



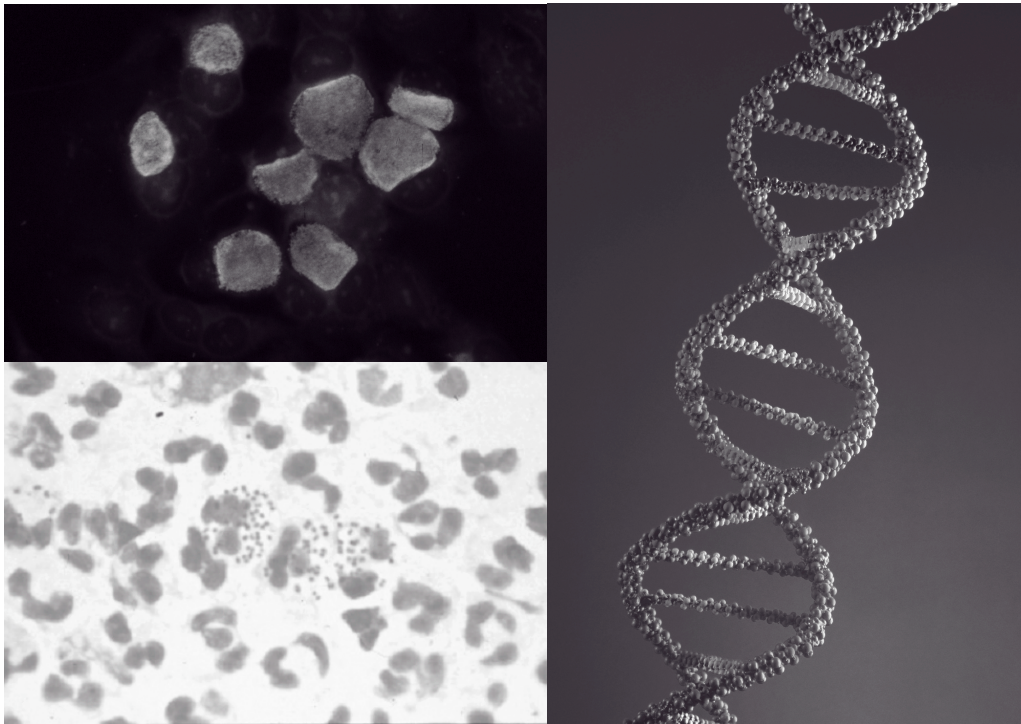
# MMWR<sup>TM</sup>

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### Screening Tests To Detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections — 2002



**INSIDE: Continuing Education Examination**

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**On the Cover:** Methods to detect *Chlamydia trachomatis* or *Neisseria gonorrhoeae* include microscopy (Gram-stain negative diplococci, lower-left), culture (intracellular inclusions, upper-left), and nucleic acid detection tests (DNA helix, right).

## CONTENTS

Introduction .....	1
Testing Technologies .....	3
Laboratory-Based Tests .....	3
Point-of-Care Tests .....	6
C. <i>trachomatis</i> and N. <i>gonorrhoeae</i> Test Performance	
When Used for Screening .....	6
C. <i>trachomatis</i> Tests for Screening Women and Men .....	7
N. <i>gonorrhoeae</i> Tests for Screening Women and Men .....	8
Selecting Screening Tests .....	9
Performance Perspective for Selecting Screening Tests .....	9
Additional Considerations in Selecting a Screening Test ..	12
Methods To Enhance Performance or Reduce Costs .....	14
Collecting, Transporting, and Storing Specimens .....	19
Collecting and Transporting Specimens for Screening .....	19
Collecting Specimens for Indications	
Other Than Screening .....	21
Laboratory Implementation of NAATS .....	21
Test of Cure, Treatment Failure, and Antimicrobial	
Resistance .....	22
Sexual Assault and Sexual Abuse .....	22
References .....	23
Appendix A .....	28
Appendix B .....	31
Appendix C .....	34
Appendix D .....	35
Appendix E .....	37
Appendix F .....	38
Continuing Education Examination .....	CE-1

This report is also available at <http://www.cdc.gov/std/labguidelines>. A limited number of copies can be ordered at that website.

# Screening Tests To Detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections — 2002

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## Summary

Since publication of CDC's 1993 guidelines (CDC. Recommendations for the prevention and management of *Chlamydia trachomatis* infections, 1993. MMWR 1993;42[No. RR-12]:1–39), nucleic acid amplification tests (NAATs) have been introduced as critical new tools to diagnose and treat *C. trachomatis* and *Neisseria gonorrhoeae* infections. NAATs for *C. trachomatis* are substantially more sensitive than previous tests. When using a NAAT, any sacrifice in performance when urine is substituted for a traditional swab specimen is limited, thus reducing dependence on invasive procedures and expanding the venues where specimens can be obtained. NAATs can also detect both *C. trachomatis* and *N. gonorrhoeae* organisms in the same specimen. However, NAATs are usually more expensive than previous tests, making test performance from an economic perspective a key consideration.

This report updates the 1993 guidelines for selecting laboratory tests for *C. trachomatis* with an emphasis on screening men and women in the United States. (In this report, screening refers to testing persons in the absence of symptoms or signs indicating *C. trachomatis* or *N. gonorrhoeae* infection.) In addition, these guidelines consider tests from an economic perspective and expand the previous guidelines to address detection of *N. gonorrhoeae* as well as *C. trachomatis* infections. Because of the increased cost of NAATs, certain laboratories are modifying manufacturers' procedures to improve test sensitivity without incurring the full cost associated with screening with a NAAT. Such approaches addressed in these guidelines are pooling of specimens before testing with a NAAT and additional testing of specimens whose non-NAAT test result is within a gray zone. This report also addresses the need for additional testing after a positive screening test to improve the specificity of a final diagnosis.

To prepare these guidelines, CDC staff identified pertinent concerns, compiled the related literature published during 1990 or later, prepared tables of evidence, and drafted recommendations. Consultants, selected for their expertise or disciplinary and organizational affiliations, reviewed the draft recommendations. These final guidelines are the recommendations of CDC staff who considered contributions from scientific consultants. These guidelines are intended for laboratorians, clinicians, and managers 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 material in this report originated in the National Center for HIV, STD, and TB Prevention, Harold W. Jaffe, M.D., Acting Director, and the Division of Sexually Transmitted Diseases Prevention, Harold W. Jaffe, M.D., Acting Director; and the National Center for Infectious Diseases, James M. Hughes, M.D., Director, and the Division of AIDS, STD, and TB Laboratory Research, Jonathan E. Kaplan, M.D., Acting Director.

## Introduction

An estimated 3 million *Chlamydia trachomatis* infections occur annually among sexually active adolescents and young adults in the United States (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.

Untreated, *C. trachomatis* infections can lead to serious complications. In certain studies,  $\leq 40\%$  of women with untreated *C. trachomatis* infections experience pelvic inflammatory disease (PID) (3,4). Of these, the majority have symptoms that are too mild or nonspecific for them to seek medical treatment. Regardless of symptom severity, the consequences of PID are severe. Of those with PID, 20% will become infertile; 18% will experience debilitating, chronic pelvic pain; and 9% will have a life-threatening tubal pregnancy (5). *C. trachomatis* infection during pregnancy leads to infant conjunctivitis and pneumonia and maternal postpartum endometritis.

Among men, urethritis is the most common illness resulting from *C. trachomatis* infection. Complications (e.g., epididymitis) affect a minority of infected men and rarely result in sequelae. Among men who engage in receptive anal intercourse, the rectum is a common site of *C. trachomatis* infection. Rectal infections are usually asymptomatic, but can cause symptoms of proctitis or proctocolitis. *C. trachomatis* can cause conjunctivitis among adults and is a cause of sexually acquired reactive arthritis.\*

Estimated tangible costs of *C. trachomatis* illness in the United States exceed \$2.4 billion annually (6). Also critical are the intangible costs, including the psychological and emotional injury caused by infertility and ectopic pregnancy.

As of December 2000, all 50 states and the District of Columbia had enacted laws requiring the reporting of *C. trachomatis* cases. In 2001, *C. trachomatis* infections were the most commonly reported communicable infections, with a total of 783,242 reports to CDC (7). Rates of *C. trachomatis* infection for women are highest for adolescents (2,536/100,000 among women aged 15–19 years) and young adults (2,447/100,000 among women aged 20–24 years). These age groups had the highest rates of infection for men as well, although the peak rate occurred among men aged 20–24 years (605/100,000).

Reported rates of *C. trachomatis* infections have risen significantly during 1987–2001 (51–278 cases/100,000 persons) (7). This increase is probably caused by a combination of factors, including an increased awareness of the need to screen women for *C. trachomatis* infection, resulting in the initiation of screening programs in both public and private health-care settings, improvement in the sensitivity of diagnostic tests, improved surveillance and reporting systems, and continued high infection rates.

Introduction of large-scale screening programs (e.g., one initiated in the Department of Health and Human Services Region X [Alaska, Idaho, Oregon, and Washington] family planning clinics in 1988) have been followed by a reduction in *C. trachomatis* positivity rates by  $\leq 60\%$  (7–9). *C. trachomatis* screening programs have been initiated throughout the United States that are based on such demonstration projects.

In 2001, *Neisseria gonorrhoeae* was second in frequency only to *C. trachomatis* among reported communicable infections in the United States, with 361,705 reported cases (7). The age distribution of *N. gonorrhoeae* infections is similar to that for *C. trachomatis* infections. Also similar to *C. trachomatis*, uncomplicated *N. gonorrhoeae* infection is usually confined to the mucosa of the cervix, urethra, rectum, and throat; *N. gonorrhoeae* infection is often asymptomatic among females; and, if untreated, *N. gonorrhoeae* infection can lead to PID, tubal infertility, ectopic pregnancy, and chronic pelvic pain (10). *N. gonorrhoeae* usually causes symptomatic urethritis among males, and occasionally results in epididymitis. Rarely, local infection disseminates to cause an acute dermatitis tenosynovitis syndrome, which can be complicated by arthritis, meningitis, or endocarditis (10). Also, similar to *C. trachomatis*, *N. gonorrhoeae* can be acquired at birth. *N. gonorrhoeae* neonatal infection can cause severe conjunctivitis, which can result in blindness if untreated and, rarely, sepsis with associated meningitis, endocarditis, or arthritis. After the introduction of a national control program in the mid-1970s, the overall rate of reported *N. gonorrhoeae* infection had declined by 74% during 1975–1997 (7,11). However, the rate increased by 8.1% during 1997–1999, followed by a limited decline in 2000–2001.

Culture testing for *C. trachomatis* and *N. gonorrhoeae* has been the reference standard against which all other tests have been compared. However, other tests have been needed because culture methods for *C. trachomatis* are difficult to standardize, technically demanding, and expensive. Culture for either agent is associated with problems in maintaining the viability of organisms during transport and storage in the diverse settings in which testing is indicated. Thus, diagnostic test manufacturers have developed nonculture tests that do not require viable organisms, including tests that can be automated. The first nonculture screening 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

\*These guidelines do not address trachoma and lymphogranuloma venereum, which rarely occur in the United States.

ribonucleic acid (RNA) sequences. With the availability of these nonculture tests, screening programs for *C. trachomatis* were initiated, and screening programs for *N. gonorrhoeae* began to change from culture to using the more convenient and, in remote settings, more reliable nonculture methods. The primary drawback of these tests, chiefly for *C. trachomatis*, is that they fail to detect a substantial proportion of infections (12–23). Consequently, a new generation of nonculture tests, called nucleic acid amplification tests (NAATs), were developed that amplify and detect *C. trachomatis*-specific or *N. gonorrhoeae*-specific DNA or RNA sequences. These tests are substantially more sensitive than the first generation nonculture tests (12–23).

These guidelines are intended to assist laboratorians, clinicians, and managers 1) select screening tests for *C. trachomatis* or *N. gonorrhoeae* from the complex array of tests available; 2) establish standard operating procedures for collecting, processing, and analyzing specimens; and 3) interpret test results for laboratory reporting, counseling, and treating patients. The guidelines were developed through literature reviews and extended consultation with non-CDC sexually transmitted disease (STD) specialists.

## Testing Technologies

The following discussion is a review of the complex array of technologies now available for laboratory diagnosis of *C. trachomatis* and *N. gonorrhoeae* infections. For this report, technologies are subdivided into those that are designed for 1) batch testing in a laboratory or 2) point-of-care testing as single tests or a limited number of tests performed while patients await results. Laboratory-based tests include culture, NAATs, nucleic acid hybridization and transformation tests, EIAs, and DFA tests. Point-of-care tests have long included the Gram-stained smear for *N. gonorrhoeae*. Point-of-care tests for *C. trachomatis* include solid-phase EIAs and a solid-phase optical immunoassay. The leukocyte esterase test (LET) is a dipstick test that is applied to urine specimens to screen for urinary tract inflammation (see Methods To Enhance Performance or Reduce Costs). Personnel, quality assurance, and quality control requirements relating to the use of all tests for medical care are published in the Clinical Laboratory Improvement Amendments of 1988 (CLIA) regulations (24) and are linked to testing complexity.

## Laboratory-Based Tests

### Culture Tests

***C. trachomatis* Culture.** Cell culture for *C. trachomatis* involves inoculating a confluent monolayer of susceptible cells

with an appropriately collected and transported specimen. After 48–72 hours of growth, infected cells develop characteristic intracytoplasmic inclusions that contain substantial numbers of *C. trachomatis* elementary and reticulate bodies. These unique inclusions are detected by staining with a fluorescein-conjugated monoclonal antibody that is specific for the major outer membrane protein (MOMP) of *C. trachomatis*.

Cell culture methods vary among laboratories, leading to probable substantial interlaboratory variation in performance (25). For example, because of a larger inoculum and reduced risk of cross-contamination, the shell vial method of culture is more sensitive and specific than the 96-well microtiter plate method (26,27). 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 another cycle of growth (28).

Tissue culture detection of *C. trachomatis* is highly specific if a *C. trachomatis*-MOMP-specific stain is used, because stained *C. trachomatis* inclusions have a unique appearance. Less specific inclusion-detection methods using EIA, iodine, and Giemsa are not recommended (29,30). Certain CDC consultants believe that commercial stains employing monoclonal antibodies directed against lipopolysaccharide (LPS), which are genus-specific rather than species-specific, are more sensitive and more economical than species-specific monoclonal antibody stains directed against MOMP. Such stains might be suitable for routine use, but a species-specific stain would be preferable in situations requiring increased specificity.

The high specificity of cell culture and ability to retain the isolate make cell culture the first choice when the results will be used as evidence in legal investigations. In addition, cell culture is the only method by which a clinical isolate can be obtained for antimicrobial susceptibility testing. The relatively low sensitivity, long turnaround time, difficulties in standardization, labor intensity, technical complexity, stringent transport requirements, and relatively high cost are the primary disadvantages of cell culture isolation of *C. trachomatis*. Additional information regarding cell culture for *C. trachomatis* is available elsewhere (14,28,31).

***N. gonorrhoeae* Culture.** Methods of gonococcal culture have been well-described elsewhere (12,32). Specimens are streaked on a selective (e.g., Thayer-Martin or Martin-Lewis) or nonselective (e.g., chocolate agar) medium if specimens are from nonsterile or sterile sites, respectively. Inoculated media are incubated at 35°C–36.5°C in an atmosphere supplemented with 5% CO<sub>2</sub> and examined at 24-hour intervals for ≤72 hours. Culture media for *N. gonorrhoeae* isolation include a base medium supplemented with chocolate equine or bovine

blood to support the growth of the gonococcus; selective media differ from routine culture media in that they contain antimicrobial agents (i.e., vancomycin, colistin, and an anti-fungal agent), which inhibit the growth of other bacteria and fungi. Supplemental CO<sub>2</sub> can be supplied by a CO<sub>2</sub> incubator, candle-extinction jar, or CO<sub>2</sub>-generating tablets.

A presumptive identification of *N. gonorrhoeae* isolates recovered from a genital specimen on selective medium can be made with a Gram stain and oxidase test. A presumptive identification indicates only that a Gram-negative, oxidase-positive diplococcus (e.g., a *Neisseria* species or *Branhamella* [*Moraxella*] *catarrhalis*) has been isolated from a specimen. Certain coccobacilli, including *Kingella denitrificans*, might appear to be Gram-negative diplococci in Gram-stained smears. A confirmed laboratory diagnosis of *N. gonorrhoeae* cannot be made on the basis of these tests alone. A presumptive test result is sufficient to initiate antimicrobial therapy, but additional tests must be performed to confirm the identity of an isolate as *N. gonorrhoeae*. Culture isolation is also suitable for nongenital tract specimens. Using selective media is necessary if the anatomic source of the specimen normally contains other bacterial species.

The sensitivity of culture can be monitored (i.e., quality-controlled) by evaluating results among males with urethral discharge. Culture results are compared with results obtained by using Gram-stained smear or by using nonselective medium. Vancomycin-sensitive strains, which were relatively common in certain areas in the past, now appear to be an uncommon cause of false-negative cultures (10,12,33,34).

The advantages of culture are high sensitivity and specificity, low cost, suitability for use with different types of specimens, and the ability to retain the isolate for additional testing. Retention of the isolate for additional testing might be indicated for medicolegal purposes, antimicrobial susceptibility determination, and subtyping of isolates. The major disadvantage of culture for *N. gonorrhoeae* is that specimens must be transported under conditions adequate to maintain the viability of organisms. Another disadvantage is that a minimum of 24–72 hours is required from specimen collection to the report of a presumptive culture result.

