

# Current Methods of Laboratory Diagnosis of *Chlamydia trachomatis* Infections

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## EPIDEMIOLOGY AND CLINICAL MANIFESTATIONS

### Biology

The chlamydiae are nonmotile, gram-negative bacterial pathogens that were once mistakenly thought to be viruses because of their obligate intracellular life cycle. Chlamydiae are metabolically deficient in their ability to synthesize ATP and thus require an exogenous source of this high-energy compound. Chlamydiae undergo a unique biphasic developmental cycle, forming distinctive intracellular inclusions that permit identification by light or fluorescence microscopy. Chlamydiae are susceptible to broad-spectrum antibiotics, particularly tetracyclines and macrolides of the erythromycin class. Because the chlamydial cell wall is different from that of many bacteria,

beta-lactam antibiotics such as penicillin lack bactericidal activity in vitro against these microorganisms.

There are four recognized species of *Chlamydia*: *Chlamydia trachomatis*, *C. psittaci*, *C. pneumoniae*, and *C. pecorum*. *C. trachomatis* includes the agents of trachoma, lymphogranuloma venereum (LGV), urogenital tract disease, and inclusion conjunctivitis. Within the *C. trachomatis* species, there are three biovars or clusters based on etiologic potential for disease categories. These are the trachoma, LGV, and murine biovars. The murine biovar is much less closely related genetically to the other biovars and is now thought by many investigators to be misclassified within the species *C. trachomatis* (205). The species is also divided into 15 well-characterized serotypes known as serovars, as well as several additional serovariants that have been identified (110, 207).

No attempt has been made to comprehensively review the biology, pathogenesis, or epidemiology of *C. trachomatis* infections, since several excellent reviews have been published on these subjects (26, 154, 230).

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### Epidemiology

Although *C. trachomatis* infection did not become a fully reportable disease in the United States until 1996, it is known to be the most common bacterial sexually transmitted disease (STD). The actual incidence of chlamydial infection is not yet known due to lack of reporting in all 50 states up to 1996; however, national trends have been estimated by using data from states that reported cases prior to 1996, sentinel surveillance, surveys, and models based on proxies of infection (26, 228, 229). It is currently estimated that about 4 million new chlamydial infections occur each year in the United States at an estimated annual cost exceeding 2.4 billion dollars (30, 228, 229). Worldwide, it is estimated that there are more than 50 million new cases of *C. trachomatis* infection annually (107). *C. trachomatis* infections are among the STDs known to increase the risk for human immunodeficiency virus (HIV) infection (109); thus, treatment of chlamydial infections could delay the spread of HIV in some groups.

Although the major impact of disease caused by *C. trachomatis* is on the female reproductive tract, this agent also causes infections in men and children. The prevalence of *C. trachomatis* infection in sexually active adolescent women, the population considered most at risk, generally exceeds 10%, and in some adolescent and STD clinic populations of women, the prevalence can reach 40% (11, 30, 178). The prevalence of *C. trachomatis* infection ranges from 4 to 10% in asymptomatic men (133, 166) and from 15 to 20% in men attending STD clinics (199). Chlamydial infections in newborns occur as a result of perinatal exposure; approximately 65% of babies born from infected mothers become infected during vaginal delivery (16, 180).

The biggest challenge to the control of chlamydial disease is that as many as 70 to 80% of women and up to 50% of men who are infected do not experience any symptoms (184, 197, 245). This results in a large reservoir of unrecognized, infected individuals who are capable of transmitting the infection to sexual partners. Contributing to this challenge is the fact that immunity following infection is thought to be type specific and only partially protective; therefore, recurrent infections are common (154). Among adolescent females infected with *C. trachomatis*, a retrospective study in Wisconsin reported that 54% of those under age 15 at initial infection and 30% of those between 15 and 19 developed recurrent infections (77), and in another study, 38% had recurrent infection within 3 years (23). Evidence suggests that the risk of developing sequelae such as ectopic pregnancy or infertility increases with each successive episode of infection (77, 128).

### Risk and Demographic Factors for *C. trachomatis* Infections

The most common demographic correlate of infection with *C. trachomatis* in women is young age (<20 years). The biologic basis for this association is thought to be anatomic differences in the cervix of younger women, wherein the squamocolumnar junction, a primary host target for *C. trachomatis*, is everted and thus more exposed, a condition known as ectopy. Demographic factors associated with older women include unmarried status, nulliparity, black race, and poor socioeconomic conditions (26, 230). Higher numbers of sexual partners, a new sexual partner, lack of use of barrier contraceptive devices, and concurrent gonococcal infection are also consistently associated with chlamydial infections (120). The use of oral contraceptives is associated with cervical chlamydial infections but not pelvic inflammatory disease (PID) (48, 71, 74); this is believed to be a result of induced ectopy; however, the rela-

tionship may be confounded in some studies by behavioral factors and has thus been controversial in recent years (230).

### Clinical Sequelae of *C. trachomatis* Infections in Women

Although most infections caused by *C. trachomatis* in women are asymptomatic, clinical manifestations include cervicitis, urethritis, endometritis, PID, or abscess of the Bartholin glands (22, 30). Although the initial site of infection is usually the cervix, the urethra and rectum may also be infected (53). Culture studies have shown that among women infected with *C. trachomatis*, 50 to 60% are infected at both the cervix and urethra, 30% have only cervical infections, and 5 to 30% have only urethral infections (142, 151, 189). PID, which results from ascending infection, is responsible for most of the morbidity and cost resulting from chlamydial infection.

When symptoms do occur, they most commonly consist of vaginal discharge and dysuria. Postcoital bleeding is often reported. Symptoms of chlamydial PID, which may be subtle, include pelvic, uterine, or adnexal pain. Clinical signs associated with chlamydial infection include mucopurulent cervicitis, cervical friability, culture-negative pyuria, and more than 10 polymorphonuclear leukocytes (PMNs) per oil immersion field of a Gram-stained cervical smear (17, 184).

Asymptomatic chlamydial infections are an important cause of PID and ectopic pregnancy. In both Europe and North America, a higher proportion of PID has been found to be attributable to *C. trachomatis* than to *Neisseria gonorrhoeae* (26). Paradoxically, chlamydial infections can apparently cause more severe tubal immunopathology than other agents in spite of the absence of overt symptoms (208). This is most probably due to the greater chronicity and fulminating character of chlamydial infections compared with more acute infections such as gonorrhea. Silent and untreated salpingitis is now recognized as a major cause of infertility; more than 50% of women with documented tubal occlusion report no history of PID but show serologic evidence of previous *C. trachomatis* infection (230). Similarly, multiple studies have shown associations between previous chlamydial infection, both symptomatic and asymptomatic, and ectopic pregnancy (26, 233).

The prevalence of *C. trachomatis* infection in pregnant women ranges from 2 to 35% (129, 172, 209). Pregnant women with chlamydial infections are at increased risk for adverse outcomes of pregnancy, and postpartum PID. In one study by Martin et al., pregnant women with *C. trachomatis* infection were 10-fold more likely to have outcomes of stillbirth and neonatal death (129). Gestation periods were also significantly shorter in infected women. Other studies have not confirmed this association (72, 73, 215); however, a subpopulation of women with recent or invasive infection indicated by significant immunoglobulin M (IgM) antibody titers against *C. trachomatis* were found in two studies to be at higher risk for preterm delivery and premature rupture of the membranes (73, 209). Diagnosis and treatment of women who are infected with *C. trachomatis* during pregnancy and their sexual partners will prevent these adverse outcomes, as well as postpartum and perinatal disease.

Ideally, women with symptoms or clinical signs should be tested for *C. trachomatis* infection and treated, as should be their sexual partners. However, some conditions such as PID and gonococcal infection require immediate treatment which should include an antibiotic regimen for coverage of *C. trachomatis* infection (30). Presumptive treatment of women with mucopurulent cervicitis and/or other clinical signs is a reasonable approach based on the increased prevalence of *C. trachomatis* infection in women with these conditions, but whenever

possible, this decision should be based on the results of local screening programs or estimates of prevalence (30, 230). The increased availability of accurate and cost-effective diagnostic tests for *C. trachomatis* infection resulted in the reversal in 1993 of an earlier Centers for Disease Control and Prevention (CDC) recommendation to treat presumptively without testing (30). Testing with or without empiric therapy is now encouraged since a positive test can facilitate referral and treatment of sexual partners and can provide guidance for management of patients who do not respond as expected to therapy.

#### Clinical Sequelae of *C. trachomatis* Infections in Infants

*C. trachomatis* is the most common cause of neonatal conjunctivitis and one of the most common causes of pneumonia in early infancy (45, 69). Prophylactic treatment of the eyes with silver nitrate does not prevent chlamydial infection; 15 to 25% of treated infants who were exposed at birth develop conjunctivitis, and 3 to 16% develop pneumonia (161). Symptoms of conjunctivitis usually develop within 2 weeks of delivery, and if the infection is untreated, chlamydial pneumonia can develop at 4 to 17 weeks after delivery (45). These conditions are occasionally difficult to treat, and prolonged hospitalization may be necessary (30). Infants with chlamydial pneumonia are at increased risk for later pulmonary dysfunction and possibly for chronic respiratory disease (231).

#### Clinical Sequelae of *C. trachomatis* Infections in Men

Among heterosexual men, chlamydial infections are usually urethral and up to 50% are asymptomatic (197, 245). When symptoms do occur, usually 1 to 3 weeks following exposure, they are indistinguishable from those of gonorrhea (urethral discharge and/or pyuria). However, compared with gonococcal urethritis, chlamydial urethritis is more likely to be asymptomatic. Nongonococcal urethritis is the most common clinical syndrome seen in men in the United States; 30 to 50% of cases of nongonococcal urethritis are caused by *C. trachomatis* (79). Chlamydial urethritis is presumptively diagnosed by history, urethral discharge, and the presence of four or more PMNs per oil immersion field of a Gram-stained urethral smear or pyuria noted on urinalysis. Untreated infections may lead to arthritis or Reiter's syndrome.

Epididymitis, or infection of the sperm ducts of the testicles, is most often due to *C. trachomatis* or *N. gonorrhoeae* in young, sexually active men less than 35 years of age (18). In older men, epididymitis is more often due to other etiologies associated with urinary tract abnormalities or instrumentation rather than sexually transmitted origins. Unilateral scrotal pain is the primary symptom, and common clinical signs of this infection include scrotal swelling, tenderness, and fever. If urethral symptoms are also present, a sexually transmitted bacterial etiology is likely (154).

Among homosexual and bisexual men, the prevalence of chlamydial urethritis is about one-third of that reported in heterosexual men (199). In STD clinic populations, 4 to 8% of homosexual men have chlamydial infections of the rectum, most of which are asymptomatic (167). When they do occur, symptoms of rectal infection in men and women who practice receptive anal intercourse may include rectal discharge and pain during defecation (91). In homosexual men with chlamydial proctitis, the same symptoms are found, together with tenesmus, diarrhea, and rectal bleeding. A presumptive diagnosis of proctitis is based on history, swelling and friability of rectal mucosa, numerous PMNs in Gram-stained rectal specimens, or, if indicated, rectal biopsy specimens that show PMN infiltration into the lamina propria (154).

#### Lymphogranuloma Venereum

LGV is a systemic disease caused by *C. trachomatis* serovars L<sub>1</sub> to L<sub>3</sub>. LGV is uncommon in the United States but is highly prevalent in parts of Africa, Asia, and South America and occurs in both men and women (156). The LGV serovars of *C. trachomatis* are more invasive than other genital serovars, resulting in infection of the epithelial layers and underlying soft tissue. The primary symptom is a painless genital ulcer or papule. In homosexual men and women who practice receptive anal intercourse, the ulcer is often accompanied by proctocolitis with symptoms resembling inflammatory bowel disease. The most common manifestation of the secondary stage of LGV in men, and the reason most men seek treatment, is inflammation and swelling of the inguinal lymph nodes (156). Women tend to be less symptomatic at this stage: only 20 to 30% of women present with inguinal lymphadenopathy and approximately one-third of women without proctocolitis present with lower abdominal and back pain (156). The secondary stage of infection is characterized by systemic symptoms including fever, malaise, chills, anorexia, myalgia, and arthralgia (154). Untreated infections can lead to late complications including ulceration and hypertrophy of the genitalia, arthritis, and fistula formation involving the rectum, bladder, vagina, or vulva (156).

#### LABORATORY TESTING FOR *C. TRACHOMATIS*

The traditional approach to laboratory diagnostic testing for *C. trachomatis* infections has consisted of cell culture of inocula prepared from urogenital specimens. Antigen and nucleic acid detection technologies were developed during the 1980s and have found widespread application in diagnosis due to lesser demands of cost, expertise, preservation of infectivity during transport, and time required to obtain results. Most recently, nucleic acid amplification technologies have been developed, and application of these tests has taught us that culture is not as sensitive and that the prevalence of *C. trachomatis* infection is higher in most populations than were previously believed. Modern approaches to laboratory diagnostic testing for *C. trachomatis* have become complex, sometimes involving the use of combinations of different test technologies for screening and confirmation. Issues such as ensuring the adequacy of specimen collection, the importance of positive predictive value of tests in low prevalence populations, the need for an improved "gold standard," and the value of screening asymptomatic populations have evolved as important concerns for the present. The following sections describe methods of specimen collection and laboratory testing approved for use today in the United States for diagnosis of *C. trachomatis* infections. This is followed by discussions of some of the current challenges in laboratory testing, such as assessment of adequacy of specimens, confirmatory testing, test selection for screening versus diagnosis, testing for test-of-cure, and testing of sexual assault and abuse victims.

#### Specimen Collection

Proficiency in specimen collection and transport is paramount to accuracy in diagnostic testing for *C. trachomatis*. Both the sensitivity and the specificity of diagnostic tests for *C. trachomatis* have been shown to be directly related to the adequacy of the specimen (82, 95, 137, 159, 187). The lack of specimen adequacy remains a serious shortcoming in many screening programs and research studies. In some of the public health screening programs funded by the CDC for the prevention of infertility due to STDs, as many as 30% of specimens



were inadequate in spite of extensive clinician training. Clinicians must be trained in the proper techniques and reassessed frequently.

Because chlamydiae are obligate intracellular pathogens, the objective of specimen collection should usually be to include the host cells that harbor the organism. Specimens that contain secretions or exudate but lack the cells that harbor chlamydiae (urethral or endocervical columnar cells) are not satisfactory. The most sensitive technologies such as DNA amplification may not require intact chlamydial elementary bodies since in theory only a few gene copies are needed for a positive result; however, a recent study has shown that even a DNA amplification test can be affected by specimen adequacy measured as the presence of host cells (97). Because the requirements of specimen collection and transport are very different depending on whether the viability of the chlamydiae must be maintained, these topics are treated separately.

**Collection and transport of specimens for culture.** Specimens that are collected in a medium designed to maintain the viability of chlamydiae should be assumed to also carry the potential to contain other infectious agents, such as hepatitis B virus or HIV. Universal precautions should be followed when handling such specimens, and laboratory workers should be vaccinated against hepatitis B virus (27).

The most common anatomic site used to obtain specimens for the isolation of *C. trachomatis* from women is the endocervix, which is sampled with a swab or cytologic brush. The type of swab used to collect the specimen is an important consideration since some types have been reported to cause toxicity to cell cultures or to inhibit chlamydial growth within cells (122). Dacron, cotton, rayon, and calcium alginate-tipped swabs may be used, and individual lots should be tested for toxicity to cell culture (122, 127). Swabs with wooden shafts must be avoided. The swab should be inserted into the cervical os past the squamocolumnar junction, about 1 to 2 cm deep, rotated for 15 to 30 s, and removed without touching the vaginal mucosa. Cytologic brushes collect more cells than swabs and thus are thought by some investigators to improve isolation rates (140); however, the brushes are more invasive, induce bleeding, which may inhibit some nonculture tests (1), and cannot be used for pregnant women. In cases where the clinician is well trained in collecting specimens, the use of a cytologic brush is probably not advantageous (96).

Specimens are collected following the removal of secretions and discharge from the cervix, which decreases bacterial contamination and toxicity for culture and improves the appearance of direct fluorescent-antibody (DFA) stains (55, 78). Specimens for *C. trachomatis* detection should also be obtained following any needed specimens for Gram-stained smears or for the culture of *N. gonorrhoeae*. Since 1993, the CDC has recommended that specimens for Papanicolaou (Pap) smears be collected before obtaining an endocervical specimen for *C. trachomatis* culture (30); however, cytologic brushes used for the Pap smear induce bleeding, which may affect the performance of nonculture tests. To avoid this problem, some public health clinics have performed the sampling for the Pap smear after collecting samples for *N. gonorrhoeae* and *C. trachomatis*; however, there are insufficient data to address whether this practice diminishes the performance of Pap smears.

The pooling of a urethral swab specimen with the endocervical swab specimen increases culture sensitivity by 23% (90). The same swab type that is used for male urethral specimens is suitable for this purpose. The swab should be inserted 1 cm into the female urethra, rotated once prior to removal, and

placed in culture transport medium, either together with the endocervical swab or in a separate tube.

The preferred site of sample collection from males is the anterior urethra. A dry swab is placed 3 to 4 cm into the urethra and rotated prior to removal. The subject should not have urinated within the previous hour, since urination will reduce sensitivity of most diagnostic tests by washing out infected columnar cells.

For conjunctival specimens, as for endocervical specimens, any purulent exudate should be removed before collecting epithelial cells by rubbing a dry swab over the everted palpebral conjunctiva. For suspected LGV infections, bubo pus, saline aspirates of the bubo, swabs of the rectum, or biopsy specimens of the lower gastrointestinal tract aided by anoscopy are best. Optimal sites within the gastrointestinal tract are hypertrophic or ulcerative lesions (57).

The likelihood of isolation is optimized if specimens are refrigerated immediately after collection at 2 to 8°C and kept at this temperature during transport to the laboratory. The time between collection and laboratory processing of specimens for culture should ideally be less than 48 h; however, specimens that cannot be processed within this time may be frozen at -70°C until processed. Freezing specimens is likely to result in at least a 20% loss of viability (122, 165). Freezing specimens or cultures at -20°C has a deleterious effect on the viability and antigens of *C. trachomatis* and should be avoided (149).

Traditionally, media for the transport of specimens for chlamydial culture have consisted of variations of medium formulations originally developed for the transport of rickettsiae, most commonly 2-sucrose phosphate (2SP) (64, 146) or sucrose-glutamate phosphate (24). The addition of 2 to 5% fetal bovine serum is favored by some investigators and helps to preserve the viability of chlamydiae in specimens that must be frozen. Antimicrobial agents to which chlamydiae are not susceptible are often added to the transport medium to inhibit or prevent the growth of fungi and bacteria present in clinical specimens. Broad-spectrum antibiotics such as tetracyclines, penicillins, or macrolides should be avoided. Gentamicin (10 µg/ml) or vancomycin (100 µg/ml) for bacterial contaminants and amphotericin B (2.5 to 4.0 µg/ml) or nystatin (25 to 50 U/ml) for fungal contaminants are most commonly used. Viral transport media are not suitable substitutes, since these usually contain penicillin. Recently, synthetic transport media for culture and some nonculture tests have been developed and approved for diagnostic use (M4 transport media [MicroTest, Inc.] and FlexTrans media [Bartels Diagnostics]). Limited studies have found that the new M4 synthetic or "universal" medium was comparable to 2SP for culture and was equivalent or superior to other commercial formulations for nonculture tests such as enzyme immunoassay (EIA) and PCR (175). The lack of standardization of traditional culture transport media formulations and their unsuitability for nonculture tests make the new "universal" media attractive. Both 2SP and the commercial "universal" media are effective for use in the PCR test, making it possible to perform both culture and DNA amplification from a single swab specimen (175, 222).

**Collection of specimens for nonculture tests.** Collection and transport of specimens for commercially licensed nonculture tests should be performed as instructed by the manufacturer and are generally performed as described for culture tests. With the advent of combination tests for both *C. trachomatis* and *N. gonorrhoeae* infections, a single endocervical or urethral swab specimen has been found to be sufficient for testing for both agents once vaginal secretions are removed (81, 114). Commercial diagnostic tests should be used only with the types

of specimens approved for the test as defined in the package insert. Specimens for which nonculture methods have not been developed, approved, or adequately evaluated should be tested by the culture method. Examples of specimens that are generally not acceptable for testing by nonculture methods are vaginal, rectal, nasopharyngeal, or female urethral specimens (30). In cases of suspected sexual assault or abuse, only culture tests should be used regardless of the specimen site (30).

Since infection of the urethra occurs commonly during *C. trachomatis* and *N. gonorrhoeae* infections, copies of target DNA remain present in urine and are detectable by nucleic acid amplification tests. Development of these improved urine tests for the detection of *C. trachomatis* has been a major advance and allows access to asymptomatic populations for purposes of noninvasive screening. In addition to being noninvasive, urine specimens offer considerable advantage because issues concerning the adequacy of collection are less important than for endocervical or urethral specimens. Urine specimens are not, however, free from concerns about adequacy, since test manufacturers specify that urine specimens should be collected as first catch, should be of appropriate volume, and should be obtained within no less than 1 to 2 h of previous urination. In contrast to nucleic acid amplification tests, culture of urine specimens has historically not been useful, and antigen detection tests for urine have been relatively insensitive compared to DNA amplification tests, performing best with urine specimens from symptomatic males (35, 40). Nucleic acid amplification testing with urine specimens from females can also detect cervical infections (111), presumably as a result of the urine washing over mucosal surfaces that have been contacted by cervical or vaginal secretions during collection.

