

Recommendations for the Laboratory-Based Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* — 2014



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Front cover photo: Laboratorians testing bacterial isolates for antimicrobial resistance (Photo/Association of Public Health Laboratories).

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Recommendations for the Laboratory-Based Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* — 2014

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Summary

This report updates CDC's 2002 recommendations regarding screening tests to detect Chlamydia trachomatis and Neisseria gonorrhoeae infections (CDC. Screening tests to detect Chlamydia trachomatis and Neisseria gonorrhoeae infections—2002. MMWR 2002;51[No. RR-15]) 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 nonculture 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 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 (1). 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 (2). 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) (3). 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 (4). 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 (5).

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) (6,7). 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

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experience debilitating, chronic pelvic pain; and 9% will have a life-threatening tubal pregnancy (8). 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 (9,10). *C. trachomatis* infection during pregnancy might lead to infant conjunctivitis and pneumonia and maternal postpartum endometritis (11). 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 (12).

C. trachomatis infections of the rectum can result from unprotected anal intercourse and are typically asymptomatic but might progress to proctocolitis (13,14). Ocular infections can result in conjunctivitis in neonates and adults (15). Sexually acquired reactive arthritis also has been reported as a possible consequence of *C. trachomatis* infection (16).

Neisseria gonorrhoeae

N. gonorrhoeae infections are the second most common notifiable communicable disease in the United States with 309,341 cases being reported to CDC in 2010 (1). Gonococcal infections tend to cause a stronger inflammatory response than *C. trachomatis* but are typically asymptomatic in women until complications such as PID develop (17). In men, the majority of urethral infections cause urethritis with painful urination and, less commonly, epididymitis or disseminated gonococcal infection (17).

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 (18). CDC recommends routine laboratory screening of genital and extragenital sites for all sexually active MSM at risk for infection (19).

Purpose of This Report

This report updates CDC's 2002 recommendations regarding screening tests to detect *C. trachomatis* and *N. gonorrhoeae* infections (20) and provides new recommendations regarding optimal specimen types, the use of tests to detect rectal and oropharyngeal *C. trachomatis* and *N. gonorrhoeae* infections, and information regarding when supplemental testing is indicated (Box 1). These recommendations 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.

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 non-gonococcal *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 testing 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.

Methods

In 2008, CDC and the Association of Public Health Laboratories (APHL) convened an independent work group to evaluate available information and make suggestions for CDC to consider in the development of recommendations for the laboratory diagnosis of *C. trachomatis* and *N. gonorrhoeae* in the United States. Members of the work group* were selected on the basis of published expertise in the field of *C. trachomatis* and *N. gonorrhoeae* diagnostics or were public health laboratory directors, sexually transmitted diseases (STD) clinicians, CDC's Division of STD Prevention, and representatives of the Food and Drug Administration (FDA) or the Centers for Medicaid Services (CMS). Four members of the work group, including three who served as co-authors, previously had published papers in which they acknowledged receiving financial support from diagnostic test manufacturers for test evaluations. These potential conflicts of interest were disclosed and managed in accordance with the editorial standards of the journals that published the scientific reports. In addition, in August 2013, to maintain objectivity and to confirm that the recommendations were evidence based, a second independent panel of microbiologic, statistical, and clinical experts reviewed the draft recommendations. Approximately 6 months before a meeting held at CDC during January 13–15, 2009, work group members were asked by conference call to identify key questions regarding chlamydia and gonorrhea laboratory diagnostics that emerged from literature reviews and discussed the information available to answer those questions. Work group members were assigned key questions to research (see Appendix) and, with the assistance of CDC and APHL staff, conducted an extensive Medline database of peer-reviewed literature published during January 1, 2000–January 1, 2009. The Medline database was searched using the terms “*Chlamydia trachomatis*” or “chlamydia” “*Neisseria gonorrhoeae*” or “gonorrhea” or “lymphogranuloma venereum” or “LGV” and “infection” or “reproductive tract” or “specimen” or “urogenital specimen” or “urine” or “rectum” or “pharynx” or “oropharynx” or “culture” or “nucleic acid amplification” or “nucleic acid probe” or “enzyme immunoassay” or “detection” or “performance” or “screening” or “adolescent” or “prevalence” or “confirmation” or “repeat testing” or “pediatric” or “sexual assault” or “sexual abuse” or “point of care” or “serology.” The key questions were categorized into three principal areas of laboratory diagnostics: 1) performance characteristics of tests, 2) screening applications, and 3) laboratory confirmation of test results. Monthly conference calls or e-mail message exchanges were conducted with work group members researching key questions in each principal area to ensure progress and adequate

support in obtaining relevant publications. Work group members assigned to address key questions developed tables of evidence from peer-reviewed publications and presented these tables at the in-person meeting held in January 2009. Each key question was introduced, and publications were discussed in terms of strengths, weaknesses, and overall relevance of the data to the key questions. Scientific publications with findings derived from studies with an analytic plan involving a patient's infected status were included in developing these recommendations. Studies using discrepant analysis were excluded. All work group members agreed with these inclusion and exclusion criteria because they approach design characteristics used by FDA when evaluating diagnostic tests for marketing in the United States. During the meeting, each topic was presented by the assigned work group member, and an open forum followed to allow all work group members and ad hoc attendees to discuss the merits of publications used to address the key questions. At the end of each discussion, a recommendation was proposed and adopted for consideration by CDC if there were no objections from the work group members. Following the in-person meeting, the same database was searched periodically for subsequently published articles for the work group to consider by e-mail and/or teleconference calls. A writing team was formed to draft the recommendations generated from these discussions, and the senior CDC author was responsible for the overall content.

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).

* A list of the members of the work group appears on page 19.