## NAATs

The common characteristic among NAATs is that they are designed to amplify 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 ability to produce a positive signal from as little as a single copy of the target DNA or RNA. Commercial tests differ in their amplification methods and their target nucleic acid sequences. The Roche

Amplicor<sup>®</sup> (manufactured by Roche Diagnostics Corporation, Basel, Switzerland) test uses polymerase chain reaction (PCR); the Abbott LCx<sup>®</sup> (Abbott Laboratories, Abbott Park, Illinois) test uses ligase chain reaction (LCR); and the Becton Dickinson BDProbeTec<sup>™</sup> ET (Becton, Dickinson and Company, Franklin Lakes, New Jersey) test uses strand displacement amplification to amplify *C. trachomatis* DNA sequences in the cryptic plasmid that is found in >99% of strains of *C. trachomatis*. The Gen-Probe APTIMA<sup>™</sup> (Gen-Probe, Incorporated, San Diego, California) assay for *C. trachomatis* uses transcription-mediated amplification (TMA) to detect a specific 23S ribosomal RNA target. These nucleic acid amplification methods are also used to detect *N. gonorrhoeae*. The target for the Roche Amplicor test for *N. gonorrhoeae* is a 201 base pair sequence within the cytosine methyltransferase gene M:Ngo P11. The Abbott LCx test for *N. gonorrhoeae* detects a 48 base-pair sequence in the Opa genes, ≤11 copies of which occur per cell, whereas the BDProbeTec ET for *N. gonorrhoeae* detects a DNA sequence that is within the multicopy pilin gene-inverting protein homologue. The Gen-Probe APTIMA Combo 2 version of TMA detects the 16S ribosomal RNA (rRNA) of *N. gonorrhoeae*. Additional overviews of the various amplification methods can be found in other published reports (16,35,36) and in materials available from the manufacturers.

The majority of commercial NAATs have been cleared<sup>†</sup> by the Food and Drug Administration (FDA) to detect *C. trachomatis* and *N. gonorrhoeae* in endocervical swabs from women, urethral swabs from men, and urine from both men and women. In addition, other specimens (e.g., those from the vagina [37–50] and eye [51–53]) have been used with satisfactory performance, although these applications have not been cleared by FDA. Testing of rectal and oropharyngeal specimens with NAATs has had limited evaluation and is not recommended.

The ability of NAATs to detect *C. trachomatis* and *N. gonorrhoeae* without a pelvic examination or intraurethral swab specimen (for males) (e.g., by testing urine) is a key advantage of NAATs, and this ability facilitates screening males and females in other than traditional screening venues (e.g., STD and family planning clinics). A disadvantage of NAATs is that specimens can contain amplification inhibitors that result in false-negative results. Certain manufacturers provide

<sup>†</sup> The term *cleared* is used by the Food and Drug Administration (FDA) to describe the process they use to review applications to market the class of diagnostic tests that includes *C. trachomatis* and *N. gonorrhoeae* tests discussed in these guidelines. The term *approved* is used by FDA to describe a more rigorous process they use to review applications to market classes of diagnostic tests that involve, for example, higher levels of risk if the test result is erroneous than is the case for *C. trachomatis* or *N. gonorrhoeae*.

amplification controls to detect inhibition. Selected NAATs might be substantially less sensitive than non-NAATs when performed on urine than when performed on endocervical specimens (54–56) or male urethral swabs (54,56). Although not documented by published head-to-head studies, the majority of CDC consultants believe that non-NAATs are substantially less sensitive than NAATs when used on urine specimens. The nucleic acid primers employed by commercial NAATs for *C. trachomatis* are not known to cross-react with DNA from other bacteria found in humans. However, the primers employed by certain NAATs for *N. gonorrhoeae* might cross-react with nongonococcal *Neisseria* species (54,56,57). NAATs are also more susceptible than non-NAATs to false-positive results because of contamination if strict quality control procedures are not applied.

### Nucleic Acid Hybridization (Nucleic Acid Probe) Tests

Two nucleic acid hybridization assays are FDA-cleared to detect *C. trachomatis* or *N. gonorrhoeae*: the Gen-Probe PACE<sup>®</sup> 2 and the Digene Hybrid Capture<sup>®</sup> II assays. Both the PACE and Hybrid Capture assays can detect *C. trachomatis* or *N. gonorrhoeae* in a single specimen. The PACE 2C test and the Hybrid Capture II CT/GC versions of these tests do not differentiate between the two organisms and, when positive, should be followed by tests for each organism to obtain an organism-specific result. In the Gen-Probe hybridization assays, a DNA probe that is complementary to a specific sequence of *C. trachomatis* or *N. gonorrhoeae* rRNA hybridizes with any complementary rRNA that is present in the specimen (58). A competitive probe version of the PACE 2 assay is commercially available to augment specificity. In this version, the test is repeated on initially positive specimens with and without adding an unlabeled probe. The unlabeled probe competitively inhibits binding of the labeled probe; a reduction in signal when the assay is performed with the unlabeled probe is interpreted as verification of the initial positive test result.

RNA hybridization probes in the Digene assay are specific for DNA sequences of *C. trachomatis* and *N. gonorrhoeae*, including both genomic DNA and cryptic plasmid DNA (59). Technical requirements and expertise necessary for performing nucleic acid hybridization tests are similar to those for the EIAs, which are described in this report. One of the advantages of the nucleic acid hybridization tests is the ability to store and transport specimens for  $\leq 7$  days without refrigeration before receipt and testing by the laboratory.

### Nucleic Acid Genetic Transformation Tests

The Gonostat<sup>®</sup> test (Sierra Diagnostics, Incorporated, Sonoma, California) uses a gonococcal mutant that grows when transformed by DNA extracted from a swab specimen con-

taining *N. gonorrhoeae*. *N. meningitidis* causes false-positive results (60). The test has received limited evaluation in published studies (61–64), which include an evaluation of its use with mailed specimens (62). A genetic transformation test is not available for detection of *C. trachomatis* infection.

### EIA Tests

A substantial number of EIA tests have been marketed for detecting *C. trachomatis* infection. By contrast, the performance and cost characteristics of EIA tests for *N. gonorrhoeae* infection have not made them competitive with culture (32). *C. trachomatis* EIA tests detect chlamydial LPS with a monoclonal or polyclonal antibody that has been labeled with an enzyme. The enzyme converts a colorless substrate into a colored product, which is detected by a spectrophotometer. Specimens can be stored and transported without refrigeration and should be processed within the time indicated by the manufacturer. One disadvantage of the EIA methods that detect LPS is the potential for false-positive results caused by cross-reaction with LPS of other microorganisms, including other *Chlamydia* species (28,30,65,66). Manufacturers have developed blocking assays that verify positive EIA test results. The test is repeated on positive specimens with the addition of a monoclonal antibody specific for chlamydia LPS. The monoclonal antibody competitively inhibits chlamydia-specific binding by the enzyme-labeled antibody; a negative test result when using the blocking antibody is interpreted as verification of the initial positive test result. EIA tests should not be used with rectal specimens because of cross-reactions with fecal bacteria.

### DFA Tests

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 slide should be processed by the laboratory in  $\leq 7$  days after the specimen has been obtained. Staining consists of covering 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 used in certain commercial kits can cross-react with nonchlamydial bacterial species, as well as with LPS of *C. pneumoniae* and *C. psittaci*. DFA with a *C. trachomatis*-specific anti-MOMP monoclonal antibody is considered to be highly specific, when performed by an experienced

microscopist. An additional advantage of DFA is that the quality of endocervical smears can be assessed by checking for the presence of columnar cells (see Collecting and Transporting Specimens for Screening). DFA requires that the laboratorian be competent in fluorescent microscopy and adequately trained in identifying fluorescein-stained *C. trachomatis* elementary bodies. DFA is best suited for laboratories that test a limited number of specimens, because the procedure is fatiguing and time-consuming. DFA tests have not been established as an initial test for the direct detection of *N. gonorrhoeae* in clinical specimens.

### Serology Tests

Serology has limited value in testing for uncomplicated genital *C. trachomatis* infection and should not be used for screening because previous chlamydial infection frequently elicits long-lasting antibodies that cannot be easily distinguished from the antibodies produced in a current infection. More specific information regarding serologic assays for *C. trachomatis* antibody has been reported elsewhere (14). A serologic screening or diagnostic assay is not available for *N. gonorrhoeae*.

### Point-of-Care Tests

#### C. trachomatis Point-of-Care Tests

Tests for *C. trachomatis* have been developed that can be performed within 30 minutes, do not require expensive or sophisticated equipment, and are packaged as single units. The results are read qualitatively. These so-called rapid or stat tests can offer advantages in physicians' offices, small clinics and hospitals, detention centers, and other settings where results are needed immediately (e.g., when decisions need to be made regarding additional testing or treatment while the patient is still present). These tests are classified under CLIA as tests of moderate complexity (24). Accordingly, personnel standards, quality control, quality assurance, and proficiency testing requirements apply when performing FDA-cleared *C. trachomatis* tests that are rapid enough to qualify as point-of-care tests. In addition, these tests are usually less sensitive and more expensive than laboratory-based *C. trachomatis* tests that require longer to perform. Similar to EIAs, these tests use antibodies against LPS that detect all three *Chlamydia* species that infect humans and are subject to the same potential for false-positive results caused by cross-reactions with other microorganisms. A point-of-care test should not be selected if it is performed in a laboratory after the patient's visit. Such use of point-of-care tests is inappropriate because sensitivity and specificity are typically less, controls less rigorous, and costs higher than for tests designed for laboratory use (67).

#### Gram Stain for *N. gonorrhoeae*

Gram stain is a key tool for the diagnosis of gonococcal urethritis in men, but its application to screening is limited because of the requirement for an intraurethral swab specimen if discharge is not present at the urethral meatus. A Gram stain for the presumptive diagnosis of *N. gonorrhoeae* infection is performed on thin smears of urethral exudate from men and is presumptively positive if the smear contains typical Gram-negative diplococci within polymorphonuclear (PMN) leukocytes. Unfortunately, other *Neisseria* species have a similar appearance. Although commensal *Neisseria* species are not normal flora of anogenital sites, isolates of *Neisseria meningitidis* and nonpathogenic *Neisseria* species have been reported occasionally from anogenital sites among both men and women (12).

As a point-of-care test, Gram stain is most reliable for the presumptive identification of *N. gonorrhoeae* in urethral exudates from men. The sensitivity and specificity of a Gram stain for males with symptomatic urethritis are comparable to culture isolation followed by oxidase testing and Gram staining of the isolate (32,68,69). Gram-negative extracellular diplococci without any intracellular diplococci might be observed in smears from men with early symptomatic infections. The sensitivity of Gram stain for males with asymptomatic urethral infection has not been determined. Gram-negative intracellular diplococci might be observed with certain infections caused by nongonococcal *Neisseria* species (e.g., *N. cinerea*).

The sensitivity of Gram stains of endocervical specimens is lower than for urethral specimens from men with symptomatic gonorrhea (10,32,69), and adequate specificity requires a skilled microscopist. For these reasons, Gram stain is not recommended for testing for *N. gonorrhoeae* infection among women. Gram stains of pharyngeal specimens are not recommended because *N. meningitidis* and commensal *Neisseria* species colonize the pharynx (12). As with point-of-care tests for *C. trachomatis*, the Gram-stained smear is classified under CLIA as a moderate complexity test for urethral and endocervical specimens (24). Gram stains from all other body sites are categorized as high complexity. A trained laboratorian is required for preparing and reading high-complexity Gram-stained smears.

#### C. trachomatis and N. gonorrhoeae Test Performance When Used for Screening

Numerous evaluations of *C. trachomatis* and *N. gonorrhoeae* tests when used for screening have been published. NAAT sensitivities have consistently exceeded the sensitivities of non-NAATs. However, the majority of published estimates of



test performance are uncertain because of probable bias from 1) misclassification of study subjects' infection status by reference standard tests; 2) using reference standard tests that are more suitable for determining whether a study subject is infected at one anatomic site (e.g., the endocervix) versus determining whether the study subject is infected at any anatomic site; and 3) predominance of studies of female and symptomatic male patients from STD clinics who might not be representative of populations who would be targeted for screening. Estimates of the differences in the performance of NAATs and non-NAATs are also uncertain because only a limited number of evaluations have been conducted that evaluated the two sets of tests by using the same set of study subjects (i.e., a head-to-head study).

Identifying a suitable standard for classifying study subjects with respect to true infection status has been difficult. Customary reference standards (e.g., culture) are highly specific but lack sensitivity. When such standards are used, evaluated test specificity is probably underestimated. Truly infected subjects who are misclassified as uninfected by the reference standard are more likely to be determined positive (and thus, inappropriately considered falsely positive) by the evaluated test than are the uninfected subjects. Misclassification of evaluated test results as false-positives occurs more frequently if the evaluated test (e.g., a NAAT) is more sensitive than the reference test (e.g., culture). The majority of evaluators of *C. trachomatis* and *N. gonorrhoeae* tests have attempted to address this problem by using a discrepant analysis procedure. This procedure has been strongly contested by statisticians and clinical epidemiologists because reference tests are applied dependent upon evaluated test results. This dependency contributes a positive bias to estimates of sensitivity and does not eliminate ambiguity regarding specificity estimates (70–75).

Identifying a reference standard that accurately classifies study subjects who can be infected at multiple anatomic sites is also problematic, chiefly for women. The majority of test evaluations for women use an endocervical reference standard only. Estimates of evaluated test sensitivity are probably higher and specificity lower, when the reference standard for infection is based on a positive result from a single anatomic site (e.g., an endocervical swab) rather than from multiple potentially infected sites (e.g., an endocervical swab or urine specimens) (54,76,77). The former standard, which produces estimates by using a specimen from one anatomic site, can be considered a specimen standard and addresses the concern of test performance regarding identification of infection at that site. The latter standard can be considered a patient standard.

The following sections summarize *C. trachomatis* and *N. gonorrhoeae* test performance on the basis of recent test

evaluation studies, conducted without discrepant analysis, and consultation with specialists in test evaluation.

## C. *trachomatis* Tests for Screening Women and Men

### Sensitivity

Reviews of screening tests for *C. trachomatis* conclude that sensitivities of commercial NAATs exceed those of non-NAATs (14–23). A substantial proportion of the published evaluations of NAATs cited in these reviews have relied on discrepant analysis. However, certain studies permit the calculation and comparison of NAAT and non-NAAT sensitivities by using culture as an independent reference standard. A five-center study reported sensitivities of NAATs for endocervical specimens that exceeded the sensitivity of a nonamplified nucleic acid hybridization test by 19.7% (95% confidence interval [CI] = 12.9%–26.6%) for LCR and 12.4% (95% CI = 2.1%–22.7%) for PCR (78). The sensitivities of LCR and PCR were slightly lower when performed on urine specimens than on endocervical specimens (83.4% versus 91.4% and 79.5% versus 84.0%, respectively). The sensitivity of the nonamplified hybridization test (71.6%) was similar to that reported in an earlier study (75.3%) that compared the hybridization test, three EIAs, and a DFA performed on endocervical swab specimens by using culture as the reference standard (79). Sensitivities of the tests evaluated in that study were 61.9%–75.3%.

Culture, a NAAT (LCR), and the nonamplified hybridization test have also been compared by using an independent reference standard (78). For this evaluation, the reference standard was a positive PCR performed on an endocervical or urine specimen. For endocervical swab specimens, the sensitivity of LCR (85.5%) exceeded that of culture (74.7%) by 10.8% and that of the hybridization test (61.9%) by 23.6%. For the urine specimen, the sensitivity of LCR was 80.8%.

A limited number of studies have used an independent reference standard to compare the sensitivities of tests for detection of *C. trachomatis* infection in asymptomatic men. One five-center study compared LCR and PCR performed on urine from asymptomatic males by using culture of intraurethral swab specimens as the independent reference standard (80). The sensitivities of LCR (84.4%) and PCR (85.4%) were similar.

The majority of *C. trachomatis* test evaluation specialists consulted for these guidelines believe that the sensitivities of the point-of-care tests for *C. trachomatis* are substantially less than the sensitivities of tests with longer processing times. However, published studies do not provide a basis for specifying the magnitude of the difference.

## Specificity and Positive Predictive Value

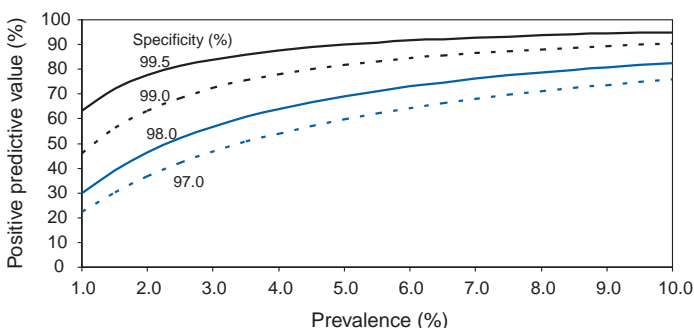
At the prevalences of *C. trachomatis* infection typical of populations who are screened (e.g., 2%–10%), the specificity of the screening test and the infection prevalence are key variables because they strongly influence the proportion of positive test results that reflect infection. The term used for this proportion is the positive predictive value (PPV) (81,82).<sup>§</sup> At lower prevalences, a positive screening test might need to be followed by an additional test to ensure an adequate PPV.

Published evaluations of NAATs that have used alternative target NAATs<sup>¶</sup> to perform discrepant analysis have reported increased specificities (e.g., exceeding 99.0%–99.5%); such reports have addressed NAATs for detection of *C. trachomatis* in endocervical specimens, male urethral specimens, and male or female urine. However, statisticians and clinical epidemiologists have criticized these estimation procedures also. Published studies or studies described in package inserts that have used DFA to perform discrepant analysis or have used culture as the standard without performing discrepant analysis have reported specificities of 94.1%–99.5% (75). In 1993, CDC reported a similar range of specificities for nonamplified nucleic acid probe and EIA tests to detect *C. trachomatis* (31). This report includes a graph of PPVs associated with a test with a sensitivity of 85% across a range of test specificities and prevalences typical of those reported for *C. trachomatis* screening applications (Figure).