Urine specimens for antigen detection and DNA amplification tests (PCR or ligase chain reaction [LCR]) should be collected as directed by the test manufacturer. Subjects should not have urinated within the previous hour, and females should be instructed not to clean the perineum prior to urinating. The first catch of 10 to 20 ml of urine should be collected in a clean collection cup and refrigerated immediately at 2 to 8°C. However, midstream-catch urine has been reported to be effective for testing by LCR in a recent study of men attending an STD clinic (36). Results of the same study suggested that the time since the last void has less impact on the performance of LCR than on other non-DNA amplification tests. Times since the last void of >3 h dramatically decrease the sensitivity of antigen detection tests for urine from women (188) but not from men (188, 210). Ambient-temperature storage of fresh unprocessed urine should be minimized, since the low pH and high urea content rapidly denature DNA present in the specimen, especially at 25°C and above. Several studies of LCR performed on frozen urine specimens have been reported, but none of these include direct comparisons of the sensitivity of LCR on fresh and frozen urine (10, 42, 111). PCR is currently not approved for use on frozen urine specimens; however, freezing and thawing may improve the sensitivity of PCR for urine specimens in which transient inhibitory factors are present (193). Once processed as specified by the manufacturer, urine specimens can be stored at 2 to 8°C for up to 4 days for PCR. Combination DNA amplification tests that will allow the amplification of both *C. trachomatis* and *N. gonorrhoeae* from the same urine specimen are being developed.

When specimens obtained by both invasive and noninvasive methods are available for testing, the sensitivity of detection may be improved by collection and testing of both types. In a study of endocervical and first-catch urine specimens collected from mostly asymptomatic women, no single test by either culture, DFA testing, PCR, or LCR on a single specimen

identified all of the infected women (41). Results on whether endocervical specimens detect more or fewer *C. trachomatis* infections than do first-catch urine specimens from the same women when both are tested by DNA amplification methods have been discrepant among laboratories (41, 56, 141). These discrepancies could not be explained by differences in the prevalences or symptomatic states of the populations studied. Differences between study sites in adequacy of endocervical specimens or in the site of infection, whether cervical and/or urethral, could account for the discrepancy in results.

A new and promising approach to noninvasive testing is the collection of vaginal introitus or vulval specimens for testing by nucleic acid amplification. Non-nucleic acid amplification test methods have not proven satisfactory with vaginal or extragenital specimens. However, preliminary reports have shown that in one study, PCR testing of vaginal introitus specimens was as sensitive as culture of cervical specimens (234) and in another study, LCR testing of vulval specimens was as sensitive as testing of cervical specimens (201). The adequacy of the cervical specimens, if assessed, was not reported in the latter study. Studies comparing the recovery of *C. trachomatis* DNA from different specimen sites could underestimate the performance of cervical specimens if they are not collected adequately. An intriguing fact is that in one of these studies (234), the vaginal introitus specimens were self-collected by the patients. This method might prove to be useful in settings where women do not normally seek clinical care and could collect and mail in their own specimens for testing. Further studies are needed to determine the utility of these unconventional sampling sites for *C. trachomatis* tests.

**Quality assurance of specimen collection.** Several studies have shown that without quality assurance of specimen adequacy, more than 10% of specimens will be unsatisfactory because they contain exudate and lack urethral or endocervical columnar cells (95, 96, 118). Two methods have been developed for the determination of specimen adequacy by visualization of columnar cells: (i) performing a direct specimen smear, staining with a chlamydia-specific fluorescent antibody followed by a counterstain or by Giemsa stain, and (ii) centrifugation of an aliquot of a specimen in non-detergent-based transport medium, such as that used for culture or EIA, onto a microscope slide and staining by a DFA test (33, 63). For determination of specimen adequacy, many laboratorians find a rapid differential hematology stain such as Diff-Quik (Baxter Diagnostics, McGaw Park, Ill.) easier to read than fluorescent antibody-stained slides due to improved differentiation of cell types. A specimen is considered adequate if it contains at least one columnar or metaplastic cell per slide. A specimen would be determined to be inadequate if any one of the following conditions exist: no cellular components, no columnar or metaplastic cells, or the presence of only squamous epithelial cells or PMNs. Detergent-based transport media such as those conventionally used for DNA probe or DNA amplification tests will lyse chlamydial elementary bodies and columnar cells and cannot be used for assessment of specimen adequacy. Routine performance of DFA tests for *C. trachomatis* makes it possible to continuously monitor specimen adequacy. When using non-DFA tests, periodic cytologic examination of specimen quality is recommended by the CDC to ensure that specimen collection remains adequate over time (30).

### Laboratory Methods

**Culture methods.** Until recently, culture was considered the gold standard for detection of *C. trachomatis* in urogenital specimens because it has a specificity that approaches 100%.

Since culture detects only viable infectious chlamydial elementary bodies and has minimal potential for contamination, it remains the standard for medicolegal issues such as sexual assault and child abuse. The disadvantages of using culture as a gold standard include its relative insensitivity (70 to 85% in the hands of experienced laboratory personnel) compared with DNA amplification techniques (37, 38, 111), the requirement for a stringent cold chain of transportation of specimens, and limited availability to clinicians due to the expense, high level of technical expertise necessary, and time required to obtain results (3 to 7 days). An advantage of culture is that it preserves the organism for additional studies such as genotyping or antimicrobial susceptibility testing. With the advent of DNA amplification techniques, culture tests are used less frequently and are generally now performed only in specialized reference laboratories. Currently, CDC recommends that culture be used for the detection of *C. trachomatis* in urethral specimens from women and asymptomatic men, nasopharyngeal specimens from infants, rectal specimens from all patients, and vaginal specimens from prepubertal girls (30). In cases of suspected sexual abuse or assault, only culture tests should be used to diagnose *C. trachomatis* infection.

Culture is performed by inoculating specimens onto cell culture monolayers. If sufficient numbers of viable chlamydial elementary bodies are present, they infect the cells and grow to form intracytoplasmic inclusions. The inclusions are visualized following 48 to 72 h of incubation by staining with fluorescently labeled antibodies that bind chlamydial lipopolysaccharide (LPS) to recognize all chlamydial species, or major outer membrane protein (MOMP) for *C. trachomatis*-specific recognition. The preferred method for identification of inclusions is to stain infected monolayers with species-specific, anti-MOMP fluorescein-labeled monoclonal antibodies. The direct visualization of inclusions that possess very distinctive morphology contributes to the near 100% specificity of culture tests. Culture results should not be assessed by an antigen detection method such as EIA, since this results in the loss of the high specificity produced when inclusions are directly visualized and introduces the possibility of false-positive results (28).

Historically, other stains including Gram, Giemsa, or iodine staining have been used to visualize chlamydial inclusions in cell culture, but these are not commonly used today due to their lack of sensitivity and specificity compared to fluorescent-antibody staining (185, 200, 206). Although the chlamydiae are classified as gram negative, the Gram stain is of little practical use because the staining reaction is so variable. In some Giemsa stain preparations, *C. trachomatis* can be distinguished from other organisms by the color reaction, morphology, and location of the inclusion; however, such distinction requires an experienced microscopist, and inclusions are easily confused with artifacts created by the staining process. Iodine is a selective stain for glycogen. Since glycogen is present only during certain phases of the developmental cycle of *C. trachomatis*, iodine staining has a low sensitivity and, more importantly, is of no value for the evaluation of endocervical specimens, since normal cervical cells contain glycogen.

Cell monolayers for culture of *C. trachomatis* are grown in dram or shell vials on glass coverslips or in the wells of multiwell cell culture dishes (132). Traditionally, McCoy (195, 200, 242), HeLa 229 (108, 170), and, more recently, BGMK (89, 106) cells have been used to support the growth of *C. trachomatis*. The shell vial method of culture is more sensitive for clinical specimens than the use of 96-well microculture plates when McCoy cells and growth medium with cycloheximide are used (200, 242). In addition, the increased opportunity for cross-contamination with use of 96-well microculture plates

may adversely affect the specificity of culture as compared with the shell vial method.

The susceptibility of HeLa 229 cells to infection is increased by pretreatment with DEAE-dextran (108, 170). The same effect is seen with uncentrifuged McCoy cell monolayers; however, the benefit of DEAE is precluded when centrifugation is used to assist inoculation (195).

Prior to inoculation, clinical specimens should be sonicated to disrupt host cells and inclusions and to separate chlamydial elementary bodies (226). Sonication can also benefit by reducing bacterial contamination. For safety, sonication should be performed only in biological safety cabinets and preferably with the use of sonicators equipped with cup horns so that the specimen tubes can remain capped. If a sonicator is not available, specimens should be vortexed thoroughly or can be disrupted with glass beads.

To inoculate the cell cultures, the overlying culture medium should first be removed and replaced with enough of the specimen in culture transport medium to cover the monolayer and prevent drying during subsequent centrifugation. Following inoculation of the clinical specimen, centrifugation of cell monolayers significantly enhances culture sensitivity (171). This is a result of alteration of the host cell membrane and is not due to sedimentation of chlamydial particles onto the cell monolayer. Centrifugation at  $2,500$  to  $3,000 \times g$  at  $30$  to  $35^\circ\text{C}$  for 1 h increases the number of inclusions in an infected cell monolayer by 100- to 1,000-fold for strains of the trachoma biovar (171). Centrifugation of microculture plates is performed at a maximum of approximately  $1,500 \times g$  due to limitations of the structural stability of the polystyrene polymer. The potential for a cytotoxic effect of the specimen on the monolayer is minimized by removal of the residual specimen after centrifugation by aspiration and overlaying the monolayer with fresh cell culture medium.

The most commonly used growth medium is Eagle's minimal essential medium (EMEM) supplemented with amino acids and vitamins, fetal calf serum (5 to 10%), extra glucose (0.056 M), and L-glutamine (2 mM). HEPES buffer (10 mM) may be added to help maintain a neutral pH. Dulbecco's modification of EMEM without cycloheximide has been reported to yield larger and a higher number of inclusions than EMEM without cycloheximide, but the two media produced similar results when both were supplemented with cycloheximide (76). Use of the same antibiotics as in culture transport medium will reduce bacterial and fungal contamination in the culture. Once inoculated, host monolayers should be incubated in growth medium containing cycloheximide to selectively inhibit host cell protein synthesis. Optimal concentrations of cycloheximide range from 0.5 to 1.5  $\mu\text{g}/\text{ml}$ ; however, manufacturer lots should be tested individually by culture of reference *C. trachomatis* strains.

Blind passages of sonicated monolayers following 48 h of incubation have been reported to result in the recovery of an additional 3 to 10% of isolates (8); however, due to the delay in reporting results, this procedure may not be feasible in most laboratories. A study of another chlamydial species, *C. pneumoniae*, has shown that extended incubation times may be as important for recovery of organisms as blind passage (236); however, it is uncertain if this also applies to the faster-growing *C. trachomatis*.

**Nonculture methods. (i) General considerations and the evolving "gold standard."** Nonculture, non-nucleic acid amplification technologies are based on direct visualization of the chlamydial organism by staining with fluorescein-labeled specific antibodies (direct cytologic examination or DFA), immunohistochemical detection of antigen (EIAs and rapid tests),



TABLE 1. Recent studies evaluating EIA (Chlamydiazyme) detection of endocervical or male urethral *C. trachomatis* infection by using a culture standard or an expanded culture standard

Authors, yr (reference)	No. of subjects (gender)	Prevalence (%)	Sensitivity (%)	Specificity (%)	Expanded standard method <sup>a</sup>	Positive predictive value (%)	Negative predictive value (%)
Biro et al., 1994 (20) <sup>b</sup>	479 (F)	16	65	100 <sup>c</sup>	DFA, consensus <sup>d</sup>	100	95
Chernesky et al., 1994 (37) <sup>b</sup>	447 (F)	6	78	100 <sup>c</sup>	LCR	100	98
Wiesenfeld et al., 1994 (235)	474 (F)	13	43	100 <sup>c</sup>	PCR <sup>e</sup>	100	92
Clarke et al., 1993 (47) <sup>b</sup>	217 (F)	22	78	98.8	DFA	93	94
Warren et al., 1993 (227) <sup>b</sup>	1,037 (F)	5	80	99.3	DFA	86	99
Altaie et al., 1992 (2) <sup>b</sup>	1,240 (F)	9	80	100	DFA	100	97
Mills et al., 1992 (136)	502 (F)	11	91	98	None	86	99
Van Dyck et al., 1992 (219)	670 (F)	15	54	96 <sup>c</sup>	None	70	92
Genc et al., 1991 (63)	245 (F)	12	85	99 <sup>c</sup>	DFA	92	97
	196 (M)	11	62	96 <sup>c</sup>	DFA	70	94
Gaydos et al., 1990 (60) <sup>b</sup>	307 (F + M)	15	77	98	DFA	95	83
Kluytmans et al., 1990 (103)	611 (F)	8	68	96	None	58	97
	280 (M)	14	92	92	None	66	98.6
Moncada et al., 1990 (137) <sup>f</sup>	2,891 (F)	9	81	99	DFA	99	97.8

<sup>a</sup> Method added to culture for expanded definition of true positive. If none, culture alone was used as the standard.

<sup>b</sup> Also compared with other nonculture tests.

<sup>c</sup> With use of blocking antibody reagent.

<sup>d</sup> Two consensus nonculture test positives included in definition of true positive.

<sup>e</sup> PCR used as standard; no culture performed.

<sup>f</sup> Multicenter study.

detection of hybridization to a DNA probe, and nonspecifically by measuring a marker of infection in urine (leukocyte esterase test). The advantages of nonculture, non-nucleic acid amplification technologies include access to testing for laboratories lacking the expertise or facilities to perform culture, reduced requirements for specimen transport, and standardization of technology. Although culture methods lack standardization and vary widely among laboratories, the combination of 100% specificity and the ability to detect only viable chlamydiae as opposed to residual antigen or nucleic acid causes culture to remain, at least for the present, the standard of diagnosis for legal applications such as sexual assault and abuse. Culture also continues to play a role in quality assurance of antigen and nucleic acid detection methods in that it is currently used as a component of the expanded reference standard for evaluations of test performance used by the U.S. Food and Drug Administration (FDA) in licensing new diagnostic tests for *C. trachomatis*.

In the past, numerous investigators have evaluated the performance of nonculture tests for *C. trachomatis* compared to culture as a standard for diagnosis. However, culture alone is an inadequate standard in that it detects only 75 to 85% of infections in excellent laboratories (181). Thus, when nonculture tests are compared with culture alone, and especially when the culture method is not ideal, specificities of the test under evaluation may be underestimated because apparent false-positive results are actually culture misses. Conversely, the sensitivities of nonculture tests may be overestimated when both culture and the nonculture test miss a true infection. The worst-case scenario would be for a test manufacturer to submit clinical site performance data to the FDA from laboratories that all performed culture poorly, thereby making the new test appear much more sensitive than it actually is, since both culture and the new test have missed cases of infection. Unfortunately, the inaccuracy of this performance data would not be apparent and would have ended up in the test manufacturer's package insert if the test became licensed. To overcome these problems, many investigators and the FDA now use an expanded definition of a true positive result based on the

results of a combination of culture and nonculture tests (182). This expanded standard is particularly important to use when evaluating new technology that is inherently more sensitive than existing technology, e.g., nucleic acid amplification tests. Most commonly, the combination of a culture-positive or culture-negative result and a positive DFA test on deposited specimens has been used as a standard for comparison (Tables 1 to 9). With the advent of new nucleic acid amplification technologies with potential for significantly greater sensitivity than culture and DFA test combined, the definition of true positive has been expanded further to include an alternate target DNA amplification test (MOMP-PCR or MOMP-LCR) (Tables 6 to 8). Expanding gold standards in this way has had the effect of lowering the sensitivity of tests under evaluation (86); thus, all evaluations performed in comparison with culture alone (all early studies) have probably overestimated sensitivity. In the test evaluation tables shown, it should be noted that differences exist in the definition of true positives and in the prevalence of infection in study populations and that culture sensitivities may vary widely between studies.

Nonculture, non-nucleic acid amplification technologies such as EIA, DNA hybridization probe, and DFA tests are the most accessible and commonly used diagnostic methods for *C. trachomatis*, but they require additional consideration in interpretation due to reduced sensitivities and specificities relative to culture. Testing of specimens from populations with a low prevalence of infection, generally defined as  $\leq 5\%$ , is particularly prone to error, since the proportion of false-positive results with any test that has a specificity of  $<100\%$  will rise as the prevalence of infection in the population decreases (173). Contributing to this problem is the fact that specimens from most women in low-prevalence populations contain fewer chlamydial organisms than do specimens from most women in high-prevalence populations (115, 121). In one study in which deposits from centrifuged cervical and urine specimens from women in a high-prevalence population were tested by a DFA assay, one-fifth (cervical) to one-half (urine) of the specimens contained fewer than 10 chlamydial elementary bodies (75). Thus, the performance demands for an accurate diagnostic test

TABLE 2. Recent studies evaluating EIA (Chlamydiazyme) detection of *C. trachomatis* in urine from men by using culture with and without nonculture tests of urethral specimens as a standard

Authors, yr (reference)	No. of subjects	Prevalence (%)	Sensitivity (%)	Specificity (%)	Expanded standard method <sup>a</sup>	Positive predictive value (%)	Negative predictive value (%)
Chernesky et al., 1994 (37)	305	18	68	100 <sup>c</sup>	LCR	100	93
Talbot et al., 1994 (210) <sup>b</sup>	318	15	76	100 <sup>c</sup>	DFA	100	96
Moncada et al., 1994 (139) <sup>b</sup>	1,341	15	76	97	DFA	80	96
Backman et al., 1993 (7)	167	34	55	98	None	94	81
Ehret et al., 1993 (54)	540	14	83	96	None	76	97
Genc et al., 1991 (63)	196	11	77	94 <sup>c</sup>	DFA	67	96
Mumtaz et al., 1991 (145)	312	11	78	100 <sup>c</sup>	DFA	100	97

<sup>a</sup> Method added to culture for expanded definition of true positive. If none, culture alone was used as the standard.

<sup>b</sup> Also compared with other nonculture tests.

<sup>c</sup> With use of blocking antibody reagent.

for *C. trachomatis* are considerable, and the interpretation of results for any test with a specificity of <100% should be made with caution, particularly for diagnosis of infections such as *C. trachomatis* that carry serious medical, legal, and social implications.

**(ii) Antigen detection methods.** (a) *DFA test.* The DFA technique adds the considerable advantage of chlamydia-specific antibody staining to direct examination of specimens and remains one of the most useful diagnostic techniques available. With the use of monoclonal antibody reagents specific for the MOMP of *C. trachomatis*, the sensitivity of DFA is 80 to 90% and the specificity is 98 to 99% relative to culture when both are performed optimally (43, 162, 194). Like culture, the DFA test has very high specificity due to its dependence on visualization of the distinctive morphology and staining characteristics of chlamydial inclusions and elementary bodies. The DFA test is the only diagnostic test available that permits simultaneous assessment of specimen adequacy by visualization of cuboidal columnar epithelial cells. An inadequate specimen contains no columnar epithelial cells, excessive cervical mucus, or a predominance of squamous epithelial cells. The DFA test is relatively rapid (about 30 min) and does not require refrigeration of specimens during transport.

The first antibody reagents commercially available for the DFA test were polyclonal and were relatively nonspecific in that they tended to cross-react with other bacteria. Several different monoclonal antibody reagent preparations that are directed against MOMP and LPS antigens are now commercially available. Antibodies to LPS react with all species of

*Chlamydia* and exhibit inferior staining characteristics compared with those of antibodies to MOMP because of the uneven distribution of LPS on the surface of elementary bodies. Monoclonal antibodies to the species-specific epitope of MOMP are thus the preferred reagent for detection of *C. trachomatis* infections by the DFA test. A test kit system for the DFA test that employs anti-MOMP monoclonal antibody and includes positive and negative control slides is commercially available (Microtrak DFA; Behring Diagnostics, San Jose, Calif.); monoclonal and polyclonal fluorescence-labeled antibody reagents are also commercially available from various companies for use in the DFA test.

The DFA test has been used to detect *C. trachomatis* infection in conjunctival (15, 164), urethral (198, 211), and rectal (168) smears and in respiratory specimens from infants (59, 152) but is used primarily for endocervical smears (211, 212, 237). The sensitivity of the test is apparently lower with male urethral specimens than with endocervical specimens (about 70%) for inapparent reasons and, in one study, regardless of the presence of symptoms of urethritis (43).

Although the DFA staining method is rapid, microscopic evaluation of each specimen is laborious and requires highly trained and experienced personnel. In 1986, the College of American Pathologists initiated a survey for proficiency testing of the DFA method of detection of *C. trachomatis*. In a summary of results of the College of American Pathologists program from 1986 to 1992, performance on positive specimens was found to vary with the specimen fixation method, the number of elementary bodies present, the serotype of the an-

TABLE 3. Recent studies evaluating EIA (Microtrak) detection of endocervical or male urethral *C. trachomatis* infection by using an expanded culture standard

Authors, yr (reference)	No. of subjects (gender)	Prevalence (%)	Sensitivity (%)	Specificity (%)	Expanded standard method <sup>a</sup>	Positive predictive value (%)	Negative predictive value (%)
Biro et al., 1994 (20) <sup>b</sup>	479 (F)	16	80	98	DFA, consensus <sup>c</sup>	90	96
Chan et al., 1994 (33)	32,495 (F)	7	96	99	DFA	92	99
	5,113 (M)	15	98	97	DFA	84	99
Skulnick et al., 1994 (193) <sup>b</sup>	993 (F)	2	61	100	PCR, MOMP	92	99
Thomas et al., 1994 (213)	151 (F)	24	74	100	DFA	100	92
Clarke et al., 1993 (47) <sup>b</sup>	217 (F)	22	80	99	DFA	93	94
Altaie et al., 1992 (2) <sup>b</sup>	1,240 (F)	9	94	100	DFA	100	99
Moncada et al., 1992 (138) <sup>b</sup>	1,254 (F)	10	93	99	DFA	94	99
Gaydos et al., 1990 (60) <sup>b</sup>	792 (F)	13	89	96	DFA	80	98
	240 (M)	10	97	98	DFA	91	99

<sup>a</sup> Method added to culture for expanded definition of true positive.

<sup>b</sup> Also compared with other nonculture tests.

<sup>c</sup> Two consensus nonculture test positives included in definition of true positive.