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 for the diagnosis of chlamydial and gonococcal infections (21–30). 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 *C. trachomatis* and *N. gonorrhoeae* 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-detection tests were followed by nucleic acid hybridization tests, which detect *C. trachomatis*-specific or *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 *C. trachomatis* were initiated, and screening programs for *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 *C. trachomatis*, was that they failed to detect a substantial proportion of infections (30–39). This changed with the introduction of NAATs that amplify and detect *C. trachomatis*-specific or *N. gonorrhoeae*-specific DNA or RNA sequences. These tests are approximately 20%–35% more sensitive than the earlier nonculture tests (30–39).

This report emphasizes the importance of maintaining the capability to culture for both *N. gonorrhoeae* and *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. *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 *C. trachomatis* such as the type recently described in Sweden (40,41).

Recommendations

Tests to Detect *C. trachomatis* and *N. gonorrhoeae*

Isolation and identification of *Chlamydia trachomatis*.

Specimen collection swabs for *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 *C. trachomatis* culture is invasive requiring insertion of a swab 2–3 cm into the male urethral 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 media 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 media containing the specimen should be stored at -70°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 *C. trachomatis* (42–46). Once the specimen has been inoculated, $2\mu\text{g}/\text{ml}$ of cycloheximide should be added to the growth medium to suppress protein synthesis by the host eukaryotic cell (47). Inoculated cells are harvested after 48–72 hours of growth; infected cells develop characteristic intracytoplasmic inclusions that contain substantial numbers of *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 *C. trachomatis* is highly specific if a *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 (48–50). Less specific inclusion-detection methods using iodine or Giemsa stain are not recommended (48–50).

Cell culture methods vary among laboratories, resulting in substantial interlaboratory variation in performance (51). The shell vial method of culture uses a larger inoculum with a reduced risk for crosscontamination and therefore provides better accuracy than the 96-well microtiter plate method (42,43). 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 (49).

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 (28,52). 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 diplococci 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 (39). 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 exudate 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 (53–55). 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) (39). 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.

Methods of gonococcal culture have been described elsewhere (39,56). 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 media for *N. gonorrhoeae* isolation include a base medium supplemented with chocolized (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 (57). 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* (39). 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* (39).

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 time of specimen collection. The primary advantage of isolating *N. gonorrhoeae* by culture is the ability to characterize the isolate further by antimicrobial susceptibility

TABLE 1. Characteristics of gram-negative, oxidase-positive *Neisseria* and related species of human origin

Species	Acid from					Superoxol	Reduction of nitrate	Polysaccharide from sucrose	Tributyryl hydrolysis
	Glucose	Maltose	Lactose	Sucrose	Fructose				
<i>N. gonorrhoeae</i>	+	-	-	-	-	+	-	-	-
<i>N. meningitidis</i>	+	+	-	-	-	+	-	-	-
<i>N. lactamica</i>	+	+	+	-	-	+	-	-	-
<i>N. cinerea</i> *	-	-	-	-	-	+	-	-	-
<i>N. polysaccharea</i>	+	+	-	-	-	+	-	-	-
<i>N. subflava</i> †	+	+	-	V	V	+	-	V	-
<i>N. sicca</i>	+	+	-	+	+	+	-	+	-
<i>N. mucosa</i>	+	+	-	+	+	+	+	+	-
<i>N. flavescens</i>	-	-	-	-	-	+	-	+	-
<i>N. elongata</i> ‡	-	-	-	-	-	-	-	-	-
<i>Branhamella catarrhalis</i>	-	-	-	-	-	+	+	-	+
<i>Kingella denitrificans</i>	+	-	-	-	-	-	+	-	-

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 subflava, flava, and perflava. *N. subflava* 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 coccobacillus.

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>) (19), and the availability of gonococcal culture capacity at the local level is an important consideration if a patient fails therapy (58).

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 β -lactamase that destroys penicillin (59,60). Resistance to tetracycline also might occur when the organism acquires an extrachromosomal gene from streptococcus, the *tetM* gene that allows for ribosomal protein synthesis that is normally impaired by tetracycline (61). 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 (62,63). Testing specimens for genetic alterations in the chromosome requires a complete understanding of the complex and multiple mechanisms associated with resistance. For example, chromosomal-mediated resistance to penicillin can alter penicillin binding, penetration, or efflux (64). Resistance to fluoroquinolones results from mutations in DNA gyrase (*gyrA*) or topoisomerase (*parC*) resulting in decreased drug penetration and increased efflux (65,66). Penicillin-, tetracycline-, and fluoroquinolone-resistant *N. gonorrhoeae* isolates now are disseminated widely throughout the United States and globally (67). These antimicrobial agents no longer are 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 (68) (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 (69). 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 had commercially available and FDA-cleared NAAT assay platforms for the detection of *C. trachomatis* and *N. gonorrhoeae* in the United States. NAAT assays are recommended for detection of urogenital infections caused by *C. trachomatis* and *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 (70–72). 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), Amplicor 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) (Table 2). 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 *C. trachomatis* DNA sequences in the cryptic plasmid that is found in >99% of strains of *C. trachomatis*. The Hologic/Gen-Probe Aptima Combo 2 assay for *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 *C. trachomatis* (nvCT) strain. These nucleic acid amplification methods also are used to detect *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 *C. trachomatis* are not known to detect DNA from other bacteria found in humans. However, the primers employed by the Becton Dickinson *N. gonorrhoeae* NAATs might detect nongonococcal *Neisseria* species (73–76) (Table 3). Most commercial NAATs have been cleared by FDA to detect *C. trachomatis* and *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, 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.

Performance of Tests to Detect *C. trachomatis* and *N. gonorrhoeae*

Studies assessing the performance of NAATs might include test algorithms that use multiple NAATs, nonculture and culture tests as reference standards. Regardless of the analytic study design, the performance characteristics are relative to the standards used at the time of evaluation. When less sensitive methods are used as the reference standard, the specificity of the test under evaluation is likely to be underestimated. Conversely, the sensitivity of older assays was likely overestimated because of the relative poor performance of the assays used as standards at the time.