<sup>§</sup> For example, when a test with a specificity of 99% and a sensitivity of 85% is used to screen a population of 10,000 patients with a *C. trachomatis* prevalence of 10% (i.e., 1,000 patients have an infection), an average of 940 test results will be positive: 850 patients with a positive result will actually be infected, and 90 will not be infected (i.e., false-positives). PPV is  $850/940 = 0.904$ . When this same test is used to screen 10,000 patients with a chlamydia prevalence of only 2% (i.e., 200 patients have an infection), an average of 268 test results will be positive: 170 patients will be infected and 98 will not. The PPV is  $170/268 = 0.634$ .

<sup>¶</sup> Published evaluations of commercial NAATs have augmented reference tests with an alternative target NAAT performed by the manufacturer that employs the same amplification method as the commercial NAAT, except for substitution of a different set of primers.

**FIGURE. Positive predictive values of a test with a sensitivity of 85% across a range of values of specificity and prevalence of infection**



All positive tests should be considered presumptive evidence of infection. Regardless of screening setting, screening location, patient population, and patient characteristics, a false-positive test result for *C. trachomatis* can have adverse medical, social, and psychological impacts for a patient. In such a case, consideration should be given to increasing specificity by performing an additional test after a positive screening test and requiring that both the screening test and additional test be positive to make a diagnosis of *C. trachomatis* infection (83). PPV of the diagnosis will be increased unless the screening and additional tests are falsely positive for the same reasons (e.g., both are mislabeled or both cross-react with a nonchlamydial organism). However, an additional test does not fully resolve all concerns because it might provide a false-negative result. Because therapy for *C. trachomatis* is safe and should not be delayed, therapy can be offered while awaiting additional test results or even if the additional test is negative. Patients with positive screening test results require counseling regarding both the risks of delaying therapy and the possibility of a false-positive test result. Consideration should be given to routine additional testing for persons with positive *C. trachomatis* screening tests when risk factor information or actual surveys indicate that the prevalence is low, resulting in an inadequate PPV (e.g., <90%) (see Additional Considerations in Selecting a Screening Test and also, Methods To Enhance Performance or Reduce Costs).

## *N. gonorrhoeae* Tests for Screening Women and Men

### Sensitivity

As for *C. trachomatis*, a substantial proportion of published evaluations of NAATs for *N. gonorrhoeae* have relied on discrepant analysis for calculation of test sensitivities, which might have positively biased the estimates. However, a meta-analysis of evaluations of *N. gonorrhoeae* summarizes sensitivity estimates for those studies for which sensitivity estimates could be based on culture as the independent reference standard (13). By using a culture standard, sensitivities were similar for an endocervical swab nucleic acid hybridization test (92.1%) and an FDA-cleared NAAT (96.7%).

Package inserts for NAATs recently cleared by FDA now include results of studies conducted by using independent reference standards without employing discrepant analysis. Two such package inserts include the results of culture, a previously FDA-cleared NAAT, and the candidate NAAT (84,85). When the independent reference standard for infection was a previously FDA-cleared NAAT positive on either endocervical swab or urine specimens, the sensitivities of endocervical swab culture were less than for the Becton Dickinson

BDProbeTec (83.7% versus 90.2%) and Gen-Probe APTIMA (89.3% versus 100.0%) tests; the sensitivities of the NAATs were lower when performed on urine than on endocervical swabs (78.2% versus 90.2% and 91.7% versus 100.0%, respectively). Compared with tests for *C. trachomatis*, differences in sensitivities among NAATs, nucleic acid hybridization tests without nucleic acid amplification, and culture are reduced when using endocervical swabs. The exception is that culture sensitivity can decline when transport or storage conditions compromise organism viability. Gram-stain sensitivity is not presented for men because Gram stain of urethral swab smears is seldom used for screening men outside STD clinic settings, and its performance for that purpose has not been well-evaluated. Gram stain is also not widely used for screening women because of decreased sensitivity and variable specificity.

### Specificity and PPV

As with *C. trachomatis*, test specificities and prevalences among populations screened for *N. gonorrhoeae* are key determinants of PPV of positive screening test results. Reported specificities of *N. gonorrhoeae* screening tests are similar to those for *C. trachomatis*. Unlike *C. trachomatis* tests, cross-reactivity between *N. gonorrhoeae* and pathogenic and non-pathogenic *Neisseria* has been demonstrated for certain NAATs (54,56,57). This cross-reactivity has not been reported for the Abbott LCx and Gen-Probe APTIMA and PACE 2 tests. However, nongonococcal *Neisseria* are infrequently recovered from the genitourinary tract. As with *C. trachomatis* tests, all positive screening tests should be considered presumptive evidence of infection, and routine additional testing after a positive *N. gonorrhoeae* screening test result would usually be indicated when screening among a low-prevalence population. Prevalences of *N. gonorrhoeae* are usually lower than for *C. trachomatis*, possibly resulting in lower PPVs. The decision to screen and conduct additional tests after a positive screening test should be made separately for the two organisms (see Additional Considerations in Selecting a Screening Test and also, Methods To Enhance Performance or Reduce Costs).

## Selecting Screening Tests

Multiple considerations affect the selection of a screening test. Test sensitivity is emphasized to minimize occurrence of false-negative tests, which can result in complications of untreated infection and ongoing transmission. However, additional considerations might lead to the selection of a different test (see Additional Considerations in Selecting a Screening Test). For example, a goal of maximizing test sensitivity to avoid missing the opportunity to treat infected persons might warrant tolerating a limited number of false-positive diagnoses.

However, consideration must also be given to reducing the rate and consequences of false-positive tests and to cost (see Methods To Enhance Performance or Reduce Costs). This report focuses on screening applications of tests for *C. trachomatis* and *N. gonorrhoeae* infections. This report also contains a listing of additional indications for *C. trachomatis* testing and recommendations for choice of test and type of specimen (Appendix A); similar information for *N. gonorrhoeae* is also included (Appendix B).

## Performance Perspective for Selecting Screening Tests

On the basis of sensitivity, ease of specimen collection, and ability to assess antimicrobial susceptibility (*N. gonorrhoeae*), recommendations for screening women and men for *C. trachomatis* and *N. gonorrhoeae* genitourinary tract infections are outlined in this section. Recommended screening tests will, compared with less sensitive tests, minimize the risk for disease sequelae and continued transmission of infections as a result of false-negative screening tests. Clinicians should be aware of the potential for adverse consequences caused by a false-positive test result (e.g., substantial psychosocial or legal consequences); patients with positive results should be counseled regarding the potential for false-positive results, and additional testing should be considered. Because such a result might itself be falsely negative and therapies for *C. trachomatis* and *N. gonorrhoeae* are safe and effective, treatment might be offered while awaiting results from additional testing or even if an additional test is negative. At lower prevalences, consideration should be given to routine additional testing after a positive screening test. Testing strategies have been proposed that can increase specificity and constrain costs by combining use of NAATs and non-NAATs. A particularly promising strategy called gray-zone testing involves screening with a non-NAAT and using a lower cutoff value than that established by the manufacturer as a criterion for a positive result. A NAAT is then performed as an additional test when the non-NAAT screening test results are in a zone above the new cutoff value. This strategy could achieve greater sensitivity than would be provided by using a non-NAAT by itself and greater specificity than would be provided by the separate use of either a non-NAAT or a NAAT, and cost less than using a NAAT by itself. This strategy might be useful for screening among lower prevalence populations for which both PPV and cost of detecting an infection are of increased concern. This strategy warrants further evaluation (see Additional Considerations in Selecting a Screening Test and also, Methods To Enhance Performance or Reduce Costs).

## Screening Women for *C. trachomatis* and *N. gonorrhoeae* Genitourinary Tract Infections

The following recommendations are based on sensitivity, ease of specimen collection, and ability to assess antimicrobial susceptibility (*N. gonorrhoeae*) (Box 1). Additional concerns, including cost and PPV, are addressed in subsequent sections (see Additional Considerations in Selecting a Screening Test and also, Methods to Enhance Performance or Reduce Costs).

### *C. trachomatis*.

1. A *C. trachomatis* NAAT performed on an endocervical swab specimen provides the highest sensitivity and might be preferred if a pelvic examination is acceptable; otherwise, a NAAT can be performed on urine. The sensitivity of NAATs when using urine to detect *C. trachomatis* in women is similar, or only slightly inferior, to their sensitivity when using endocervical swabs (78,86). By using urine as a specimen, screening persons in such venues as family planning clinics can now be extended beyond women undergoing pelvic examinations. Screening can be performed also in nontraditional venues where pelvic examinations are not performed (e.g., schools, jails and detention centers, and street HIV or STD outreach programs).
2. Although less sensitive than NAATs, unamplified nucleic acid hybridization tests, EIAs, and DFA performed on an endocervical swab specimen are acceptable for screening (see Methods To Enhance Performance and Reduce Costs).

### BOX 1. Tests used for screening women for genitourinary tract infection

#### ***Chlamydia trachomatis***

- A nucleic acid amplification test (NAAT) performed on an endocervical swab specimen, if a pelvic examination is acceptable; otherwise, a NAAT performed on urine.
- An unamplified nucleic acid hybridization test, an enzyme immunoassay, or direct fluorescent antibody test performed on an endocervical swab specimen.
- Culture performed on an endocervical swab specimen.

#### ***Neisseria gonorrhoeae***

- Culture performed on an endocervical swab specimen. If transport and storage conditions are not conducive to maintaining the viability of *N. gonorrhoeae*, a NAAT or nucleic acid hybridization test can be performed on an endocervical swab specimen.
- A NAAT performed on urine.

3. *C. trachomatis* culture performed on an endocervical swab specimen is a suitable test for screening. Specificity of culture tests is high; however, sensitivity is less than for NAATs and variable because of technical complexity, lack of standardization, and the challenge of maintaining viable organisms.

No tests have been cleared by FDA for use with vaginal specimens. However, studies have assessed using vaginal specimens for screening by NAATs (37–49), including vaginal specimens collected by the patient (37,38,40,42,44,47–49). The results of these studies are promising. Unless manufacturers obtain FDA clearance for this intended use, individual laboratories testing vaginal specimens will need to conduct a study to establish, rather than just to verify, test performance characteristics to satisfy CLIA requirements (Appendix C). The sensitivity of non-NAATs with urine or vaginal swab specimens is suboptimal. In the case of EIAs, specificity with vaginal swab and urine specimens is also lower than with endocervical swab specimens (65). Such uses of non-NAATs are not recommended.

### *N. gonorrhoeae*.

1. An *N. gonorrhoeae* culture test performed on an endocervical swab specimen might be preferred on the basis of ease of additional testing and on the continuing need for antimicrobial-resistance monitoring. When culture sensitivity might be compromised because of problems in maintaining appropriate transport and storage conditions, a NAAT or nucleic acid hybridization test can be performed on an endocervical swab specimen.
2. As with *C. trachomatis*, screening for *N. gonorrhoeae* can be extended to nontraditional clinical settings by using a NAAT performed on urine. The sensitivity of NAATs to detect *N. gonorrhoeae* might be less when using urine than when using an endocervical swab specimen (54–56). This decrease might be greater for certain NAATs than others (54–56).

As with *C. trachomatis*, no tests are FDA-cleared for use with vaginal specimens, although a study that assessed using patient-obtained vaginal specimens for screening by NAATs yielded promising results (50). Unless manufacturers obtain FDA clearance for this intended use, laboratories testing vaginal specimens will need to conduct a study to establish, rather than just to verify, test performance characteristics to satisfy CLIA requirements (Appendix C). The sensitivity of non-NAAT tests to detect *N. gonorrhoeae* with urine or vaginal swab specimens is suboptimal. Such uses of non-NAATs are not recommended.

## Screening Men for *C. trachomatis* and *N. gonorrhoeae* Urethral Infections

This section includes recommendations for tests for screening men for *C. trachomatis* and *N. gonorrhoeae* urethral infections. These recommendations are based on sensitivity, ease of specimen collection, and ability to assess antimicrobial susceptibility (*N. gonorrhoeae*) (Box 2). Additional concerns, including cost and PPV, are addressed in subsequent sections (see Additional Considerations in Selecting a Screening Test and Methods to Enhance Performance or Reduce Costs).

### *C. trachomatis*.

1. A NAAT for *C. trachomatis* performed on an intraurethral swab or urine specimen is the preferred test. According to STD specialists, NAATs might be more sensitive for detecting *C. trachomatis* infection of asymptomatic men when performed on an intraurethral swab specimen than on urine, although limited published evaluations demonstrate no superiority for swab specimens (77,87,88). However, intraurethral swab specimens are often unacceptable to males who do not have symptoms of urethritis (89–91). NAATs performed on urine to detect *C. trachomatis* have adequate sensitivity to be used for screening. The sensitivity of non-NAATs to detect *C. trachomatis* in urine from men is too low for them to be recommended for screening.
2. A non-NAAT or culture for *C. trachomatis* performed on an intraurethral swab specimen is acceptable.

### *N. gonorrhoeae*.

1. Culture performed on an intraurethral swab specimen is preferred in settings where collecting such specimens is acceptable and transport and storage conditions are

suitable for culture. Collection of exudate for *N. gonorrhoeae* culture at the urethral meatus without insertion of the swab into the urethra is sufficient when exudate is present. Culture performed on an intraurethral swab specimen is preferred because it facilitates additional testing, which is critical for monitoring for antimicrobial resistance. When culture sensitivity might be compromised because of problems in maintaining appropriate transport and storage conditions, a NAAT or nucleic acid hybridization test can be performed on an intraurethral swab specimen.

2. A NAAT or nucleic acid hybridization test performed on an intraurethral swab specimen or a NAAT performed on urine are acceptable tests for screening. Intraurethral swab specimens are often unacceptable to males who do not have symptoms of urethritis (89–91). In such cases, NAATs performed on urine to detect *N. gonorrhoeae* have adequate sensitivity for use in screening. Only a limited number of published studies have addressed whether NAATs perform better with urethral swab specimens than with urine in males with asymptomatic infection. One study of asymptomatic males reported that a NAAT was substantially less sensitive with urine than with urethral swabs; furthermore, NAATs were less sensitive with either specimen among males with asymptomatic infection than with symptomatic infection (54,56).

## Screening Women or Men with Possible Rectal or Pharyngeal Exposure to *C. trachomatis* or *N. gonorrhoeae* Infection

Selecting tests for screening for *C. trachomatis* and *N. gonorrhoeae* rectal or pharyngeal infections is difficult because of limited experience with nonculture tests and increased potential for cross-reactivity with other organisms (Box 3).

### *C. trachomatis*.

1. Culture isolation is acceptable for detecting *C. trachomatis* in rectal or pharyngeal swab specimens. A *C. trachomatis*-MOMP-specific stain should be used with culture of rectal or pharyngeal specimens to detect *C. trachomatis*. Less-specific inclusion-detection methods by using EIA, iodine, Giemsa, or LPS-specific monoclonal antibodies, are not recommended (29,30).
2. DFA can be performed on rectal or pharyngeal swab specimens. A *C. trachomatis*-MOMP-specific stain should be used.

### *N. gonorrhoeae*.

1. Culture isolation is well established for detecting *N. gonorrhoeae* in rectal and pharyngeal swab specimens. After isolation from rectal or pharyngeal specimens of

### BOX 2. Tests used for screening men for urethral infection

#### ***Chlamydia trachomatis***

- A nucleic acid amplification test (NAAT) performed on an intraurethral swab specimen if collecting such a specimen is acceptable; otherwise, a NAAT performed on urine.
- A non-NAAT or culture performed on an intraurethral swab specimen.

#### ***Neisseria gonorrhoeae***

- Culture performed on an intraurethral swab specimen if collecting such a specimen is acceptable and transport and storage conditions are suitable for culture.
- A NAAT or nucleic acid hybridization test performed on an intraurethral swab specimen if collecting such a specimen is acceptable; otherwise, a NAAT performed on urine.

### BOX 3. Tests for screening women or men for rectal or pharyngeal infection

#### ***Chlamydia trachomatis***

- Culture performed on rectal or pharyngeal swab specimens; a *C. trachomatis*-major outer membrane protein (MOMP)-specific stain should be used.
- Direct fluorescent antibody test performed on rectal or pharyngeal swab specimens; a *C. trachomatis*-MOMP-specific stain should be used.

#### ***Neisseria gonorrhoeae***

- Culture performed on rectal or pharyngeal swab specimens; a selective medium should be used with additional testing on colonies of typical oxidase-positive, Gram-negative diplococci.

typical oxidase-positive, Gram-negative diplococci on selective media, definitive identification of *N. gonorrhoeae* requires additional testing because of the common occurrence of other pathogenic and nonpathogenic *Neisseria* species in the pharynx and their occasional occurrence in the rectum (12,92,93) (see Methods To Enhance Performance or Reduce Costs).

If the foregoing methods are not available, screening for *C. trachomatis* and *N. gonorrhoeae* in rectal or pharyngeal specimens is not recommended. Only limited evaluations have been published of nonculture tests to detect *C. trachomatis* or *N. gonorrhoeae* oropharyngeal or rectal infections (32,94,95).

### **Additional Considerations in Selecting a Screening Test**

In addition to test sensitivity, ease of specimen collection, and assessment for antimicrobial susceptibility (*N. gonorrhoeae*), other considerations in choosing a screening test include 1) the relatively high cost of NAATs (i.e., economic considerations); 2) laboratory environmental changes necessary to implement NAATs; 3) the need for additional testing to support *C. trachomatis* or *N. gonorrhoeae* diagnoses; and 4) the likelihood of screening-test-positive persons returning for treatment. The ability of some tests to detect *C. trachomatis* and *N. gonorrhoeae* in the same specimen might also affect the choice of test (see Methods To Enhance Performance or Reduce Costs). Recommendations for transporting and storing specimens must also be considered (Appendix D).