TABLE 4. Recent studies evaluating EIA (Microtrak) detection of *C. trachomatis* in urine from males by using culture with or without nonculture tests of urethral specimens as a standard

Authors, yr (reference)	No. of subjects	Prevalence (%)	Sensitivity (%)	Specificity (%)	Expanded standard method <sup>a</sup>	Positive predictive value (%)	Negative predictive value (%)
Domeika et al., 1994 (52)	184	18	85	100	PCR, MOMP <sup>b</sup>	100	77.5
Moncada et al., 1994 (139) <sup>c</sup>	1,341	15	86	99	DFA	94	97
Talbot et al., 1994 (210) <sup>c</sup>	318	15	78	99	DFA	97	97
Matthews et al., 1993 (130)	304	33	82	98	None	95	92

<sup>a</sup> Method added to culture for expanded definition of true positive. If none, culture alone was used as the standard.

<sup>b</sup> PCR used as standard; no culture was performed. A second PCR was performed with alternate target primers specific for the MOMP.

<sup>c</sup> Also compared with other non-culture tests.

tigen, and the antibody staining product used (237). Performance was also affected by the level of experience of laboratory personnel with the DFA test method (237).

The minimum number of elementary bodies required for a DFA test result to be considered positive has varied in different studies from 1 to 10; however, the presence of 10 elementary bodies or more is generally accepted for the test to be positive for clinical diagnostic purposes. Lowering this cutoff point (160) or performing centrifugation of specimens (212) has been found to modestly increase the sensitivity. Laboratories that reduce the positive cutoff to <10 elementary bodies should consider the possible accompanying reduction in specificity and positive predictive value of the test (116).

Although the DFA test is still used in many laboratories as a primary diagnostic test for *C. trachomatis*, the labor and skill required to perform the test limit its use to low volumes of specimens. As it has lost favor as a primary diagnostic test, the DFA test has become popular as a confirmation test for positive results of other nonculture *C. trachomatis* tests (33, 98) and in discrepancy analysis schemes for DNA amplification tests because of its high specificity (Tables 6 to 8). In confirmation and discrepancy analyses, the cutoff for a positive test is generally 2 elementary bodies. Protocols for performing DFA tests as confirmatory tests vary but generally consist of centrifugation of the original specimen in transport buffer onto a microscope slide followed by fixation and staining by the conventional DFA method (33, 98). This procedure has been most often used to confirm the results of EIA for antigen detection and to assess the adequacy of the specimens for EIA, but it has also been used to examine sediments of centrifuged

urine (111, 202). DFA tests on urine sediment from males may be of limited use other than as confirmatory tests, since their sensitivity relative to DFA testing of urethral swabs has been reported to be <70% (202).

(b) EIA. Diagnostic tests based on immunochemical detection of LPS genus-specific antigen were developed during the late 1980s, and this technology has produced the greatest number of commercial tests available today. In direct EIAs, enzyme-labeled antibodies that recognize all species of *Chlamydia* bind to LPS extracted from elementary bodies in the specimen. In indirect EIAs, a primary anti-LPS antibody (usually murine IgG) is used as the detector reagent, followed by a secondary enzyme-linked, usually anti-murine IgG antibody. LPS is used with this technology since it is more abundant and more soluble than MOMP. The enzyme component of specifically bound antibodies converts a colorless substrate to a colored product that is detected by a spectrophotometric reader. Alternatively, the conjugated enzyme may convert a fluorescence-generating substrate to a signal detected by a fluorescence reader. Total processing time for manual EIAs is 3 to 4 hours. A disadvantage of the EIA is that antibodies to LPS may cross-react with the LPS of other gram-negative bacteria to produce false-positive results (8, 28, 94, 196). To improve the specificity, some manufacturers have developed blocking assays that are used to verify positive EIA results. The blocking test is performed by repeating initially positive EIAs in the presence of monoclonal antibodies specific for chlamydial LPS. The monoclonal antibody competitively inhibits, or blocks, the LPS epitope bound by the detector antibody; thus, a reduced signal with the use of blocking antibody is interpreted as ver-

TABLE 5. Recent studies evaluating DNA probe (PACE 2) detection of endocervical or male urethral *C. trachomatis* infection by using a culture standard or an expanded culture standard

Authors, yr (reference)	No. of subjects (gender)	Prevalence (%)	Sensitivity (%)	Specificity (%)	Expanded standard method <sup>a</sup>	Positive predictive value (%)	Negative predictive value (%)
Altwegg et al., 1994 (3)	222 (F)	10	77	100	PCR	100	97
Biro et al., 1994 (20) <sup>b</sup>	479 (F)	16	67	96	DFA, consensus <sup>c</sup>	75	94
Blanding et al., 1993 (21) <sup>b</sup>	940 (F)	4	76	97	Consensus <sup>c</sup>	52	98
Warren et al., 1993 (227) <sup>b</sup>	1,037 (F)	5	96	99	DFA	74	99
Hosein et al., 1992 (80)	246 (F)	13	94	99	None	94	99
Limberger et al., 1992 (114)	398 (F)	5	95	100	None	100	99
Kluytmans et al., 1991 (102)	482 (F)	9	93	98	PCR	83	99
	260 (M)	13	77	99	PCR	96	96
Iwen et al., 1991 (85)	318 (F)	9	93	98	None	85	99
Lees et al., 1991 (112)	909 (F)	3	86	99	None	96	99
Yang et al., 1991 (241)	426 (F)	10	86	99 <sup>d</sup>	None	91	98

<sup>a</sup> Method added to culture for expanded definition of true positive. If none, culture alone was used as the standard.

<sup>b</sup> Also compared with other nonculture tests.

<sup>c</sup> Two consensus nonculture test positives were included in the definition of true positive.

<sup>d</sup> With use of competition probe assay.

TABLE 6. Recent studies evaluating PCR (Amplicor) detection of endocervical *C. trachomatis* infections by using an expanded culture standard

Authors, yr (reference)	No. of subjects	Prevalence (%)	Sensitivity (%)	Specificity (%)	Expanded standard method <sup>a</sup>	Positive predictive value (%)	Negative predictive value (%)
Altwegg et al., 1994 (3) <sup>b</sup>	222	10	95	96	MOMP, <sup>c</sup> consensus <sup>d</sup>	73	99
Bianchi et al., 1994 (19)	290	4	92	100	MOMP	92	99
Mahony et al., 1994 (126)	770	4	91	100	MOMP	100	99
Skulnick et al., 1994 (193) <sup>b</sup>	993	2	89	99	MOMP	84	99
Bass et al., 1993 (9)	945	8	96	100	MOMP	100	99
Bauwens et al., 1993 (13)	587 <sup>e</sup>	10	88	100	MOMP	99	99
	362	8	64	99	MOMP	95	99

<sup>a</sup> Method added to culture for expanded definition of true positive.

<sup>b</sup> Also compared with other nonculture tests.

<sup>c</sup> A second PCR was performed with alternate target primers specific for MOMP.

<sup>d</sup> Two consensus nonculture test positives are included in the definition of true positive; no culture was performed.

<sup>e</sup> Two separate studies were performed as preclinical and clinical evaluations, respectively.

ification of the initial positive test result. To achieve a confirmed test, a minimum cutoff value of percent inhibition of positive signal with the use of blocking antibody is calculated based on the ratio of optical density of the initial positive test to that of the blocked test. Blocking reagents have significantly improved the specificity of EIAs and are highly recommended when EIAs are used as screening tests (147).

Relatively few studies that compare multiple EIAs directly have been performed (139, 147) (Table 9), and most published studies have compared a single commercial EIA to a standard of culture (Tables 1 to 4). Most EIAs to date are less sensitive than culture (Tables 1 to 4), and some EIAs are less sensitive than others. Without the use of an antibody blocking reagent for confirmation, the specificity varies by product but for most EIA tests is about 97%. With confirmation by antibody blocking reagent, the specificity is improved to >99% (Table 9). Thus, EIA should not be used without confirmation by blocking reagent in screening populations with a low prevalence of infection since the predictive value of a positive test is low. Blocking antibody reagent should be used only with specimens with which it has been validated. An alternative confirmation procedure for EIA is to perform a DFA test on the centrifuged specimen (14, 33, 148).

The first-developed and most extensively evaluated commercial EIA is Chlamydiazyme (Abbott Diagnostics, North Chicago, Ill.). Data shown in Tables 1 and 2 summarize recent performance evaluations for Chlamydiazyme relative to culture and expanded culture standards for endocervical, male urethral, and male and female urine specimens. Based on these studies, the overall sensitivity and specificity of Chlamydiazyme are 73 and 98%, respectively, for urogenital specimens from women and men, and 73 and 97%, respectively, for symptomatic male urine specimens. The sensitivity of Chlamydiazyme

improves with the use of either DFA or blocking antibody for confirmation of "gray-zone" specimens below the absorbance cutoff (98). The use of confirmation with blocking antibody reagent has been reported in several studies to reduce the proportion of false-positive results with Chlamydiazyme (33, 39, 94) and is mandatory when testing urine specimens because of the presence of contaminating gram-negative bacteria (6, 50). The blocking antibody reagent used for Chlamydiazyme has been found in one study to agree well with confirmation by DFA tests with MOMP-specific monoclonal antibody (67), but in another study, 24% of EIA-reactive, blocking test-negative specimens were positive by DFA (244). Since LPS is more soluble than MOMP and significant quantities of LPS are deposited on the surface of infected cells during acute infection (93), the concentration of blocking antibody contained in the commercial reagent may be insufficient to suppress reactivity in some specimens. Regardless of the use of blocking reagent, Chlamydiazyme is reported not to be useful for female urine specimens due to its lack of sensitivity and its high rate of false-positives (39, 99).

Another widely used and more recently developed EIA for *C. trachomatis* is the Microtrak EIA (Behring). The performance characteristics of Microtrak EIA for urogenital and urine specimens are summarized in Tables 3 and 4. Based on these studies, the overall sensitivity and specificity of Microtrak EIA compared with culture or an expanded culture standard are 83 and 98 to 99%, respectively, with endocervical specimens; 97 and 97 to 98%, respectively, with male urethral specimens; and 82 and 99%, respectively, with male urine specimens. The specificity of the Microtrak EIA is in the range of other EIAs and can be improved by confirmation with the Microtrak DFA test (14). The performance of the Microtrak

TABLE 7. Recent studies evaluating PCR (Amplicor) detection of *C. trachomatis* in urine from men and women by using culture with nonculture tests of urethral or endocervical specimens as a standard

Authors, yr (reference)	No. of subjects (gender)	Prevalence (%)	Sensitivity (%)	Specificity (%)	Expanded standard method <sup>a</sup>	Positive predictive value (%)	Negative predictive value (%)
Bianchi et al., 1994 (19)	466 (M)	14	93	99	MOMP <sup>b</sup>	97	97
Skulnick et al., 1994 (193)	394 (F)	3	92	99	MOMP	85	99
Bauwens et al., 1993 (12)	365 (M)	9	96	98	MOMP	82	99
Jaschek et al., 1993 (87)	530 (M)	9	95	99	DFA, MOMP	99	99

<sup>a</sup> Method added to culture for expanded definition of true positive.

<sup>b</sup> A second PCR was performed with alternate target primers specific for the MOMP.

TABLE 8. Recent studies evaluating LCR (Abbott) detection of *C. trachomatis* infection with endocervical or urine specimens by using culture with nonculture tests of endocervical or urethral specimens as a standard

Authors, yr (reference)	No. of subjects (gender, sample site)	Prevalence (%)	Sensitivity (%)	Specificity (%)	Expanded standard method <sup>a</sup>	Positive predictive value (%)	Negative predictive value (%)
Bassiri et al., 1995 (10)	447 (F, cx <sup>b</sup> )	3	87	100	MOMP <sup>c</sup>	100	99
Lee et al., 1995 (111) <sup>d</sup>	1,937 (F, urine)	8	94	99	DFA, MOMP	99	99
Chernesky et al., 1994 (37)	447 (F, urine)	6	96	100	DFA, MOMP	100	99
	305 (M, urine)	18	96	100	DFA, MOMP	100	99
Chernesky et al., 1994 (42) <sup>d</sup>	542 (M, ur <sup>e</sup> )	18	98	100	DFA, MOMP	100	99
	1,043 (M, urine)	14	93	99	DFA, MOMP	98	98
Schachter et al., 1994 (183) <sup>d</sup>	2,132 (F, cx <sup>b</sup> )	11	94	100	DFA, MOMP	100	99

<sup>a</sup> Method added to culture for expanded definition of true positive.

<sup>b</sup> Endocervical swab specimens.

<sup>c</sup> Second PCR performed with alternate target primers specific for the major outer membrane protein.

<sup>d</sup> Multicenter study.

<sup>e</sup> Urethral swab specimens.

EIA has been shown in multiple direct-comparison studies to be superior to that of Chlamydiazyme (2, 147, 148).

Several other EIAs for detection of *C. trachomatis* are commercially available, but they have not been as well evaluated as Chlamydiazyme and Microtrak. All employ an LPS-specific antibody and are thus subject to the same limitations. Examples include IDEIA (Dako Diagnostics, Carpinteria, Calif.) (49, 63, 75, 113, 139, 210), Kallestad Pathfinder EIA (Sanofi Diagnostics Pasteur, Minneapolis, Minn.) (147), Prima EIA (Baxter Diagnostics, Inc., McGaw Park, Ill.) (formerly Northumbria AntigEnz) (46, 176), and Pharmacia EIA (Pharmacia, Franklin, Ohio) (34, 105, 227).

In recent years, there has been considerable interest in the use of EIA tests with urine specimens (Tables 2 and 4). For this purpose, urine must be centrifuged and the sediment must be resuspended in the manufacturer's diluent buffer before the test is performed. The performance of IDEIA has been reported in one comparison study to be similar to that of Microtrak EIA but superior to Chlamydiazyme for male urine specimens (113), whereas in another study of male urine, these three EIAs performed similarly (139). Prima EIA performed on urine sediments from asymptomatic males was reported to be more sensitive than previously reported values for Microtrak or Chlamydiazyme EIA, but the study did not directly compare the tests (176). In general, EIAs have been reported to perform best with urine specimens from symptomatic males and are less sensitive than DNA amplification tests performed on urine specimens (Tables 2, 4, 7, and 8).

EIAs with improved performance characteristics based on advanced technology and/or on automation of procedures have

TABLE 9. Head-to-head evaluation of five different nonculture *C. trachomatis* tests by using an expanded culture standard in 4,553 women with a 4% prevalence of infection<sup>a</sup>

Test	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
PACE 2 (Gen-Probe)	75.3	99.9	99	99
DFA (Syva Microtrak)	74.9	99.8	95	99
EIA (Syva Microtrak)	70.7	99.9	99	99
EIA (Sanofi)	61.1	99.9	99	98
EIA (Chlamydiazyme)	60.6	100	100	98

<sup>a</sup> True positive defined as culture positive or consensus positive in two nonculture tests excluding positive by multiple EIAs unless the cytospin DFA test is also positive. All tests were confirmed by the manufacturer's confirmation test except for the DFA test. Data courtesy of W. J. Newhall V.

been recently approved; examples include Microtrak XL (Syva) (131), Access Chlamydia (Sanofi) (25), IMx Select (Abbott) (40), and VIDAS Chlamydia (BioMerieux, Hazelwood, Mo.) (204); others are under development. In one preliminary study, the IMx Select was reported to perform better than culture (40). It should be noted, however, that in this evaluation the sensitivity of culture was only about 60%, serving as an example of how culture as an inadequate standard can make a test appear more sensitive than it actually is and how culture performance can vary among laboratories. Further studies are needed that compare the performance of multiple approved EIA tests for detection of *C. trachomatis*; no study has yet compared all tests that are currently commercially available. Performance varies widely among the commercially available EIAs. If EIA technology is chosen for use as a diagnostic test for *C. trachomatis* infection, the performance characteristics of individual EIAs should be carefully compared based on head-to-head evaluation studies and preferably with use of an expanded gold standard that includes nucleic acid amplification tests.

(c) *Rapid or "point-of-care" tests.* Rapid tests, also called "point-of-care" or "near-to-patient" tests, for *C. trachomatis* employ EIA technology in formats based primarily on membrane capture or latex immunodiffusion. Rapid tests are performed in physician's offices, do not require sophisticated equipment, and can be completed in about 30 min. Results are read visually and are thus qualitative. Like the traditional laboratory-performed EIAs, the rapid tests use antibodies against LPS that detect all chlamydial species and are subject to the same potential for false-positive results due to cross-reactivity with LPS from other microorganisms.

Commercially available examples of rapid tests include Clearview (Unipath Ltd., Bedford, United Kingdom) (21, 100, 193), TestPack (Abbott) (66, 189), and SureCell (Johnson & Johnson, Rochester, N.Y.) (58, 139). None have been well evaluated, but several studies have shown that, in general, the rapid tests are significantly less sensitive and specific than laboratory-performed EIAs (58, 66, 100, 213, 214) and the PACE 2 DNA probe (21). Reported sensitivities of rapid tests relative to culture range from 52 to 85% for endocervical swabs (21, 58, 66, 100) and 65 to 85% for male urethral swabs (66, 186); specificities are over 95%. It should be noted that published evaluations of rapid tests have been performed by experienced laboratory staff and may not necessarily reflect the performance of the tests when performed by nonlaboratory personnel in physician's offices. Since the rapid tests are designed to be performed by nonlaboratory personnel, quality assurance is



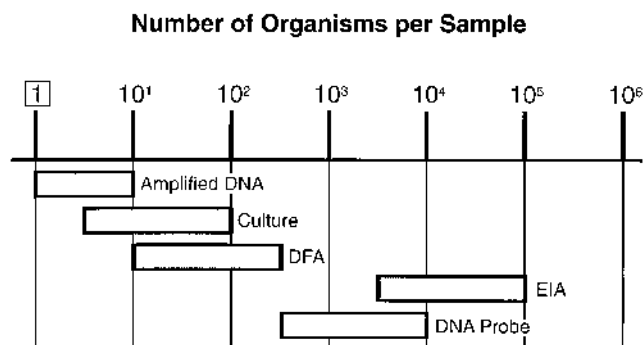


FIG. 1. Relative limits of detection of different technologies used to diagnose *C. trachomatis*. The data are log numbers of chlamydial elementary bodies detected. Data are from references 150, 155, and 239.

essential. Because laboratory-performed tests for *C. trachomatis* are readily available and superior in performance, rapid tests offer an advantage only when results are required immediately for patient management. However, rapid tests should not be used in a low-prevalence population or for asymptomatic individuals due to the potential for false-positive results. The results of a rapid test should always be considered presumptive and, if positive, should be confirmed by a laboratory-performed test.

(iii) **Nucleic acid detection methods.** (a) *DNA hybridization probe.* Currently, there is only a single commercially available DNA probe for detection of *C. trachomatis*: the PACE 2 test (Gen-Probe, San Diego, Calif.). The PACE 2 test is probably the most commonly used test for *C. trachomatis* in public health laboratories in the United States. This test employs a chemiluminescent DNA probe. The probe hybridizes to a species-specific sequence of chlamydial 16S rRNA. Once the DNA-rRNA hybrid is formed, it is adsorbed onto a magnetic bead and the chemiluminescent response is detected quantitatively with a luminometer. Since actively dividing chlamydiae contain up to  $10^4$  copies of 16S rRNA, the PACE 2 test should theoretically be more sensitive than antigen detection systems. In evaluations of analytical sensitivity with purified chlamydial organisms, the DNA probe is about 1 log unit more sensitive than EIAs and can detect approximately  $10^3$  chlamydial elementary bodies (Fig. 1). The clinical sensitivity of PACE 2 is similar to the best EIAs (Table 9). A summary of recent studies comparing the performance of PACE 2 with culture or an expanded culture standard is shown in Table 5. Based on these studies, the overall sensitivity and specificity of PACE 2 are 85 and 98 to 99%, respectively. The sensitivity of PACE 2 relative to a DNA amplification standard has not yet been well evaluated but has been reported to be 77 to 93% (3, 102).

Although the specificity of PACE 2 is relatively high, a confirmation assay was developed by the manufacturer based on probe competition (probe competition assay [PCA]). As expected, the PCA has been reported to confirm a high rate of PACE 2-positive results (114, 203). Similar to the EIA and blocking antibody test, the PCA uses the same target for confirmation that is detected in PACE 2, which, arguably, is less ideal for excluding false-positive results than is an alternative target confirmation test. The sensitivity of PACE 2 for male urethral specimens was reported to be significantly improved by modifications, including the use of a more rigid swab type, a 50% reduction in the amount of transport medium, and performance of the PCA on all specimens with gray-zone results of  $>0.6$  and  $<1.0$  times the cutoff factor (101).

Grossly bloody specimens can produce false-positive results in the PACE 2 test due to autofluorescence; however, use of the PCA will correct the problem by failing to confirm the positive result. Such specimens should be encountered only rarely since the autofluorescent effect that results in false-positive results requires at least 6 to 8% blood in the specimen. Laboratories that do not use the PCA should set up blood standards prepared by a series of dilutions of 1, 2, 4, 6, and 8% blood in saline for purposes of color comparison to bloody specimens. If positive, results of any specimens containing at least 6% blood should be confirmed by PCA. If PCA is not available, the specimen should be considered inadequate for testing and a replacement specimen should be obtained.

Advantages of the PACE 2 test are that it can be used in conjunction with a probe for detection of *N. gonorrhoeae* because a single swab specimen can be used for both tests. Like all of the nonculture tests, the PACE 2 test lacks dependence on cold-chain transportation, and specimens remain stable in storage. Total processing time is 2 to 3 h, and the technical expertise required to perform the test is similar to that of the EIAs. Instruments that automate the testing procedure are available from the manufacturer. Disadvantages of the PACE 2 test are that it is less sensitive than DNA amplification tests and that positive results in low-prevalence populations should be confirmed according to CDC recommendations (also true for EIAs) (30). Confirmation of positive and negative gray-zone results, if performed, adds to the cost of performing PACE 2 tests.

Recently, Gen-Probe developed another DNA probe test that simultaneously detects both *C. trachomatis* and *N. gonorrhoeae* from a single specimen (PACE 2C). A positive result indicates the presence of either or both organisms and requires additional testing specific for the detection of *C. trachomatis* and *N. gonorrhoeae* for identification and confirmation of the precise etiology of infection. In locations where it is required to report cases of these two infections separately to STD control program agencies, the combined PACE 2C test may not offer much advantage. In a preliminary evaluation of PACE 2C with culture as a standard, sensitivities were reported to be  $>89\%$  and specificities were  $>95\%$  for detection of *C. trachomatis* and *N. gonorrhoeae* infections in a high-prevalence population of men and women (134).