Because no gold standard exists, researchers compared two versions of the patient-infected-status algorithm (PISA) to assess the performance of NAATs. Using simulations with latent-class models, these researchers concluded that PISA-based methods can produce biased estimates of sensitivity and specificity that changed markedly as the true prevalence changes (77). However, there is no consensus on the optimal approach to evaluating the performance of NAATs, and better methods are needed (78). Until better methods become available, these recommendations support continuing reliance on NAATs based on their approval by FDA for indicated clinical use.

Simply quoting sensitivity and specificity data from package inserts or published studies is not useful because the numbers are estimates and are valid only within the context of the particular evaluation. Variables that can impact on these numbers include what comparison tests were used, in which population the evaluation was performed, and whether calculations were made on the basis of an infected patient standard or a direct comparison of specimens.

TABLE 2. Food and Drug Administration–cleared* specimen types and requirements for the transport and storage of specimens for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by nucleic acid amplification test (NAAT) type

FDA-cleared NAAT	FDA-cleared specimen types	Specimen transport and storage conditions
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 Aptima CT assay Aptima GC assay (Hologic/Gen-Probe Inc., San Diego, CA)	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)
BD ProbeTec 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 Q ^X CT Amplified DNA assay BD ProbeTec Q ^X GC 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 BDSurePath 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°C (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°–30°C (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)
Xpert CT/NG assay (Cepheid, Sunnyvale, CA)	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 at 2°–30°C (swab in Xpert CT/NG Swab Transport Reagent tube)
cobas CT/NG test (Roche Diagnostics, Indianapolis, IN)	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 (≥1 mL) 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

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

Nevertheless, despite the absence of a criterion standard, valid generalizations can be made. All diagnostic tests including NAATs can generate inaccurate results, and it is important for laboratorians and clinicians to understand test limitations. Certain false positives and false negatives can occur as a consequence of specimen collection, test operation, and laboratory environment. However, NAATs are far superior in overall performance compared with other *C. trachomatis* and *N. gonorrhoeae* culture and nonculture diagnostic methods. NAATs offer greatly expanded sensitivities of detection, usually well above 90%, while maintaining very high specificity, usually ≥99%. NAATs typically detect 20%–50% more chlamydial infections than could be detected by culture or earlier nonculture tests (20). The increment for detection of gonococcal infections is somewhat less.

Detection of Genitourinary *C. trachomatis* and *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 (79,80). 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 (81).

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 is 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). USPSTF does not recommend screening for gonorrhea in women who are at low risk for infection (81).

For female screening, specimens obtained with a vaginal swab are the preferred specimen type. Vaginal swab specimens are as sensitive as cervical swab specimens, and there is no difference in specificity (82–87). Self-collected vaginal swabs are equivalent in sensitivity and specificity to those collected by a clinician (83,88). 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 (Table 2). 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 (82,87,89) (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 (11), no such consensus has been reached regarding control program definitions in men who have sex with women (12). Although there are no recommendations to screen heterosexual men, it USPSTF suggests testing to test sexually active 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 (81).

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 (81). However,

BOX2. *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.

it is not recommended to screen for gonorrhea infections in men at low risk for infection (81).

Overwhelming evidence described the performance of male first catch urine samples as equivalent to, and in some situations superior to, urethral swabs (23,90). Use of urine samples is highly acceptable and might improve the likelihood of uptake 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 (91), 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 (91) used Becton Dickinson's ProbeTec NAAT, which had been validated previously for such use. Among 6,434 MSM attending an STD clinic 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*

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 an 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.

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* (92).

In the United Kingdom, some studies on screening MSMs have been performed using NAATs (93,94), 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 (94).

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 (95). For oropharyngeal *N. gonorrhoeae*, sensitivities were 41% for culture, 72% for SDA, and 84% for AC2; and for rectal *N. gonorrhoeae*, sensitivities were 43% for culture, 78% for SDA, and 93% for AC2. For oropharyngeal

infections with *C. trachomatis* (for which only nine infections were detected), sensitivities were 44% for culture, 67% for SDA, and 100% for AC2; for rectal *C. trachomatis*, sensitivities were 27% for culture, 63% for SDA, and 93% for AC2. Specificities were $>99.4\%$ for all specimens, tests, and anatomic sites. 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 (36,75,76).

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 (Box 4). 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 (96). 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).

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 sexual activity after treatment.

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 (19). 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 (97,98). 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

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 $\geq 1:64$ typically can be measured in the serum of patients with bubonic LGV (99). 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 the CFT (99). 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 crossreactive MIF titers that are often greater than 1:256 (99). 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 been published (19).

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 (100–104).

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* (20). 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* (105–107). Therefore, routine additional testing following a positive NAAT screening test for *C. trachomatis* no longer is 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 most 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 (108,109). 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 (110). 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 (111–113). 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. The number of specimens pooled to achieve the greatest cost savings for a particular prevalence can be calculated (111). 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 (111). Savings from reduced reagent costs have ranged from 40% to 60% (111). 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 *C. trachomatis* infections. Depending on the commercial product used, the antigen that is detected by the antibody in the *C. trachomatis* DFA procedure is either the MOMP or LPS molecule. Specimen material is obtained with a swab or endocervical brush, which is then rolled over the specimen well of a slide. After the slide has dried and the fixative applied, the slide can be stored or shipped at ambient temperature. The laboratory should process the slide <7 days after the specimen has been obtained. Staining consists of flooding the smear with fluorescein-labeled monoclonal antibody that binds to *C. trachomatis* elementary bodies. Stained elementary bodies are then identified by fluorescence microscopy. Only *C. trachomatis* organisms will stain with the anti-MOMP antibodies used in commercial kits. The anti-LPS monoclonal antibodies will react with family-specific epitopes found within the LPS of *Chlamydiaceae* and might cross-react with LPS of other bacteria. The procedure requires an experienced microscopist and is labor-intensive and time-consuming. No DFA tests exist for the direct detection of *N. gonorrhoeae* in clinical specimens.