### **Economic Analysis**

Economic analyses can assist in selecting the optimal tests and strategy for *C. trachomatis* and *N. gonorrhoeae* screening. Moreover, economic analyses can be relevant for STD screen-

ing because, in the absence of symptoms or signs, the cost of screening tests, including the costs of labor and other nonkit costs, is often not reimbursed by insurance plans or other payers. Thus, knowing the costs and benefits of a screening program that might not be fully reimbursed can be critical to decision makers (Box 4).

### **Laboratory Environment**

The laboratory must consider how candidate tests match the laboratory environment. For example, laboratories must satisfy the requirements for physical space, separation of work areas (e.g., sample processing and amplification), and use of any special equipment (e.g., biosafety cabinets) as recommended by the test kit manufacturer. Differences in throughput among different NAATs must be considered. Additional considerations include technician time, turnaround time, technical difficulty, equipment costs, and time required to maintain equipment. Laboratories are encouraged to investigate these concerns and their impact on cost-effectiveness when choosing among the available testing methods.

### **Need for Additional Testing To Support *C. trachomatis* or *N. gonorrhoeae* Diagnoses**

Efforts to maximize test sensitivity to avoid missing the opportunity to identify and treat infected persons might warrant tolerating a certain number of false-positive diagnoses. However, consideration must also be given to reducing the rate and consequences of false-positive tests. All tests, including culture for *C. trachomatis*, occasionally generate false-positive results. For these reasons, all positive tests are considered to be presumptive evidence of infection (Box 5). Regardless of health-care provider setting, patient population, and patient characteristics, a false-positive test result for *C. trachomatis* or *N. gonorrhoeae* can have adverse medical, social, and psychological impacts for a patient. In such cases, consideration should be given to performing an additional test to verify a positive screening test.

The potential for false-positive test results complicates the interpretation of positive tests among patients in populations with a low prevalence of infection. This occurs because the proportion of total positive tests that are truly positive (i.e., PPV) is lower among such a population. Consideration should be given to routine additional testing for persons with positive *C. trachomatis* or *N. gonorrhoeae* screening tests when risk-factor information or actual surveys indicate that the prevalence is low, resulting in a lower PPV (e.g., <90%) (see *C. trachomatis* and *N. gonorrhoeae* Test Performance When Used for Screening).

Economic analysis of additional testing to increase the specificity of a screening test is problematic because the primary benefit of such additional testing is in averting costs that are

**Box 4. Performing an economic analysis**

The information in this box is not intended as a guide to conducting economic analyses, which is beyond the scope of these guidelines. Resources are available that explain the theory and process in detail.\* The simplest economic analysis is a cost-analysis in which the costs of delivering a service or operating a program are determined. Cost-effectiveness analysis (CEA) estimates the cost of an intervention per unit outcome that the intervention generates (e.g., infections treated or cases of pelvic inflammatory disease [PID] avoided). CEA is most useful for comparing different interventions that achieve the same outcomes.†

The terms cost-effective and cost-saving are commonly used interchangeably, but they do not mean the same thing. For example, if a program is cost-saving, that means the cost of implementing a program is less than the health-care costs the program prevents. A program is cost-effective if it uses resources efficiently, compared with alternative uses for the same resources.§

**Calculating Screening Costs and Benefits**

Programs considering an economic analysis of their screening activities should consider direct medical costs, including test kits, reagents, and specimen-collection materials. They should also consider health-care providers' time for collecting specimens, administration, overhead and facilities costs, and specimen packing and transport. The testing facility must also consider costs incurred in changing test technology (i.e., startup equipment, laboratory remodeling costs, or such recurring costs as training) and any changes in number or level of staff.

To measure screening benefits, programs should consider the savings realized through averted medical costs (e.g., costs associated with cases of PID averted through detecting and treating *C. trachomatis* and *N. gonorrhoeae* infections). Other health outcomes that might be of interest are epididymitis in men, uncured infections in men that might lead to reinfection of sex partners, and neonatal complications. Other averted costs include indirect costs (e.g., lost productivity because of disease) or intangible costs (e.g., patient pain and suffering). However, the indirect and intangible costs of sexually transmitted diseases are inadequately researched.

When calculating costs and benefits of screening, programs should define the perspective of interest (e.g., a specific provider or practice, the sexually transmitted disease [STD] program of a state health department, a commercial laboratory, a managed care organization, the entire health-care system, or society). The perspective determines which costs and benefits are included in the calculations. For example, a societal perspective CEA includes all costs and benefits associated with a program, whereas a health-care-system perspective CEA includes direct medical costs but excludes costs borne solely by patients (e.g., transportation and lost productivity). Published CEAs usually use one of these two perspectives.¶

An interactive computer program named SOCRATES (Screening Optimally for Chlamydia: Resource Allocation, Testing, and Evaluation Software) is now available at the CDC website to aid in test selection and methods to reduce costs for screening for *C. trachomatis* infection among asymptomatic women. SOCRATES is a simple cost-effectiveness model that uses a broad health-care-system perspective to analyze the costs involved in using nucleic acid amplification, nucleic acid hybridization, and enzyme immunoassay tests, singly or with gray-zone testing, among patient populations with positivity rates that can be set and changed by the user. Costs of testing and treatment can also be set and changed by the user. This program is available at <http://www.cdc.gov/nchstp/dstd/HEDIS.htm>.

**Role of Economic Analysis**

Economic analysis should rarely be the driving force for test selection and screening strategies, but it should be considered with other factors (e.g., test performance, ease of specimen collection, laboratory capacity, population prevalence, and disease prevention goals of the screening program). Certain testing approaches that are recommended in these guidelines (see Methods To Enhance Performance or Reduce Costs) are difficult to assess by using CEA, because such concerns as the repercussions of delivering a false-positive test result to a patient are difficult to value in monetary terms.

\* **Sources:** Haddix AC, Teutsch SM, Shaffer PA, Duñet DO. Prevention effectiveness: a guide to decision analysis and economic evaluation. New York, NY: Oxford University Press, 1996. Gold MR, Siegel JE, Russell LB, Weinstein MC. Cost-effectiveness in health and medicine. New York, NY: Oxford University Press, 1996.

† Other common economic analyses include cost-utility and cost-benefit. Cost-utility analysis converts cost-effectiveness outcomes to quality-adjusted life years (QALYs) or disability-adjusted life years (DALYs). Cost-utility analyses are rare in sexually transmitted diseases (STD) because the QALY or DALY impacts of STD and their sequelae are unresearched. Cost-benefit analysis converts the outcomes achieved by a program to dollar values so that the effects of different programs (e.g., STD screening and environmental health) can be directly compared (**Source:** Haddix AC, Teutsch SM, Shaffer PA, Duñet DO. Prevention effectiveness: a guide to decision analysis and economic evaluation. New York, NY: Oxford University Press, 1996).

§ For example, if a program incurs \$1,000 for screening and treating women for *C. trachomatis* and, therefore, avoids \$2,000 in treatment costs for PID, the program is cost-saving. However, the program might still be cost-effective even if the program does not avert enough sequelae costs to be cost-saving. A program that has a net cost (program costs minus averted sequelae costs) of \$500/case of PID averted might be considered cost-effective, compared with the first program, although it is more expensive, if it prevented more cases. The determination of whether such a program is cost-effective requires that a judgement be made regarding the value of the health outcomes in question. No universally applicable rules exist.

¶ The choice of perspective can influence the conclusions of a cost-effectiveness analysis. In the previous example, done from a health-care-system perspective, program costs were \$1,000/woman, and averted PID costs were \$2,000, making the program cost-saving. Certain PID costs (e.g., those for infertility treatments) routinely occur after the initial infection, and the program conducting the testing might never incur them. The program doing the testing might only reduce its costs for sequelae by \$800 (for treatment of acute PID) for every \$1,000 spent on screening. From the perspective of the individual program, screening might not be cost-saving. A societal perspective provides the truest indication of the cost-effectiveness of a program, but a more limited perspective can point to the impact on a given provider or program, including the barriers to implementation of new tests or screening programs.

#### BOX 5. Additional testing and patient management after a positive screening test

- All positive screening tests should be considered presumptive evidence of infection.
- An additional test should be considered after a positive screening test if a false-positive screening test would result in substantial adverse medical, social, or psychological impact for a patient.
- Consideration should be given to routinely performing an additional test after a positive screening test if the positive predictive value is considered low (e.g., <90%).
- Patients should be counseled regarding prompt treatment after a positive screening test because an additional test might be falsely negative.

intangible. These include psychosocial costs that can result from a false-positive test result. These costs vary according to setting and are not well-researched. The benefits of reducing these intangible costs must be weighed against the tangible direct costs of performing the confirmatory tests. Fortunately, these costs apply only to patients who initially test positive and frequently constitute only a limited proportion of total costs per positive test, particularly in low prevalence settings where the additional testing is typically needed (see Methods To Enhance Performance or Reduce Costs).

#### Consideration of Point-of-Care Testing

Point-of-care tests for *C. trachomatis* screening are less sensitive than laboratory-based tests but should be considered in situations where screening-test-positive persons might fail to return for treatment or return after substantial delays. Point-of-care tests are not a cost-effective option if they are processed after the patient visit because they are relatively insensitive and require labor-intensive processing. Each health-care provider needs to compare the sensitivities, costs, and treatment rates for point-of-care and laboratory-based tests. Providers need to determine whether the opportunity to provide treatment to certain patients who would otherwise go untreated warrants the additional cost and less favorable sensitivity of point-of-care testing. FDA-cleared *C. trachomatis* and *N. gonorrhoeae* tests that can be performed rapidly enough to qualify as point-of-care tests must be performed in a CLIA-certified laboratory because they are classified under CLIA as moderate complexity tests (24).

#### Methods To Enhance Performance or Reduce Costs

Different approaches have been used to increase the efficiency of standard screening methods. Although selective screening is not a laboratory method, using selective screening criteria is included in the following discussion because the predictive values and cost to detect an infection are strongly influenced by infection prevalence. Another approach is to use a NAAT to test specimens that yield results from an EIA or unamplified nucleic acid probe test that fall in a zone around the cutoff (i.e., gray zone). This technique warrants further evaluation as a method to decrease the gap in sensitivity between NAATs and other tests without incurring the full additional cost of testing all specimens with a NAAT. Interest in pooling specimens for testing by a NAAT is similarly motivated. Augmenting screening tests with additional testing to improve test specificity is of increasing importance because *C. trachomatis* and *N. gonorrhoeae* prevalences have declined after the introduction of screening programs and because *C. trachomatis* screening has expanded into lower prevalence populations. Using test formats (e.g., nucleic acid probe tests or NAATs) that permit testing for both *C. trachomatis* and *N. gonorrhoeae* might reduce costs. Finally, the urine leukocyte esterase test, which has a low sensitivity but is inexpensive, has been used to select specimens for testing with a specific *C. trachomatis* or *N. gonorrhoeae* test when use of a more sensitive initial test was not feasible.

#### Selective Screening To Increase the Percentage of Positive Tests

Selecting persons for testing who are at high risk can increase the prevalence of infection among tested persons, thereby reducing screening costs to detect persons with *C. trachomatis* infection. Symptoms or signs are not included as screening criteria because they warrant immediate diagnostic testing. Evaluations of screening algorithms for *C. trachomatis*, including an algorithm recommended by CDC in 1993 (31), have been published (96–104). The third U.S. Preventive Services Task Force (USPSTF) reviewed this information and published *C. trachomatis* screening recommendations (105) (available at <http://www.ahrq.gov/clinic/uspstfix.htm>) (Appendix E). USPSTF also concluded that satisfactory urine screening tests for men had been introduced too recently for sufficient evidence to have been developed to make screening recommendations. Using criteria to select women or men to screen for *N. gonorrhoeae* infection has received only limited evaluation, although the prevalence of *N. gonorrhoeae* is usually lower than the prevalence of *C. trachomatis*. Thus, concerns regarding the cost to detect an



infection and PPV might be greater for *N. gonorrhoeae* than for *C. trachomatis* (106).

### Gray-Zone Testing To Improve Test Performance

To establish the positive-negative cutoff value for a diagnostic test, a manufacturer attempts to achieve the highest sensitivity while keeping the specificity at or near 100%. This is done by setting the cutoff at the lowest level possible while minimizing false-positive results. Certain test readings from truly infected persons will fall below this cutoff value (false-negatives). Often, a substantial proportion of the readings for persons with false-negative results cluster just below the cutoff, whereas the readings for persons with truly negative results are clustered farther below the cutoff. Consequently, a relatively narrow zone below the cutoff might exist within which the proportion of readings that are from truly infected persons is substantially higher than the proportion of readings farther below the cutoff. The ratio of true positives to true negatives in this zone is affected by the prevalence of infection as well as by test performance among the tested population. Similarly, false-positive results are clustered just above the cutoff, whereas the readings for persons with truly positive results are clustered farther above the cutoff. A relatively narrow zone above the cutoff might exist within which the proportion of readings that are from truly uninfected persons is substantially higher than the proportion of readings farther above the cutoff. When screening test results are in these zones, commonly called the negative and positive gray zones, respectively, a laboratory has the option of retesting specimens with another test.

By using a NAAT to retest specimens with gray-zone results from less sensitive and less expensive screening tests, the sensitivity and specificity of the screening test might be improved at less cost than switching entirely to the NAAT. However, such a restricted use of a NAAT might be less sensitive than testing the entire population with a NAAT. A NAAT is also theoretically preferable to a competition or blocking antibody assay for additional testing of specimens from persons with positive non-NAAT screening tests (see Additional Testing To Improve Test Specificity). Testing strategies that combine use of a NAAT by itself in higher prevalence populations and as an additional test after gray-zone non-NAAT results among lower prevalence populations warrant further evaluation. Laboratories should establish feasibility, cost-effectiveness, and gray-zone limits before implementation of the strategy.

Although gray-zone testing with a NAAT has been evaluated in multiple studies (107–113), these studies have not employed independent reference standards. In certain cases, the second test was performed on the specimen that was tested

by the screening test. If the second test has not been cleared by FDA for use with that type of specimen, CLIA requires that the laboratory conduct a study to establish the performance of the second test when conducted on the screening test specimen (Appendix C). This regulatory requirement is critical because NAATs might generate more false-positive and false-negative results when performed on specimens collected for tests designed with less stringent protection against contamination and amplification enzyme inhibitors. Gray-zone testing must be evaluated in appropriately designed research studies by using an independent reference standard before a recommendation is made regarding its utility in routine practice.

### Pooling Specimens To Reduce Costs

Because of the high sensitivity of NAATs, pooling specimens before testing for *C. trachomatis* and *N. gonorrhoeae* with LCR and PCR has been proposed as a method of reducing costs (114–120). 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 at lower prevalences without increasing the probability of a pool testing positive. Available evidence indicates that pooling might be a cost-effective alternative to testing individual specimens with minimal if any loss of sensitivity or specificity (114–120). However, insufficient data exist from published, peer-reviewed studies that address the implementation or performance concerns raised by pooling to make a recommendation concerning testing of pooled specimens.

Theoretically, pooling could either increase or decrease the sensitivity and specificity of a NAAT compared with processing individual specimens. To be reported as positive, pooled specimens must be positive according to the pooled result and according to the individual test. This requirement should contribute an increase in specificity and a decrease in sensitivity compared with individual testing. In addition, pooling could reduce NAAT sensitivity because the majority of pooling protocols result in a reduced amount of an individual specimen being tested. Moreover, pooling can permit inhibitors present in a single specimen to cause specimens that would have been truly positive when tested individually to be falsely negative when tested in a pool. Such inhibition will go undetected if an internal control for inhibition is not included when testing pooled specimens. Conversely, dilution of specimens by pooling can decrease the effect of inhibitors. Pooling adds testing and data-recording steps that can result in errors and report-

ing of additional false-positive or false-negative results. In addition, pooling results in a higher number of specimens in a run being positive. This increases the chances for suboptimal technique (e.g., by pipetting) to result in cross-contamination and false-positive results.

Published studies of pooling using PCR and LCR tests for *C. trachomatis* and *N. gonorrhoeae* are still limited, and the majority of such studies have been conducted without an independent reference standard. Nevertheless, published studies indicate that pooling 4–10 urine or endocervical specimens before testing can substantially reduce costs and improve throughput while maintaining the high performance characteristics associated with NAATs (114–120). Savings from reduced reagent costs have ranged from 40% to 60%. Because of the increased complexity of the pooling protocol, savings in personnel time are proportionately less than the savings in reagent costs.

In addition to these performance concerns, the following concerns should be addressed when considering use of pooling:

- what procedures to follow when all the individual specimens from a positive pool provide negative results (e.g., test for inhibition);
- use of internal amplification controls to detect inhibition;
- the need to inform the provider or patient that specimens are being tested by using a pooling protocol;
- how to report test results; and
- reimbursement by third-party payers.

Because pooling is a modification of FDA-cleared procedures, laboratories that implement pooling of patient specimens must satisfy CLIA requirements by establishing performance specifications (Appendix C).

### Additional Testing To Improve Test Specificity

An additional test might be indicated for a person with a positive screening test result, if a false-positive result would have a serious adverse consequence (Box 5). Because treatments for *C. trachomatis* and *N. gonorrhoeae* are safe and relatively inexpensive, the person might wish to receive and complete treatment while additional testing is being done, or even if the additional test is negative. Routine additional testing to improve the predictive value of a positive screening test should be considered when the prevalence of either *C. trachomatis* or *N. gonorrhoeae* infection is low, resulting in a low PPV (e.g., <90%) (see *C. trachomatis* and *N. gonorrhoeae* Test Performance When Used for Screening and also, Additional Considerations in Selecting a Screening Test).

