# The laboratory diagnosis of *Chlamydia trachomatis* infections

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Lower genital tract infections with *Chlamydia trachomatis* are predominantly asymptomatic in men and women. Diagnostic technology has provided several approaches to the diagnosis of C *trachomatis*. Outside of cells, *Chlamydia* can die or degrade without optimal storage and transportation. Because some of the other assays perform better on certain specimen types, it is important for laboratories to recognize these differences and provide advice to physicians and nurses collecting patient specimens, with the objective of diagnosing lower genital tract infections to prevent transmission and upper tract damage. Most invasive specimens, such as cervical or urethral swabs, may be collected for culture, antigen or nucleic acid detection. Noninvasive samples such as first-void urine and vaginal swabs can be easily collected by the patient; these samples must be tested by more sensitive nucleic acid amplification tests. These newer investigative strategies should enable implementation of screening programs to identify and treat partners. Serology has not been particularly useful for the diagnosis of acute C *trachomatis* infections in adults. Presently, it appears that antibiotic-resistant C *trachomatis* is not a clinical problem. Laboratories providing C *trachomatis* diagnosis require participation in continuous quality improvement programs.

**Key Words:** *Antigen detection; Appropriate specimens; Cell culture; Diagnostic methods; Nucleic acid amplification*

## Le diagnostic en laboratoire des infections à *Chlamydia trachomatis*

Les infections des voies génitales inférieures par le *Chlamydia trachomatis* sont en grande partie asymptomatiques chez les hommes et les femmes. La technologie diagnostique a permis d'utiliser plusieurs démarches diagnostiques du C *trachomatis*. À l'extérieur des cellules, le chlamydia peut mourir ou se dégrader si son entreposage et son transport ne sont pas optimaux. Puisque d'autres dosages réagissent mieux sur certains types d'échantillons, il est important que les laboratoires admettent ces différences et conseillent les médecins et les infirmières qui recueillent les échantillons auprès des patients, afin de diagnostiquer les infections des voies génitales inférieures et de prévenir la transmission et les lésions des voies supérieures. La plupart des échantillons effractifs, tels que les écouvillons cervicaux et urétraux, peuvent être recueillis pour les besoins des cultures et de détection des antigènes et des acides nucléiques. Les échantillons non effractifs, tels que le premier jet des urines et les frottis vaginaux, peuvent facilement être prélevés par les patients, mais doivent être testés au moyen d'épreuves par amplification d'acides nucléiques plus sensibles. Ces stratégies d'investigation plus récentes devraient permettre l'implantation de programmes de dépistage pour repérer et traiter les partenaires. La sérologie n'a pas été particulièrement utile pour diagnostiquer les infections à C *trachomatis* aiguës chez les adultes. Pour l'instant, il semble que le C *trachomatis* antibiorésistant ne constitue pas un problème clinique. Les laboratoires qui offrent le diagnostic du C *trachomatis* exigent une participation à des programmes d'amélioration continue de la qualité.

With an estimated 90 million cases of *Chlamydia trachomatis* worldwide, including four million new infections occurring each year in North America, the challenge for Canadians is to apply useful diagnostic tests to screen sexually active populations to treat and prevent transmission and upper genital tract sequelae.

C *trachomatis* serovars A, B, Ba and C are associated with endemic trachoma, which is the most common preventable form of blindness in certain parts of the Mediterranean and Middle East. Serovars L1, L2 and L3 are associated with lymphogranuloma venereum (LGV) in tropical settings. Canadian physicians may occasionally encounter patients with trachoma or LGV, but serovars D through K are the major organisms diagnosed in Canadian laboratories. Serovars D through K cause nongonococcal urethritis and epididymitis in men, Reiter's syndrome or proctitis, conjunctivitis in both men and women, and cervicitis, urethritis, endometritis, salpingitis and perihepatitis in women (1). Between one-half and two-thirds of chlamydial infections in men and women may be asymptomatic and remain undiagnosed and untreated. In women, this may lead to late sequelae such as endometritis, salpingitis, pelvic inflammatory disease, ectopic pregnancy or tubal factor infertility. C *trachomatis* in the cervix may be transmitted to a neonate during vaginal delivery, resulting in conjunctivitis and neonatal pneumonia (2). Vaginal, pharyngeal and enteric infections in neonates have also been recognized. Both adult and neonatal infections are easily treated.

Infections with C *trachomatis* have been associated with increased rates of transmission of HIV (3).

Laboratory services for the diagnosis of C *trachomatis* became feasible in the early 1980s when cell culture systems were developed for the inoculation of clinical specimens. These systems complemented cytological methods and moved the diagnostic field forward. Throughout the 1980s and 1990s, because of an effort by commercial companies to develop kits for detecting antigens and nucleic acids, we are now in a position to provide diagnostic testing and screening with noninvasively collected specimens (4,5).