Nucleic acid hybridization/probe tests. Two nucleic acid hybridization assays are FDA-cleared to detect *C. trachomatis* or *N. gonorrhoeae*: the Hologic/Gen-Probe PACE 2 and the Digene Hybrid Capture II assays. Both the PACE and Hybrid Capture assays can detect *C. trachomatis* or *N. gonorrhoeae* in a single specimen. 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 *N. gonorrhoeae*. *N. meningitidis* causes false-positive results (114). The test has received limited evaluation in published studies (115–118), which included an evaluation of its use with mailed specimens (117). Amplified and hybridization tests that detect *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 *N. gonorrhoeae* NAATs. A genetic transformation test is not available for detection of *C. trachomatis* infection.

Enzyme immunoassay (EIA) tests. A substantial number of EIA tests have been marketed for detecting *C. trachomatis*

infection. The performance and cost characteristics of EIA tests for *N. gonorrhoeae* infection have not made them competitive with other available tests (56). *C. trachomatis* EIA tests detect chlamydial LPS, and there is the potential for false-positive results caused by crossreaction with LPS of other microorganisms. Manufacturers have developed blocking assays that verify positive EIA test results to improve specificity. 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 *C. trachomatis* infection. It should not be used for screening because previous chlamydial infection might or might not elicit a systemic antibody response. Infections caused by LGV serovars of *C. trachomatis* tend to invade to the draining lymph nodes resulting in a greater likelihood of detectable systemic antibody response and might aid in diagnosis of inguinal (but not rectal) disease (99). The complement fixation test was classically used for this purpose but has been replaced by the more sensitive species-specific microimmunofluorescence test. A serologic screening or diagnostic assay is not available for *N. gonorrhoeae*.

Conclusion

Technological evolution in clinical laboratory diagnostics has advanced considerably 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 to use NAATs for the detection of chlamydia and gonorrhea as the standard laboratory test remain. These updated 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 clinic settings.

Future revisions to these recommendations will be influenced by the development and marketing of new laboratory tests, or indications 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 updates to these recommendations will be available on the CDC Division of STD Prevention website (<http://www.cdc.gov/std>).