False-positive results might occur for multiple reasons, including the following:

- the nucleic acid target, antigen, or phenotype (e.g., colistin resistance, oxidase production) detected by the test is possessed by organisms in the specimen other than *C. trachomatis* or *N. gonorrhoeae*;
- the detection system generates a signal in the absence of target;
- the specimen becomes contaminated; and
- staff make data entry errors.

Approaches to detect false-positive results by applying an additional test can be ordered by preference on the basis of theoretical considerations (Box 6).

Theoretically, testing a second specimen with a different type of test is least likely to confirm a false-positive result, whereas repeating the original test on the original specimen is most likely to do so. Using a NAAT as an additional test after a positive non-NAAT test might be an effective and highly economical approach that deserves additional evaluation. However, except when culture is used to obtain an isolate, a non-NAAT should not be used as an additional test after a NAAT because of the lower sensitivity of non-NAATs. Such additional testing could be extended to persons with non-NAAT screening test results in the gray zone (see Gray-Zone Testing To Improve Test Performance). An advantage of isolating the organism by culture is flexibility in the choice of multiple additional testing procedures, and compared with NAATs, a reduced risk of error caused by contamination during initial or subsequent testing. Using an FDA-cleared test as an additional test to verify the positive results of a screening test does not by itself introduce additional requirements under CLIA (24). However, if the additional test is performed on the original screening test specimen and the additional test has not been cleared by FDA for use with that type of specimen, CLIA requirements for establishing performance characteristics (24) need to be satisfied (Appendix C).

**Additional Testing After a Positive *C. trachomatis* Screening Test.** Selection of an additional test to verify a positive *C. trachomatis* screening test is straightforward when a second

#### BOX 6. Approaches to additional testing, in order by theoretical consideration, after a positive screening test

- Test a second specimen with a different test that uses a different target, antigen, or phenotype and a different format.
- Test the original specimen with a different test that uses a different target, antigen, or phenotype and a different format.
- Repeat the original test on the original specimen with a blocking antibody or competitive probe.
- Repeat the original test on the original specimen.

specimen can be collected, as when a patient returns for screening test results or when the need for additional testing can be anticipated when the screening specimen is collected (Box 7). Using culture with a *C. trachomatis*-specific anti-MOMP stain as the additional test has the advantage of high specificity and flexibility in choice of additional testing, if warranted. However, culture lacks sensitivity and necessitates obtaining and maintaining a suitable sample either at the initial or a subsequent visit. This fact has made culture less desirable. In addition, finding a laboratory to perform culture can be problematic. If obtaining a separate specimen for additional testing is difficult, the additional test must perform adequately with the transport medium used for the screening test.

Using a blocking antibody format to verify a positive EIA screening test and using a competitive probe format to verify a positive nucleic acid probe screening test have been the additional tests most widely used. The blocking antibody or competitive probe additional test is usually performed on the original screening specimen. These formats have been widely used for additional testing, despite not being the theoretically preferred approaches. This is because approaches preferred on theoretical grounds introduce the logistical and economic challenges of establishing a format for the additional test that differs from that of the screening test. Collecting a separate

**BOX 7. Additional testing after a positive *Chlamydia trachomatis* screening test**

**Positive Nonnucleic Acid Amplification Tests (Non-NAAT)**

- Culture with a *C. trachomatis*-specific anti-MOMP (major outer membrane protein) stain can be used after a positive non-NAAT because of the high specificity and the flexibility for additional testing, but culture poses increased difficulties in specimen transport and storage.
- Competitive probe and blocking antibody formats can be used after positive nucleic acid probe tests and enzyme immunoassays, respectively, but this approach is less likely, theoretically, to detect a false-positive result.
- A NAAT has high potential as an additional test after non-NAAT tests because of the increased sensitivity; however, this use of NAATs has received limited evaluation.

**Positive NAAT**

- Only another NAAT has a sufficiently high sensitivity to serve as an additional test after a positive NAAT; however, such an approach to additional testing has received limited evaluation.

specimen for the additional test also presents logistical difficulties and increases cost. Studies have not been conducted to compare application of such different approaches.

Because of the greater sensitivity of NAATs, a NAAT is the only recommended additional test to verify a result from another NAAT and is, potentially, a superior additional test to verify a non-NAAT positive *C. trachomatis* screening test. Except for using culture to obtain an isolate, a non-NAAT should not be used as an additional test after a NAAT because of the lower sensitivity of the non-NAAT. Using a NAAT as an additional test has received limited evaluation. In particular, determining that NAAT specificity and sensitivity are not reduced is critical if a NAAT is performed on specimens collected, transported, and possibly processed by using non-NAAT procedures that might be less stringent with respect to preventing contamination and inhibition of amplification enzymes.

Manufacturers of NAATs for *C. trachomatis* infection have developed alternate target (e.g., MOMP targets) versions of their commercial tests that the manufacturers have employed for resolving discrepant results in evaluation studies. Using the same format for the additional test as for the screening test offers logistical and economic advantages analogous to those offered by the blocking antibody and competitive probe approaches for non-NAAT tests. Such assays are not offered commercially by NAAT manufacturers but might be in the future.

**Additional Testing After a Positive *N. gonorrhoeae* Screening Test.** Methods to confirm that Gram-negative, oxidase-positive, bacteria isolated on a selective culture medium are *N. gonorrhoeae* have been reviewed (Box 8) (12,32). Acid production from carbohydrates and the nucleic acid probe culture confirmation test (AccuProbe,<sup>®</sup> manufactured by Gen-Probe, San Diego, California) are the most sensitive and specific methods. PACE 2 is also FDA-cleared for identifying *N. gonorrhoeae*.

Pure growth on subculture is required for acid production tests, but not for the AccuProbe test. AccuProbe is not known to cross-react with other organisms found in humans; however, variant strains of certain pathogenic and commensal *Neisseria* species can provide false-positive acid production results, and certain strains of *N. gonorrhoeae* might provide false-negative acid production results. Additional tests will be required to differentiate between species that might produce acid from glucose but not from maltose, sucrose, or lactose (Appendix F) or to identify variant gonococcal isolates that fail to produce acid from glucose.

Although nongonococcal pathogenic and commensal *Neisseria* species are commonly found in the oropharynx, they are unusual in genitourinary specimens. Either the acid production or the nucleic acid probe methods could be used for

### BOX 8. Additional testing after a positive *Neisseria gonorrhoeae* screening test

#### Presumptively Positive Culture

- Acid production from carbohydrates or the Gen-Probe AccuProbe<sup>®</sup> or PACE 2<sup>®</sup> tests are the preferred methods to confirm that typical, Gram-negative, oxidase-positive colonies are *N. gonorrhoeae*.
- Requiring that both the acid production and nucleic acid probe methods be positive for *N. gonorrhoeae* ensures a high specificity.

#### Positive Nonculture Test

- Culture with confirmation as described previously is the preferred additional test after a positive nonculture test if specimen transport and storage conditions are suitable.
- A competitive probe format might be used after a positive nucleic acid probe test, but this approach is less likely, theoretically, to detect a false-positive result.
- A NAAT as an additional test after a nonculture test has received limited evaluation, and certain NAATs might cross-react with nongonococcal *Neisseria* species.\*

\* **Sources:** 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. 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. Becton Dickinson. BDProbeTec<sup>™</sup> ET *Chlamydia trachomatis* and *Neisseria gonorrhoeae* amplified DNA assays [Package insert]. Sparks, MD: Becton Dickinson, 1999.

routine additional testing after presumptively positive screening cultures. Requiring that both types of tests be positive should guarantee an increased level of specificity. A detailed discussion of problems in differentiating *N. gonorrhoeae* from other *Neisseria* and related species is located at <http://www.cdc.gov/ncidod/dastlr/gcdir/NeIdent/Index.html>. If an isolate cannot be conclusively identified as *N. gonorrhoeae* at a local laboratory, the isolate should be sent to a reference laboratory (e.g., a city, county, or state public health laboratory) for confirmation, chiefly in cases of alleged sexual assault or rape.

Antibody tests (i.e., tests that detect gonococcal antigens) are not recommended for detection of *N. gonorrhoeae*. Coagglutination tests, which might cross-react with nongonococcal strains, are less sensitive or specific than acid production tests or the AccuProbe or PACE 2 tests. The monoclonal fluorescent antibody test for *N. gonorrhoeae* does not detect all gonococcal isolates and is not recommended as the primary test for detecting *N. gonorrhoeae*.

Additional testing after positive nonculture screening tests for *N. gonorrhoeae* involves the same approaches that are described in the previous section for *C. trachomatis*. Because verification of positive nonculture tests for *N. gonorrhoeae* has received only limited evaluation and certain NAATs might cross-react with nongonococcal *Neisseria* species (54,56,57), substantial effort is warranted to arrange for culture and the assistance of a reference laboratory if a false-positive diagnosis of *N. gonorrhoeae* would have serious adverse consequences, as in testing of children.

**Reporting Test Results.** When additional testing has been performed, the laboratory should report the results of both the screening test and the additional tests, as well as the overall interpretation. The laboratory has the responsibility to educate clinicians regarding the importance of all laboratory results, including both screening and additional test results. In particular, clinicians need to be aware of the limitations of the additional tests, including the possibility that they yield false-negative results when the screening test is positive. Because serious side effects from therapies for *C. trachomatis* and *N. gonorrhoeae* are uncommon, clinicians might recommend treatment after a positive screening test for a person at risk for infection, pending additional testing or even when a positive screening test is not verified by additional testing.

#### Cotesting for *C. trachomatis* and *N. gonorrhoeae* To Reduce Costs

Multiple tests permit testing for both organisms by using the same specimen. The prevalence of *N. gonorrhoeae* is less than *C. trachomatis* in the majority of areas of the United States; however, the prevalence of each varies widely, even within such limited areas as cities or counties. Usually, screening for *N. gonorrhoeae* will not be justified unless screening for *C. trachomatis* is also warranted. Decisions regarding screening for either or both organisms should not be made without a careful evaluation of the local epidemiology of *N. gonorrhoeae* and *C. trachomatis*.

Cotesting for *C. trachomatis* and *N. gonorrhoeae* by using tests specially designed for such cotesting should be considered, if transport conditions would reduce the sensitivity of *N. gonorrhoeae* culture or if using such tests reduces the cost. However, provision should be made to perform an additional test to improve test specificity whenever indicated (see Additional Testing To Improve Test Specificity) and to obtain isolates for antimicrobial susceptibility testing in the case of a repeated treatment failure (see Test of Cure, Treatment Failure, and Antimicrobial Resistance).

## Using LET to Select for *C. trachomatis* or *N. gonorrhoeae* Tests

LET, which has a low sensitivity but is inexpensive, has been used to select specimens for testing with a specific *C. trachomatis* or *N. gonorrhoeae* test when universal testing with a more sensitive initial test is not feasible. Studies have demonstrated that using a positive LET to select patients for screening with a more sensitive and specific test can be cost-effective. Published studies have focused primarily on asymptomatic men and have reported that this approach is more cost-effective when the prevalence is low (89,91,121–124). One study (121) determined that the negative predictive value of LET among asymptomatic men aged  $\geq 30$  exceeded 98%, thereby providing support for no additional *C. trachomatis* testing among that population. Among men aged  $< 30$  years, whether a negative result is a valid basis on which to exclude that population from testing with more sensitive tests is uncertain. Because the test does not detect either *C. trachomatis* or *N. gonorrhoeae*, but rather nonspecific inflammatory enzymes, a positive LET should be followed by specific tests for *C. trachomatis* and *N. gonorrhoeae*. The importance of a positive LET that is followed by negative tests for *C. trachomatis* and *N. gonorrhoeae* is unknown, but could indicate infection with other organisms (e.g., *Trichomonas vaginalis*, *Ureaplasma urealyticum*, or *Mycoplasma genitalium*).

## Collecting, Transporting, and Storing Specimens

Correct specimen collection and handling techniques are critical for all methods used to identify *C. trachomatis* and *N. gonorrhoeae*. Even diagnostic tests with the highest performance characteristics cannot produce accurate results when specimens submitted to the laboratory are incorrectly collected. Recommendations for transporting and storing specimens are summarized in this report (Appendix D).

Clinicians require training and periodic assessment to maintain correct technique for specimen collection (125–127). The presence of columnar epithelial cells has been associated with increased sensitivity in the majority of studies that evaluated collection of endocervical specimens for *C. trachomatis* screening with NAATs as well as other types of tests (125–132). How these results apply to other types of specimens is unclear. Obtaining columnar cells is less critical for detecting *N. gonorrhoeae* than for detecting *C. trachomatis*.

## Collecting and Transporting Specimens for Screening

### Specimen Collection Recommendations Applicable to Culture and Nonculture Tests

Although the requirement for columnar endocervical cells applies less to *N. gonorrhoeae* than to *C. trachomatis*, guidelines included in this report are appropriate for both organisms (Box 9).

### Specific Requirements for *C. trachomatis* Culture

Swabs with plastic or wire shafts can be used to obtain specimens for cell culture (133–135). Swab tips can be made of cotton, rayon, or Dacron,<sup>®</sup> but should not be made of calcium alginate (133–136). Swabs with wooden shafts should not be used because the wood might contain substances that are toxic to *C. trachomatis* or tissue culture cells (133–135). As part of routine quality control, samples of each lot of swabs that are used to collect specimens for *C. trachomatis* isolation should be screened for possible inhibition of *C. trachomatis* growth and toxicity to tissue culture cells.

The substitution of an endocervical brush for a swab might increase the sensitivity of culture for endocervical specimens from nonpregnant women (137). However, using an endocervical brush might induce bleeding. Although such bleeding does not interfere with the isolation of *C. trachomatis*, patients should be advised regarding possible spotting.

When culture isolation of *C. trachomatis* from women is to be performed, processing a specimen from the urethra as well as the endocervix can increase sensitivity by 23% (138). Placing the two specimens in the same transport container is acceptable. The viability of *C. trachomatis* organisms must be maintained during transport to the laboratory (28).

- When the elapsed time between specimen collection and inoculation is  $\leq 24$  hours, specimens should be stored at 4°C and inoculated in cell culture as quickly as possible.
- When specimens cannot be inoculated in  $\leq 24$  hours, the specimens should be maintained at  $\leq -70^\circ\text{C}$ .
- Specimens for culture should never be stored at  $-20^\circ\text{C}$  or in frost-free freezers.

### Specific Requirements for *N. gonorrhoeae* Culture

Swabs with plastic or wire shafts can be used to obtain specimens for culture. Swab tips can be made of rayon, Dacron, or calcium alginate. Obtaining a second cervical specimen for *N. gonorrhoeae* culture is associated with an increase in sensitivity (139). Inoculating the two specimens on the same culture plate is acceptable.

**BOX 9. Specimen-collection guidelines****Endocervical Specimens**

- Nonculture specimens should be obtained as directed by the test manufacturer in the package insert.
- By established practice, specimens for *C. trachomatis* tests are obtained after specimens for Gram-stained smear or *N. gonorrhoeae* culture. When a Papanicolaou smear is to be collected, whether specimens for *C. trachomatis* or *N. gonorrhoeae* should be collected first or last is unknown. Bleeding can occur when a Papanicolaou smear is obtained first, and gross blood interferes with certain tests for *C. trachomatis* and *N. gonorrhoeae*.
- Before obtaining a specimen, a sponge or large swab should be used to remove all secretions and discharge from the cervical os.
- For nonculture tests, the swab supplied or specified by the test manufacturer should be used.
- The appropriate swab or endocervical brush should be inserted 1–2 cm into the endocervical canal (i.e., past the squamocolumnar junction). The swab should be rotated against the wall of the endocervical canal  $\geq 2$  times or for the period of time recommended by the manufacturer. The swab should be withdrawn without touching any vaginal surfaces and placed in the appropriate transport medium.

**Urethral Specimens**

- Specimens should be obtained as directed by the test manufacturer in the package insert.
- If possible, obtaining specimens should be delayed until  $\geq 1$  hour after the patient has voided.
- Specimens should be obtained for *C. trachomatis* tests after obtaining specimens for a Gram-stained smear or *N. gonorrhoeae* culture.
- For nonculture tests, the swab supplied or specified by the manufacturer should be used.
- The urogenital swab should be inserted gently into the urethra (females, 1–2 cm; males, 2–4 cm). The swab should be rotated in one direction for  $\geq 1$  revolutions and withdrawn. For males or females with urethral discharge, exudate collected from the urethral meatus is sufficient for *N. gonorrhoeae* culture. An intraurethral specimen is required for *C. trachomatis* testing, regardless of the presence of exudate at the meatus.

**Urine Specimens**

- Specimens should be obtained as directed by the test manufacturer in the package insert.
- If possible, specimen collection should be delayed until  $\geq 1$  hour after the patient has voided.\*
- First-catch urine (e.g., the first 10–30 cc voided after initiating the stream) should be used.

The viability of *N. gonorrhoeae* organisms must be maintained during transport to the laboratory; therefore, the following recommendations are made for transporting specimens:

- Specimens should be inoculated directly onto a selective or nonselective (if the specimen is from a sterile site) nutritive medium (e.g., Martin-Lewis or chocolate agar containing IsoVitaleX,<sup>®</sup> respectively).
- Specimens should be incubated at 35°C–36.5°C in a CO<sub>2</sub>-enriched atmosphere immediately after collection.
- For transport to a local laboratory, placement of the inoculated plates in a CO<sub>2</sub>-enriched atmosphere is more critical than incubation at 35°C–36.5°C (140–142). Inoculated media can be held at room temperature in a CO<sub>2</sub>-enriched atmosphere for  $\leq 5$  hours without a loss of viability.
- For transport to a remote laboratory, specimens should be inoculated onto commercial transport media (e.g., JEMBEC<sup>®</sup> or Transgrow media). Inoculated media should be incubated for 18–24 hours before being transported, and the specimen should arrive  $\leq 48$  hours after collection from the patient. Specimens should not be transported in Stuart's, Amies', or other such media; specimens are diluted and organisms might lose viability if delays in shipping occur. If specimens must be transported in extreme hot or cold conditions, the specimens should be placed in an insulated Styrofoam<sup>®</sup> container. Detailed directions for collection of specimens for culture of *N. gonorrhoeae* have been described elsewhere (12,143,144).

