Transport Reagent tube)  
45 days at 2°–15°C (female urine specimen in Xpert CT/NG Urine Transport Reagent tube)  
45 days at 2°–30°C (male urine specimen in Xpert CT/NG Urine Transport Reagent tube)  
45 days 2°–30°C (swab in Xpert CT/NG Swab Transport Reagent tube) |
| **cobas CT/NG test**         | Asymptomatic women: endocervical swab, patient-collected vaginal swab in a clinical setting, clinician-collected vaginal swab, gynecologic specimens collected in PreservCyt solution and urine.  
Asymptomatic men: urine  
Symptomatic women: endocervical swab, patient-collected vaginal swab in a clinical setting, clinician-collected vaginal swab, gynecologic specimens collected in PreservCyt solution and urine.  
Symptomatic men: urine. | ≤1 yr at 2°–30°C (swab or urine specimen in cobas PCR media)  
24 hrs at 2°–30°C (Neat male urine specimen prior to addition to cobas PCR media)  
Cervical specimens collected in PreservCyt Solution may be stored at 2°–30°C for up to 12 months. Aliquots (≥1mL) of cervical specimens collected in PreservCyt Solution may be stored in 13 mL round-based Sarstedt tubes for up to 4 weeks at 2°–30°C. |

*FDA-cleared NAATs and specimen types as of January 1, 2014.
Table 3. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Nucleic Acid Amplification Test (NAAT) target sequences and possible false reactions, by test type

<table>
<thead>
<tr>
<th>FDA-cleared NAAT</th>
<th>Nucleic acid target for <em>C. trachomatis</em></th>
<th>Nucleic acid target for <em>N. gonorrhoeae</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott RealTime CT/NG (Abbott Laboratories, Abbott Park, IL)</td>
<td>Two distinct specific sequence regions within the 7,500 base pair <em>C. trachomatis</em> cryptic plasmid DNA.</td>
<td>48 base pair sequence within the <em>Opa</em> gene of <em>N. gonorrhoeae</em>.</td>
</tr>
<tr>
<td></td>
<td>The test does not detect plasmid free <em>C. trachomatis</em>.</td>
<td>No false-positive tests based on analytical specificity testing.</td>
</tr>
<tr>
<td>Aptima COMBO 2 assay</td>
<td>Specific region within the 23S rRNA from <em>C. trachomatis</em> (Aptima Combo 2 assay)</td>
<td>Specific region within the 16S rRNA from <em>N. gonorrhoeae</em> (Aptima Combo 2 assay)</td>
</tr>
<tr>
<td>Aptima CT assay</td>
<td>Specific region with the 16S rRNA from <em>C. trachomatis</em> (Aptima CT assay)</td>
<td>Specific region with the 16S rRNA from <em>N. gonorrhoeae</em> that is distinct from the Aptima Combo 2 assay target (Aptima GC assay).</td>
</tr>
<tr>
<td>Aptima GC assay</td>
<td>Both the Aptima Combo 2 assay and APTIMA CT assay detects nCT.</td>
<td>No false-positive tests based on analytical specificity testing.</td>
</tr>
<tr>
<td>(Hologic/Gen-Probe Inc., San Diego, CA)</td>
<td>The test does not detect plasmid-free <em>C. trachomatis</em>.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No false-positive tests based on analytical specificity testing.</td>
<td></td>
</tr>
<tr>
<td>BD ProbeTec ET CT/GC Amplified DNA assay (Becton Dickinson and Company, Sparks, MD)</td>
<td>One distinct sequence within the 7,500 base pair <em>C. trachomatis</em> cryptic plasmid DNA.</td>
<td>Chromosomal pilin gene-inverting protein homologue.</td>
</tr>
<tr>
<td></td>
<td>The test does not detect plasmid-free <em>C. trachomatis</em>.</td>
<td><em>Neisseria cinerea, Neisseria subflava</em> and <em>Neisseria lactamica</em> might result in false-positive test results based on analytical specificity testing.</td>
</tr>
<tr>
<td></td>
<td>No false-positive tests based on analytical specificity testing.</td>
<td></td>
</tr>
<tr>
<td>BD ProbeTec QX CT Amplified DNA Assay</td>
<td>One distinct sequence within the 7,500 base pair <em>C. trachomatis</em> cryptic plasmid DNA.</td>
<td></td>
</tr>
<tr>
<td>BD ProbeTec QX GC Amplified DNA Assay (Becton Dickinson and Company, Sparks, MD)</td>
<td>The test does not detect plasmid-free <em>C. trachomatis</em>.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No false-positive tests based on analytical specificity testing.</td>
<td></td>
</tr>
<tr>
<td>X pert CT/NG Assay (Cepheid, Sunnyvale, CA)</td>
<td>One distinct <em>C. trachomatis</em> chromosomal DNA sequence.</td>
<td>Two distinct chromosomal sequences each with a different reporter. Both sequences have to be detected to obtain a positive <em>N. gonorrhoeae</em> result.</td>
</tr>
<tr>
<td></td>
<td>No false-positive tests based on analytical specificity testing.</td>
<td>No false-positives based on analytical specificity testing.</td>
</tr>
<tr>
<td></td>
<td>The test detects plasmid-free <em>C. trachomatis</em>.</td>
<td></td>
</tr>
<tr>
<td>cobasCT/NG test (Roche Diagnostics, Indianapolis, IN)</td>
<td>CT primers CP102 and CP103 to define a sequence of approximately 206 nucleotides within the cryptic plasmid DNA of <em>C. trachomatis</em>.</td>
<td>NG primers NG514 and NG519 to define a sequence of approximately 190 nucleotides (DR-9A) from the DR-9 region.</td>
</tr>
<tr>
<td></td>
<td>CT primers CTMP101 and CTMP102 to define a sequence of approximately 182 nucleotides within the cryptic plasmid DNA of <em>C. trachomatis</em>.</td>
<td>NG primers, NG552 and NG579, to define a second sequence of approximately 215 nucleotides (DR-9B) from the DR-9 region.</td>
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<td>No false-positive tests based on analytical specificity testing.</td>
<td>No false-positive tests based on analytical specificity testing.</td>
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<td>The test detects plasmid-free <em>C. trachomatis</em>.</td>
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</table>
Box 1. Summary of recommendations