References

1. CDC. Sexually transmitted disease surveillance, 2010. Atlanta, GA: US Department of Health and Human Services, CDC; 2011.
2. Stamm WE. *Chlamydia trachomatis* infections of the adult. In: Holmes KK, Sparling FF, Stamm WE., et al., eds. Sexually transmitted diseases. 4th ed. New York, NY: McGraw-Hill Companies, Inc.; 2007:575–94.
3. Owusu-Eduesei K, Chesson HW, Gift TL, et al. The estimated direct medical cost of selected sexually transmitted infections in the United States, 2008. *Sex Transm Dis* 2013;40:197–201.
4. Hoover KW, Tao G, Kent CK. Trends in the diagnosis and treatment of ectopic pregnancy in the United States. *Obstet Gynecol* 2010;115:495–502.
5. Blandford JM, Gift TL. Productivity losses attributable to untreated chlamydial infection and associated pelvic inflammatory disease in reproductive-aged women. *Sex Transm Dis* 2006;33(Suppl):S117–21.
6. Stamm WE, Guinan ME, Johnson C, Starcher T, Holmes KK, McCormack WM. Effect of treatment regimens for *Neisseria gonorrhoeae* on simultaneous infection with *Chlamydia trachomatis*. *N Engl J Med* 1984;310:545–9.
7. Rees E. Treatment of pelvic inflammatory disease. *Am J Obstet Gynecol* 1980;138:1042–7.
8. Westrom L, Joesoef R, Reynolds G, Hadgu A, Thompson SE. Pelvic inflammatory disease and fertility: a cohort study of 1,844 women with laparoscopically verified disease and 657 control women with normal laparoscopy results. *Sex Transm Dis* 1992;19:185–92.
9. Chow JM, Yonekura L, Richwald GA, Greenland S, Sweet RL, Schachter J. The association between *Chlamydia trachomatis* and ectopic pregnancy: a matched-pair, case-control study. *JAMA* 1990;263:3164–7.
10. Miettinen A, Heinonen PK, Teisala K, Hakkarainen K, Punnonen R. Serologic evidence for the role of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma hominis* in the etiology of tubal factor infertility and ectopic pregnancy. *Sex Transm Dis* 1990;17:10–4.
11. US Preventive Services Task Force. Screening for chlamydial infection: recommendation statement. *Ann Intern Med* 2007;147:128–34.
12. CDC. Male chlamydia screening consultation, Atlanta, Georgia, March 28–29, 2006. Meeting Report, May 22, 2007. Atlanta, GA: US Department of Health and Human Services, CDC; 2007. Available at <http://www.cdc.gov/std/chlamydia/ChlamydiaScreening-males.pdf>.
13. Boisvert J-F, Koutsky LA, Suchland RJ, Stamm WE. Clinical features of *Chlamydia trachomatis* rectal infection by serovar among homosexually active men. *Sex Transm Dis* 1999;26:392–8.
14. Quinn TC, Goodell SE, Mkrtrichian PA-C, et al. *Chlamydia trachomatis* proctitis. *N Engl J Med* 1981;305:195–200.
15. Jones BR, Al-Hussaini MK, Dunlop EM. Infection of the eye and the genital tract by the TRIC agent. *Br J Vener Dis* 1964;40:19–24.
16. Carter JD, Hudson AP. The evolving story of *Chlamydia*-induced reactive arthritis. *Curr Opin Rheumatol* 2010;22:424–30.
17. Hook EW III, Handsfield HH. Gonococcal infections in the adult. In: Holmes KK, Sparling FF, Stamm WE, et al., eds. Sexually transmitted diseases. 4th ed. New York, NY: McGraw-Hill Companies, Inc.; 2007:627–45.
18. CDC. HIV prevention through early detection and treatment of other sexually transmitted diseases—United States: recommendations of the Advisory Committee for HIV and STD Prevention. *MMWR* 1998;47(No. RR-12).
19. CDC. Sexually transmitted diseases treatment guidelines, 2010. *MMWR* 2010;59(No. RR-12).
20. CDC. Screening tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections—2002. *MMWR* 2002;51(No. RR-15).
21. Gaydos CA, Quinn TC, Willis D, et al. Performance of the APTIMA Combo 2 assay for the multiplex detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in female urine and endocervical swab specimens. *J Clin Microbiol* 2003;41:304–9.
22. Van Der Pol B, Ferrero DV, Buck-Barrington L, et al. Multicenter evaluation of the BDProbeTec ET system for the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine specimens, female endocervical swabs, and male urethral swabs. *J Clin Microbiol* 2001;39:1008–16.
23. Martin DH, Cammarata C, Van Der Pol B, et al. Multicenter evaluation of AMPLICOR and automated COBAS AMPLICOR CT/NG tests for *Neisseria gonorrhoeae*. *J Clin Microbiol* 2000;38:3544–9.
24. Van Der Pol B, Quinn TC, Gaydos CA, et al. Multi-center evaluation of the AMPLICOR and automated COBAS AMPLICOR CT/NG Tests for the detection of *Chlamydia trachomatis*. *J Clin Microbiol* 2000;38:1105–12.
25. Alary M, Gbenafa-Agossa C, Aïna G, et al. Evaluation of a rapid point of care test for the detection of gonococcal infection among female sex workers in Benin. *Sex Transm Infect* 2006;82:29–32.
26. Lee HH, Chernesky MA, Schachter J, et al. Diagnosis of *Chlamydia trachomatis* genitourinary infection in women by ligase chain reaction assay of urine. *Lancet* 1995;345:213–6.
27. Jaschek G, Gaydos CA, Welsh L, Quinn TC. Direct detection of *Chlamydia trachomatis* in urine specimens from symptomatic and asymptomatic men by using a rapid polymerase chain reaction assay. *J Clin Microbiol* 1993;31:1209–12.
28. Black CM, Marrazzo J, Johnson RE, et al. Head-to-head multicenter comparison of DNA probe and nucleic acid amplification tests for *Chlamydia trachomatis* in women with use of an improved reference standard. *J Clin Microbiol* 2002;40:3757–63.
29. Marrazzo JM, Johnson RE, Green TA, et al. Impact of patient characteristics on performance of NAATs and DNA probe of *Chlamydia trachomatis* in women with genital infections. *J Clin Microbiol* 2005;43:577–84.
30. Gaydos CA, Theodore M, Dalesio N, Wood BJ, Quinn TC. Comparison of three nucleic acid amplification tests for the detection of *Chlamydia trachomatis* in urine specimens. *J Clin Microbiol* 2004;42:3041–5.
31. Koumans EH, Johnson RE, Knapp JS, St. Louis ME. Laboratory testing for *Neisseria gonorrhoeae* by recently introduced nonculture tests: a performance review with clinical and public health considerations. *Clin Infect Dis* 1998;27:1171–80.
32. Chernesky M, Morse S, Schachter J. Newly available and future laboratory tests for sexually transmitted diseases (STDs) other than HIV. *Sex Transm Dis* 1999;26(Suppl):S8–11.
33. Chernesky MA. Nucleic acid tests for the diagnosis of sexually transmitted diseases. *FEMS Immunol Med Microbiol* 1999;24:437–46.
34. Davies PO, Ridgway GL. Role of polymerase chain reaction and ligase chain reaction for the detection of *Chlamydia trachomatis*. *Int J STD AIDS* 1997;8:731–8.
35. Domeika M. Diagnosis of infections due to *Chlamydia trachomatis*. *Acta Obstet Gynecol Scand* 1997;164(Suppl):121–7.
36. Schachter J. DFA, EIA, PCR, LCR and other technologies: what tests should be used for diagnosis of chlamydia infections? *Immunol Invest* 1997;26:157–61.
37. Taylor-Robinson D. Evaluation and comparison of tests to diagnose *Chlamydia trachomatis* genital infections. *Hum Reprod* 1997;12(Suppl):113–20.
38. Guaschino S, De Seta F. Update on *Chlamydia trachomatis* [Review]. *Ann N Y Acad Sci* 2000;900:293–300.