**Adequacy of Endocervical Specimens**

Without endocervical specimen quality assurance,  $\geq 10\%$  of specimens collected for *C. trachomatis* testing are probably unsatisfactory because they contain secretions or exudate, but lack endocervical cells (126,128,145). A substantially reduced likelihood exists of detecting *C. trachomatis* in inadequate specimens by all tests, including NAATs (125–132).

**Assessing Endocervical Specimen Quality.** Health-care providers and laboratorians are encouraged to evaluate the effect on endocervical *C. trachomatis* test positivity rates of monitoring the endocervical columnar cell content of specimens and then providing feedback and, as indicated, training to clinicians. Until proven unnecessary, routine or periodic assessment of endocervical specimen quality is recommended for all types of *C. trachomatis* tests. A report presenting alternative methods of measuring specimen quality has been developed by the National Chlamydia Laboratory Committee for the CDC-supported *C. trachomatis* screening program and distributed to constituent screening programs (additional information is available at [http://www.aphl.org/chlamydia\\_lab.cfm](http://www.aphl.org/chlamydia_lab.cfm)). The majority of published studies have

\* Source: Sellors J, Chernesky M, Pickard L, et al. Effect of time elapsed since previous voiding on the detection of *Chlamydia trachomatis* antigens in urine. *Eur J Clin Microbiol Infect Dis* 1993;12:285–9.

categorized specimens dichotomously as either containing the appropriate endocervical cells or not. In one study, a dose-response association between quantity of the appropriate endocervical cells and the *C. trachomatis* positivity rate was demonstrated by using a multiple-category scale for quantity of appropriate cells (132). This study also prescribed a systematic process for reviewing slides. Diff-Quick stain has been described as efficient and inexpensive (125).

The principal purpose of assessing the quality of endocervical specimens is to determine whether the sensitivity of *C. trachomatis* tests would be enhanced by providing feedback and training to clinicians. No evaluations have been published of the cost-effectiveness of alternative approaches to measuring the quality of endocervical cell specimens and improving specimen-collection practices, and no studies have correlated measures of specimen adequacy with test positivity for specimens collected for *N. gonorrhoeae* testing.

**Reporting and Follow-Up of Inadequate Specimens.** An inadequate specimen should be reported as inadequate. Whenever possible, a second specimen should be collected for repeat testing.

## Collecting Specimens for Indications Other Than Screening

Anatomic site-specific recommendations for application of *C. trachomatis* and *N. gonorrhoeae* tests, including for medicolegal applications, are summarized in this report (Appendices A and B). Multiple sources provide directions for collecting specimens for *N. gonorrhoeae* testing (12,143,144).

## Laboratory Implementation of NAATS

NAATs require more attention to procedural details and development and maintenance of quality control systems than other nonculture screening tests (e.g., EIAs and nonamplified nucleic acid hybridization assays). An increase in such requirements results from the susceptibility of NAAT amplification enzymes to inhibition and the potential of NAATs to generate cross-contaminating target and to detect limited quantities of target present as a contaminant. Despite close attention to quality control, concerns regarding consistency of test performance and reproducibility persist, as indicated by reports of heterogeneity of results in clinical trials (78,80) and varying rates of reproducing positive results (146–151).

As laboratories and health-care providers transition to amplification tests, certain critical concerns should be addressed, including

- clinician training whenever a change occurs in the testing method. Training should address
  - indications for test use (e.g., appropriate types of specimens);
  - general instruction in obtaining adequate specimens from any site and specific instruction in obtaining a proper endocervical specimen (i.e., one that contains endocervical cells rather than ectocervical cells or vaginal material);
  - requirements for storage and transport; and
  - interpretation of test results.
- monitoring of specimen collection and transport and periodic reinforcement of staff training.
- development of standard laboratory operating procedures and quality-assurance protocols based on package inserts and any supplementary manufacturer instructions. Procedures and protocols should address
  - adoption of prescribed work areas and specimen handling procedures to avoid cross-contamination, which is of heightened importance because of the inherent sensitivity of amplification tests;
  - use of positive and negative controls, including a positive control from culture stock or known positive clinical specimens in addition to the control provided in the commercial kit;
  - trade-offs of using amplification controls to identify inhibitors (e.g., reducing false-negative results but decreasing throughput); and
  - creation of a data system that alerts laboratorians when a run includes an unusual number of positive specimens or when positive specimens are clustered within a run.
- manufacturer-based training of laboratory staff with periodic retraining.
- CLIA requirements for verifying or establishing test performance characteristics. If a laboratory is adopting an FDA-cleared test that is classified under CLIA as a high-complexity test, CLIA requires conducting a study to verify that the test performs according to the manufacturer's package insert claims (24). If the laboratory is adopting a test that has not been cleared by FDA or is adopting a modification of an FDA-cleared test, CLIA requires a more extensive study to establish performance specifications, because FDA-cleared package insert specifications are lacking (24) (Appendix C).
- participation in a proficiency testing program.

## Test of Cure, Treatment Failure, and Antimicrobial Resistance

Test-of-cure is not recommended as a routine procedure after therapy for *C. trachomatis* or *N. gonorrhoeae* infection with first-line CDC-recommended treatment regimens, except after *C. trachomatis* therapy during pregnancy (152). Nonculture tests that are performed <3 weeks after completion of antimicrobial therapy might be falsely positive because of the presence of nonviable organisms; this applies in particular to NAATs (153–160).

CDC recommends that clinicians contact their local or state health department for guidance and to arrange for antimicrobial susceptibility testing of isolates from patients apparently failing CDC-recommended therapy for *C. trachomatis* infection or CDC-recommended or FDA-approved therapy for *N. gonorrhoeae* infection. For this purpose, a patient's infection is considered to have failed therapy if the patient is laboratory-test-positive for *C. trachomatis* or *N. gonorrhoeae* after treatment and the patient provides a history of having complied with the prescribed therapy and denies posttreatment sexual exposure to an untreated or new sex partner.

Because knowledge is limited regarding the ability of *C. trachomatis* to develop antimicrobial resistance, CDC encourages health departments to inform CDC of treatment failures and, if possible, arrange for shipment of a swab specimen suitable for tissue culture for test confirmation. To report apparent *C. trachomatis* treatment failure, contact the following:

Surveillance and Special Studies Section, Mail Stop E-02  
Epidemiology and Surveillance Branch  
Division of STD Prevention  
Centers for Disease Control and Prevention  
1600 Clifton Rd., N.E.  
Atlanta, GA 30333  
Phone: 404-639-8371  
Attention: Susan Wang, M.D.  
or Hillard Weinstock, M.D.

To submit specimens for *C. trachomatis* testing, contact the following:

Chlamydia Laboratory  
Syphilis and Chlamydia Branch, Mail Stop D-13  
Centers for Disease Control and Prevention  
1600 Clifton Rd., NE  
Atlanta, GA 30333  
Phone: 404-639-3785  
Attention: John Papp, Ph.D.

CDC encourages health departments to inform CDC of *N. gonorrhoeae* treatment failures and, if possible, arrange for *N. gonorrhoeae* culture and testing of any isolate for suscepti-

bility to CDC-recommended regimens used to treat patients. If susceptibility testing cannot be performed at a local or state laboratory, isolates should be submitted to CDC as should any isolate that is resistant to a CDC-recommended therapy. Antimicrobial susceptibility testing should be performed according to recommendations of the National Committee for Clinical Laboratory Standards (NCCLS), and results should be interpreted according to criteria designated by NCCLS. If NCCLS has not provided criteria for resistance for a CDC-recommended therapy (e.g., cephalosporins), an isolate is considered to be resistant if the isolate fails to meet the NCCLS criteria for susceptibility. NCCLS might not have designated criteria for definition of a susceptible category for antimicrobial agents that are used for gonorrhea treatment. When no NCCLS criterion is available, consult <http://www.cdc.gov/ncidod/dastlr/gcdir/Resist/diskdif.html> for additional information or contact the Neisseria Reference Laboratory for further guidance. To report apparent *N. gonorrhoeae* treatment failure, contact the following:

Surveillance and Special Studies Section, Mail Stop E-02  
Epidemiology and Surveillance Branch  
Division of STD Prevention  
Centers for Disease Control and Prevention  
1600 Clifton Rd., N.E.  
Atlanta, GA 30333  
Phone: 404-639-8371  
Attention: Susan Wang, M.D.  
or Hillard Weinstock, M.D.

To submit specimens for *N. gonorrhoeae* testing, contact the following:

Neisseria Reference Laboratory, Unit 31  
Gonorrhea Research Branch, Mail Stop C-13  
Bldg. 1 South/Room B260  
Centers for Disease Control and Prevention  
1600 Clifton Rd., N.E.  
Atlanta, GA 30333  
Attention: David Trees, Ph.D., 404-639-2134  
or Joan S. Knapp, Ph.D., 404-639-3470

In addition, statewide programs should be maintained to routinely isolate gonococcal strains and monitor antimicrobial susceptibilities to CDC-recommended therapies and to other FDA-cleared therapies with established usage in the state.

## Sexual Assault and Sexual Abuse

Detailed information concerning evaluation and treatment of suspected victims of sexual assault or abuse can be obtained from the 2002 STD treatment guidelines (152). Presented here are general guidelines pertaining only to *C. trachomatis* and *N. gonorrhoeae* infections (Box 10). Examination of victims is



**BOX 10. General guidelines for testing specimens related to possible sexual assault or abuse**

- Endocervical specimens are appropriate for diagnosing *C. trachomatis* and *N. gonorrhoeae* infection of sexually active females. However, the immature vaginal epithelium of prepubescent females might be infected, and specimens can be taken from the vagina of these patients.
- Culture is the recommended method for detecting *C. trachomatis* in urogenital, pharyngeal, and rectal specimens.
  - Only cell culture using standard methods that employ *C. trachomatis*-specific antibodies to detect intracytoplasmic inclusions should be used.
  - Nonculture/nonamplification tests for *C. trachomatis* are not sufficiently sensitive and specific for them to be used among either victims or alleged assailants implicated in a sexual assault.
  - Data, experience, and court cases are insufficient to assess the applicability of NAATs to detect *C. trachomatis* or *N. gonorrhoeae* in investigating sexual assault and abuse. However, certain researchers have indicated that NAATs for *C. trachomatis* could be used as an alternative to cell culture if cell culture is unavailable and if another NAAT that targets a different sequence can be performed as an additional test if the initial NAAT test is positive.
- Culture is the recommended method for detecting *N. gonorrhoeae* in urogenital, pharyngeal, or rectal swab specimens.
  - Gram-negative diplococci isolated on gonococcal selective medium from vaginal, pharyngeal, or rectal specimens must be identified by the methods described previously (see Additional Testing After a Positive *N. gonorrhoeae* Screening Test) to obtain a confirmed identification.
  - Nonculture tests for *N. gonorrhoeae* are not sufficiently sensitive and specific for them to be used among either victims or alleged assailants implicated in sexual assaults.
  - Gram-stained smear of swab specimens should not be used to detect *N. gonorrhoeae* among victims of sexual assault or abuse.
- All specimens and isolates from both suspected victims and alleged assailants should be stored at  $\leq -70^{\circ}\text{C}$  in the event additional testing is needed.

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. Because of the health and legal implications of test results, the additional time, labor, and cost of performing tests that are sensitive and highly specific are justified. Using highly specific tests is critical with preadolescent children for whom the diagnosis of a sexually transmitted infection might lead to initiation of an investigation for child abuse. Local legal requirements and guidance should be sought for maintaining and documenting a chain of custody for specimens and results that might be used in a legal investigation.

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## Appendix A

### Indications for *Chlamydia trachomatis* Testing and Test Selection by Specimen Type

Readers are cautioned to refer to the manufacturers' test kit inserts for specific details. Information in this appendix represents general conditions for comparative purposes.

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#### Endocervical swabs/urethral swabs from males

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##### Indications

- Screening
  - Females: When pelvic examination is indicated
  - Males: Urine might be more acceptable to asymptomatic males
- Endocervicitis
- Urethritis (males)
- Diseases at other anatomic locations possibly caused by sexually acquired *C. trachomatis* infection
  - Pelvic inflammatory disease
  - Urethral syndrome
  - Bartholinitis
  - Epididymitis
  - Perihepatitis (Fitz-Hugh-Curtis syndrome) (females)
  - Proctitis
  - Reactive arthritis/Reiter syndrome
  - Conjunctivitis
- Not recommended for prepubertal children

##### Test selection

- Nucleic acid amplification tests (NAATs)
  - Preferred because of high sensitivity relative to other tests
- Nonculture/non-NAAT
  - Recommended when a NAAT is not available or not economical
- Culture
  - Preferred when an isolate is needed (e.g., sexual abuse or treatment failure)
- Point-of-care tests
  - Recommended only when the patient is likely to be lost to follow-up and when the test will be performed while the patient waits for results and possible treatment
- Additional testing is recommended after an initial positive screening test if a low positive predictive value can be expected or if a false-positive result would have serious psychosocial or legal consequences

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#### Urethral swabs from females

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##### Indication

- Used with endocervical swab to increase sensitivity of culture for screening
- Urethral syndrome

##### Test selection

- Culture
  - Increases sensitivity of culture of endocervical swab for screening by  $\leq 23\%^*$
  - Among *C. trachomatis*-infected persons, dysuria is associated with a high frequency of a positive urethral culture<sup>†</sup>
- Nonculture tests are not recommended

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#### Urine

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##### Indication

- Females: Screening or testing
- Males: Screening

##### Test selection

- NAAT
  - Recommended on the basis of increased sensitivity and ease of use
  - For males, sensitivity with urine has been lower than with urethral swab in the majority of studies,<sup>§</sup> but not all<sup>¶</sup>
  - Other tests are not recommended because of low sensitivity and, in the case of enzyme immunoassay (EIA) and lipopolysaccharide (LPS)-specific direct fluorescent antibody (DFA) tests, lower specificity
  - Additional testing is recommended after an initial positive screening test if a low positive predictive value can be expected or if a false-positive result would have serious psychosocial or legal consequences

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**Vaginal swabs, postmenarcheal adolescents and adults**


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**Indication**

- Screening/testing of women when pelvic examination is not otherwise indicated

**Test selection**

- No test is recommended for use with vaginal swab specimens
  - The Food and Drug Administration (FDA) has not cleared any nonculture test for use with vaginal specimens
  - NAAT
    - Additional review is needed before a recommendation can be made; however, in multiple studies, sensitivity and specificity with a provider- or client-collected vaginal swab has been similar to screening with endocervical or urine specimens
    - Additional testing is recommended after an initial positive screening test if a low positive predictive value can be expected or if a positive result would have serious psychosocial or legal consequences
  - Culture
    - Not recommended for adults because of suboptimal sensitivity
  - Other tests are not recommended because of low sensitivity and, in case of EIA and LPS-specific DFA, low specificity

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**Vaginal swabs, prepubescent females**


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**Indication**

- Possible sexual abuse, children

**Test selection**

- Culture
  - Preferred for possibly sexually abused children because of presence of vaginal epithelium that is susceptible to *C. trachomatis* infection, high specificity, and ability to retain isolate for additional testing
- FDA has not cleared any nonculture test for use with vaginal specimens
- NAAT
  - When culture is not available, certain *C. trachomatis* specialists support using a NAAT if a positive result can be verified by another NAAT
- Other tests are not recommended because of low sensitivity and, in the case of EIA and LPS-specific DFA, low specificity

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**Rectal swabs**


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**Indication**

- Patients with history of receptive anal intercourse
- Proctitis
- Possible sexual abuse, children

**Test selection**

- Culture
    - Preferred when an isolate is needed (e.g., sexual abuse)
    - Sensitivity not well-defined; high specificity, especially if *C. trachomatis*-specific stain is used
  - DFA
    - FDA-cleared for use with rectal specimens
    - Limited evaluation in published studies
    - Sensitivity not well-defined; potentially high specificity if *C. trachomatis*-specific stain is used
  - Other tests are not recommended
    - NAAT
      - Although cross-reactivity with other rectal bacteria has not been reported for NAATs, they have received only limited evaluation in published studies
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**Pharyngeal swabs**


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**Indication**

- Patients concerned regarding exposure during fellatio or cunnilingus
- Newborns or infants (nasopharyngeal specimens)
  - Neonatal conjunctivitis
  - Pneumonia consistent with *C. trachomatis* etiology
- Possible sexual abuse, children

**Test selection**

- Culture
  - Preferred method
  - Necessary when an isolate is needed (e.g., sexual abuse)
  - Sensitivity not well-defined; high specificity, including if *C. trachomatis*-specific stain is used
- DFA
  - FDA-cleared for use with pharyngeal specimens
  - Limited evaluation in published studies
  - Sensitivity not well-defined; potentially high specificity if *C. trachomatis*-specific stain is used
- Other tests are not recommended
  - NAAT
    - Although cross-reactivity with other pharyngeal bacteria has not been reported for NAATs, they have received only limited evaluation in published studies\*\*

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**Conjunctival swabs**


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**Indication**

- Conjunctivitis among adults
- Newborns or infants
  - Neonatal conjunctivitis
  - Pneumonia consistent with *C. trachomatis* etiology

**Test selection**

- Culture
  - Preferred, when available, because of high sensitivity and specificity
- EIA, nucleic acid probe, and DFA tests
  - EIA, nucleic acid probe, and DFA tests that are FDA-cleared for use with conjunctival specimens have had uniformly high sensitivities with conjunctival specimens from newborns;†† evaluation studies are more limited for conjunctival specimens from adults with conjunctivitis
  - Specificities of tests on conjunctival specimens have also been high,†† although the potential for cross-reaction with other bacteria exists for EIA and for culture and DFA if used with stains that are not specific for *C. trachomatis*
- Other tests are not recommended

\* Source: Jones RB, Katz BP, Van der Pol B, Caine VA, Batteiger BE, Newhall WJ. Effect of blind passage and multiple sampling on recovery of *Chlamydia trachomatis* from urogenital specimens. *J Clin Microbiol* 1986;24:1029–33.