(b) *Nucleic acid amplification tests.* The development of tests based on nucleic acid amplification technology has been the most important advance in the field of chlamydial diagnosis since in vitro cell culture techniques replaced the yolk sac for culture and isolation of the organism from clinical specimens. Because nucleic acid amplification is exquisitely sensitive (capable of detecting as little as a single gene copy) and highly specific, it offers the opportunity to use noninvasive sampling techniques to screen for infections in asymptomatic individuals who would not ordinarily seek clinical care. This is a critical advantage, since the majority of chlamydial infections in women and a significant proportion of infections in men are asymptomatic. In addition, DNA amplification tests using noninvasive sampling have been reported to improve upon current screening tests that use invasive sampling by as much as a 30% increase in sensitivity (111).

The most widely known of the DNA amplification technologies is PCR (144, 174). The PCR test employs two synthetic oligonucleotide primers with sequences that are complementary to flanking regions of a specific DNA segment present in the target organism. PCR can be genus, species, group, or strain specific depending on the primer design. A target DNA template in clinical specimens must be made available to the primers by detergent- or heat-mediated lysis of organisms and

denaturation of double-stranded DNA. Once the primers are hybridized to the DNA template (one per DNA strand), they are extended into DNA products of a length determined by the distance between the two primer annealing sites. The primers are extended through the activity of a thermostable DNA polymerase enzyme, most commonly *Taq* polymerase (Perkin Elmer, Norwalk, Conn.). The double-stranded PCR products become templates for a second round of primer annealing upon denaturation. Multiple cycles of denaturation, annealing, and extension of products result in a logarithmic amplification of the DNA target segment. The PCR products, also called amplicons, are detected by colorimetric capture probe assay or by electrophoresis and staining with a DNA intercalating fluorescent dye (e.g., ethidium bromide).

Since the development of noncommercial PCR technology during the 1980s, many investigators and diagnosticians initially had high hopes that this would quickly become the diagnostic test of the future. Unfortunately, many investigators found that the technology, while performing superbly under analytical conditions, did not make the transition to a clinical testing environment smoothly, and it took 8 years for the PCR to finally become an approved commercial diagnostic kit. Early in the process, it was widely feared that false-positive results due to DNA contamination would impede the progress of PCR into the clinical domain. Contrary to this impression, however, experience proved that false-negatives were actually a larger problem than false-positives, due to substances commonly present in human clinical specimens that are inhibitory to the polymerase reaction (1, 119, 218, 240). False-positive results in PCR are most likely to originate in the laboratory from poor practices such as failure to physically separate sites for reagent preparation and DNA product amplification, failure to remove gloves after handling tubes containing DNA controls or PCR products, or sloppy pipetting that results in splashover from one well to another in a microtiter well system. Once a problem with false-positive results is identified, it is easily corrected by improving procedures and decontaminating all surfaces with dilute bleach. Once these steps are taken, carryover contamination is a problem that is rarely repeated by experienced laboratories. On the other hand, false-negative results due to the presence of inhibitors are difficult to correct since they occur unpredictably and are detectable only by using amplification controls (e.g., human  $\beta$ -globin primers), by repeating PCR on diluted specimens, or by spiking duplicate samples with chlamydial DNA. None of these methods is feasible on a routine basis in a clinical laboratory due to costs. It is not known to what extent other DNA amplification technologies will be susceptible to inhibitors in clinical specimens, but this is currently an area of great interest.

Since all nucleic acid amplification technologies detect nucleic acid targets, they do not depend on either viability or an intact state of the target organism for a positive result. This limitation makes the DNA amplification tests subject to results that may be discrepant with culture results due to nonviability of target organisms, and this should be carefully considered when performing test-of-cure applications. Although it has not been well studied, the "window" for the culture-negative, PCR-positive state following therapy with doxycycline appears to last up to 3 weeks (13, 238). After this time, patient specimens become both culture and PCR negative. The ability to detect nucleic acid targets also makes these tests vulnerable to contamination with nucleic acid residues that may persist much longer than viable organisms and are much more likely to be a source of contamination in the laboratory. This limitation has contributed to the fact that culture has not yet been replaced

by nucleic acid amplification tests as the legal standard of diagnosis for forensic applications.

The PCR test for detection of *C. trachomatis* developed by Roche Diagnostics (Amplicor) was the first PCR test to be approved by the FDA in the United States (117). As the first commercial PCR, the Amplicor test has had to pave the way for tests that will follow in terms of comparison with other technologies, general acceptability, and survival of the transitions from research laboratories to private clinical laboratories to public health laboratories. PCR technology is readily available to any investigator who can acquire synthetic oligonucleotide primers, and the resulting "home brew" PCR tests widely published in the literature should not be confused with the licensed Amplicor PCR test, because they vary widely in performance and specificity. Since 1993, Amplicor PCR has been relatively well evaluated for both urogenital and urine specimens, with an overall sensitivity and specificity of 90% and 99 to 100%, respectively (Tables 6 and 7). Amplicor PCR is approved for cervical, male urethral, and male urine specimens.

In the Amplicor test, primers target a 207-bp segment of the cryptic plasmid DNA present in *C. trachomatis* strains. Although *C. trachomatis* strains that lack the cryptic plasmid have been reported (5, 157), the strains have not been cultivable or the lack of plasmid has not been consistently demonstrated by independent laboratories; thus, the clinical significance of such strains remains controversial. In any case, comparison of plasmid PCR and MOMP PCR performed in several laboratories on the same specimens has not revealed any additional plasmid-free strains (13, 117). The MOMP PCR is not available commercially and has been performed primarily by the manufacturer of Amplicor for purposes of discrepancy analysis (12, 13, 115) (Table 6). The cryptic plasmid is present in *C. trachomatis* strains at 7 to 10 copies per genome; thus, the plasmid PCR is more sensitive than the PCR for detection of the MOMP gene that is present at 1 copy per genome (125, 150, 169).

PCR product is detected in the Amplicor test by use of an immobilized oligonucleotide capture probe consisting of sequence that is complementary to the amplified target. Avidin-horseradish peroxidase conjugate is then added and binds to the biotinylated PCR product. Following a wash to eliminate unbound conjugate, peroxidase substrate is added to produce a colorimetric reaction. Results can be read on any spectrophotometric reader at 450 nm. The potential for DNA contamination of Amplicor test results has been minimized by the addition of uracil-*N*-glycosylase (UNG) to DNA templates, included as part of the Amplicor PCR kit. During the formation of DNA products, uracil is substituted for thymine since the Amplicor master mix contains dUTP and lacks 2'-dTTP. UNG catalyzes cleavage of uracil-containing DNA strands at the deoxyuridine residues by opening the deoxyribose chain. UNG has no effect on naturally occurring DNA templates. The opened chains break in the first heating step of the Amplicor test. Broken chains cannot be amplified; thus, any contaminating DNA from previous amplifications (carryover contamination) will not be amplified in the Amplicor PCR test. The UNG enzyme is inactivated at high temperatures; therefore, newly formed PCR products are unaffected by this system. The UNG system will not protect against potential contamination due to natural *C. trachomatis* DNA present prior to amplification, such as from splashover of wells or contact with surfaces contaminated with clinical specimens. Such contamination is more likely to be present in the laboratory than in the clinic setting. A recent study in which 35 different surfaces in a public health clinic in Alabama were swabbed and tested by DNA amplification methods resulted in 3 positive results (80). Two of the

three surfaces contaminated with *C. trachomatis* DNA were telephones, and one was a countertop in an examination room. It is unclear if these data are representative of clinic settings in general; however, it is unlikely that the tips of swabs used to collect patient specimens will contact any surface other than the patient. Thus, if proper specimen collection and handling techniques are observed, false-positive results due to contamination originating from the clinic should not occur.

In several studies on Amplicor PCR, a small number of false-negatives that became positive after storage and repeat testing were reported (9, 13, 117, 126, 193). Although this phenomenon has not been well studied, it is believed by many investigators to be a result of transient inhibition caused in some cases by the relatively high concentration of sodium dodecyl sulfate detergent in the Amplicor specimen transport medium. Although the specimen is diluted with buffer in the first step of swab specimen processing, the inhibitory effect of sodium dodecyl sulfate may not be fully neutralized until after some interval of exposure to the diluent. In any case, recent studies have shown that the sensitivity of Amplicor was improved by substituting a commercial "universal" transport medium that lacks detergent (175) or 2SP medium (222) for the Amplicor transport medium or by transporting dry swabs (97).

Another nucleic acid amplification technology used for the diagnosis of *C. trachomatis* infection is LCR (Abbott Laboratories) (51). The LCR test for *C. trachomatis* was FDA approved for diagnostic use in the United States in late 1995. Unlike PCR, the technology is not readily available for "home brew" applications. In the LCR test, four synthetic oligonucleotide probes (two per DNA strand) anneal at specific target sites on the cryptic plasmid. Each pair of probes hybridize close together on the target DNA template, with only a 1- to 2-nucleotide gap in between. Once the probes are annealed, the gap is filled by DNA polymerase and closed by the ligase enzyme. This two-step process of closing the gap between annealed probes makes the LCR, in theory, more specific than PCR technology. The ligated probe pairs anneal to each other and, upon denaturation, form the template for successive reaction cycles, thus producing a logarithmic amplification of the target sequence. Like PCR, the LCR product is made in a thermocycler. The LCR product is detected in an automated instrument (LCx) that uses an immunocolorimetric bead capture system. The LCx instrument is designed to minimize the risk of carryover contamination by use of a sharp probe that pierces the caps of tubes containing the LCR product, thereby avoiding the need for opening reaction tubes and pipetting DNA products into secondary vessels. At the end of the LCR assay, amplified products are inactivated by the automatic addition of a chelated metal complex and an oxidizing agent.

Early evaluations of the performance of LCR are promising, with an overall sensitivity and specificity of 94 and 99 to 100%, respectively (Table 8). Unlike the EIAs and DNA probe test, both PCR and LCR appear to be highly sensitive and specific for detection of *C. trachomatis* in urine from females as well as males, although the PCR is currently not approved for urine from females. Comparisons of the performance of PCR and LCR have not yet been adequately studied; however, preliminary data suggest that the two tests will perform similarly for both urogenital specimens and urine (41, 56).

Other novel DNA amplification technologies are evolving for *C. trachomatis* as well as for other infectious agents such as *N. gonorrhoeae*, *Mycobacterium tuberculosis*, and HIV (see the section on future directions, below).

**(iv) Direct cytologic examination.** Demonstration of typical intracellular chlamydial inclusions in direct smears can be useful in the diagnosis of *C. trachomatis* infections under certain

conditions, particularly for acute inclusion conjunctivitis of neonates (181), and has the advantage of allowing simultaneous detection of the intracellular diplococci typical of gonococcal ophthalmia neonatorum. Direct cytologic testing by Giemsa stain of fixed smears is rapid and sensitive (>90%) for detecting chlamydial conjunctivitis in newborns (181); however, this method is not recommended for diagnosing conjunctivitis or genital infections in adults due to its lack of sensitivity (57, 181).

Historically, the Giemsa stain has been used for cytologic examination of conjunctival smears (57, 179). Conjunctival scrapings are air dried on microscope slides, fixed in methanol, and stained for 1 h with freshly prepared Giemsa stain. The slides are rapidly rinsed in 95% ethanol, dried, and examined under 400 $\times$  power with confirmation of typical inclusion morphology under 1,000 $\times$  oil immersion. Control slides consisting of *C. trachomatis*-infected and uninfected cell cultures should be processed and examined in parallel. The slide should contain at least 10<sup>3</sup> epithelial cells for the specimen to be considered adequate. Inclusions will appear purple if they contain elementary bodies and more basophilic (blue) if they contain reticulate bodies. Cell nuclei will be pink. Under dark-field microscopy, inclusions will appear yellow and very granular. Recognition of chlamydial inclusions requires considerable expertise and is not recommended for laboratories that receive specimens only occasionally.

**(v) Leukocyte esterase test.** The leukocyte esterase (LE) test is a rapid dipstick test for use with urine specimens. The LE test is designed to detect urinary tract infections by detecting the enzyme produced by PMN, an inflammatory cell that accumulates in urine during an infection. The dipstick holds an absorbent patch containing indoxylcarbonate ester, which forms a purple color when hydrolyzed by LE. Thus, the LE test can diagnose urethritis but cannot identify the specific cause of infection. Positive LE test results occur with infections caused by a number of different agents including *C. trachomatis* and *N. gonorrhoeae*.

The sensitivity of the LE test for detection of *C. trachomatis* infection varies widely from 31 to 100%, and specificities range from 83 to 100% (88, 123, 133, 153, 190, 232). The LE test has performed best as a screening test for adolescent males and, according to most reports, should not be used for testing specimens from women or older men due to unsatisfactory performance (133, 153). In at least one study, the LE test was shown to be a better predictor of positive gonococcal cultures than of chlamydial infection in adolescent and young men (88). In contrast, two studies by a group of investigators in Canada in which the LE test was compared with EIA and PCR have shown the LE test to be useful in screening for asymptomatic urethritis in an urban hospital population of males aged 16 to 35 years (190) and in developing countries (123). The reasons for such discrepant results are unclear; however, it is possible that they are due to differences in the population studied, the volume of urine tested (presumably, larger volumes have a dilution effect), or the time of day that urine was voided (the first void of the day would contain the highest esterase activity). It should be noted that a positive LE test result requires additional testing specific for detection of *C. trachomatis* and *N. gonorrhoeae* to determine the etiology of the urethritis.

**Serologic tests. (i) General considerations.** Serologic tests are generally not useful in the diagnosis of genital tract infections caused by *C. trachomatis*. This is because antibodies elicited by *C. trachomatis* infection are long lived and a positive antibody test will not distinguish a previous from a current infection. The use of convalescent-phase serum specimens can be helpful, since a fourfold increase in antibody titer is predic-



tive of an active infection; however, it can take up to 1 month or longer for antibody titers to rise, and the delay is not appropriate to the time frame necessary for therapy and management of patients. The presence of IgM antibodies is an unreliable marker of acute infection in adolescents and adults since IgM is often not present, presumably because the person has been infected previously with *C. trachomatis* or possibly with another chlamydial species such as *C. pneumoniae* and is generating an anamnestic response to the most recent exposure. In spite of test manufacturers' claims, no currently available serologic test of a single serum specimen will provide conclusive evidence of current infection with *C. trachomatis*.

The two exceptions to the general lack of value of serologic tests are the IgM-based diagnosis of chlamydial pneumonitis in infants and the diagnosis of patients with LGV. Since LGV is a systemic disease, antibody responses are stronger and may develop sooner than is the case for localized genital tract infections. For such individuals, a fourfold rise in the microimmunofluorescence test (MIF) titer to LGV antigens or a complement fixation (CF) test titer of  $\geq 32$  supports a presumptive diagnosis of LGV (156). With LGV, however, it can be difficult to demonstrate rises in titer, since the natural history of the disease is such that the patient often does not seek clinical care until after the acute stage of infection.

Serologic tests that have been developed for detection of antibodies to *C. trachomatis* for diagnostic purposes are the CF test, the MIF test, and tests based on EIAs.

(ii) **CF test.** The CF test remains the most commonly used serologic test in public health laboratories for the diagnosis of psittacosis and LGV. The CF test detects complement-fixing antibodies that recognize the genus-specific LPS antigen and is not specific for any one chlamydial species.

The CF test is technically demanding and can be performed in a test tube or microtiter plate format. The test tube system is thought to be more accurate, but the microtiter system is easier for screening large numbers of serum samples. Reagents should be standardized and positive results should be confirmed in the test tube system. Historically, high-quality antigen for the CF test is produced in embryonated hen eggs infected with *C. psittaci* (163). Infected egg yolk is extracted with an organic solvent to enrich for the carbohydrate-containing LPS antigen called group antigen. Since this procedure is categorized as a Biosafety Level 3 biohazard (217), high-quality CF antigen is not readily available. Guinea pig complement used in the CF test must be carefully screened for the presence of anti-chlamydial antibodies, since *C. psittaci* is a common agent of inclusion conjunctivitis in guinea pig colonies (177).

A single CF titer of  $\geq 256$  strongly supports the diagnosis of LGV, and a titer of  $\leq 32$  rules out this diagnosis. Although a single serum specimen with a CF titer of  $\geq 64$  can be consistent with a diagnosis of infection due to any of the chlamydial species, the predictive value of this titer on a single specimen is low. Treatment with antibiotics can delay or diminish the production of CF antibody (135) and will reduce the sensitivity of the test.

(iii) **MIF test.** The MIF test, developed in the early 1970s as a tool for epidemiologic research on chlamydial infections (224, 225), is the most sensitive of the serologic tests for *Chlamydia* species and the only serologic test that detects species- and serovar-specific responses. It is the diagnostic test of choice for chlamydial pneumonitis in infants by detection of IgM antibodies. The test is highly reliable in all populations for detection of a prior exposure to chlamydiae by the presence of IgG antibodies. The MIF test is also sensitive for the detection of IgM, although this isotype is usually not present in patients with genital tract infections unless they represent the first ex-

posure of the individual. The primary disadvantages are that the MIF is quite laborious and antigens are costly and available only on a limited basis. For these reasons, the MIF test is performed in only a few research laboratories.

The species and serovar specificity of the MIF test is believed to be due to antibodies that react with species- and serovar-specific epitopes in chlamydial MOMP. The 15 serovars of *C. trachomatis* were originally described by their reactivity in the MIF test (224). More recent methods for serotyping *C. trachomatis* strains have confirmed the original 15 serovars and established the existence of several new serovariants (110, 207).

The antigens for MIF consist of formalin-fixed elementary bodies grown in an egg yolk sac or in cell culture and then suspended in a 1 to 3% suspension of yolk sac in saline. At present, MIF antigens for research are commercially available only from the Washington Research Foundation (Seattle, Wash.). The antigens are dotted with a special pen tip onto a microscope slide in a specific pattern, one dot per serovar or serovar pool. Serovars that are antigenically related can be pooled to simplify the MIF test (207). The four commonly used serovar pools are [L<sub>1</sub>, L<sub>2</sub>, and L<sub>3</sub>], [B, E, and D], [C, H, I, and J], and [G, F, and K]. Each dotted slide contains all serovars to be tested. Slides are fixed with acetone and can be stored frozen at this stage. Serial dilutions of patient serum are placed on the antigen dots and incubated for 30 min to 1 h. The slides are then washed, dried, and stained with a fluorescein-conjugated anti-human class-specific Ig. The titer of the conjugate must be predetermined with a positive reference serum standard to determine the optimal concentration for use. The slides are then washed, dried, and read by visualization under a UV-illuminated microscope. End point titers are read as the highest dilution showing an even distribution of bright-apple-green-fluorescing elementary bodies. The serovar-specific response is determined on the basis of which antigen yields the highest positive end point dilution.

Positive and negative reference sera should always be included in each MIF test and should react consistently within a reference range that is preestablished by the laboratory, usually within 1 dilution of the expected titer. The MIF test primarily detects serovar-specific responses, but if the antigen includes chlamydial reticulate bodies, chlamydial genus reactivity will also be detected (243).

(iv) **EIA for chlamydial antibodies.** Several tests that detect chlamydial antibodies by an EIA, also called ELISA, are commercially available. These tests detect reactivity to genus-specific antigen, or LPS, of chlamydial elementary or reticulate bodies. As for other serologic tests, measurement of the EIA titer on a single serum specimen will not distinguish between previous and current infections. In addition to this limitation, the EIA detects antibodies to all chlamydial species and is not specific for antibodies to *C. trachomatis*. Since the prevalence of antibodies to *C. pneumoniae* is 50 to 70% for most populations in the United States (65), a positive result in the EIA may represent a cross-reaction with antibodies to *C. pneumoniae*. In a study of an STD clinic population in England, it was reported that antibodies to *C. pneumoniae* and *C. psittaci* accounted for up to half of all chlamydia-specific IgG (143).

Although EIAs for chlamydial antibodies are less sensitive than is the MIF test, the detection of IgM in infants with chlamydial pneumonitis by EIA may be nearly as sensitive as by the MIF test (124). Other investigators have reported a lack of reliability in detecting IgM by EIA (181).

There have been relatively few published studies on commercially available EIAs. Until further information is available on the clinical utility of this technology, EIAs should be used

TABLE 10. Guidelines for diagnosis of infections with non-LGV strains of *C. trachomatis*<sup>a</sup>

Definitive (requires 1 or 2)
1. Isolation and confirmed identification of <i>C. trachomatis</i> in tissue culture from cervical, rectal, or urethral exudate and identification of characteristic intracellular inclusions
2. Identification of <i>C. trachomatis</i> by one of the following methods and confirmation by a second culture or nonculture test method
a. Identification of the organism by DFA test of exudate
b. Detection of antigen by EIA of exudate
c. Detection of nucleic acid from exudate by DNA probe or DNA amplification technique <sup>b</sup>
Presumptive (requires 1 and 2)
1. Presence or absence of clinical symptoms (e.g., mucopurulent cervicitis, urethritis, epididymitis, PID)
2. Detection of <i>C. trachomatis</i> by a nonculture test
Suggestive (requires 1 and either 2 or 3) <sup>c</sup>
1. Clinical symptoms (e.g., mucopurulent cervicitis, urethritis, epididymitis, PID)
2. Exclusion of other causes of discharge or exudate (e.g., gonorrhea)
3. Sexual exposure to a person infected with <i>C. trachomatis</i> or recently diagnosed with nongonococcal urethritis, mucopurulent cervicitis, or PID

<sup>a</sup> Modified from reference 29.

<sup>b</sup> At present, there are no standardized procedures for confirmation of DNA amplification methods.

<sup>c</sup> Testing is recommended to determine a specific diagnosis and to expedite management of sexual partners.

only for serosurveys of high-risk populations or for the detection of IgM in infants with chlamydial pneumonitis when the MIF test is not available.