39. Elias J, Frosch M, Vogel U. *Neisseria*. In: Versalovic J, Carroll KC, Funke G, Jorgensen JH Landry ML, Warnock DW, eds. Manual of clinical microbiology. 10th ed. Washington, DC: American Society of Microbiology; 2011:559–603.
40. Won H, Ramachandran P, Steece R, et al. Is there evidence of the new variant *Chlamydia trachomatis* in the United States? *Sex Transm Dis* 2013;40:352–3.
41. Ripa T, Nilsson PA. A *Chlamydia trachomatis* Strain with a 377-bp deletion in the cryptic plasmid causing false-negative nucleic acid amplification tests. *Sex Transm Dis* 2007;34:255–6.
42. Stamm WE, Tam M, Koester M, Cles L. Detection of *Chlamydia trachomatis* inclusions in McCoy cell cultures with fluorescein-conjugated monoclonal antibodies. *J Clin Microbiol* 1983;17:666–8.
43. Yoder BL, Stamm WE, Koester CM, Alexander ER. Microtest procedure for isolation of *Chlamydia trachomatis*. *J Clin Microbiol* 1981;13:1036–9.
44. Kuo C-C, Wang S-P, Wentworth BB, Grayston JT. Primary isolation of TRIC organisms in HeLa 229 cells treated with DEAE-dextran. *J Infect Dis* 1972;125:665–8.
45. Johnston SL, Siegel. Comparison of Buffalo green monkey kidney cells and McCoy cells for the isolation of *Chlamydia trachomatis* in shell vial centrifugation culture. *Diagn Microbiol Infect Dis* 1992;15:355–7.
46. Krech T, Bleckmann M, Paatz R. Comparison of buffalo green monkey kidney cells and McCoy cells for the isolation of *Chlamydia trachomatis* in a microtiter system. *J Clin Microbiol* 1989;27:2364–5.
47. Ripa KT, Mårdh P-A. Cultivation of *Chlamydia trachomatis* in cycloheximide-treated McCoy cell. *J Clin Microbiol* 1977;6:328–31.
48. CDC. False-positive results with the use of chlamydial tests in the evaluation of suspected sexual abuse—Ohio 1990. *MMWR* 1991;39:932–5.
49. Barnes RC. Laboratory diagnosis of human chlamydial infections. *Clin Microbiol Rev* 1989;2:119–36.
50. Hammerschlag MR, Aji S, Laraque D. Inappropriate use of nonculture tests for the detection of *Chlamydia trachomatis* in suspected victims of child sexual abuse: a continuing problem. *Pediatrics* 1999;104:1137–9.
51. Pate MS, Hook EW. Laboratory to laboratory variation in *Chlamydia trachomatis* culture practices. *Sex Transm Dis* 1995;22:322–6.
52. Watson EJ, Templeton A, Russell I, Paavonen J, et al. The accuracy and efficacy of screening tests for *Chlamydia trachomatis*: a systematic review. *J Med Microbiol* 2002;51:1021–31.
53. Drake CJ, Barenfanger J, Lawhorn J, Verhulst S. Comparison of easy-flow Copan liquid Stuart's and Starplex swab transport systems for recovery of fastidious aerobic bacteria. *J Clin Microbiol* 2005;43:1301–3.
54. Wade JJ, Graver MA. Six auxotypes of *Neisseria gonorrhoeae* in transport media. *J Clin Microbiol* 2003;41:1720–1.
55. Arbiq JC, Forward KR, LeBlanc J. Evaluation of four commercial transport media for the survival of *Neisseria gonorrhoeae*. *Diagn Microbiol Infect Dis* 2000;36:3596–601.
56. Jephcott AE. Microbiological diagnosis of gonorrhoea. *Genitourin Med* 1997;73:245–52.
57. Pace PJ, Catlin BW. Characteristics of *Neisseria gonorrhoeae* strains isolated on selective and nonselective media. *Sex Transm Dis* 1986;13:29–39.
58. CDC. Cephalosporin-resistant *Neisseria gonorrhoeae* public health response plan. Atlanta, GA: US Department of Health and Human Services, CDC; 2012. Available at <http://www.cdc.gov/std/treatment/Ceph-R-ResponsePlanJuly30-2012.pdf>.
59. Ashford WA, Golash RG, Hemming VG. Penicillinase-producing *Neisseria gonorrhoeae*. *Lancet* 1976;7897:657–8.
60. Philips I. Beta-lactamase-producing, penicillin-resistant gonococcus. *Lancet* 1976;7897:656–7.
61. Morse SA, Johnson SR, Biddle JW, Roberts MC. High-level tetracycline resistance in *Neisseria gonorrhoeae* is the result of acquisition of streptococcal *tetM* determinant. *Antimicrob Agents Chemother* 1986;20:117–21.
62. Knapp JS, Fox KK, Trees DL, Whittington WL. Fluoroquinolone resistance in *Neisseria gonorrhoeae*. *Emerg Infect Dis* 1997;3:33–9.
63. Whittington WL, Knapp JS. Trends in antimicrobial resistance in *Neisseria gonorrhoeae* in the United States. *Sex Transm Dis* 1988;15:202–10.
64. Ison CA. Antimicrobial agents and gonorrhoea: therapeutic choice, resistance and susceptibility testing. *Genitourin Med* 1996;72:253–7.
65. Jacoby GA. Mechanisms of resistance to quinolones. *Clin Infect Dis* 2005;41:S120–6.
66. Hooper DC. Bacterial topoisomerases, anti-topoisomerases, and anti-topoisomerase resistance. *Clin Infect Dis* 1998;27:S54–63.
67. Newman LM, Moran JS, Workowski KA. Update on the management of gonorrhoea in adults in the United States. *Clin Infect Dis* 2007;44 (Suppl 3):S84–101.
68. Clinical and Laboratory Standards Institute. Analysis and presentation of cumulative antimicrobial susceptibility data; approved Guideline. 3rd ed. CLSI document M39–A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
69. Wang SA, Papp JR, Stamm WE, Peeling RW, Martin DH, Holmes KK. Evaluation of antimicrobial resistance and treatment failures for *Chlamydia trachomatis*: a meeting report. *J Infect Dis* 2005;191:917–23.
70. Howell MR, Quinn TC, Gaydos CA. Screening for *Chlamydia trachomatis* in asymptomatic women attending family planning clinics: A cost effectiveness analysis of three preventive strategies. *Ann Intern Med* 1998;28:277–84.
71. Howell MR, Quinn TC, Brathwaite W, Gaydos CA. Screening women for *Chlamydia trachomatis* in family planning clinics: the cost-effectiveness of DNA amplification assays. *Sex Transm Dis* 1998;25:108–17.
72. Hu D, Hook EW III, Goldie SJ. Screening for *Chlamydia trachomatis* in women 15 to 29 years of age: a cost-effectiveness analysis. *Ann Intern Med* 2004;141:1501–13.
73. Martin DH, Cammarata C, Van der Pol B, et al. Multicenter evaluation of AMPLICOR and automated COBAS AMPLICOR CT/NG tests for *Neisseria gonorrhoeae*. *J Clin Microbiol* 2000;38:3544–9.
74. Van der Pol B, Martin DH, Schachter J, et al. Enhancing the specificity of the COBAS AMPLICOR CT/NG test for *Neisseria gonorrhoeae* by retesting specimens with equivocal results. *J Clin Microbiol* 2001;39:3092–8.
75. Bachmann LH, Johnson RE, Cheng H, Markowitz LE, Papp JR, Hook EW III. Nucleic acid amplification tests for diagnosis of *Neisseria gonorrhoeae* oropharyngeal infections. *J Clin Microbiol* 2009;47:902–7.
76. Bachmann LH, Johnson RE, Cheng H, et al. Nucleic acid amplification tests for diagnosis of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* rectal infections. *J Clin Microbiol* 2010;48:1827–32.
77. Hadgu A, Dendukuri N, Wang L. Evaluation of screening tests for detecting *Chlamydia trachomatis*. *Epidemiology* 2012;23:72–82.
78. Miller WC. Reference-test bias in diagnostic-test evaluation. *Epidemiology* 2012;23:83–5.
79. Scholes D, Stergachis A, Heidrich FE, et al. Prevention of pelvic inflammatory disease by screening for cervical chlamydial infection. *N Engl J Med* 1996;334:1362–6.
80. Kamwendo F, Forslin L, Bodin L, et al. Decreasing incidences of gonorrhoea- and chlamydia-associated acute pelvic inflammatory disease: a 25-year study from an urban area of central Sweden. *Sex Transm Dis* 1996;23:384–91.
81. US Preventive Services Task Force. Screening for gonorrhoea: recommendation statement. *Ann Fam Med* 2005;3:263–7.
82. Schachter J, Chernesky MA, Willis DE, et al. Vaginal swabs are the specimens of choice when screening for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: results from a multicenter evaluation of the APTIMA assays for both infections. *Sex Transm Dis* 2005;32:725–8.
83. Masek BJ, Arora N, Quinn N, et al. Performance of three nucleic acid amplification tests for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by use of self-collected vaginal swabs obtained via an Internet-based screening program. *J Clin Microbiol* 2009;47:1663–7.
84. Shafer M-A, Moncada J, Boyer CB, Betsinger K, Flinn SD, Schachter J. Comparing first-void urine specimens, self-collected vaginal swabs, and endocervical specimens to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by a nucleic acid amplification test. *J Clin Microbiol* 2003;41:4395–9.