Most requests for the laboratory diagnosis of C *trachomatis* are for the following situations: male patients with nongonococcal

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**TABLE 1**
**Recommended diagnostic methods according to specimen type for *Chlamydia trachomatis***

| Specimen | Diagnostic method | | | | |
|---|---|---|---|---|---|
| | Microscopy | Culture | AD* | NAH* | NAA† |
| Conjunctival | + | + | + | + | + |
| Nasopharyngeal | – | + | – | – | + |
| Cervical | –‡ | + | + | + | + |
| Urethral | – | + | + | + | + |
| Rectal | – | + | +§ | – | + |
| Vulval | – | – | – | – | + |
| Vaginal | – | – | – | – | + |
| Introital | – | – | – | – | + |
| Meatal | – | – | – | – | + |
| Urine | – | – | – | – | + |
| Bubo pus | – | + | – | – | + |
| Semen | – | – | – | – | +¶ |

**Samples positive by nucleic acid hybridization (NAH) or enzyme immunoassay antigen detection (AD) require confirmation by another AD method or by neutralization; †Relative to the other assays, nucleic acid amplification (NAA) tests are the most sensitive and specific; ‡Papanicolaou smear not useful; §Only direct fluorescent antibody; ¶No commercial NAA tests are approved, therefore, semen should only be used with a validated protocol. Low-level NAA results are not always repeatable*

urethritis, postgonococcal urethritis, epididymitis or Reiter's syndrome; female patients with mucopurulent cervicitis, urethral syndrome, endometritis, salpingitis, perihepatitis, ectopic pregnancy, tubal factor infertility or pelvic pain; all patients with gonorrhea; sexual contacts of symptomatic patients or patients with a positive laboratory diagnosis (contact testing); pregnant women; infants with conjunctivitis and/or pneumonia; asymptomatic sexually active patients; and finally, donors of semen for assisted fertilization. The purpose of the present document is to provide guidance for the clinician and laboratory worker and enable the most appropriate specimen collection, testing and reporting of C *trachomatis* infections, thus enabling effective patient treatment and management.

## SPECIMEN COLLECTION, TRANSPORT, STORAGE AND PROCESSING

Chlamydiae are labile bacteria, and viability can be maintained by keeping specimens cold and minimizing the time between specimen collection and processing in the laboratory. A variety of swab types can be used, but toxicity related to materials in swabs can be problematic. It is useful, therefore, to test swab types for toxicity in cell cultures or interference in nonculture assays when proprietary swabs are not provided by the manufacturer. As a general rule, swabs with wooden shafts should not be used. Cotton, Dacron and calcium alginate swabs may all be used, although toxicity has been noted with specific lots of each (6). The cytobrush has also been used to collect endocervical specimens. It appears to collect more cells than swabs and has been associated in some investigators' experiences with higher recovery rates of chlamydiae and higher rates of antigen detection by direct fluorescent antibody (DFA). Regardless of whether a cytobrush or swab is used, clinicians and other health care providers should be trained to collect adequate specimens for chlamydia detection. Table 1 summarizes recommendations for matching specimen type to diagnostic methods. For successful culture of chlamydiae, swabs, scrapings and small tissue samples should be forwarded to the laboratory in a special chlamydial transport medium such as 2SP (0.2 M sucrose-phosphate transport medium containing 10 µg of gentamicin/mL, 25 µg of vancomycin/mL and 25 U of nystatin/mL). Broad-spectrum antibiotics such as tetracyclines, macrolides or penicillin cannot be used in the transport media because they have activity against chlamydiae. Chlamydial specimens should be refrigerated on receipt in the laboratory; if specimens cannot be processed within 24 h after collection, they should be frozen at –70°C. Currently, culture is the only assay that provides acceptable results for C *trachomatis* in legal abuse cases, but collection of specimens for nucleic acid amplification (NAA) tests at the same time may enhance an investigation.

When specimens are collected for DFA, enzyme immunoassay (EIA), nucleic acid hybridization (NAH) or NAA procedures, the descriptions and procedural instructions given in the product's package insert should be followed. This includes the use of swabs or transport media specified by the manufacturers because the use of other materials may impair the sensitivity and/or specificity of the test.

For cytology, isolation in culture or antigen detection methods, epithelial cell specimens should be collected by vigorous swabbing or scraping of the involved sites. Purulent discharges that lack infected epithelial cells are inappropriate and should be cleaned from the site before the sample is collected. Appropriate sites include the conjunctiva for trachoma or inclusion conjunctivitis, the male anterior urethra (several centimetres into the urethra) and the female cervix (within the endocervical canal) or urethra. Because C *trachomatis* infects columnar or squamocolumnar cells, cervical specimens must be collected at the transitional zone or within the opening of the cervix. The organism also infects the female urethra, and recovery rates may be improved by collecting a specimen from the urethra and the cervix and sending both to the laboratory. Vaginal swabs are inappropriate for these non-NAA detection methods (Table 1). It should be remembered that the squamocolumnar transitional zone in prepubertal females is ahead of the cervix, in the vaginal area. Also, culture should be the assay of choice in such patients because of the possibility of medicolegal proceedings.