(v) **Other serologic tests.** Other serologic tests have been commercially developed. One example is the ImmunoComb Chlamydia bivalent test (Organics). ImmunoComb is a solid-phase EIA or dot blot assay incorporating LPS-extracted *C. trachomatis* and *C. pneumoniae* elementary bodies. ImmunoComb test results have been reported in a single study to correlate well with those of the MIF test (44). Until further evaluations are performed, the clinical utility of new serologic tests is uncertain.

### Confirmatory Testing

The most recent guidelines from CDC on prevention and management of *C. trachomatis* infections recommend that all positive results of nonculture tests performed on low-prevalence or low-risk populations be confirmed (30). This is due to the low positive predictive value of nonculture tests in low-prevalence populations. The positive predictive value of a test is the likelihood that a positive test represents a true infection, and this value depends not only on the sensitivity and specificity of the test but also on the prevalence of infection in the population. For example, in a high-risk population with a prevalence of 20%, a test with a sensitivity of 90% and a specificity of 98% will have a positive predictive value of 92%. In contrast, the very same test used in a low-risk population with a prevalence of 5% will have a positive predictive value of only 70%. This means that 3 of 10 positive tests will be false-positives. This represents a serious drawback when testing for an STD with medical, legal, and social implications.

Confirmation of positive results increases the specificity and, in turn, the positive predictive value of nonculture tests. Con-

firmation of nonculture tests is required for a definitive diagnosis of *C. trachomatis* infections. The *CDC Clinical Practice Guidelines* define diagnosis of *C. trachomatis* in three categories: suggestive, presumptive, and definitive (29; modified in Table 10). Suggestive diagnosis requires the presence of clinical symptoms of infection and either exclusion of other causes or sexual contact with a partner who is infected. A nonculture test gives a presumptive diagnosis. Definitive diagnosis requires a positive culture test or a positive nonculture test that has been confirmed by a second test method.

Three methods of confirming nonculture tests have been recommended by CDC in order of preference (30). The first method is to confirm by performing a culture test and detecting the growth of *C. trachomatis* with a fluorescein-conjugated species-specific antibody. The second method is to perform a second nonculture test that identifies a different target than that identified in the first test. For example, an EIA could be confirmed by centrifuging the specimen and performing a DFA test with a MOMP-specific antibody on the sediment. The third method is to use a blocking antibody for EIA or a competitive probe for the DNA probe that confirms a positive by preventing attachment of labeled antibody or probe. The third method is the least desirable since it depends on the same target as the initial test for confirmation. However, since test manufacturers are now making this type of confirmation method commercially available, it is widely used and is reported to be effective in increasing the specificity of tests (94, 114, 136, 137, 203).

When developing clinician-laboratory protocols for testing for *C. trachomatis*, the prevalence of infection in the population to be tested should be considered. If the population has a high prevalence of infection (>5%), initial positive results do not need confirmation unless requested by the clinician on the basis of an individual patient assessment of risk and the consequences of a positive test result (30). If the population has a low prevalence of infection ( $\leq 5\%$ ), an initial positive nonculture test result should be confirmed on all individuals (30). Unfortunately, the prevalence is not usually known. Many clinicians do not indicate the prevalence or risk level of the population for a particular person being tested, either because laboratory requisition slips do not request this information or because they may not be familiar with these recommendations. In such cases, the ability to confirm positive results on the same specimen without requiring a return visit by the patient lends an advantage.

If confirmation of a positive result cannot be completed rapidly on the same specimen, these patients and their sexual partners should be treated while waiting for the results of the confirmatory test (30). The clinician should postpone treatment pending a confirmation of the initial positive result only if the adverse consequences of a false-positive test outweigh the risks of transmission and disease progression (30). The probability of the patient returning for treatment on a follow-up visit should be considered in this decision. Since nonculture tests for *C. trachomatis* vary in sensitivity, the possibility of a false-negative confirmation test should also be considered.

At the time that the guidelines for confirmation of nonculture tests were developed by the CDC, DNA amplification tests were not yet approved for diagnostic use. Thus, the recommendations as written do not apply to PCR or LCR. To date, experience with PCR and LCR tests for the diagnosis of *C. trachomatis* infections has been limited primarily to research laboratories. Since the specificity of both PCR and LCR in research settings is at least 99%, the positive predictive value of these tests is high in both low- and high-prevalence populations (Tables 6 to 8). At present, no method of confirmation

for PCR or LCR has been approved for use. The only research tests reported so far for confirmation of PCR and LCR are the MOMP PCR and MOMP LCR, which are performed only by the manufacturers, or home brew MOMP PCR methods developed in research laboratories; none are generally available, and none are approved for diagnostic use. Due to the substantially greater sensitivity of PCR and LCR than of non-DNA amplification tests, none of the currently approved tests represent a good choice for purposes of confirming PCR or LCR. The best method may be to confirm PCR by LCR or vice versa. In many laboratories, this will not be feasible due to costs. In such cases and until a need for confirmation of positive nucleic acid amplification tests develops through documentation of multiple false-positive test results, positive PCR and LCR tests probably do not need to be confirmed. However, PCR and LCR tests remain under evaluation, and recommendations from the CDC regarding a need for confirmation of DNA amplification tests could develop as further data become available.

### Issues in Test Selection for Screening versus Diagnosis

Because of the considerable costs of untreated infections and their sequelae, screening for *C. trachomatis* infection has been found to be cost-effective. Due to the potential for false-positive results and the cost of detecting infections in low-prevalence groups, the CDC currently recommends that screening be limited to women who are at a higher than average risk for infection. CDC recommendations for screening include all women attending adolescent care or STD clinics, family-planning clinics, or prenatal clinics and women who are undergoing elective abortion or residing in detention facilities. Specific screening criteria recommended by CDC for selective testing for chlamydial infection in other facilities are shown in Table 11. Pregnant women should be screened only if they are in a risk category as defined by the screening criteria in Table 11.

Faced with a wide array of commercial test technologies and manufacturers, many laboratory directors and clinicians find that the selection of tests for detection of *C. trachomatis* is difficult if not bewildering. A recent issue of *CAP (College of American Pathologists) Today* listed 27 commercial products for laboratory testing for *C. trachomatis* in the United States alone (107). The availability of culture, the former gold standard of diagnosis, is diminishing due to expense, labor, and the demand for technical expertise. While culture is primarily available only in reference laboratories, nonculture tests have become popular in clinical laboratories due to decreased cost, shorter time for results, standardization, and reduced requirements for specimen transport as compared with culture. The relative analytical sensitivities of the various technologies currently available for detection of chlamydial elementary bodies are shown in Fig. 1. A comparison of the clinical sensitivities of the nonculture technologies relative to a standard that includes DNA amplification tests is shown in Fig. 2.

When choosing a test for screening asymptomatic persons, it is important to consider that due to the low prevalence of infection in such populations, the positive predictive value will be low unless the specificity of the test is high. Nonculture tests such as the DFA test, EIA, or DNA probe must be confirmed in low-prevalence populations if positive, and this should be factored into the cost of screening. With appropriate confirmation by a second test or a blocking method, the best of the EIAs and the DNA probe are highly specific and relatively sensitive; thus, they represent good choices as screening tests when high volumes of tests are processed, when cost is a

TABLE 11. Screening criteria recommended by the CDC for identification of women who should be tested for *C. trachomatis* infection<sup>a</sup>

Women with mucopurulent cervicitis
Sexually active women less than 20 years of age
Women 20–24 years of age who meet either of or women over 24 years of age who meet both of the following criteria:
Inconsistent use of barrier contraception
New or more than one sexual partner during the last 3 months

<sup>a</sup> Modified from reference 30.

driving factor, and when urogenital specimens are readily obtainable. Antigen detection methods are cost-effective in screening for *C. trachomatis* cervical infection in populations with infection prevalences of 2 to 7% (158, 191, 216). When selecting a particular EIA or choosing between EIA and DNA probe, data from studies in which multiple tests have been compared head-to-head relative to a nucleic acid amplification standard should be considered more accurate than data from different studies that compare only a single test to a standard (Table 9) (139, 147). This is because standards of infection, especially culture methods, tend to vary considerably among laboratories.

For individuals from whom invasive specimens are not available, or if the highest sensitivity of detection available is desired, the nucleic acid amplification tests should be the test of choice. In general, non-DNA amplification tests have not performed well with urine specimens from asymptomatic individuals. Testing of urine specimens from asymptomatic individuals by nucleic acid amplification tests has broadened *C. trachomatis* screening to new levels, especially in asymptomatic men who are not willing to provide urethral swabs for testing. Even if noninvasive sampling is not an important issue, for example, in women who present annually for Pap smear screening, PCR or LCR tests on an endocervical specimen are the most sensitive and specific tests that can be offered. Unfortunately, they are also the most expensive. However, as new amplification tests are developed and competition increases, costs are likely to come down. The fact that many test manufacturers have established discounted public health domain pricing has been extremely helpful, and PCR and LCR are included among these. At this writing, several regional and state laboratories are now using PCR for public health testing for *C. trachomatis* infection.

Screening strategies based on using combinations of tests on urine specimens have been developed for asymptomatic males. In one study, EIA of urine testing positive with a leukocyte esterase dipstick (LE-EIA) was found to be most cost-effective for asymptomatic males (62). The same investigators later found that when DNA amplification assays were substituted for EIA in the same screening strategy, cure rates were 13% higher and costs were lower than those of LE-EIA screening above a prevalence of 4% (61).

When choosing a test for diagnosis of symptomatic persons, it should be considered that the prevalence of infection is high in this population and that positive predictive values of laboratory tests with relatively high specificity are also high. Confirmation of positive results is not necessary for symptomatic persons. Since antigen loads are typically high in symptomatic patients, any of the best EIAs, DNA probe, or DFA are good test choices. Nucleic acid amplification tests, such as PCR or LCR, are also excellent choices for use in symptomatic patients.

If there are legal implications to a positive test result, the



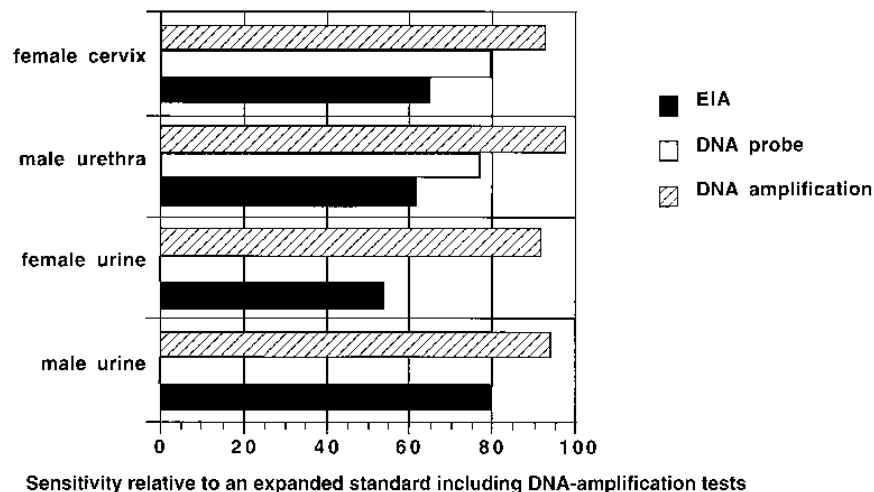


FIG. 2. Clinical sensitivities of different technologies used to detect *C. trachomatis* relative to an expanded standard that includes DNA amplification tests. Values shown for urine from females and males are relative to results of tests performed on endocervical or male urethral specimens, respectively. Values shown for EIA reflect the range observed for different test manufacturers, as shown in Tables 1 to 4. Sensitivity data are averaged from references listed in Tables 1 to 8.

only choice of laboratory test is culture, since it remains the standard for legal purposes and nonculture tests have not been well evaluated on some of the specimens that are tested in cases of suspected child abuse.

#### Laboratory Testing for Test-of-Cure

Recommendations for the treatment of genital chlamydial infections have been published by the CDC (30, 31). Treatment failure as defined by positive cultures 7 to 14 days after therapy is uncommon if compliance with recommended regimens is complete. Routine test-of-cure visits during the immediate posttreatment period are not recommended. However, if laboratory testing for test-of-cure applications is done for research or indicated for other special purposes, special considerations should guide the selection of laboratory tests. Historically, only culture has been used for this purpose and is performed 7 to 14 days after completion of therapy. Nonculture tests should be used with great caution, since there is likely to be an interval after starting therapy during which culture tests will be negative and antigen and nucleic acid detection tests will remain positive. This is thought to be a result of the antibiotic therapy, in which chlamydial organisms are initially killed but residual chlamydial antigen and nucleic acid remain at the site of infection and are not cleared by the immune system for some time after therapy is completed. For doxycycline, this interval has been reported to last up to 3 weeks after completion of therapy (13, 238). This interval has not been defined for therapy with azithromycin.

The presence of residual chlamydial antigen and nucleic acid in the absence of viable organisms can result in a positive test, depending on the test used. Such a positive result can be misinterpreted as a treatment failure. Treatment failures can be defined only on the basis of a positive culture or on the basis of a nonculture test of a specimen collected after all residual chlamydial target antigen or DNA is cleared. The latter method is complicated by the possibility of new sexual contacts and/or reinfection during the interval prior to clearance of residual targets.

Since the prevalence of infection in test-of-cure applications is extremely low, only tests with a positive predictive value near or equal to 100%, e.g., culture, should be used. Although the

nucleic acid amplification tests, such as PCR or LCR, also have high positive predictive values, they are less useful for test-of-cure applications than culture unless they are used after an interval of 3 weeks following therapy as a result of the potential for ambiguous results from detection of residual DNA targets following clearance of infection.

#### Laboratory Testing for Sexual Assault and Abuse Victims

The diagnosis of one or more of the STDs is considered a marker of sexual abuse in prepubertal children without a history of prior sexual activity and if perinatal transmission can be excluded. In three studies of sexually abused children who were routinely cultured for *C. trachomatis*, rectogenital infections were identified in 2 to 13% (70, 83, 84). An important confounding variable is that perinatal maternal-infant transmission resulting in vaginal or rectal infection has been documented to produce prolonged infection up to 3 years of age (68).

Diagnosis of *C. trachomatis* infections in sexual assault victims and in children suspected of being victims of sexual abuse requires special procedures and considerations. It is important to correctly identify the STD agent(s) from such cases for several reasons: to provide appropriate therapy to the victims and to prevent complications of infection; to initiate appropriate investigations of sexual abuse when an STD pathogen is diagnosed in a child; and to culture individuals who have had contact with the child to match pathogens that would indicate those individuals who should be considered suspects in the investigation (104). Due to the nature of sexual abuse, procedures and sites for collection of clinical specimens vary from sexually active adolescents and adults. Genital examination under anesthesia is sometimes indicated for collection of specimens. Table 12 lists the recommended sites for the recovery of *C. trachomatis* in neonates and children. Following perinatal transmission, the eye, nasopharynx, vagina, and rectum can all be colonized for up to 3 years if the child is untreated. Due to the presence of maternal antibody, the sensitivity of diagnostic tests may be lowered from birth to 3 months or longer (92).

In cases with legal implications due to assault or suspected sexual abuse, culture is the only recommended method of

TABLE 12. Recommended sites for the recovery of *C. trachomatis* in neonates and children<sup>a</sup>

Patient category	Site
Girls	Vagina
	Anal canal
	Oropharynx
Boys	Urethra
	Anal canal
	Oropharynx
Neonates	Conjunctiva
	Nasopharynx
	Orogastric fluid
	Urethra (if there is a discharge)
	Vagina

<sup>a</sup> Modified from reference 92.

diagnosis of *C. trachomatis* infection. It is also the recommended method for detection of *C. trachomatis* in the urethra of asymptomatic boys, the anorectum of patients of all ages, the nasopharynx of infants, and the vagina of prepubertal girls because of the lack of sufficient evaluation of the performance of nonculture tests, including nucleic acid amplification tests, on these specimens (30). Specimens must be transported in medium specifically designed for culture of *C. trachomatis*, maintained throughout at 4°C, and inoculated into cell culture within 24 h of collection. If initial exposure has been recent, a sufficient number of organisms to produce a positive culture result may not be present. A follow-up examination with collection of additional specimens should be scheduled approximately 2 weeks later. Culture results must be obtained with the use of species-specific monoclonal anti-MOMP antibody reagents, since *C. pneumoniae* can cause respiratory infections that are not sexually transmitted. Optimal processing of specimens for culture testing requires access to laboratories with considerable experience in this method. It is not sufficient to diagnose *C. trachomatis* infection clinically or to use presumptive or definitive criteria other than culture that may be used when identifying infection in a sexually active population.

Many hospitals and laboratories have standard procedures for screening victims of suspected sexual abuse. Such protocols should include a list of sexually transmitted pathogens and the procedures for collecting and transporting specimens and identifying pathogens. Specific details will vary depending on state laws. For example, many states require that specimens be transported by a police officer to maintain the chain of evidence. In any case, original specimens and isolates should be preserved as evidence.

### FUTURE DIRECTIONS

Laboratory testing technologies will continue to be refined, and performance in sensitivity and specificity of available tests will most probably improve to the stage whereby noninvasive testing will provide rapid definitive diagnosis of sexually transmitted chlamydial infections. In early 1994, the Rockefeller Foundation offered a \$1 million prize to anyone who could develop a rapid and accurate test for *C. trachomatis* and *N. gonorrhoeae* infections that is applicable in resource-poor developing countries. The criteria for the award are stringent, but the challenge should hasten the arrival of the next significant advance in the field: a rapid noninvasive test that is accurate for detection of infection in asymptomatic individuals. The

Rockefeller prize thus sets a goal that may be beyond that of which the technology is now capable.

The most promising of the recently developed technologies for detection of *C. trachomatis* are the nucleic acid amplification tests. With the PCR test approved since 1993, followed by the LCR test, many other nucleic acid amplification technologies currently under development may soon be available for *C. trachomatis*. Next in line for the detection of *C. trachomatis* is most probably the transcription-mediated amplification (TMA) test (Gen-Probe) (32). The TMA test, in its present format, targets 16S rRNA and works as an isothermal system that uses enzymatic target amplification and chemiluminescent detection in a single tube format. The only instrumentation required is a luminometer for detection of the product. Many laboratories that are now performing the PACE 2 DNA probe test already have the instrumentation necessary for the TMA test.

Another nucleic acid amplification test under development for detection of *C. trachomatis* is the Q-beta (QB) replicase assay (Gene-Track) (192). QB replicase is probably the most technically complex of the nucleic acid amplification technologies. The QB replicase enzyme is an RNA-directed RNA polymerase that replicates the RNA of the bacteriophage virus Q-beta. In the QB replicase test, two different probes are used. The reporter probe consists of QB RNA synthetically linked to a *C. trachomatis* rRNA tail, and the capture probe consists of *C. trachomatis* DNA with a polyadenine tail. Both probes hybridize to adjacent regions of *C. trachomatis* 16S rRNA. The hybridized complex is captured by beads coated with polythymidine, which is complementary to the polyadenine tail of the capture probe. QB replicase amplifies the QB RNA attached to the complex, and products are detected by a fluorimetric system. The entire process is isothermal at 38°C, and a fully automated system has been developed by the manufacturer. A preliminary comparison of the QB replicase assay with semi-quantitative PCR showed that while both assays exhibited similar analytical sensitivity, the QB replicase assay was less subject to inhibitory factors present in endocervical specimens (4).

Two additional nucleic acid amplification technologies that are under development for the detection of one or more infectious disease agents are nucleic acid sequence-based amplification (NASBA; Organon Teknika) (221) and strand displacement amplification (SDA; Becton Dickinson) (223). NASBA, formerly called 3SR (self-sustaining sequencing reaction), uses technology that is similar to TMA in that it is an isothermal, RNA-based method. The first primer contains a T7 RNA polymerase promoter along with a sequence targeting chlamydial RNA. The enzyme reverse transcriptase extends the primer to make a DNA copy of the target sequence and forms an RNA-DNA hybrid. The RNA strand of the hybrid molecule is degraded by RNase H, and then the remaining single DNA strand forms a template for a second primer. Extension of the annealed second primer by reverse transcriptase forms a double-stranded DNA which acts as the template for further RNA transcripts by T7 RNA polymerase. Multiple cycles of RNA transcript processing by RNase H and reverse transcriptase result in logarithmic amplification of the target RNA sequence. The analytical sensitivity of NASBA has been reported to be 10 molecules, and the clinical sensitivity for HIV is 400 copies (220). The first NASBA product was designed to quantitatively detect HIV and is available for investigational use only in the United States at this time.

SDA employs a primer that incorporates a restriction site, an exonuclease-deficient fragment of DNA polymerase, and the restriction enzyme *HincIII* to make one copy of the DNA target. When both sense and antisense primers are included in

this scheme, the reaction proceeds logarithmically and is isothermal at 41°C except for an initial requirement for heat to separate double-stranded DNA target. The first strand displacement assay will be a multiplex test that amplifies two mycobacterial targets.

Of these new nucleic acid amplification technologies, it is not clear which will become products for the detection of *C. trachomatis*. Since the field is already highly competitive, with at least four different DNA amplification tests, it is likely that manufacturers will steer new products more toward the diagnosis of other infectious agents. With only two licensed DNA amplification tests for detection of *C. trachomatis* as yet, we are at the brink of a new diagnostic era and have much to learn before such tests completely replace culture and non-amplified DNA technologies. The evidence strongly suggests, however, that it is just a matter of time before they will.

In the future, there is much work to be done. Examples of some of the additional studies that are needed include research on the cost-effectiveness of different screening and intervention strategies, the challenges of converting DNA amplification technologies to tests that can be performed near to the patient in 30 min or less, and identification and elimination of factors in clinical specimens that inhibit DNA amplification tests and produce false-negative results.