85. Schachter J, McCormack WM, Chernesky MA, et al. Vaginal swabs are appropriate specimens for diagnosis of genital tract infection with *Chlamydia trachomatis*. *J Clin Microbiol* 2003;41:3784–9.
86. Hsieh Y-H, Howell MR, Gaydos JC, McKee KT Jr, Quinn TC, Gaydos CA. Preference among female army recruits for use of self-administered vaginal swabs or urine to screen for *Chlamydia trachomatis* genital infections. *Sex Transm Dis* 2003;30:769–73.
87. Michel CC, Sonnex C, Carne CA, et al. *Chlamydia trachomatis* load at matched anatomical sites: Implications for screening strategies. *J Clin Microbiol* 2007;45:1395–402.
88. Knox J, Tabrizi SN, Miller P, et al. Evaluation of self-collected samples in contrast to practitioner-collected samples for detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* by polymerase chain reaction among women living in remote areas. *Sex Transm Dis* 2002;29:647–54.
89. Falk L, Coble BI, Mjörnberg PA, Fredlund H. Sampling for *Chlamydia trachomatis* infection—a comparison of vaginal, first-catch urine, combined vaginal and first-catch urine and endocervical sampling. *Int J STD AIDS* 2010;21:283–7.
90. Gaydos CA, Ferrero DV, Papp JR. Laboratory aspects of screening men for *Chlamydia trachomatis* in the new millennium. *Sex Transm Dis* 2008;35(Suppl):S44–50.
91. Kent CK, Chaw JK, Wong W, et al. Prevalence of rectal, urethral, and pharyngeal chlamydia and gonorrhea detected in 2 clinical settings among men who have sex with men: San Francisco, California, 2003. *Clin Infect Dis* 2005;41:67–74.
92. CDC. Clinic-based testing for rectal and pharyngeal *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infections by community-based organizations—five cities, United States, 2007. *MMWR* 2009;58:716–9.
93. Manavi K, McMillan A, Young H. The prevalence of rectal chlamydial infection amongst men who have sex with men attending the genitourinary medicine clinic in Edinburgh. *Int J STD AIDS* 2004;15:162–4.
94. Annan NT, Sullivan AK, Nori A, et al. Rectal chlamydia—a reservoir of undiagnosed infection in men who have sex with men. *Sex Transm Infect* 2009;85:176–9.
95. Schachter J, Moncada J, Liska S, Shayevich C, Klausner JD. Nucleic acid amplification tests in the diagnosis of chlamydial and gonococcal infections of the oropharynx and rectum in men who have sex with men. *Sex Transm Dis* 2008;35:637–42.
96. Centers for Medicare and Medicaid Services. 42 C.F.R. Part 493 Laboratory requirements: Clinical Laboratory Improvement Amendments of 1988.
97. Girardet RG, Lahoti S, Howard LA, et al. Epidemiology of sexually transmitted infections in suspected child victims of sexual assault. *Pediatrics* 2009;124:79–86.
98. Black CM, Driebe EM, Howard LA, et al. Multicenter study of nucleic acid amplification tests for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in children being evaluated for sexual abuse. *Pediatr Infect Dis J* 2009;28:608–13.
99. Mabey D, Peeling RW. lymphogranuloma venereum. *Sex Transm Infect* 2002;78:90–2.
100. Alexander S, Martin IMC, Ison C. A comparison of two methods for the diagnosis of lymphogranuloma venereum. *J Med Microbiol* 2008;57:962–5.
101. Chen CY, Chi KH, Alexander S, et al. The molecular diagnosis of lymphogranuloma venereum: evaluation of a real-time multiplex polymerase chain reaction test using rectal and urethral specimens. *Sex Transm Dis* 2007;34:451–5.
102. Morré SA, Spaargaren J, Fennema JSA, de Vries HJC, Coutinho RA, Peña AS. Real-time polymerase chain reaction to diagnose lymphogranuloma venereum. *Emerg Infect Dis* 2005;11:1311.
103. Spaargaren J, Fennema HS, Morre SA, de Vries HJ, Coutinho RA. New lymphogranuloma venereum *Chlamydia trachomatis* variant, Amsterdam. *Emerg Infect Dis* 2005;11:1090–2.
104. Halse TA, Musser KA, Limberge RJ. A multiplexed real-time PCR assay for rapid detection of *Chlamydia trachomatis* and identification of serovar L-2, the major cause of lymphogranuloma venereum in New York. *Mol Cell Probes* 2006;20:290–7.
105. Schachter J, Hook EW, Martin DH, et al. Confirming positive results of nucleic acid amplification tests (NAATs) for *Chlamydia trachomatis*: all NAATs are not created equal. *J Clin Microbiol* 2005;43:1372–3.
106. Schachter J, Chow JM, Howard H, Bolan G, Moncada J. Detection of *Chlamydia trachomatis* by nucleic acid amplification testing: our evaluation suggests that CDC-recommended approaches for confirmatory testing are ill-advised. *J Clin Microbiol* 2006;44:2512–7.
107. Moncada J, Donegan E, Schachter J. Evaluation of CDC-recommended approaches for confirmatory testing of *Neisseria gonorrhoeae* nucleic acid amplification results. *J Clin Microbiol* 2008;46:1614–9.
108. Gaydos CA, Crotchfelt KA, Howell MR, Kralian S, Hauptman P, Quinn TC. Molecular amplification assays to detect chlamydial infections in urine specimens from high school female students and to monitor the persistence of chlamydial DNA after therapy. *J Infect Dis* 1998;177:417–24.
109. Workowski KA, Lampe MF, Wong KG, Watts MB, Stamm WE. Long-term eradication of *Chlamydia trachomatis* genital infection after antimicrobial therapy. *JAMA* 1993;270:2071–5.
110. Bachmann LH, Desmond RA, Stephens J, Hughes A, Hook EW III. Duration of persistence of gonococcal DNA detected by ligase chain reaction in men and women following recommended therapy for uncomplicated gonorrhea. *J Clin Microbiol* 2002;40:3596–601.
111. Kacena KA, Quinn SB, Howell MR, Madico GE, Quinn TC, Gaydos CA. Pooling urine samples for ligase chain reaction screening for genital *Chlamydia trachomatis* infection in asymptomatic women. *J Clin Microbiol* 1998;36:481–5.
112. Lindan C, Mathur M, Jerajani H, et al. Utility of Pooled Urine Specimens for Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in Men Attending Public Sexually Transmitted Infection Clinics in Mumbai, India, by PCR. *J Clin Microbiol* 2005;43:1674–7.
113. Currie MJ, McNiven M, Yee T, Schiemer U, Bowden FJ. Pooling of Clinical Specimens Prior to Testing for *Chlamydia trachomatis* by PCR Is Accurate and Cost Saving. *J Clin Microbiol* 2004;42:4866–7.
114. Zubrzycki L. Non-culture tests for the diagnosis of gonorrhea [Review]. *Adv Exp Med Biol* 1990;263:77–88.
115. Janik A, Juni E, Heym GA. Genetic transformation as a tool for detection of *Neisseria gonorrhoeae*. *J Clin Microbiol* 1976;4:71–81.
116. Jaffe HW, Kraus SJ, Edwards TA, Weinberger SS, Zubrzycki L. Diagnosis of gonorrhea using a genetic transformation test on mailed clinical specimens. *J Infect Dis* 1982;146:275–9.
117. Butler LO, Knight RDJ. Laboratory diagnosis of gonococcal infection by genetic transformation. *J Clin Microbiol* 1982;15:810–4.
118. Zubrzycki L, Weinberger SS. Laboratory diagnosis of gonorrhoea by a simple transformation test with a temperature-sensitive mutant of *Neisseria gonorrhoeae*. *Sex Transm Dis* 1980;7:183–7.