† Source: Paavonen J. *Chlamydia trachomatis*-induced urethritis in female partners of men with nongonococcal urethritis. *Sex Transm Dis* 1979;6:69–71.

§ Sources: Stary A, Schuh E, Kerschbaumer M, Gotz B, Lee H. Performance of transcription-mediated amplification and ligase chain reaction assays for detection of chlamydial infection in urogenital samples obtained by invasive and noninvasive methods. *J Clin Microbiol* 1998;36:2666–70. Crotchfelt KA, Welsh LE, DeBonville D, Rosenstraus M, Quinn TC. Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in genitourinary specimens from men and women by a coamplification PCR assay. *J Clin Microbiol* 1997;35:1536–40. Buimer M, van Doornum GJJ, Ching S, et al. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by ligase chain reaction-based assays with clinical specimens from various sites: implications for diagnostic testing and screening. *J Clin Microbiol* 1996;34:2395–2400. Carroll KC, Aldeen WE, Morrison M, Anderson R, Lee D, Mottice S. Evaluation of the Abbott LCx Ligase Chain Reaction Assay for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine and genital swab specimens from a sexually transmitted disease clinic population. *J Clin Microbiol* 1998;36:1630–3. Ferrero DV, Meyers HN, Schultz DE, Willis SA. Performance of the Gen-Probe AMPLIFIED Chlamydia Trachomatis Assay in detecting *Chlamydia trachomatis* in endocervical and urine specimens from women and urethral and urine specimens from men attending sexually transmitted disease and family planning clinics. *J Clin Microbiol* 1998;36:3230–3. Ossewaarde JM, van Doornum GJJ, Buimer M, Choueiri B, Stary A. Differences in the sensitivity of the Amplicor *Chlamydia trachomatis* PCR assay. *Genitourin Med* 1997;73:207–11. Puolakkainen M, Hiltunen-Back E, Reunala T, et al. Comparison of performances of two commercially available tests, a PCR assay and a ligase chain reaction test, in detection of urogenital *Chlamydia trachomatis* infection. *J Clin Microbiol* 1998;36:1489–93. Stary A, Choueiri B, Hörting-Müller I, Halisch P, Teodorowicz L. Detection of *Chlamydia trachomatis* in urethral and urine samples from symptomatic and asymptomatic male patients by the polymerase chain reaction. *Eur J Clin Microbiol Infect Dis* 1996;15:465–71.

¶ Sources: Van der Pol B, Quinn TC, Gaydos CA, et al. Multicenter evaluation of the AMPLICOR and automated COBAS AMPLICOR CT/NG tests for detection of *Chlamydia trachomatis*. *J Clin Microbiol* 2000;38:1105–12. Toye B, Peeling RW, Jessamine P, Claman P, Gemmill I. Diagnosis of *Chlamydia trachomatis* infections in asymptomatic men and women by PCR assay. *J Clin Microbiol* 1996;34:1396–1400. Vincelette J, Schirm J, Bogard M, et al. Multicenter evaluation of the fully automated COBAS AMPLICOR PCR test for detection of *Chlamydia trachomatis* in urogenital specimens. *J Clin Microbiol* 1999;37:74–80. Wiesenfeld HC, Uhrin M, Dixon BW, Sweet RL. Diagnosis of male *Chlamydia trachomatis* urethritis by polymerase chain reaction. *Sex Transm Dis* 1994;21:268–71. Young H, Moyes A, Horn K, Scott GR, Patrizio C, Sutherland S. PCR testing of genital and urine specimens compared with culture for the diagnosis of chlamydial infection in men and women. *Int J STD AIDS* 1998;9:661–5.

\*\*Source: Hammerschlag MR, Roblin PM, Gelling M, Tsumura N, Jule JE, Kutlin A. Use of polymerase chain reaction for the detection of *Chlamydia trachomatis* in ocular and nasopharyngeal specimens from infants with conjunctivitis. *Pediatr Infect Dis J* 1997;16:293–7.

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## Appendix B

### Indications for *Neisseria gonorrhoeae* Testing and Test Selection by Specimen Type

Readers are cautioned to refer to the manufacturers' test kit inserts for specific details. Information in this appendix represents general conditions for comparative purposes.

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#### Endocervical swabs/urethral swabs from males

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##### Indication

- Screening
  - Females: When pelvic examination is indicated
  - Males: Urine might be more acceptable to asymptomatic males
- Endocervicitis
- Urethritis (males)
- Diseases at other anatomic locations possibly caused by sexually acquired *N. gonorrhoeae* infection
  - Pelvic inflammatory disease
  - Urethral syndrome
  - Bartholinitis
  - Epididymitis
  - Perihepatitis (Fitz-Hugh-Curtis syndrome) (females)
  - Proctitis
  - Disseminated gonococcal infections
  - Conjunctivitis
- Not recommended for prepubertal children

##### Test selection

- Gram-stained smear as a point-of-care test for males with urethral discharge
  - For males with urethral discharge, the sensitivity and specificity are similar to culture with oxidase testing and Gram-staining of any colonies with *N. gonorrhoeae* morphology
  - Culture after a positive Gram-stained smear might be useful for quality assurance, but additional testing is not usually otherwise indicated
- Culture
  - Preferred if ambient conditions during holding and transport of inoculated media are adequate to maintain the viability of organisms
  - Sensitivity and specificity of culture with additional testing approaches or surpasses that of other tests
  - A culture isolate should be tested for antimicrobial resistance if a patient fails therapy
  - Culture allows monitoring for antimicrobial resistance
- Nucleic acid amplification tests (NAATs) or nucleic acid hybridization tests
  - Recommended when conditions during holding and transport of inoculated culture media are not adequate to maintain the viability of organisms
  - Commercial polymerase chain reaction (PCR)\* and strand displacement assays<sup>†</sup> have cross-reacted with nongonococcal *Neisseria*; such cross-reactivity has not been reported for commercial ligase chain reaction (LCR) and unamplified probe assays
- Additional testing is recommended after an initial positive screening test if a low positive predictive value can be expected or if a false-positive result would have serious psychosocial or legal consequences

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#### Urine

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##### Indication

- Males: Screening
- Females: Screening when pelvic examination is not indicated

##### Test selection

- NAATs
    - Sensitivity with urine might be lower than with urethral (males) or endocervical swabs<sup>§</sup>
    - Other tests are not recommended because of low sensitivity
    - Additional testing is recommended after an initial positive screening test if a low positive predictive value can be expected because of a low prevalence or if a false-positive result would have serious psychosocial or legal consequences for a person
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**Vaginal swabs, postmenarcheal adolescents and adults**


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**Indication**

- Screening/testing of women when pelvic examination is not otherwise indicated

**Test selection**

- No test is recommended for use with vaginal swab specimens.
  - The Food and Drug Administration (FDA) has not cleared any nonculture test for use with vaginal specimens
  - NAAT
    - Additional review is needed before a recommendation can be made; however, in one study,<sup>6</sup> sensitivity and specificity with a provider- or client-collected vaginal swab was similar to screening with endocervical or urine specimens
    - Additional testing is recommended after an initial positive screening test if a low positive predictive value can be expected or if a false-positive result would have serious psychosocial or legal consequences
  - Culture
    - Not recommended for adults because of suboptimal sensitivity
  - Other tests are not recommended because of low sensitivity

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**Vaginal swabs, prepubescent children**


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**Indication**

- Possible sexual abuse, children

**Test selection**

- Culture
  - Preferred for possibly sexually abused children because of presence of vaginal epithelium that is susceptible to *N. gonorrhoeae* infection, high specificity, and ability to retain isolate for additional testing
- FDA has not cleared any nonculture test for use with vaginal specimens
- NAATs
  - When culture is not available, certain specialists support use of a NAAT if a positive result can be verified by another NAAT
- Other tests are not recommended because of low sensitivity and specificity

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**Rectal swabs**


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**Indication**

- Patients with history of receptive anal intercourse
- Proctitis
- Possible sexual abuse, children

**Test selection**

- Culture
  - Preferred
    - The sensitivity of culture is not well-defined; isolates that are oxidase-positive and Gram-negative diplococci should receive additional testing to verify an initial presumptive *N. gonorrhoeae* diagnosis, if a false-positive result would have serious medical, psychosocial, or legal consequences
    - A culture isolate should be tested for antimicrobial resistance if a patient fails therapy
- Other tests are not recommended

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**Pharyngeal swabs**


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**Indication**

- Patients concerned regarding exposure during fellatio or cunnilingus
- Newborn or infant (nasopharyngeal specimens)
  - Neonatal conjunctivitis
- Possible sexual abuse, children

**Test selection**

- Culture
    - Preferred
      - Sensitivity of culture for pharyngeal specimens is not well-defined
      - Isolates that are oxidase-positive and contain Gram-negative diplococci should receive additional testing to verify an initial presumptive *N. gonorrhoeae* diagnosis because of the common occurrence of nongonococcal *Neisseria* in the pharynx
      - A culture isolate should be tested for antimicrobial resistance if a patient fails therapy
  - Other tests are not recommended
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**Conjunctival swabs**


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**Indication**

- Conjunctivitis among adults
- Newborn or infant
  - Neonatal conjunctivitis

**Test selection**

- Gram stain as point-of-care test
  - Recommended to establish a presumptive diagnosis of *N. gonorrhoeae* during a patient's visit for conjunctivitis
  - Adequate sensitivity because of high concentration of organisms
  - Gram stain should be followed by laboratory-based testing because Gram-negative diplococci other than *N. gonorrhoeae* are occasionally isolated from conjunctiva
- Culture
  - Preferred
  - High sensitivity because of high concentration of organisms
  - Oxidase-positive and Gram-stain-positive bacteria other than *N. gonorrhoeae* occasionally isolated from conjunctiva
  - Inoculation onto nonselective media might increase sensitivity
- Only a limited number, if any, nonculture tests are FDA-cleared for conjunctival specimens

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**Diagnosing disseminated gonococcal infection, adults or neonates**


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**Test selection**

- Gram stain as point-of-care test
  - Recommended on any synovial fluid and cerebrospinal fluid collected for other tests and on endocervical and urethral swab specimens
  - Gram stain should be followed by laboratory-based testing, which is more sensitive and specific
- Culture
  - Preferred
  - Blood, synovial fluid from affected joints, and, if indicated, cerebrospinal fluid specimens should be inoculated onto nonselective as well as selective media
  - Swab specimens from the endocervix (adult female), vagina (neonate), urethra (adult male), rectum, and pharynx should be inoculated onto selective media
  - Additional testing recommended after an initial positive test to verify diagnosis
- No other tests are recommended

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\* **Source:** 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.

† **Source:** Becton Dickinson. BDProbeTec™ ET Chlamydia trachomatis and *Neisseria gonorrhoeae* amplified DNA assays [Package insert]. Sparks, MD: Becton Dickinson, 2001.

§ **Sources:** 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. Crotchfelt KA, Welsh LE, DeBonville D, Rosenstraus M, Quinn TC. Detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in genitourinary specimens from men and women by a coamplification PCR assay. *J Clin Microbiol* 1997;35:1536–40.

¶ **Source:** Hook EW III, Ching SF, Stephens J, Hardy KF, Smith KR, Lee HH. Diagnosis of *Neisseria gonorrhoeae* infections in women by using the ligase chain reaction on patient-obtained vaginal swabs. *J Clin Microbiol* 1997;35:2129–32.

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## Appendix C

### Conducting Studies To Evaluate *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Tests, Including Studies Required by the Regulations of the Clinical Laboratory Improvement Amendments of 1988 for Verifying or Establishing Test Performance Characteristics

When a test is a candidate to replace an established test, the sensitivities and specificities of the candidate and established tests should be compared. Recommendations for the conduct of test evaluation studies have been reviewed elsewhere (C-1–C-4). The imperfect sensitivity and specificity of reference standards complicate evaluation of *C. trachomatis* and *N. gonorrhoeae* tests. Discrepant analysis has been used universally to address this problem in the past. However, this approach has been criticized because of the likelihood of producing biased estimates of test characteristics (C-5–C-8). Key to advancing the evaluation of *C. trachomatis* and *N. gonorrhoeae* tests is the introduction of study designs that compare candidate and established tests in the same study by using an independent reference standard or by using approaches that do not require designation of a reference standard. Investigation of such designs for evaluation of *C. trachomatis* tests has begun (C-6, C-9–C-15).

The Clinical Laboratory Improvement Amendments of 1988 (CLIA) requires that a clinical laboratory verify or establish the performance of a test to be used in the medical care of persons when it is introduced into that laboratory (C-16). Verification of performance under CLIA applies to the introduction of tests that are Food and Drug Administration (FDA)-cleared, and the CLIA requirement is that the laboratory demonstrate that test performance (i.e., accuracy, precision, reportable range of results, and appropriate normal values) in that laboratory be equivalent to the performance claimed by the manufacturer in the FDA-cleared package insert. Establishing performance under CLIA applies to tests or modifications of tests that have not been FDA-cleared for a particular use. When a modification or use of a test has not been FDA-cleared, CLIA requires that the laboratory establish the performance characteristics of the test for that use. In addition to the performance specifications noted previously, establishing performance must include determinations of analytical sensitivity and specificity, to include interfering substances.

Such verification or establishment studies are relatively straightforward when the candidate test performs similarly to the established test. In such cases, the laboratory can verify or establish performance by using the established test as the reference standard if discrepancies between the candidate and established test are uncommon. If the candidate test performs differently from the established test, the laboratory is faced with the economic and logistical challenge of verifying or establishing the performance of the candidate test by introducing an independent reference standard. If the laboratory lacks an independent reference standard, a reference laboratory with an independent reference standard might be enlisted to 1) blindly test a suitable sample of verification or establishment study specimens with the reference standard tests or 2) supply a panel of specimens previously characterized by the reference laboratory. The reader is referred to

the American Society for Microbiology CUMITECH series for detailed guidance regarding conduct of verification and establishment studies (C-17).

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- C-2. Noe DA. Evaluation of the medical utility of classification studies [Chapter 4]. In: Noe DA, Rock RC, Grochow LB, Kahn SB, Perlin E, eds. *Laboratory medicine: the selection and interpretation of clinical laboratory studies*. Baltimore, MD: Williams & Wilkins, 1994:44–52.
- C-3. Noe DA. Diagnostic classification [Chapter 3]. In: Noe DA, Rock RC, Grochow LB, Kahn SB, Perlin E, eds. *Laboratory medicine: the selection and interpretation of clinical laboratory studies*. Baltimore, MD: Williams & Wilkins, 1994:27–43.
- C-4. Reid MC, Lachs MS, Feinstein AR. Use of methodological standards in diagnostic test research: getting better but still not good. *JAMA* 1995;274:645–51.
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- C-8. McAdam AJ. Discrepant analysis: how can we test a test? *J Clin Microbiol* 2000;38:2027–9.
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- C-13. Qu Y, Hadgu A. Model for evaluating sensitivity and specificity for correlated diagnostic tests in efficacy studies with an imperfect reference test. *Journal of the American Statistical Association* 1998;93:920–8.
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- C-16. Code of Federal Regulations. Title 42, Chapter IV, part 493—Laboratory requirements.
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## Appendix D

### Recommendations for Transport and Storage of Specimens for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Testing, by Testing Procedure Type

Readers are cautioned to refer to the manufacturers' test kit inserts for specific details. Information in this appendix represents general conditions for comparative purposes.