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#### REFERENCES

- Akane, A., K. Matsubara, H. Nakamura, S. Takahashi, and K. Kimura. 1994. Identification of the heme compound copurified with deoxyribonucleic acid (DNA) from bloodstains, a major inhibitor of polymerase chain reaction amplification. *J. Forensic Sci.* **39**:362-372.
- Altaie, S. S., F. A. Meier, R. M. Centor, M. Wakabongo, D. Toksoz, K. M. Harvey, E. Basinger, B. A. Johnson, R. R. Brookman, and H. P. Dalton. 1992. Evaluation of two ELISA's for detecting *Chlamydia trachomatis* from endocervical swabs. *Diagn. Microbiol. Infect. Dis.* **15**:579-586.
- Altwegg, M., D. Burger, U. Lauper, and G. Schär. 1994. Comparison of Gen-Probe PACE 2, AmpliCor Roche, and a conventional PCR for the detection of *Chlamydia trachomatis* in genital specimens. *Med. Microbiol. Lett.* **3**:181-187.
- An, Q., J. Liu, W. O'Brien, G. Radcliffe, D. Buxton, S. Popoff, W. King, M. Vera-Garcia, L. Lu, J. Shah, et al. 1995. Comparison of characteristics of Q beta replicase-amplified assay with competitive PCR assay for *Chlamydia trachomatis*. *J. Clin. Microbiol.* **33**:58-63.
- An, Q., G. Radcliffe, R. Vassallo, D. Buxton, W. J. O'Brien, D. A. Pelletier, W. G. Weisburg, J. D. Klinger, and D. M. Olive. 1992. Infection with a plasmid-free variant *Chlamydia* related to *Chlamydia trachomatis* identified by using multiple assays for nucleic acid detection. *J. Clin. Microbiol.* **30**:2814-2821.
- Anderson, J. R., G. Mumtaz, P. Mulhare, J. Poddar, and G. L. Ridgway. 1993. Mandatory use of confirmation stage with Chlamydiazyme during urinary sediment analysis. *J. Clin. Pathol.* **46**:896-897.
- Backman, M., A. K. Ruden, O. Ringertz, and E. G. Sandstrom. 1993. Detection of *Chlamydia trachomatis* in urine from men with urethritis. *Eur. J. Clin. Microbiol. Infect. Dis.* **12**:447-449.
- Barnes, R. C. 1989. Laboratory diagnosis of human chlamydial infections. *Clin. Microbiol. Rev.* **2**:119-136.
- Bass, C. A., D. L. Jungkind, N. S. Silverman, and J. M. Bondi. 1993. Clinical evaluation of a new polymerase chain reaction assay for detection of *Chlamydia trachomatis* in endocervical specimens. *J. Clin. Microbiol.* **31**:2648-2653.
- Bassiri, M., H. Y. Yu, M. A. Domeika, J. Burczak, L.-O. Svensson, H. H. Lee, and P.-A. Mårdh. 1995. Detection of *Chlamydia trachomatis* in urine specimens from women by ligase chain reaction. *J. Clin. Microbiol.* **33**:898-900.
- Batteiger, B. E., and R. B. Jones. 1987. Chlamydial infections. *Infect. Dis. Clin. North Am.* **1**:55-81.
- Bauwens, J. E., A. M. Clark, M. J. Loeffelholz, S. A. Herman, and W. E. Stamm. 1993. Diagnosis of *Chlamydia trachomatis* urethritis in men by polymerase chain reaction assay of first-catch urine. *J. Clin. Microbiol.* **31**:3013-3016.
- Bauwens, J. E., A. M. Clark, and W. E. Stamm. 1993. Diagnosis of *Chlamydia trachomatis* endocervical infections by a commercial polymerase chain reaction assay. *J. Clin. Microbiol.* **31**:3023-3027.
- Beebe, J. L., M. P. Rau, and K. D. Albrecht. 1993. Confirmatory testing of *Chlamydia trachomatis* Syva enzyme immunoassay gray zone specimens by Syva direct fluorescent antibody test. *Sex. Transm. Dis.* **20**:140-142.
- Bell, T. A., C.-C. Kuo, W. E. Stamm, M. R. Tam, R. S. Stephens, K. K. Holmes, and J. T. Grayston. 1984. Direct fluorescent monoclonal antibody stain for rapid detection of infant *Chlamydia trachomatis* infections. *Pediatrics* **74**:224-228.
- Bell, T. A., K. I. Sandstrom, M. G. Gravett, et al. 1987. Delayed appearance of *Chlamydia trachomatis* infections acquired at birth. *Pediatr. Infect. Dis. J.* **6**:928-931.
- Berg, A. O., F. E. Heidrich, S. D. Fihn, J. J. Bergman, R. W. Wood, W. E. Stamm, and K. K. Holmes. 1984. Establishing the cause of genitourinary symptoms in women in a family practice. *JAMA* **251**:620-625.
- Berger, R. E., E. R. Alexander, J. P. Harnish, C. A. Paulsen, G. D. Monda, J. Ansell, and K. K. Holmes. 1979. Etiology and therapy of acute epididymitis: prospective study of 50 cases. *J. Urol.* **121**:750-754.
- Bianchi, A., C. Scieux, N. Brunat, D. Vexiau, M. Kermanach, P. Pezin, M. Janier, P. Morel, and P. H. Lagrange. 1994. An evaluation of the polymerase chain reaction AmpliCor *Chlamydia trachomatis* in male urine and female urogenital specimens. *Sex. Transm. Dis.* **21**:196-200.
- Biro, F. M., S. F. Reising, J. A. Doughman, L. M. Kollar, and S. L. Rosenthal. 1994. A comparison of diagnostic methods in adolescent girls with and without symptoms of *Chlamydia* urogenital infection. *Pediatrics* **93**:476-480.
- Blanding, J., L. Hirsch, N. Stranton, T. Wright, S. Aarnaes, L. M. de la Maza, and E. M. Peterson. 1993. Comparison of the Clearview *Chlamydia*, the PACE 2 assay, and culture for detection of *Chlamydia trachomatis* from cervical specimens in a low-prevalence population. *J. Clin. Microbiol.* **31**:1622-1625.
- Bleker, O. P., D. J. Smalbraak, and M. F. Shutte. 1990. Bartholin's abscess: the role of *Chlamydia trachomatis*. *Genitourin. Med.* **66**:24-25.
- Blythe, M. J., B. P. Katz, B. E. Batteiger, J. A. Ganser, and R. B. Jones. 1992. Recurrent genitourinary chlamydial infections in sexually active female adolescents. *J. Pediatr.* **121**:487-493.
- Bovarnick, M. R., J. C. Miller, and J. C. Snyder. 1950. The influence of certain salts, amino acids, sugars, and proteins on the stability of rickettsiae. *J. Bacteriol.* **59**:509-522.
- Brandt, K., H. Stoneham, G. B. Horsman, and E. L. Chan. 1995. Evaluation of Sanofi Diagnostics Pasteur ACCESS *Chlamydia* in comparison with Syva Microtrak II *Chlamydia* EIA assay, abstr. C-477. *In Abstracts of the 95th General Meeting of the American Society for Microbiology 1995*. American Society for Microbiology, Washington, D.C.
- Cates, W., Jr., and J. N. Wasserheit. 1991. Genital chlamydial infections: epidemiology and reproductive sequelae. *Am. J. Obstet. Gynecol.* **164**:1771-1781.
- Centers for Disease Control and Prevention. 1989. Guidelines for prevention of transmission of human immunodeficiency virus and hepatitis B virus to health care and public safety workers. *Morbidity and Mortality Weekly Report* **38**:1-37.
- Centers for Disease Control and Prevention. 1991. False-positive results with the use of chlamydial tests in the evaluation of suspected sexual abuse. *Morbidity and Mortality Weekly Report* **39**:932-935.
- Centers for Disease Control and Prevention. 1991. Sexually transmitted diseases clinical practice guidelines. Centers for Disease Control and Prevention, Atlanta, Ga.
- Centers for Disease Control and Prevention. 1993. Recommendations for the prevention and management of *Chlamydia trachomatis* infections. *Morbidity and Mortality Weekly Report* **42**(No. RR-12):1-39.
- Centers for Disease Control and Prevention. 1993. 1993 sexually transmitted diseases treatment guidelines. *Morbidity and Mortality Weekly Report* **42**(No. RR-14):1-102.
- Chambers, B., T. Quigley, B. Weinbaum, S. Wolf, and K. Clark. 1995. Detection of *Chlamydia trachomatis* in male and female urine specimens using transcription-mediated amplification (TMA), abstr. C-488. *In Abstracts of the 95th Annual Meeting of the American Society for Microbiology 1995*. American Society for Microbiology, Washington, D.C.
- Chan, E. L., K. Brandt, and G. B. Horsman. 1994. A 1-year evaluation of Syva Microtrak *Chlamydia* enzyme immunoassay with selective confirmation by direct fluorescent-antibody assay in a high-volume laboratory. *J. Clin. Microbiol.* **32**:2208-2211.
- Chan, S. W. 1993. Evaluation of Pharmacia *Chlamydia* EIA for diagnosis of genital infections caused by *Chlamydia trachomatis*. *Pathology* **25**:68-70.
- Chernesky, M., S. Castriciano, J. Sellors, I. Stewart, I. Cunningham, S. Landis, et al. 1990. Detection of *Chlamydia trachomatis* antigens in urine as an alternative to swabs and cultures. *J. Infect. Dis.* **161**:124-126.
- Chernesky, M. A., D. Jang, S. Chong, H. Lee, S. Sellors, and J. B. Mahony. 1995. Order of urine collection affects the diagnosis of *Chlamydia trachomatis* infection in men by ligase chain reaction, GenProbe Pace II, Chlamydiazyme, and leukocyte esterase testing, abstr. 316, p. 180. *In Abstracts of the 11th Meeting of the International Society for STD Research 1995*.



37. Chernesky, M. A., D. Jang, H. Lee, J. D. Burczak, H. Hu, J. Sellors, S. J. Tomazic-Allen, and J. B. Mahony. 1994. Diagnosis of *Chlamydia trachomatis* infections in men and women by testing first-void urine by ligase chain reaction. *J. Clin. Microbiol.* **32**:2682–2685.
38. Chernesky, M. A., D. Jang, K. Luinstra, S. Chong, L. Pickard, J. Sellors, and J. B. Mahony. 1994. Ability of ligase chain reaction and polymerase chain reaction to diagnose female lower genitourinary *Chlamydia trachomatis* infection by testing cervical swabs and first void urine, p. 326–329. *In* J. Orfila, G. I. Byrne, M. A. Chernesky, J. T. Grayston, R. B. Jones, G. L. Ridgway, P. Saikku, J. Schachter, W. E. Stamm, and R. S. Stephens (ed.), *Chlamydial infections*. Proceedings of the Eighth International Symposium on Human Chlamydial Infections. Societa Editrice Esculapio, Bologna, Italy.
39. Chernesky, M. A., D. Jang, J. Sellors, S. Castriciano, L. Pickard, K. Luinstra, and J. Mahony. 1993. Confirmatory testing demonstrates that false-positive rates in the Chlamydiazyme assay are influenced by gender and genital specimen type. *Sex. Transm. Dis.* **20**:301–306.
40. Chernesky, M. A., D. Jang, J. Sellors, P. Coleman, J. Bodner, I. Hrusovsky, S. Chong, and J. B. Mahony. 1995. Detection of *Chlamydia trachomatis* antigens in male urethral swabs and urines with a microparticle enzyme immunoassay. *Sex. Transm. Dis.* **22**:55–59.
41. Chernesky, M. A., H. Lee, D. Jang, K. Luinstra, S. Chong, J. Sellors, L. Pickard, and J. Mahony. 1995. Female *Chlamydia trachomatis* genitourinary infections diagnosed by testing cervical swabs and first void urine by polymerase chain reaction and ligase chain reaction assays, abstr. D105, p. 85. *In* Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
42. Chernesky, M. A., H. Lee, J. Schachter, J. D. Burczak, W. E. Stamm, W. M. McCormack, and T. C. Quinn. 1994. Diagnosis of *Chlamydia trachomatis* urethral infection in symptomatic and asymptomatic men by testing first-void urine in a ligase chain reaction assay. *J. Infect. Dis.* **170**:1308–1311.
43. Chernesky, M. A., J. B. Mahoney, S. Castriciano, M. Mores, I. O. Stewart, S. F. Landis, W. Seidelman, et al. 1986. Detection of *Chlamydia trachomatis* antigens by enzyme immunoassay and immunofluorescence in genital specimens from symptomatic and asymptomatic men and women. *J. Infect. Dis.* **154**:141–148.
44. Clad, A., H. Freidank, J. Plunnecke, B. Jung, and E. E. Petersen. 1994. *Chlamydia trachomatis* species-specific serology: ImmunoComb Chlamydia bivalent versus microimmunofluorescence (MIF). *Infection* **22**:165–173.
45. Claesson, B. A., B. Trollfors, I. Brolin, M. Granstrom, J. Henrichsen, U. Jodal, P. Juto, I. Kallings, K. Kanclerski, T. Lagergard, et al. 1989. Etiology of community-acquired pneumonia in children based on antibody responses to bacterial and viral antigens. *Pediatr. Infect. Dis. J.* **8**:856–862.
46. Clark, A., W. E. Stamm, C. Gaydos, L. Welsh, T. C. Quinn, J. Schachter, and J. Moncada. 1992. Multicenter evaluation of the AntigEnz chlamydia enzyme immunoassay for diagnosis of *Chlamydia trachomatis* genital infection. *J. Clin. Microbiol.* **30**:2762–2764.
47. Clarke, L. M., M. F. Sierra, B. J. Daidone, N. Lopez, J. M. Covino, and W. M. McCormack. 1993. Comparison of the Syva Microtrak enzyme immunoassay and Gen-Probe PACE 2 with cell culture for diagnosis of cervical *Chlamydia trachomatis* infection in a high-prevalence female population. *J. Clin. Microbiol.* **31**:968–971.
48. Cottingham, J., and D. Hunter. 1992. *Chlamydia trachomatis* and oral contraceptive use: a quantitative review. *Genitourin. Med.* **68**:209–216.
49. Crowley, T., D. Milne, J. T. Arumainayagam, I. D. Paul, and E. O. Caul. 1992. The laboratory diagnosis of male *Chlamydia trachomatis* infections—a time for change? *J. Infect.* **25**(Suppl. 1):69–75.
50. Demaio, J., R. S. Boyd, R. Rensi, and A. Clark. 1991. False-positive Chlamydiazyme results during urine sediment analysis due to bacterial urinary tract infections. *J. Clin. Microbiol.* **29**:1436–1438.
51. Dille, B. J., C. C. Butzen, and L. G. Birkenmeyer. 1993. Amplification of *Chlamydia trachomatis* DNA by ligase chain reaction. *J. Clin. Microbiol.* **31**:729–731.
52. Domeika, M., M. Bassiri, and P.-A. Mårdh. 1994. Diagnosis of genital *Chlamydia trachomatis* infections in asymptomatic males by testing urine by PCR. *J. Clin. Microbiol.* **32**:2350–2352.
53. Dunlop, E. M. C., B. T. Goh, S. Darougar, and R. Woodland. 1985. Triple culture tests for the diagnosis of Chlamydial infection of the female genital tract. *Sex. Transm. Dis.* **12**:68–71.
54. Ehret, J. M., J. C. Leszczynski, J. M. Douglas, S. L. Genova, M. A. Chernesky, J. Moncada, and J. Schachter. 1993. Evaluation of chlamydiazyme enzyme immunoassay for detection of *Chlamydia trachomatis* in urine specimens from men. *J. Clin. Microbiol.* **31**:2702–2705.
55. Embil, J. A., H. J. Thiebaut, F. R. Manuel, L. H. Pereira, and S. W. MacDonald. 1983. Sequential cervical specimens and the isolation of *Chlamydia trachomatis*: factors affecting detection. *Sex. Transm. Dis.* **10**:62–66.
56. Farshy, C., J. Bullard, C. Woodfill, E. Urdez, C. M. Black, Z. Simmons, B. Jones, D. Blackburn, E. Ginsburg, and C. Sague. 1995. Urine testing for cervical *Chlamydia trachomatis* infections in women attending an adolescent clinic. *In* Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
57. Fedorko, D. P., and T. F. Smith. 1991. Chlamydial infections, p. 95–125. *In* B. B. Wentworth, F. N. Judson, and M. J. R. Gilchrist (ed.), *Laboratory methods for the diagnosis of sexually transmitted diseases*, 2nd ed. American Public Health Association, Washington, D.C.
58. Ferris, D. G., and W. H. Martin. 1992. A comparison of three rapid chlamydial tests in pregnant and nonpregnant women. *J. Fam. Pract.* **34**:593–597.
59. Friis, B., C.-C. Kuo, S.-P. Wang, C. H. Mordhorst, and J. T. Grayston. 1984. Rapid diagnosis of *Chlamydia trachomatis* pneumonia in infants. *Acta Pathol. Microbiol. Immunol. Scand. Sect. B* **92**:139–143.
60. Gaydos, C. A., C. A. Reichart, J. M. Long, L. E. Welsh, T. M. Neumann, E. W. Hook III, and T. C. Quinn. 1990. Evaluation of Syva enzyme immunoassay for detection of *Chlamydia trachomatis* in genital specimens. *J. Clin. Microbiol.* **28**:1541–1544.
61. Genc, M., M. Domeika, and P.-A. Mårdh. 1994. Cost-effectiveness of screening for Chlamydia using DNA amplification. *JAMA* **271**:1741.
62. Genc, M., L. Ruusuvaara, and P.-A. Mårdh. 1993. An economic evaluation of screening for *Chlamydia trachomatis* in adolescent males. *JAMA* **270**:2057–2064.
63. Genc, M., A. Stary, S. Bergman, and P.-A. Mårdh. 1991. Detection of *Chlamydia trachomatis* in first-void urine collected from men and women attending a venereal clinic. *APMIS* **99**:455–459.
64. Gordon, F. B., I. A. Harper, A. L. Quan, J. D. Treharne, R. S. Dwyer, and J. A. Garland. 1969. Detection of Chlamydia (Bedsonia) in certain infections of man. 1. Laboratory procedures: comparisons of yolk sac and cell culture for detection and isolation. *J. Infect. Dis.* **120**:451–462.
65. Grayston, J. T. 1992. Infections caused by *Chlamydia pneumoniae* strain TWAR. *Clin. Infect. Dis.* **15**:757–763.
66. Grossman, J. H., III, M. E. Rivlin, and J. C. Morrison. 1991. Diagnosis of chlamydial infection in pregnant women using the Testpack Chlamydia diagnostic kit. *Obstet. Gynecol.* **77**:801–803.
67. Hallander, H., P. Jonsson, and B. Gastrin. 1992. Value of confirmation of Chlamydiazyme enzyme immunoassay results in the detection of *Chlamydia trachomatis*. *Eur. J. Clin. Microbiol. Infect. Dis.* **11**:550–552.
68. Hammerschlag, M. R. 1994. *Chlamydia trachomatis* in children. *Pediatr. Ann.* **23**:349–353.
69. Hammerschlag, M. R., C. Cummings, P. M. Roblin, T. H. Williams, and I. Delke. 1989. Efficacy of neonatal ocular prophylaxis for the prevention of chlamydial and gonococcal conjunctivitis. *N. Engl. J. Med.* **320**:769–772.
70. Hammerschlag, M. R., B. Doraiswamy, E. R. Alexander, et al. 1984. Are rectogenital chlamydial infections a marker of sexual abuse in children? *Pediatr. Infect. Dis.* **3**:100–104.
71. Han, Y., D. L. Morse, C. E. Lawrence, D. Murphy, and S. Hipp. 1993. Risk profile for Chlamydia infection in women from public health clinics in New York State. *J. Community Health* **18**:1–9.
72. Hardy, P. H., J. B. Hardy, E. E. Nell, et al. 1984. Prevalence of six sexually transmitted disease agents among pregnant inner city adolescents and pregnancy outcome. *Lancet* **ii**:333–337.
73. Harrison, H. R., E. R. Alexander, L. Weinstein, et al. 1983. Cervical *Chlamydia trachomatis* and mycoplasmal infections in pregnancy: epidemiology and outcomes. *JAMA* **250**:1721–1727.
74. Harrison, H. R., M. Costin, J. B. Meder, L. M. Bounds, D. A. Sims, M. Lewis, and E. R. Alexander. 1985. Cervical *Chlamydia trachomatis* infection in university women: relationship to history, contraception, ectopy, and cervicitis. *Am. J. Obstet. Gynecol.* **153**:244–251.
75. Hay, P. E., B. J. Thomas, P. J. Horner, E. MacLeod, A. M. Renton, and D. Taylor-Robinson. 1994. *Chlamydia trachomatis* in women: the more you look, the more you find. *Genitourin. Med.* **70**:97–100.
76. Herbrink, P., M. Zuyderwijk-Zwinkels, B. Niesters, J. Wagenvoort, and W. Van Dijk. 1991. Comparison of different culture media for isolation of *Chlamydia trachomatis* by cell culture on HeLa cells. *Eur. J. Clin. Microbiol. Infect. Dis.* **10**:656–659.
77. Hillis, S. D., A. Nakashima, P. A. Marchbanks, D. G. Addiss, and J. P. Davis. 1994. Risk factors for recurrent *C. trachomatis* infections in women. *Am. J. Obstet. Gynecol.* **170**:801–806.
78. Hobson, D., P. Karayiannis, R. E. Byng, E. Rees, I. A. Tait, and J. A. Davies. 1980. Quantitative aspects of chlamydial infection of the cervix. *Br. J. Vener. Dis.* **56**:156–162.
79. Holmes, K. K., H. H. Handsfield, S. P. Wang, B. B. Wentworth, M. Turck, J. B. Anderson, and E. R. Alexander. 1975. Etiology of nongonococcal urethritis. *N. Engl. J. Med.* **292**:1199–1205.
80. Hook, E., III. Personal communication.
81. Hosen, I. K., A. M. Kaunitz, and S. J. Craft. 1992. Detection of cervical *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with deoxyribonucleic acid probe assays in obstetric patients. *Am. J. Obstet. Gynecol.* **167**:588–591.
82. Howard, C., D. L. Friedman, J. K. Leete, and M. L. Christensen. 1991. Correlation of the percent of positive *Chlamydia trachomatis* direct fluorescent antibody detection tests with the adequacy of specimen collection. *Diagn. Microbiol. Infect. Dis.* **14**:233–237.