Appendix

Key Questions Reviewed by the Work Group and Member(s) Responsible for Literature Summary

Performance Characteristics

- Does the sensitivity and specificity of available tests for *C. trachomatis* and *N. gonorrhoeae* vary with respect to anatomic site from which the specimen was collected and/or specimen type?
 - Joan Chow, DrPH, California Department of Public Health, Richmond, California
 - Katherine Whitaker, PhD, Food and Drug Administration, Rockville, Maryland
- What recommendations should be made for the detection of *C. trachomatis* and *N. gonorrhoeae* infections of the genital tract, rectum, and throat?
 - Gary Budnick, MHS, Connecticut Department of Public Health Laboratory, Hartford, Connecticut
 - Steven Shapiro, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, CDC
 - Rick Steece, PhD, Infertility Prevention Project Laboratory Consultant, Pierre, South Dakota
- What test recommendations should be made for the diagnosis of suspected LGV infections (inguinal and rectal presentations)?
 - Lisa Steele, PhD, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, CDC

Screening Applications

- What specimen types are optimal for of *C. trachomatis* and *N. gonorrhoeae* screening purposes?
 - Charlotte Gaydos, DrPH, Johns Hopkins University, Baltimore, Maryland
 - Sarah Guerry, MD, Los Angeles County Department of Health Services, Los Angeles, California
 - Barbara Van Der Pol, PhD, Indiana University School of Public Health, Bloomington, Indiana
- What parameters should be considered when selecting a *C. trachomatis* and *N. gonorrhoeae* test for screening purposes?
 - George Dizikes, PhD, Illinois Department of Public Health, Chicago, Illinois
 - Robert E Johnson, MD, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, CDC
 - Scott Zimmerman, DrPH, Erie County Public Health Laboratory, Buffalo, New York

Economic Considerations

- What economic considerations might influence laboratory testing?
 - Thomas Gift, PhD, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, CDC

Laboratory Confirmation

- What approaches, if any, can be used to derive a definitive laboratory confirmation of *C. trachomatis* and *N. gonorrhoeae*?
 - Yetunde Fakile, PhD, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, CDC
 - Dennis Ferrero, MPH, Department of Biological Sciences, University of the Pacific, Stockton, California
 - Rick Steece, PhD, Infertility Prevention Project Laboratory Consultant, Pierre, South Dakota
 - Barbara Van Der Pol, PhD, Indiana University School of Public Health, Bloomington, Indiana

Work Group Members

As of January 2009

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