- Nucleic acid amplification tests (NAATs) that are cleared by the Food and Drug Administration (FDA) are recommended for detection of genital tract infections caused by *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in men and women with and without symptoms. For detecting these infections of the genital tract, optimal specimen types for NAATs are vaginal swabs from women and first catch urine from men. Older nonculture tests and non-NAATs have inferior sensitivity and specificity characteristics and no longer are recommended.

- NAATs have not been cleared by FDA for the detection of rectal and oropharyngeal infections caused by *C. trachomatis* and *N. gonorrhoeae*. CDC is recommending NAATs to test for these extragenital infections based on increased sensitivity, ease of specimen transport, and processing. Because these specimen types have not been cleared by FDA for use with NAATs, laboratories must establish performance specifications when using these specimens to meet Clinical Laboratory Improvement Amendments (CLIA) regulatory requirements and local or state regulations as applicable prior to reporting results for patient management. Positive reactions with nongonococcal *Neisseria* species have been reported with some NAATs, particularly with oropharyngeal specimens. Alternate target testing using NAATs without reported crossreactivity might be needed to avoid false positive gonorrhea results when using these tests with these specimens.

- Routine repeat testing of NAAT-positive genital tract specimens is not recommended because the practice does not improve the positive predictive value of the test.

- Laboratory interpretation of test results should be consistent with product inserts for FDA-cleared tests or have met all federal and state regulations for a modified procedure if the laboratory has changed the cutoff values or tests algorithm. This approach provides the most appropriate information to the clinician, who is ultimately responsible for assessing test results to guide patient and partner management.

- *N. gonorrhoeae* culture capacity is still needed for evaluating suspected cases of treatment failure and monitoring antimicrobial susceptibility.

- *C. trachomatis* and *N. gonorrhoeae* culture capacity might still be needed in instances of child sexual assault in boys and extragenital infections in girls.

BOX 2. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* testing in women

- Nucleic acid amplification tests (NAATs) are the recommended test method.
- A self- or clinician-collected vaginal swab is the recommended sample type. Self-collected vaginal swab specimens are an option for screening women when a pelvic exam is not otherwise indicated.
- An endocervical swab is acceptable when a pelvic examination is indicated.
- A first catch urine specimen is acceptable but might detect up to 10% fewer infections when compared with vaginal and endocervical swab samples.
- An endocervical swab specimen for *N. gonorrhoeae* culture should be obtained and evaluated for antibiotic susceptibility in patients that have received CDC-recommended antimicrobial regimen as treatment, and subsequently had a positive *N. gonorrhoeae* test result (positive NAAT >7 days after treatment), and did not engage in sexual activity after treatment.

BOX 3. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* testing in men

- Nucleic acid amplification tests (NAATs) are the recommended test method.
- A first catch urine is the recommended sample type and is equivalent to a urethral swab in detecting infection.
- A urethral swab specimen for *N. gonorrhoeae* culture should be obtained and evaluated for antibiotic susceptibility in patients who have received CDC-recommended antimicrobial regimen as treatment, and subsequently had a positive *N. gonorrhoeae* test result (Positive NAAT >7 days after treatment), and did not engage in sexual activity after treatment.