83. Ingram, D. L., D. K. Runyan, A. D. Collins, et al. 1984. Vaginal *Chlamydia trachomatis* infection in children with sexual contact. *Pediatr. Infect. Dis.* **3**:97-99.
84. Ingram, D. L., S. T. White, A. R. Occhiuti, et al. 1986. Childhood vaginal infections: association of *Chlamydia trachomatis* with sexual contact. *Pediatr. Infect. Dis.* **5**:226-229.
85. Iwen, P. C., T. M. Blair, and G. L. Woods. 1991. Comparison of the Gen-Probe PACE 2 system, direct fluorescent antibody, and cell culture for detecting *Chlamydia trachomatis* in cervical specimens. *Am. J. Clin. Pathol.* **95**:578-582.
86. Jang, D., J. W. Sellors, J. B. Mahony, L. Pickard, and M. A. Chernesky. 1992. Effects of broadening the gold standard on the performance of a chemiluminometric immunoassay to detect *Chlamydia trachomatis* antigens in centrifuged first void urine and urethral swab samples from men. *Sex. Transm. Dis.* **19**:315-319.
87. Jaschek, G., C. A. Gaydos, L. E. Welsh, and T. C. Quinn. 1993. Direct detection of *Chlamydia trachomatis* in urine specimens from symptomatic and asymptomatic men by using a rapid polymerase chain reaction assay. *J. Clin. Microbiol.* **31**:1209-1212.
88. Johnson, J., B. Neas, D. E. Parker, J. D. Fortenberry, and L. D. Cowan. 1994. Screening for urethral infection in adolescent and young adult males. *J. Adolesc. Health* **14**:356-361.
89. Johnston, S. L., and C. Siegel. 1992. Comparison of Buffalo Green Monkey Kidney cells and McCoy cells for the isolation of *Chlamydia trachomatis* in shell vial centrifugation culture. *Diagn. Microbiol. Infect. Dis.* **15**:355-357.
90. Jones, R. B., B. P. Katz, B. VanderPol, V. A. Caine, B. E. Batteiger, and W. J. Newhall. 1986. Effect of blind passage and multiple sampling on recovery of *Chlamydia trachomatis* from urogenital specimens. *J. Clin. Microbiol.* **24**:1029-1033.
91. Jones, R. B., R. A. Rabinovitch, B. P. Katz, et al. 1985. *Chlamydia trachomatis* in the pharynx and rectum of heterosexual patients at risk for genital infection. *Ann. Intern. Med.* **102**:757-762.
92. Judson, F. N., and J. Ehret. 1994. Laboratory diagnosis of sexually transmitted infections. *Pediatr. Ann.* **23**:361-369.
93. Karimi, S. T., R. H. Schloemer, and C. E. Wilde III. 1989. Accumulation of chlamydial lipopolysaccharide antigen in the plasma membranes of infected cells. *Infect. Immun.* **57**:1780-1785.
94. Kellogg, J. A., J. W. Seiple, and M. E. Hick. 1992. Cross-reaction of clinical isolates of bacteria and yeasts with the chlamydiazyme test for chlamydial antigen, before and after use of a blocking agent. *Am. J. Clin. Pathol.* **97**:309-312.
95. Kellogg, J. A., J. W. Seiple, J. L. Klinedinst, and J. S. Levisky. 1991. Impact of endocervical specimen quality on apparent prevalence of *Chlamydia trachomatis* infections diagnosed using an enzyme-linked immunosorbent assay method. *Arch. Pathol. Lab. Med.* **115**:1223-1227.
96. Kellogg, J. A., J. W. Seiple, J. L. Klinedinst, and J. S. Levisky. 1992. Comparison of cytobrushes with swabs for recovery of endocervical cells and for Chlamydiazyme detection of *Chlamydia trachomatis*. *J. Clin. Microbiol.* **30**:2988-2990.
97. Kellogg, J. A., J. W. Seiple, J. L. Klinedinst, and E. S. Stroll. 1995. Impact of endocervical specimen quality on apparent prevalence of *Chlamydia trachomatis* infections diagnosed using polymerase chain reaction, abstr. C-494, p. 86. In Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
98. Kellogg, J. A., J. W. Seiple, and E. S. Stroll. 1993. Direct fluorescent-antibody confirmation of chlamydial antigen below the detection threshold of the Chlamydiazyme enzyme-linked immunosorbent assay. *J. Clin. Microbiol.* **31**:1646-1647.
99. Kellogg, J. A., B. T. Vanderhoff, J. W. Seiple, and M. E. Hick. 1994. Comparison of first-voided urine specimens with endocervical swab specimens for enzyme-linked immunosorbent assay detection of *Chlamydia trachomatis* in women. *Arch. Fam. Med.* **3**:672-675.
100. Kluytmans, J. A., W. H. Goossens, J. W. Mouton, J. H. van Rijsoort-Vos, H. G. Niesters, W. G. Quint, L. Habbema, E. Stolz, and J. H. Wagenvoort. 1993. Evaluation of Clearview and Magic Lite tests, polymerase chain reaction, and cell culture for detection of *Chlamydia trachomatis* in urogenital specimens. *J. Clin. Microbiol.* **31**:3204-3210.
101. Kluytmans, J. A., W. H. Goossens, J. H. van Rijsoort-Vos, H. G. Niesters, and E. Stolz. 1994. Improved performance of PACE 2 with modified collection system in combination with probe competition assay for detection of *Chlamydia trachomatis* in urethral specimens from males. *J. Clin. Microbiol.* **32**:568-570.
102. Kluytmans, J. A., H. G. Niesters, J. W. Mouton, W. G. Quint, J. A. Ijpelaar, J. H. van Rijsoort, L. Habbema, E. Stolz, M. F. Michel, and J. H. Wagenvoort. 1991. Performance of a nonisotopic DNA probe for detection of *Chlamydia trachomatis* in urogenital specimens. *J. Clin. Microbiol.* **29**:2685-2689.
103. Kluytmans, J. A., A. H. van der Willigen, B. Y. van Heyst, W. I. van der Meyden, E. Stolz, and J. H. Wagenvoort. 1990. Evaluation of an enzyme immunoassay for detection of *Chlamydia trachomatis* in urogenital specimens. *Int. J. STD AIDS* **1**:49-52.
104. Knapp, J. S., and S. A. Morse. 1990. Laboratory evaluation of victims of sexual abuse for sexually transmitted diseases, p. A1.3-A1.9. In S. A. Morse, A. A. Moreland, and S. E. Thompson (ed.), Atlas of sexually transmitted diseases. J. B. Lippincott Co., Philadelphia, Pa.
105. Kok, T. W., L. E. Payne, S. E. Bailey, and R. G. Waddell. 1993. Urine and the laboratory diagnosis of *Chlamydia trachomatis* in males. *Genitourin. Med.* **69**:51-53.
106. Krech, T., M. Bleckmann, and R. Paatz. 1989. Comparison of Buffalo Green Monkey cells and McCoy cells for isolation of *Chlamydia trachomatis* in a microtiter system. *J. Clin. Microbiol.* **27**:2364-2365.
107. Krul, K. G. 1995. Closing in on Chlamydia. *CAP Today* **9**:1-20.
108. Kuo, C.-C., S.-P. Wang, B. B. Wentworth, and J. T. Grayston. 1972. Primary isolation of TRIC organisms in HeLa 229 cells treated with DEAE-dextran. *J. Infect. Dis.* **125**:665-668.
109. Laga, M., A. Manoka, M. Kivuvu, B. Malele, M. Tuliza, N. Nzila, J. Gorman, et al. 1993. Non-ulcerative sexually transmitted diseases as risk factors for HIV-1 transmission in women: results from a cohort study. *AIDS* **7**:95-102.
110. Lampe, M. F., R. J. Suchland, and W. E. Stamm. 1993. Nucleotide sequence of the variable domains within the major outer membrane protein gene from serovariants of *Chlamydia trachomatis*. *Infect. Immun.* **61**:213-219.
111. Lee, H. H., M. A. Chernesky, J. Schachter, J. D. Burczak, W. W. Andrews, S. Muldoon, G. Leckie, and W. E. Stamm. 1995. Diagnosis of *Chlamydia trachomatis* genitourinary infection in women by ligase chain reaction assay of urine. *Lancet* **345**:213-216.
112. Lees, M. I., D. M. Newnan, and S. M. Garland. 1991. Comparison of a DNA probe assay with culture for the detection of *Chlamydia trachomatis*. *J. Med. Microbiol.* **35**:159-161.
113. Leonardi, G. P., M. Seitz, R. Edstrom, J. Cruz, P. Costello, and K. Szabo. 1992. Evaluation of three immunoassays for detection of *Chlamydia trachomatis* in urine specimens from asymptomatic males. *J. Clin. Microbiol.* **30**:2793-2796.
114. Limberger, R. J., R. Biega, A. Evancoe, L. McCarthy, L. Sliwinski, and M. Kirkwood. 1992. Evaluation of culture and the Gen-Probe PACE 2 assay for detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in endocervical specimens transported to a state health laboratory. *J. Clin. Microbiol.* **30**:1162-1166.
115. Lin, J. S., W. E. Jones, L. Yan, K. A. Wirthwein, E. E. Flaherty, R. M. Haivanis, and P. A. Rice. 1992. Underdiagnosis of *Chlamydia trachomatis* infection. Diagnostic limitations in patients with low level infection. *Sex. Transm. Dis.* **19**:259-265.
116. Lipkin, E. S., J. V. Moncada, M.-A. Shafer, T. E. Wilson, and J. Schachter. 1986. Comparison of monoclonal antibody staining and culture in diagnosing cervical chlamydial infections. *J. Clin. Microbiol.* **23**:114-117.
117. Loeffelholz, M. J., C. A. Lewinski, S. R. Silver, A. P. Purohit, S. A. Herman, D. A. Buonagurio, and E. A. Dragon. 1992. Detection of *Chlamydia trachomatis* in endocervical specimens by polymerase chain reaction. *J. Clin. Microbiol.* **30**:2847-2851.
118. Lossick, J., S. DeLisle, D. Fine, D. Mosure, V. Lee, and C. Smith. 1990. Regional program for widespread screening for *Chlamydia trachomatis* in family planning clinics, p. 575-579. In W. R. Bowie, H. D. Caldwell, R. P. Jones, et al. (ed.), Chlamydial infections. Proceedings of the Seventh International Symposium on Human Chlamydial Infections. Cambridge University Press, Cambridge, England.
119. Maas, M., and K. Dalhoff. 1994. Comparison of sample preparation methods for detection of *Chlamydia pneumoniae* in bronchoalveolar lavage fluid by PCR. *J. Clin. Microbiol.* **32**:2616-2619.
120. Magder, L. S., H. R. Harrison, J. M. Ehret, T. S. Anderson, and F. N. Judson. 1988. Factors related to genital *Chlamydia trachomatis* and its diagnosis by culture in a sexually transmitted disease clinic. *Am. J. Epidemiol.* **28**:298-308.
121. Magder, L. S., K. C. Klontz, L. H. Bush, and R. C. Barnes. 1990. Effect of patient characteristics on performance of an enzyme immunoassay for detecting cervical *Chlamydia trachomatis* infection. *J. Clin. Microbiol.* **28**:781-784.
122. Mahony, J. B., and M. A. Chernesky. 1985. Effect of swab type and storage temperature on the isolation of *Chlamydia trachomatis* from clinical specimens. *J. Clin. Microbiol.* **22**:865-867.
123. Mahony, J. B., and M. A. Chernesky. 1994. Leukocyte esterase urine strips for the screening of men with urethritis—use in developing countries. *Genitourin. Med.* **70**:3-6.
124. Mahony, J. B., M. A. Chernesky, K. Bromberg, and J. Schachter. 1986. Accuracy of immunoglobulin M immunoassay for diagnosis of chlamydial infections in infants and adults. *J. Clin. Microbiol.* **24**:731-735.
125. Mahony, J. B., K. E. Luinstra, J. W. Sellors, and M. A. Chernesky. 1993. Comparison of plasmid- and chromosome-based polymerase chain reaction assays for detecting *Chlamydia trachomatis* nucleic acids. *J. Clin. Microbiol.* **31**:1753-1758.
126. Mahony, J. B., K. E. Luinstra, J. W. Sellors, L. Pickard, S. Chong, D. Jang, and M. A. Chernesky. 1994. Role of confirmatory PCRs in determining performance of Chlamydia AmpliCor PCR with endocervical specimens

- from women with a low prevalence of infection. *J. Clin. Microbiol.* **32**:2490–2493.
127. **Mårdh, P.-A., and B. Zeeberg.** 1981. Toxic effect of sampling swabs and transportation test tubes on the formation of intracytoplasmic inclusions of *Chlamydia trachomatis* in McCoy's cell cultures. *Br. J. Vener. Dis.* **57**:268–272.
  128. **Martin, D. H.** 1990. Chlamydial infections. *Med. Clin. North Am.* **74**:1367–1387.
  129. **Martin, D. H., L. Koutsky, D. A. Eschenbach, J. R. Daling, E. R. Alexander, J. K. Benedetti, and K. K. Holmes.** 1982. Prematurity and perinatal mortality in pregnancies complicated by maternal *Chlamydia trachomatis* infections. *JAMA* **247**:1585–1588.
  130. **Matthews, R. S., P. G. Pandit, S. D. Bonigal, R. Wise, and K. W. Radcliffe.** 1993. Evaluation of an enzyme-linked immunoassay and confirmatory test for the detection of *Chlamydia trachomatis* in male urine samples. *Genitourin. Med.* **69**:47–50.
  131. **McCarthy, M. D., S. Holloway, and L. Jones.** 1995. Comparison of the semiautomated Syva Micro Trak EIA system with the fully automated Micro Trak XL system for the detection of *Chlamydia trachomatis*, abstr. C-479. In Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
  132. **McComb, D. E., and C. I. Puzniak.** 1974. Micro cell culture method for isolation of *Chlamydia trachomatis*. *Appl. Microbiol.* **28**:727–729.
  133. **McNagly, S. E., R. M. Parker, J. M. Zenilman, and J. S. Lewis.** 1992. Urinary leukocyte esterase test: A screening method for the detection of asymptomatic chlamydial and gonococcal infections in men. *J. Infect. Dis.* **165**:573–576.
  134. **Melton, M., Y. Hale, M. Pawlowicz, D. Halstead, and S. Wright.** 1995. Evaluation of the Gen-Probe PACE 2C system for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in a high-prevalence population, abstr. C482, p. 138. In Abstracts of the 95th General Meeting of the American Society for Microbiology 1995. American Society for Microbiology, Washington, D.C.
  135. **Meyer, K. F., and B. Eddie.** 1956. The influence of tetracycline compounds on the development of antibodies in psittacosis. *Am. Rev. Tuberc.* **74**:566–571.
  136. **Mills, R. D., A. Young, K. Cain, T. M. Blair, M. A. Sitorius, and G. L. Woods.** 1992. Chlamydiazyme plus blocking assay to detect *Chlamydia trachomatis* in endocervical specimens. *Am. J. Clin. Pathol.* **97**:209–212.
  137. **Moncada, J., J. Schachter, G. Bolan, J. Engelman, L. Howard, I. Mushahwar, G. Ridgway, G. Mumtaz, W. Stamm, and A. Clark.** 1990. Confirmatory assay increases specificity of the Chlamydiazyme test for *Chlamydia trachomatis* infection of the cervix. *J. Clin. Microbiol.* **28**:1770–1773.
  138. **Moncada, J., J. Schachter, G. Bolan, J. Nathan, M.-A. Shafer, A. Clark, J. Schwebke, W. Stamm, T. Mroczkowski, and D. Martin.** 1992. Evaluation of Syva's enzyme immunoassay for the detection of *Chlamydia trachomatis* in urogenital specimens. *Diagn. Microbiol. Infect. Dis.* **15**:663–668.
  139. **Moncada, J., J. Schachter, M.-A. Shafer, E. Williams, L. Gourlay, B. Lavin, and G. Bolan.** 1994. Detection of *Chlamydia trachomatis* in first catch urine samples from symptomatic and asymptomatic males. *Sex. Transm. Dis.* **21**:8–12.
  140. **Moncada, J., J. Schachter, M. Shipp, G. Bolan, and J. Wilber.** 1989. Cytobrush in collection of cervical specimens for detection of *Chlamydia trachomatis*. *J. Clin. Microbiol.* **27**:1863–1866.
  141. **Moncada, J., E. Williams, and J. Schachter.** 1995. Use of AmpliCor CT to identify *Chlamydia trachomatis* in urogenital specimens collected from men and women, abstr. K81, p. 302. In Program and Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
  142. **Morris, R., J. Legault, and C. Baker.** 1993. Prevalence of isolated urethral asymptomatic *Chlamydia trachomatis* infection in the absence of cervical infection of incarcerated adolescent girls. *Sex. Transm. Dis.* **20**:198–200.
  143. **Moss, T. R., S. Darougar, R. M. Woodland, M. Nathan, R. J. Dines, and V. Cathrine.** 1993. Antibodies to *Chlamydia* species in patients attending a genitourinary clinic and the impact of antibodies to *C. pneumoniae* and *C. psittaci* on the sensitivity and the specificity of *C. trachomatis* serology tests. *Sex. Transm. Dis.* **20**:61–65.
  144. **Mullis, K. B., and F. A. Faloona.** 1987. Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. *Methods Enzymol.* **55**:335–350.
  145. **Mumtaz, G., G. L. Ridgway, S. Clark, and E. Allason-Jones.** 1991. Evaluation of an enzyme immunoassay (Chlamydiazyme) with confirmatory test for the detection of chlamydial antigen in urine from men. *Int. J. STD AIDS* **2**:359–361.
  146. **Nash, P., and M. M. Krenz.** 1991. Culture media, p. 1226–1288. In A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
  147. **Newhall, W. J., S. DeLisle, D. Fine, R. E. Johnson, A. Hadgu, B. Matsuda, D. Osmond, J. Campbell, and W. E. Stamm.** 1994. Head-to-head evaluation of five different nonculture chlamydia tests relative to a quality-assured culture standard. *Sex. Transm. Dis.* **21**:S165–S166. (Abstract.)
  148. **Olsen, M. A., A. R. Sambol, and V. A. Bohnert.** 1995. Comparison of the Syva Microtrak enzyme immunoassay and Abbott Chlamydiazyme in the detection of chlamydial infections in women. *Arch. Pathol. Lab. Med.* **119**:153–156.
  149. **Ossewaarde, J. M., and M. Rieffe.** 1989. Storage conditions of *Chlamydia trachomatis* antigens. *Eur. J. Clin. Microbiol. Infect. Dis.* **8**:658–660.
  150. **Ossewaarde, J. M., M. Rieffe, M. Rozenberg-Arska, P. M. Ossenkoppele, R. P. Nawrocki, and A. M. van Loon.** 1992. Development and clinical evaluation of a polymerase chain reaction test for detection of *Chlamydia trachomatis*. *J. Clin. Microbiol.* **30**:2122–2128.
  151. **Paavonen, J., and E. Vesterinen.** 1982. *Chlamydia trachomatis* in cervicitis and urethritis in women. *Scand. J. Infect. Dis.* **32**(Suppl.):45–54.
  152. **Paisley, J. W., B. A. Lauer, P. Melinkovich, B. A. Gitterman, D. J. Feiten, and S. Berman.** 1986. Rapid diagnosis of *Chlamydia trachomatis* pneumonia in infants by direct immunofluorescence microscopy of nasopharyngeal secretions. *J. Pediatr.* **109**:653–655.
  153. **Patrick, D. M., M. L. Rekart, and L. Knowles.** 1994. Unsatisfactory performance of the leukocyte esterase test of first voided urine for rapid diagnosis of urethritis. *Genitourin. Med.* **70**:187–190.
  154. **Pearlman, M. D., and S. G. McNeeley.** 1992. A review of the microbiology, immunology, and clinical implications of *Chlamydia trachomatis* infections. *Obstet. Gynecol. Surv.* **47**:448–461.
  155. **Peeling, R. W., and R. C. Brunham.** 1994. Molecular techniques for the laboratory identification of *Chlamydia trachomatis*. *J. Int. Fed. Clin. Chem.* **6**:78–81.
  156. **Perine, P. L., and A. O. Osoba.** 1990. Lymphogranuloma venereum, p. 195–204. In K. K. Holmes, P.-A. Mårdh, P. F. Sparling, and P. J. Wiesner (ed.), *Sexually transmitted diseases*. McGraw Hill Book Co., New York, N.Y.
  157. **Peterson, E. M., B. A. Markoff, J. Schachter, and L. M. de la Maza.** 1990. The 7.5-kb plasmid present in *Chlamydia trachomatis* is not essential for the growth of this microorganism. *Plasmid* **23**:144–148.
  158. **Phillips, R. S., M. D. Aronson, W. C. Taylor, and C. Safran.** 1987. Should tests for *Chlamydia trachomatis* cervical infection be done during routine gynecologic visits? An analysis of the costs of alternative strategies. *Ann. Intern. Med.* **107**:188–194.
  159. **Phillips, R. S., P. A. Hanff, R. S. Kauffman, and M. D. Aronson.** 1987. Use of a direct fluorescent antibody test for detecting *Chlamydia trachomatis* cervical infection in women seeking routine gynecologic care. *J. Infect. Dis.* **156**:575–581.
  160. **Pothier, P., and A. Kazmierczak.** 1986. Comparison of cell culture with two different chlamydia tests using immunofluorescence or enzyme-linked immunosorbent assay. *Eur. J. Clin. Microbiol.* **5**:569–572.
  161. **Preece, P. M., J. M. Anderson, and R. G. Thompson.** 1989. *Chlamydia trachomatis* infection in infants: a prospective study. *Arch. Dis. Child* **64**:525–529.
  162. **Quinn, T. C., P. K. Gupta, R. T. Burkman, E. W. Kappus, M. Barbacci, and M. R. Spence.** 1987. Detection of *Chlamydia trachomatis* cervical infection: a comparison of Papanicolaou and immunofluorescent staining with cell culture. *Am. J. Obstet. Gynecol.* **157**:394–399.
  163. **Rake, G., M. McKee, and M. F. Shaffer.** 1940. Agent of lymphogranuloma venereum in the yolk sac of the developing chick embryo. *Proc. Soc. Exp. Biol. Med.* **43**:332–334.
  164. **Rapoza, P. A., T. C. Quinn, L. A. Kiessling, W. R. Green, and H. R. Taylor.** 1986. Assessment of neonatal conjunctivitis with a direct fluorescent monoclonal antibody stain for Chlamydia. *JAMA* **255**:3369–3373.
  165. **Reeve, P., J. Owen, and J. D. Oriol.** 1975. Laboratory procedures for the isolation of *Chlamydia trachomatis* from the human genital tract. *J. Clin. Pathol.* **28**:910–914.
  166. **Rietmeijer, C. A. M., F. N. Judson, M. B. van Hensbroek, J. M. Ehret, and J. M. Douglas, Jr.** 1991. Unsuspected *Chlamydia trachomatis* infection in heterosexual men attending a sexually transmitted diseases clinic: evaluation of risk factors and screening methods. *Sex. Transm. Dis.* **18**:28–35.
  167. **Rompalo, A. M., P. Roberts, K. Johnson, et al.** 1988. Empirical therapy for the management of acute proctitis in homosexual men. *JAMA* **260**:348–353.
  168. **Rompalo, A. M., R. J. Suchland, C. B. Price, and W. E. Stamm.** 1987. Rapid diagnosis of *Chlamydia trachomatis* rectal infection by direct fluorescence staining. *J. Infect. Dis.* **155**:1075–1076.
  169. **Roosendaal, R., J. M. Walboomers, O. R. Veltman, I. Melgers, C. Burger, O. P. Bleker, D. M. MacClaren, C. J. Meijer, and A. J. van den Brule.** 1993. Comparison of different primer sets for detection of *Chlamydia trachomatis* by the polymerase chain reaction. *J. Med. Microbiol.* **38**:426–433.
  170. **Rota, T. R., and R. L. Nichols.** 1971. Infection of cell culture by trachoma agent. Enhancement by DEAE-dextran. *J. Infect. Dis.* **124**:419–421.
  171. **Rota, T. R., and R. L. Nichols.** 1973. *Chlamydia trachomatis* in cell culture. I. Comparison of efficiencies of infection in several chemically defined media, at various pH and temperature values, and after exposure to diethylaminoethyl-dextran. *Appl. Microbiol.* **26**:560–565.
  172. **Ryan, G., T. N. Abdella, S. G. McNeeley, V. S. Baselski, and D. E. Drummond.** 1990. *Chlamydia trachomatis* infection in pregnancy and effect of treatment on pregnancy outcome. *Am. J. Obstet. Gynecol.* **162**:34–39.
  173. **Sackett, D. L., R. B. Haynes, and P. Tugwell.** 1985. *Clinical epidemiology*, p. 59–138. Little, Brown & Co., Boston.