Intended Laboratory Procedure	Specimen	Transport	Storage
Culture isolation • <i>C. trachomatis</i>	Swab* • Cotton • Rayon • Dacron® • Cytobrush	<ul style="list-style-type: none"> <li>• ≤24 hours at 4°C</li> <li>• If delayed &gt;24 hours, freeze to -70°C</li> <li>• Transport media† Sucrose phosphate (2SP) or sucrose phosphate glutamate (SPG), supplemented with 5% fetal bovine serum, 10 µg/mL gentamicin, 100 µg/mL vancomycin, and 1 µg/mL amphotericin B Micro test Multi-Microbe Media (M4; manufactured by Micro Test, Inc., Lilburn, GA)</li> </ul>	<ul style="list-style-type: none"> <li>• Frozen to -70°C in transport media</li> <li>• Viability remains after ≥2 years, if frozen correctly</li> <li>• Avoid storage at -20°C or in frost-free freezers</li> </ul>
Culture isolation • <i>N. gonorrhoeae</i>	Swab • Rayon • Dacron • Calcium alginate	<ul style="list-style-type: none"> <li>• ≤5 hours Inoculate selective or nonselective medium and incubate at 35°C–36.5°C and place immediately in a CO<sub>2</sub>-enriched atmosphere</li> <li>• 5–48 hours Can be transported in ambient air and temperature if inoculated medium has been incubated at 35°C–36.5°C in a CO<sub>2</sub>-enriched atmosphere for 18–24 hours before transport</li> <li>• Do not refrigerate</li> </ul>	<ul style="list-style-type: none"> <li>• Must be subcultured every 18–24 hours to maintain viability until frozen</li> <li>• Should be stored frozen to ≤-70°C in trypticase soy broth plus 20% glycerine</li> </ul>
Direct fluorescent antibody • <i>C. trachomatis</i>	Swab • Cotton • Rayon • Dacron • Calcium alginate • Cytobrush	<ul style="list-style-type: none"> <li>• Prepare slides immediately after specimen collection</li> <li>• Air dry and fix slide for 10 minutes in alcohol or acetone</li> <li>• Transport to laboratory ≤7 days at room temperature or 4°C</li> </ul>	<ul style="list-style-type: none"> <li>• Fixed and unstained slides can be stored at -20°C for ≥2 years</li> </ul>
Immunoassays (enzyme immunoassay or optical immunoassay) • <i>C. trachomatis</i>	Swab • Manufacturer-supplied	<ul style="list-style-type: none"> <li>• ≤24 hours at room temperature</li> <li>• ≤5 days at 2°C–8°C</li> <li>• Do not freeze specimens</li> </ul>	<ul style="list-style-type: none"> <li>• Specimens must be processed ≤5 days after collection</li> <li>• Storage after processing is not applicable</li> </ul>

**Intended Laboratory**

<b>Procedure</b>	<b>Specimen</b>	<b>Transport</b>	<b>Storage</b>
Nucleic acid probes • <i>C. trachomatis</i> • <i>N. gonorrhoeae</i>	Swab • Manufacturer-supplied	• ≤7 days at 2°C–25°C • Swab must be immersed in manufacturer-supplied medium	• Specimens can be frozen to ≤–20°C and tested ≤60 days after collection
Nucleic acid amplification • <i>C. trachomatis</i> • <i>N. gonorrhoeae</i>	Swab • Manufacturer-supplied	• ≤5 days at 2°C–27°C; ≤7 days if maintained at 2°C–8°C • Swab must be immersed in manufacturer-supplied medium or in isolation transport medium	• Specimens can be frozen to –70°C and tested ≤60 days after collection
—	Urine	• ≤24 hours at room temperature • ≤5 days at 2°C–8°C	• Specimens can be frozen to –20°C and tested ≤60 days after collection • Manufacturer for the strand displacement assay does not recommend freezing specimens

\* New lots of swabs should be checked for toxicity to *C. trachomatis* isolation. Swabs with wooden shafts might be toxic to *C. trachomatis* or tissue culture cells.

† Not intended as a compendium of commercially available transport media.

## Appendix E

### *Chlamydia trachomatis* Screening Recommendations

The U.S. Preventive Services Task Force (USPSTF) has published recommendations for screening certain groups of women for *C. trachomatis* infection (E-1). The following summarizes those recommendations:

- Routinely screen all sexually active women aged  $\leq 25$  years for *C. trachomatis* infection, whether or not they are pregnant. Women and adolescents aged  $\leq 20$  years are at highest risk for chlamydial infection, but the majority of reported data indicate that infection is prevalent among women aged 20–25 years. More targeted screening might be indicated by local prevalence data.
- Screening of women aged  $>25$  years for *C. trachomatis* should also be considered if they are at increased risk.
- Prevalence of *C. trachomatis* infection varies widely among communities and patient populations. Knowledge of the patient population is the best guide to developing a screening strategy. Certain risk factors should be considered, including
  - having new or multiple sex partners,\*
  - having a prior history of a sexually transmitted disease,<sup>†</sup> and
  - not using condoms consistently and correctly.
- Personal risk depends on the number of risk markers and local disease prevalence. Specific risk-based protocols need to be tested locally.
- The optimal timing of screening in pregnancy is uncertain.

- Screening early in pregnancy provides increased opportunities to improve pregnancy outcomes, including low birth weight and premature delivery.
- Screening and treatment in the third trimester might be more effective at preventing transmission of chlamydial infection to the infant during birth by reducing the risk for reinfection.
- The incremental benefit of repeated screening is unknown.

- The optimal interval for screening women with a previous negative screening test is uncertain. The interval for rescreening should be based on changes in sexual partners, young age, and other *C. trachomatis* risk factors. If evidence exists that a woman is at low risk for infection (e.g., in a mutually monogamous relationship with a previous history of negative screening tests for chlamydial infection), screening frequently might be unnecessary.

USPSTF did not address screening of women with a *C. trachomatis* infection. CDC recommends that women with *C. trachomatis* infection be screened 3–4 months after treatment is completed (E-2).

#### References

- E-1. US Preventive Services Task Force. Screening for chlamydial infection: recommendations and rationale. *Am J Prev Med* 2001;20(3Suppl):90–4.
- E-2. CDC. Sexually transmitted diseases treatment guidelines. *MMWR* 2002;51(No. RR-6):1–80.

\* USPSTF does not include a time interval for this risk factor. Having new or multiple sex partners within the past 90 days was the criterion recommended previously by CDC (Source: CDC. Recommendations for the prevention and management of *Chlamydia trachomatis* infections, 1993. *MMWR* 1993;42[No. RR-12]:1–39). A history of new or multiple sex partners since the woman's last screening would also provide a time interval for previously screened women.

<sup>†</sup> USPSTF did not include a time limitation for the risk factor of having a prior history of sexually transmitted disease. Obtaining a history of a sexually transmitted disease within the past year includes an arbitrary choice of time interval. Obtaining a history of a sexually transmitted disease since the woman's last screening would also restrict the time interval for a sexually transmitted disease in the past.

## Appendix F

### Characteristics of *Neisseria* and Related Species of Human Origin\*

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

\* *N. gonorrhoeae* contains a subspecies referred to as *N. kochii*; isolates of this subspecies exhibit characteristics of both *N. gonorrhoeae* and *N. meningitidis* but will be identified as *N. gonorrhoeae* by tests routinely used for the identification of *Neisseria* species.

† Symbols: +, strains typically positive but genetic mutants might be negative; –, negative; and V, strain-dependent.

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

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

\*\* Rod-shaped organism. Catalase test is weakly positive or negative compared with those of other *Neisseria* species (catalase positive). Results in this table are for *N. elongata* subspecies elongata. Strains of *N. elongata* subspecies glycolytica might produce a weak acid reaction from D-glucose, are catalase-positive, and do not reduce nitrate but do reduce nitrite. Strains of *N. elongata* subspecies nitroreducans (formerly CDC group M-6) might produce a weak acid reaction from D-glucose, are catalase-negative, and reduce nitrate and nitrite.









# MMWR™

## Morbidity and Mortality Weekly Report

Recommendations and Reports

October 18, 2002 / Vol. 51 / No. RR-15

### Continuing Education Activity Sponsored by CDC Screening Tests To Detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections — 2002

**EXPIRATION — October 18, 2005**

You must complete and return the response form electronically or by mail by **October 18, 2005**, to receive continuing education credit. If you answer all of the questions, you will receive an award letter for 2.0 hours Continuing Medical Education (CME) credit; 0.2 Continuing Education Unit (CEUs); or 2.4 contact

hours Continuing Nursing Education (CNE) credit. If you return the form electronically, you will receive educational credit immediately. If you mail the form, you will receive educational credit in approximately 30 days. No fees are charged for participating in this continuing education activity.

#### INSTRUCTIONS

##### By Internet

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## GOAL AND OBJECTIVES

This *MMWR* provides recommendations regarding laboratory tests for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections. These recommendations were prepared by CDC staff in the Division of AIDS, STD, and TB Laboratory Research, National Center for Infectious Diseases, and the Division of STD Prevention, National Center for HIV, STD, and TB Prevention after a literature review and consultation with specialists within and outside CDC. The goal of this report is to provide guidance regarding selecting and using laboratory tests for *C. trachomatis* and *N. gonorrhoeae* infections, with an emphasis on screening men and women in the United States. Upon completion of this educational activity, the reader should be able to 1) describe the morbidity associated with *C. trachomatis* and *N. gonorrhoeae* infections that has resulted in establishment of national prevention programs, 2) list the categories of tests available for detecting *C. trachomatis* and *N. gonorrhoeae* infections, 3) apply test performance and other criteria to selecting *C. trachomatis* and *N. gonorrhoeae* screening tests, and 4) apply recommendations for implementing and using *C. trachomatis* and *N. gonorrhoeae* screening tests.

**To receive continuing education credit, please answer all of the following questions.**

1. Which of the following conditions is not an adverse consequence of a *C. trachomatis* or *N. gonorrhoeae* infection?
  - A. Infertility.
  - B. Pelvic inflammatory disease.
  - C. Pyelonephritis.
  - D. Infant pneumonia.
  - E. Ectopic pregnancy.
2. Which of the following does not apply to the recommendations of the Third U.S. Preventive Services Task Force for screening persons for *C. trachomatis* infection?
  - A. Sexually active women aged  $\leq 25$  years should be screened, unless local data indicate otherwise.
  - B. Sexually active adolescent females are at lower risk than older women (e.g., women aged 20–25 years).
  - C. Rescreening of women with *C. trachomatis* infection might be indicated because of a high rate of reinfection.
  - D. Screening of men is of potential utility as a result of new screening tests, but screening criteria have not been adequately evaluated.
3. Which of the following is an advantage of nucleic acid amplification tests?
  - A. Higher test sensitivity.
  - B. Ability to use a urine specimen.
  - C. Lower cost per test.
  - D. Ability to test for *N. gonorrhoeae* by using the same specimen.
  - E. Reduced requirements for quality assurance.
  - F. A, B, and D are advantages, but not C and E.
4. Which of the following statements best describes the positive predictive value (PPV) of a diagnostic test?
  - A. PPV is the proportion of persons who have a positive test among those who actually have the condition for which the test is performed.
  - B. PPV is the proportion of persons who actually have the condition among all persons who have a positive test.
  - C. PPV is the proportion of persons who have a negative test among those who do not have the condition.
  - D. PPV is the proportion of persons who do not have the condition among all persons who have a negative test.
5. An additional test after a positive screening test for *C. trachomatis* or *N. gonorrhoeae* infection should be considered when the . . .
  - A. prevalence of infection is low.
  - B. sensitivity of the screening test is low.
  - C. specificity of the screening test is not sufficiently high.
  - D. PPV of the screening test is thought to be low.
  - E. A, C, and D, but not B.
  - F. B, C, and D, but not A.
6. Which of the following methods of additional testing after a positive screening test for *C. trachomatis* or *N. gonorrhoeae* infection is theoretically most accurate?
  - A. Repeat the screening test on the original specimen.
  - B. Repeat the screening test on the original specimen with the addition of a blocking or competition reagent.
  - C. Retest the original specimen with a test that identifies a different target or trait and uses a different format than the screening test.
  - D. Obtain a fresh specimen and test with an alternative test that identifies a different target or trait and uses a different format than the original screening test.
7. Which of the following statements characterizes economic analyses conducted to assist in selecting a screening test for *C. trachomatis* or *N. gonorrhoeae* infection?
  - A. The cost of additional testing after a positive screening test is usually only a limited proportion of the costs of screening and treatment.
  - B. The cost of screening tests performed in the absence of symptoms or signs of *C. trachomatis* or *N. gonorrhoeae* infection is usually reimbursed by insurance.
  - C. A screening program is cost-effective only if it is also cost-saving.
  - D. Economic analyses can be conducted from a societal or a health-care provider perspective.
  - E. A and D, but not B and C.
  - F. A, C, and D, but not B.
8. Which of the following is a reason to recommend culture for the detection of *N. gonorrhoeae* infection?
  - A. Additional testing might be required to establish a diagnosis.
  - B. Treatment failures have been reported because of decreased susceptibility of *N. gonorrhoeae* to the antibiotic used for treatment.
  - C. The location for specimen collection and the laboratory are relatively far apart geographically.
  - D. Given appropriate transport conditions, culture is as sensitive or approximately as sensitive as alternative tests.
  - E. A, B, and D but not C.
9. Which of the following is a characteristic of point-of-care tests for *C. trachomatis* infection?
  - A. Sensitivities of point-of-care tests are higher than tests designed to be performed in batch after the tested person leaves.
  - B. Point-of-care tests are useful for increasing the percent of test-positive persons who receive treatment.
  - C. A lower cost per tested person has been a principal reason for screening with point-of-care tests.
10. Which of the following statements concerning the collection of endocervical swab specimens for *C. trachomatis* tests is true?
  - A. Specimens for nonculture tests should be obtained by following the directions outlined by manufacturers in package inserts.
  - B. Studies have reported that obtaining endocervical swab specimens with adequate numbers of columnar epithelial cells is critical even for nucleic acid amplification tests.
  - C. Clinicians require training and periodic assessment to maintain correct endocervical specimen collection technique.
  - D. All of the statements are true.
11. Indicate your work setting.
  - A. State/local health department.
  - B. Other public health setting.
  - C. Hospital clinic/private practice.
  - D. Managed care organization.
  - E. Academic institution.
  - F. Other.

12. Which best describes your professional activities?
- A. Patient care — emergency/urgent care department.
  - B. Patient care — inpatient.
  - C. Patient care — primary-care clinic or office.
  - D. Laboratory/pharmacy.
  - E. Public health.
  - F. Other.
13. I plan to use these recommendations as the basis for . . . (Indicate all that apply.)
- A. health education materials.
  - B. insurance reimbursement policies.
  - C. local practice guidelines.
  - D. public policy.
  - E. other.
14. Each month, approximately how many patients do you test for *C. Trachomatis* or *N. gonorrhoeae* infections?
- A. None.
  - B. 1–10.
  - C. 11–100.
  - D. 101–1,000.
  - E. >1,000.
15. How much time did you spend reading this report and completing the exam?
- A. <2.0 hours.
  - B. ≥2.0 hours but <3.0 hours.
  - C. ≥3.0 but <4.0 hours.
  - D. ≥4.0 hours.

16. After reading this report, I am confident I can describe how to select and use laboratory tests for *C. trachomatis* and *N. gonorrhoeae* infections, with an emphasis on screening men and women in the United States.
- A. Strongly agree.
  - B. Agree.
  - C. Neither agree nor disagree.
  - D. Disagree.
  - E. Strongly disagree.
17. After reading this report, I am confident I can describe the morbidity associated with *C. trachomatis* and *N. gonorrhoeae* infections that has resulted in the establishment of national prevention programs.
- A. Strongly agree.
  - B. Agree.
  - C. Neither agree nor disagree.
  - D. Disagree.
  - E. Strongly disagree.
18. After reading this report, I am confident I can list the categories of tests available to detect *C. trachomatis* and *N. gonorrhoeae* infections.
- A. Strongly agree.
  - B. Agree.
  - C. Neither agree nor disagree.
  - D. Disagree.
  - E. Strongly disagree.

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October 18, 2005/Vol. 51/No. RR-15  
Laboratory Guidelines for Screening To Detect Chlamydia  
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Fill in the appropriate blocks to indicate your answers. Remember, you must answer all of the questions to receive continuing education credit!

- 1.  A  B  C  D  E
- 2.  A  B  C  D
- 3.  A  B  C  D  E  F
- 4.  A  B  C  D
- 5.  A  B  C  D  E  F
- 6.  A  B  C  D
- 7.  A  B  C  D  E  F
- 8.  A  B  C  D  E
- 9.  A  B  C
- 10.  A  B  C  D
- 11.  A  B  C  D  E  F
- 12.  A  B  C  D  E  F
- 13.  A  B  C  D  E
- 14.  A  B  C  D  E
- 15.  A  B  C  D
- 16.  A  B  C  D  E
- 17.  A  B  C  D  E
- 18.  A  B  C  D  E
- 19.  A  B  C  D  E
- 20.  A  B  C  D  E
- 21.  A  B  C  D  E
- 22.  A  B  C  D  E
- 23.  A  B  C  D  E
- 24.  A  B  C  D  E
- 25.  A  B  C  D  E
- 26.  A  B  C  D  E  F

Signature \_\_\_\_\_ Date I Completed Exam \_\_\_\_\_

19. After reading this report, I am confident I can apply test performance and other criteria to selecting *C. trachomatis* and *N. gonorrhoeae* screening tests.
- Strongly agree.
  - Agree.
  - Neither agree nor disagree.
  - Disagree.
  - Strongly disagree.
20. After reading this report, I am confident I can apply recommendations for the implementation and use of *C. trachomatis* and *N. gonorrhoeae* screening tests.
- Strongly agree.
  - Agree.
  - Neither agree nor disagree.
  - Disagree.
  - Strongly disagree.
21. The objectives are relevant to the goal of this report.
- Strongly agree.
  - Agree.
  - Neither agree nor disagree.
  - Disagree.
  - Strongly disagree.
22. The tables, boxes, and figure are useful.
- Strongly agree.
  - Agree.
  - Neither agree nor disagree.
  - Disagree.
  - Strongly disagree.
23. Overall, the presentation of the report enhanced my ability to understand the material.
- Strongly agree.
  - Agree.
  - Neither agree nor disagree.
  - Disagree.
  - Strongly disagree.
24. These recommendations will affect my practice.
- Strongly agree.
  - Agree.
  - Neither agree nor disagree.
  - Disagree.
  - Strongly disagree.
25. The availability of continuing education credit influenced my decision to read this report.
- Strongly agree.
  - Agree.
  - Neither agree nor disagree.
  - Disagree.
  - Strongly disagree.
26. How did you learn about this continuing education activity?
- Internet.
  - Advertisement (e.g., fact sheet, *MMWR* cover, newsletter, or journal).
  - Coworker/supervisor.
  - Conference presentation.
  - MMWR* subscription.
  - Other.

Correct answers for questions 1-10  
1. C; 2. B; 3. F; 4. B; 5. E; 6. D; 7. E; 8. E; 9. B; 10. D.

## Laboratory Guidelines for Screening To Detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Infections — 2002

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