BOX 4. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in the rectum and oropharynx

- Nucleic acid amplification tests (NAATs) are the recommended test method for rectal and oropharyngeal specimens.
- Laboratories must be in compliance with CLIA for test modifications since these tests have not been cleared by the FDA for these specimen types.
- Commensal *Neisseria* species commonly found in the oropharynx might cause false positive reactions in some NAATs, and further testing might be required for accuracy.
- A rectal or oropharyngeal swab specimen for *N. gonorrhoeae* culture should be obtained and evaluated for antibiotic susceptibility in patients who have received CDC-recommended antimicrobial regimen as treatment, had a subsequent positive *N. gonorrhoeae* test result (positive NAAT >7 days after treatment), and did not engage in sexually activity after treatment.
REVIEW QUESTIONS
Course #DL-010
Choose the one best answer

1. The most common notifiable disease in the United States is
   a. Salmonella
   b. *Chlamydia trachomatis*
   c. *Neisseria gonorrhoeae*
   d. lymphogranuloma venereum

2. Laboratories should use NAATs to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in all but which of the following:
   a. evaluating patient treatment failure
   b. screening tests for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*
   c. to determine sexual assault in women
   d. oropharyngeal specimens in possible sexual assault in girls

3. Which of the following is not a consideration when culturing for *Chlamydia trachomatis*?
   a. expense
   b. technically demanding
   c. lack of specificity
   d. loss of viability in transport

4. Monitoring developing resistance to current treatment regimes is accomplished by:
   a. NAATS
   b. nucleic acid probe tests
   c. enzyme immunoassays
   d. culture

5. The most common illness/symptom among men resulting from *Chlamydia trachomatis* infection is
   a. epididymitis
   b. conjunctivitis
   c. urethritis
   d. penile discharge

6. Which of the following is a challenge (negative aspect) of NAAT testing?
   a. contamination of workspace environment by organisms
   b. hard to standardize
   c. difficulty of maintaining viability of organisms
   d. lack of sensitivity

7. Which of the following is not a disadvantage of culture testing?
   a. inability to automate testing
   b. difficulty of maintaining viability of organisms
   c. inability to detect a significant proportion of infections
   d. tissue tests for *Chlamydia trachomatis* are expensive and technically demanding

8. Specimen collection swabs for *Chlamydia trachomatis* should not have a
   a. cotton tip
   b. plastic or wire shaft
9. A gram stain of a symptomatic male’s urethral specimen that indicate *Neisseria gonorrhoeae* infection shows
   a. intracellular Gram negative cocci in chains
   b. Gram negative diplococci attaching to squamous cells
   c. intracellular Gram negative diplococci
   d. intracellular Gram positive diplococcic

10. A positive identification of cultured *Neisseria gonorrhoeae* includes
    a. maltose neg, glucose pos, oxidase neg, lactose neg
    b. oxidase pos, glucose pos, maltose neg, lactose neg
    c. maltose neg, glucose neg, oxidase pos, lactose pos
    d. oxides neg, glucose pos, maltose neg, lactose neg

11. The antibiotic recommended by CDC for treatment of *Neisseria gonorrhoeae* is
    a. fluoroquinolone
    b. cephalosporin
    c. penicillin
    d. tetracycline

12. The preferred method of antibiotic susceptibility in *Neisseria gonorrhoeae* is
    a. agar plate
    b. disc diffusion
    c. E test
    d. tube dilution

13. Which of the following is not used for preventing contamination of specimens when performing NAATS?
    a. properly follow workflow design
    b. clean workspaces frequently
    c. include multiple positive controls in each run
    d. monitor changes in monthly trends

14. CDC recommends that Clinicians should provide *Neisseria gonorrhoeae* screening for
    a. sexually active women >25 with no risk factors
    b. all pregnant women
    c. women with multiple sex partners
    d. along with all PAP smears

15. The preferred specimen for detection of *Neisseria gonorrhoeae* or *Chlamydia trachomatis* in women is
    a. cervical specimen
    b. first catch urine specimen
    c. liquid medium for PAP screening
    d. vaginal swab specimen

16. The most common extragenital infection site in MSM is
    a. pharyngeal
    b. urethral
    c. rectal
17. Examination of prepubescent children in suspected cases of sexual assault include
   a. Gram stain of discharge
   b. specimen from rectum
   c. culture of cervical specimen
   d. intra-urethral specimen culture for *Chlamydia trachomatis*

18. Lymphogranuloma venereum infection is caused by which of the *Chlamydia trachomatis* serovars?
   a. Ab
   b. L1
   c. D
   d. F

19. All but which of the following is a negative aspect of pooling specimens?
   a. not cleared by the FDA
   b. loss of sensitivity
   c. potential for cross contamination
   d. possible increased personnel time

20. Which of the following is not a consequence of *Chlamydia trachomatis* infection in women?
   a. pelvic inflammatory disease
   b. infant conjunctivitis
   c. tubal pregnancy
   d. 20% result in cervicitis