174. Saiki, R. K., S. Scharf, F. Faloona, K. B. Mullis, G. T. Horn, H. A. Erlich, and N. Arnheim. 1985. Enzymatic amplification of  $\beta$ -globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* **230**:1350-1354.
175. Salmon, V. C., B. R. Kenyon, J. C. Overall, Jr., and R. Anderson. 1994. Use of a universal transport media in a commercial polymerase chain reaction assay for *Chlamydia trachomatis*. abstr. S7, p. 33. Abstracts of the 10th Annual Clearwater Virology Symposium 1994.
176. Sanders, J. W., E. W. Hook III, L. E. Welsh, M. E. Shepherd, and T. C. Quinn. 1994. Evaluation of an enzyme immunoassay for detection of *Chlamydia trachomatis* in urine of asymptomatic men. *J. Clin. Microbiol.* **32**:24-27.
177. Schachter, J. 1980. Chlamydiae, p. 701-706. In N. R. Rose and H. Friedman (ed.), *Manual of clinical microbiology*, 2nd ed. American Society for Microbiology, Washington, D.C.
178. Schachter, J. 1989. Why we need a program for the control of *Chlamydia trachomatis*. *N. Engl. J. Med.* **320**:802-804.
179. Schachter, J., and C. R. Dawson. 1978. Human chlamydial infections, p. 187. PGG Publishing Co., Littleton, Mass.
180. Schachter, J., M. Grossman, R. L. Sweet, J. Holt, C. Jordan, and E. Bishop. 1986. Prospective study of perinatal transmission of *Chlamydia trachomatis*. *JAMA* **255**:3374-3377.
181. Schachter, J., and W. E. Stamm. 1995. Chlamydia, p. 669-677. In P. R. Murray, M. A. Pfaller, F. C. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
182. Schachter, J., W. E. Stamm, M. A. Chernesky, E. W. Hook III, R. B. Jones, F. N. Judson, J. A. Kellogg, B. LeBar, P.-A. Mårdh, W. M. McCormack, et al. 1992. Nonculture tests for genital tract chlamydial infection. What does the package insert mean, and will it mean the same thing tomorrow? *Sex. Transm. Dis.* **19**:243-244.
183. Schachter, J., W. E. Stamm, T. C. Quinn, W. W. Andrews, J. D. Burczak, and H. H. Lee. 1994. Ligase chain reaction to detect *Chlamydia trachomatis* infection of the cervix. *J. Clin. Microbiol.* **32**:2540-2543.
184. Schachter, J., E. Stoner, and J. Moncada. 1983. Screening for chlamydial infections in women attending family planning clinics. *West. J. Med.* **138**:375-379.
185. Schoenwald, E., B. L. Schmidt, G. Steinmetz, J. Hosmann, G. Pohl-Gubo, A. Luger, and G. Gasser. 1988. Diagnosis of *Chlamydia trachomatis* infection—culture versus serology. *Eur. J. Epidemiol.* **4**:75-82.
186. Schubiner, H. H., W. D. LeBar, S. Joseph, C. Taylor, and C. Jemal. 1992. Evaluation of two rapid tests for the diagnosis of *Chlamydia trachomatis* genital infections. *Eur. J. Clin. Microbiol. Infect. Dis.* **11**:553-556.
187. Schwebke, J. R., W. E. Stamm, and H. H. Handsfield. 1990. Use of sequential enzyme immunoassay and direct fluorescent antibody tests for detection of *Chlamydia trachomatis* infections in women. *J. Clin. Microbiol.* **28**:2473-2476.
188. Sellors, J., M. Chernesky, L. Pickard, D. Jang, S. Walter, J. Krepel, and J. Mahony. 1993. Effect of time elapsed since previous voiding on the detection of *Chlamydia trachomatis* antigens in urine. *Eur. J. Clin. Microbiol. Infect. Dis.* **12**:285-289.
189. Sellors, J. W., J. B. Mahony, D. Jang, et al. 1991. Comparison of cervical, urethral, and urine specimens for the detection of *Chlamydia trachomatis* in women. *J. Infect. Dis.* **164**:205-208.
190. Sellors, J. W., J. B. Mahony, L. Pickard, D. Jang, D. Groves, K. E. Luinstra, and M. A. Chernesky. 1993. Screening urine with a leukocyte esterase strip and subsequent chlamydial testing of asymptomatic men attending primary care practitioners. *Sex. Transm. Dis.* **20**:152-157.
191. Sellors, J. W., L. Pickard, A. Gafni, C. H. Goldsmith, D. Jang, J. B. Mahony, and M. A. Chernesky. 1992. Effectiveness and efficiency of selective vs universal screening for chlamydial infection in sexually active young women. *Arch. Intern. Med.* **152**:1837-1844.
192. Shah, J. S., J. Liu, J. Smith, B. Popoff, G. Radcliffe, W. J. O'Brien, G. Serpe, D. M. Olive, and W. King. 1994. Novel, ultrasensitive, Q-beta replicase-amplified hybridization assay for detection of *Chlamydia trachomatis*. *J. Clin. Microbiol.* **32**:2718-2724.
193. Skulnick, M., R. Chua, A. E. Simor, D. E. Low, H. E. Khosid, S. Fraser, E. Lyons, E. A. Legere, and D. A. Kitching. 1994. Use of the polymerase chain reaction for the detection of *Chlamydia trachomatis* from endocervical and urine specimens in an asymptomatic low-prevalence population of women. *Diagn. Microbiol. Infect. Dis.* **20**:195-201.
194. Smith, J. W., R. E. Rogers, B. P. Katz, J. F. Brickler, P. L. Lineback, B. VanderPol, and R. B. Jones. 1987. Diagnosis of chlamydial infection in women attending antenatal and gynecologic clinics. *J. Clin. Microbiol.* **25**:868-872.
195. Smith, T. F., S. D. Brown, and L. A. Weed. 1982. Diagnosis of *Chlamydia trachomatis* infections by cell cultures and serology. *Lab. Med.* **13**:92-100.
196. Stamm, W. E. 1988. Diagnosis of *Chlamydia trachomatis* genitourinary infections. *Ann. Intern. Med.* **108**:710-717.
197. Stamm, W. E., and B. Cole. 1986. Asymptomatic *Chlamydia trachomatis* urethritis in men. *Sex. Transm. Dis.* **13**:163-165.
198. Stamm, W. E., H. R. Harrison, E. R. Alexander, L. D. Cles, M. R. Spence, and T. C. Quinn. 1984. Diagnosis of *Chlamydia trachomatis* infections by direct immunofluorescence staining of genital secretions—a multicenter trial. *Ann. Intern. Med.* **101**:638-641.
199. Stamm, W. E., L. A. Koutsky, J. K. Benedetti, J. L. Jourden, R. C. Brunham, and K. K. Holmes. 1984. *Chlamydia trachomatis* urethral infections in men. Prevalence, risk factors, and clinical manifestations. *Ann. Intern. Med.* **100**:47-51.
200. Stamm, W. E., M. Tam, M. Koester, and L. Cles. 1983. Detection of *Chlamydia trachomatis* inclusions in McCoy cell cultures with fluorescein-conjugated monoclonal antibodies. *J. Clin. Microbiol.* **17**:666-668.
201. Stary, A., B. Chouieri, and H. Lee. 1995. Implication of sensitive molecular diagnosis of *Chlamydia trachomatis* in non-invasive sample types, abstr. 041, p. 43. In Abstracts of the 11th Meeting of the International Society for STD Research 1995.
202. Stary, A., M. Genc, C. Heller-Vitouch, and P. A. Mardh. 1992. Chlamydial antigen detection in urine samples by immunofluorescence tests. *Infection* **20**:101-104.
203. Stary, A., L. Teodorowicz, I. Horting-Muller, S. Nerad, and M. Storch. 1994. Evaluation of the Gen-Probe PACE 2 and the Microtrak enzyme immunoassay for diagnosis of *Chlamydia trachomatis* in urogenital samples. *Sex. Transm. Dis.* **21**:26-30.
204. Steingrimsdottir, O., J. H. Olafsson, E. Sigvaldadottir, and R. Palsdottir. 1994. Clinical evaluation of an automated enzyme-linked fluorescent assay for the detection of chlamydial antigen in specimens from high-risk patients. *Diagn. Microbiol. Infect. Dis.* **18**:101-104.
205. Stephens, R. S. 1990. Molecular genetics of *Chlamydia*, p. 63-72. In W. R. Bowie, H. D. Caldwell, R. P. Jones, P. A. Mårdh, G. L. Ridgway, J. Schachter, W. E. Stamm, and M. E. Ward (ed.), *Chlamydial infections*. Proceedings of the 7th International Symposium on Human Chlamydial Infections. Cambridge University Press, Cambridge, England.
206. Stephens, R. S., C.-C. Kuo, and M. R. Tam. 1982. Sensitivity of immunofluorescence with monoclonal antibodies for detection of *Chlamydia trachomatis* inclusions in cell culture. *J. Clin. Microbiol.* **16**:4-7.
207. Suchland, R. J., and W. E. Stamm. 1991. Simplified microtiter well cell culture method for rapid immunotyping of *Chlamydia trachomatis*. *J. Clin. Microbiol.* **29**:1333-1338.
208. Svensson, L., L. Westrom, K. T. Ripa, and P.-A. Mårdh. 1980. Differences in some clinical and laboratory parameters in acute salpingitis related to culture and serologic findings. *Am. J. Obstet. Gynecol.* **138**:1017-1023.
209. Sweet, R. L., D. V. Landers, C. Walker, and J. Schachter. 1987. *Chlamydia trachomatis* infection and pregnancy outcome. *Am. J. Obstet. Gynecol.* **156**:824-833.
210. Talbot, H., and B. Romanowski. 1994. Factors affecting urine EIA sensitivity in the detection of *Chlamydia trachomatis* in men. *Genitourin. Med.* **70**:101-104.
211. Tam, M. R., W. E. Stamm, H. H. Handsfield, R. Stephens, C.-C. Kuo, K. K. Holmes, K. Ditzinger, M. Kreiger, and R. C. Nowinski. 1984. Culture-independent diagnosis of *Chlamydia trachomatis* using monoclonal antibodies. *N. Engl. J. Med.* **310**:1146-1150.
212. Thomas, B., C. Gilchrist, and D. Taylor-Robinson. 1991. Detection of *Chlamydia trachomatis* by direct immunofluorescence improved by centrifugation of specimens. *Eur. J. Clin. Microbiol. Infect. Dis.* **10**:659-662.
213. Thomas, B. J., E. J. MacLeod, P. E. Hay, P. J. Horner, and D. Taylor-Robinson. 1994. Limited value of two widely used enzyme immunoassays for detection of *Chlamydia trachomatis* in women. *Eur. J. Clin. Microbiol. Infect. Dis.* **13**:651-655.
214. Thomas, B. J., E. J. MacLeod, and D. Taylor-Robinson. 1993. Evaluation of sensitivity of 10 diagnostic assays for *Chlamydia trachomatis* by use of a simple laboratory procedure. *J. Clin. Pathol.* **46**:912-914.
215. Thompson, S., B. Lopez, K.-H. Wong, et al. 1982. A prospective study of chlamydial and mycoplasmal infections during pregnancy, p. 155-158. In P.-A. Mårdh, K. K. Holmes, J. D. Oriel, J. Schachter, and P. Piot (ed.), *Chlamydial infections*. Fernstrom Foundation Series. Elsevier Biomedical Press, Amsterdam, The Netherlands.
216. Trachtenberg, A. I., A. E. Washington, and S. Halldorson. 1988. A cost-based analysis for chlamydia screening in California family planning clinics. *Obstet. Gynecol.* **71**:101-108.
217. U.S. Department of Health and Human Services. 1988. Biosafety in microbiological and biomedical laboratories. HHS publication (NIH) 88-8395. U.S. Government Printing Office, Washington, D.C.
218. Vandenvelde, C., R. Scheen, M. Defoor, M. Duys, J. Dumon, and D. Van Beers. 1993. Suppression of the inhibitory effect of denatured albumin on the polymerase chain reaction by sodium octanoate: application to routine clinical detection of hepatitis B virus at its infectivity threshold in serum. *J. Virol. Methods* **42**:251-263.
219. Van Dyck, E., N. Samb, A. D. Sarr, L. Van de Velden, J. Moran, S. Mboup, I. Ndoye, J. L. Lamboray, A. Meheus, and P. Piot. 1992. Accuracy of two enzyme immunoassays and cell culture in the detection of *Chlamydia trachomatis* in low and high risk populations in Senegal. *Eur. J. Clin. Microbiol. Infect. Dis.* **11**:527-534.
220. Van Gemen, B., T. Kievits, R. Schukking, D. van Strijp, L. T. Malek, R. Sookninan, H. G. Huisman, and P. Lens. 1993. Quantification of HIV-1

- RNA in plasma using NASBA during HIV-1 primary infection. *J. Virol. Methods* **43**:177–187.
221. Van Gemen, B., R. van Beuningen, A. Nabbe, D. van Strijp, S. Jurriaans, P. Lens, and T. Kievits. 1994. A one-tube quantitative HIV-1 RNA NASBA nucleic acid amplification assay using electrochemiluminescent (ECL) labelled probes. *J. Virol. Methods* **49**:157–167.
  222. Verkooyen, R. P., A. Luijendijk, W. H. F. Goessens, W. M. Huisman, J. A. J. Kluytmans, J. H. van Rijsoort-Vos, and H. A. Verbrugh. 1995. Detection of PCR-inhibitors in cervical specimens using the Amplicor *Chlamydia trachomatis* assay, abstr. D104, p. 84. In Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
  223. Walker, G. T., M. S. Fraiser, J. L. Schram, M. C. Little, J. G. Nadeau, and D. P. Malinowski. 1992. Strand displacement amplification—an isothermal, in vitro DNA amplification technique. *Nucleic Acids Res.* **20**:1691–1696.
  224. Wang, S.-P., and J. T. Grayston. 1970. Immunologic relationship between genital TRIC, lymphogranuloma venereum, and related organisms in a new microtiter indirect immunofluorescence test. *Am. J. Ophthalmol.* **70**:367–374.
  225. Wang, S.-P., J. T. Grayston, E. R. Alexander, and K. K. Holmes. 1975. Simplified immunofluorescence test with trachoma-lymphogranuloma venereum (*Chlamydia trachomatis*) antigens for use as a screening test for antibody. *J. Clin. Microbiol.* **1**:250–255.
  226. Warford, A. L., T. L. Carter, R. A. Levy, and K. A. Rekrut. 1985. Comparison of sonicated and nonsonicated specimens for the isolation of *Chlamydia trachomatis*. *Am. J. Clin. Pathol.* **83**:625–629.
  227. Warren, R., B. Dwyer, M. Plackett, K. Pettit, N. Rizvi, and A. M. Baker. 1993. Comparative evaluation of detection assays for *Chlamydia trachomatis*. *J. Clin. Microbiol.* **31**:1663–1666.
  228. Washington, A. E., R. E. Johnson, and L. L. Sanders. 1987. *Chlamydia trachomatis* infections in the United States. What are they costing us? *JAMA* **257**:2070–2072.
  229. Washington, A. E., R. E. Johnson, L. L. Sanders, R. C. Barnes, and E. R. Alexander. 1986. Incidence of *Chlamydia trachomatis* infections in the United States using reported *Neisseria gonorrhoeae* as a surrogate, p. 487–490. In D. Oriel, G. Ridgway, J. Schachter, et al. (ed.), *Chlamydia infections. Proceedings of the Sixth International Symposium on Human Chlamydia Infections*. Cambridge University Press, Cambridge, England.
  230. Weinstock, H., D. Dean, and G. Bolan. 1994. *Chlamydia trachomatis* infections. Sexually transmitted diseases in the AIDS era, part II. *Infect. Dis. Clin. North Am.* **8**:797–819.
  231. Weiss, S. G., R. W. Newcomb, and M. O. Beem. 1986. Pulmonary assessment of children after chlamydial pneumonia of infancy. *J. Pediatr.* **108**:659–664.
  232. Werner, M. J., and F. M. Biro. 1991. Urinary leukocyte esterase screening for asymptomatic sexually transmitted disease in adolescent males. *J. Adolesc. Health* **12**:326–329.
  233. Westrom, L., L. P. H. Bengtsson, and P. A. Mårdh. 1981. Incidence, trends and risks of ectopic pregnancy in a population of women. *Br. Med. J.* **282**:15–18.
  234. Wiesenfeld, H. C., R. P. Heine, F. M. DiBiasi, C. A. Repp, A. Rideout, I. Macio, and R. L. Sweet. Self collection of vaginal introitus specimens: a novel approach to *Chlamydia trachomatis* testing in women, abstr. 040, p. 42. In Abstracts of the 11th Meeting of the International Society for STD Research 1995.
  235. Wiesenfeld, H. C., M. Uhrin, B. W. Dixon, and R. L. Sweet. 1994. Diagnosis of male *Chlamydia trachomatis* urethritis by polymerase chain reaction. *Sex. Transm. Dis.* **21**:268–271.
  236. Wong, K. H., S. K. Skelton, and Y. K. Chan. 1992. Efficient culture of *Chlamydia pneumoniae* with cell lines derived from the human respiratory tract. *J. Clin. Microbiol.* **30**:1625–1630.
  237. Woods, G. L., and J. A. Bryan. 1994. Detection of *Chlamydia trachomatis* by direct fluorescent antibody staining. Results of the College of American Pathologists proficiency testing program, 1986–1992. *Arch. Pathol. Lab. Med.* **118**:483–488.
  238. Workowski, K. A., M. F. Lampe, K. G. Wong, M. B. Watts, and W. E. Stamm. 1993. Long term eradication of *Chlamydia trachomatis* genital infection after antimicrobial therapy. Evidence against persistent infection. *JAMA* **270**:2071–2075.
  239. Wu, C.-H., M.-F. Lee, S.-C. Yin, D.-M. Yang, and S. F. Cheng. 1992. Comparison of polymerase chain reaction, monoclonal antibody based enzyme immunoassay, and cell culture for detection of *Chlamydia trachomatis* in genital specimens. *Sex. Transm. Dis.* **19**:193–197.
  240. Yang, B., R. Yolken, and R. Viscidi. 1993. Quantitative polymerase chain reaction by monitoring enzymatic activity of DNA polymerase. *Anal. Biochem.* **208**:110–116.
  241. Yang, L. I., E. S. Panke, P. A. Leist, R. J. Fry, and R. F. Lee. 1991. Detection of *Chlamydia trachomatis* endocervical infection in asymptomatic and symptomatic women: comparison of deoxyribonucleic acid probe test with tissue culture. *Am. J. Obstet. Gynecol.* **165**:1444–1453.
  242. Yoder, B. L., W. E. Stamm, C. M. Koester, and E. R. Alexander. 1981. Microtest procedure for isolation of *Chlamydia trachomatis*. *J. Clin. Microbiol.* **13**:1036–1039.
  243. Yong, E. C., J. S. Chinn, H. D. Caldwell, and C.-C. Kuo. 1979. Reticulate bodies as single antigen in *Chlamydia trachomatis* serology with microimmunofluorescence. *J. Clin. Microbiol.* **10**:351–356.
  244. Zeeberg, B., I. Thelin, and C. Schalen. 1992. *Chlamydia trachomatis* antigen detection by Chlamydiazyme combined with Chlamydia Blocking Reagent verification. *Int. J. STD AIDS* **3**:355–359.
  245. Zelin, J. M., A. J. Robinson, G. L. Ridgway, E. Allason-Jones, and P. Williams. 1995. Chlamydial urethritis in heterosexual men attending a genitourinary medicine clinic: prevalence, symptoms, condom usage and partner change. *Int. J. STD AIDS* **6**:27–30.