In women with salpingitis, samples may be collected by needle aspiration of the involved fallopian tube. Endometrial specimens have also yielded chlamydiae. In other clinical situations, the rectal mucosa, nasopharynx and throat may be

swabbed. For infants with pneumonia, swabs may be collected from the posterior nasopharynx or the throat; however, nasopharyngeal or tracheobronchial aspirates collected by intubation are better specimens. For LGV strains, bubo pus, rectal or urethral swabbing, or biopsy samples should be collected.

First-void urine (FVU) specimens from men and women, and vaginal, introital or vulvar swabs from women are excellent specimens for the detection of C *trachomatis* by NAA tests (7-10) (Table 1). FVU is the first 10 mL to 30 mL of urine, and specimens should be obtained ideally between 2 h and 6 h after the last micturition. It is not necessary and may not be advantageous to obtain the first urine specimen passed in the morning. Processing of urine specimens should follow the manufacturer's instructions.

Serum specimens are not recommended for the diagnosis of acute C *trachomatis* infections because the immune responses detected for these mucous membrane infections are often short lived or due to past infections (11). The two exceptions where serology may be helpful include chlamydial neonatal pneumonia (high immunoglobulin [Ig] M) or chlamydial tubal factor infertility (high IgG). A clotted specimen should be submitted.

General guidelines for processing specimens are listed below. Fresh samples are preferred, but frozen material (–70°C) is acceptable. Commercial tests should be used only for approved specimens (as listed in the product package insert). C *trachomatis* is a biocontainment level 2 agent and is not considered to be a particularly dangerous pathogen to handle in the laboratory. However, a number of laboratory-acquired infections, usually manifested as follicular conjunctivitis, have occurred. The LGV biovar is a more invasive organism, and severe cases of pneumonia or lymphadenitis have occurred when researchers were exposed to aerosols created by laboratory procedures such as sonication or centrifugation.

## PROCESSING SPECIMENS IN THE LABORATORY

### Ocular and genital tract

For ocular and genital tract sites, the laboratory usually receives swabs in transport medium containing antibiotics. Specimens to be assayed by commercial EIA, DFA, NAH or NAA should be handled as specified in the package insert. Homemade test protocols should be validated based on performance and consensus as per the guidelines of accrediting agencies.

### Bubo pus

To prepare bubo pus, the viscous material is ground and then suspended into nutrient broth or cell culture medium to at least 20% by weight. Even when the pus is not viscous, dilution is advisable. If the bubo is not fluctuant, sterile saline may be injected and aspirated for isolation attempts. The material should be tested for bacterial contaminants by plating on appropriate agar media, and then inoculated into cell cultures for isolation of chlamydiae.

### Nasopharyngeal swabs

The nasopharyngeal swab is placed into cell culture medium and emulsified thoroughly by shaking with glass beads in a sterile container sealed tightly with a stopper. Extracts should be centrifuged for 20 min to 30 min at 100 *g* to remove coarse material before the supernatant fluid is inoculated onto cell monolayers.

### Rectal swabs

Rectal swabs are suspended in cell culture medium containing gentamicin and vancomycin. The suspension is shaken thoroughly and centrifuged at 300 *g* for 10 min, and the supernatant fluid is used. It may be further diluted (1:2 and 1:20) with culture medium before being inoculated into cell culture. Swabs from patients practicing anal sex may also benefit from sampling for NAA testing, especially for identification of L serovars causing LGV.

### Tissue samples

Frozen tissue is thawed, minced with sterile scissors, and ground with a mortar and pestle or homogenizer. A volume of cell culture medium required to make a 10% to 20% suspension is added, and the suspension is thoroughly mixed. For tissue specimens, serial dilutions (1:10 to 1:100) are often required for inoculation to overcome toxicity.

### Semen

Prospective semen donors can donate an FVU for NAA testing. Semen (stored frozen) needs to be tested in an NAA test that has been validated for this purpose.

## DIAGNOSTIC METHODS

### Direct cytological examination

Infections of the conjunctiva can be diagnosed by the detection of typical intracytoplasmic inclusions. For Giemsa staining, the smear is air dried, fixed with absolute methanol for at least 5 min and dried again. It is then covered with the freshly prepared diluted Giemsa stain for at least 1 h. The slide is rapidly rinsed in 95% ethanol to remove excess dye and then dried and examined microscopically. The inclusions are basophilic and stain pinkish-blue. Cytological testing to detect inclusions is particularly useful in diagnosing acute inclusion conjunctivitis of the newborn; the sensitivity of this method exceeds 90%. Cytological testing is relatively insensitive when diagnosing adult conjunctival and genital tract infections.

### Isolation in cell culture

Culture is the only procedure that confirms the presence of viable organisms. Antigens, nucleic acids or antibodies can be present in the absence of viable infectious particles.

Most, if not all, chlamydiae appear to be able to grow in cell culture if the inoculum is centrifuged onto preformed, pretreated cell monolayers (12). Before inoculation and centrifugation, preformed cell monolayers can be treated with 30 µg/mL of Diethylaminoethyl-Dextran in Hanks' balanced salt solution for 20 min to change the negative charge on the cell surface and facilitate adhesion of chlamydiae to the cell monolayer. This is not necessary for LGV serovars but facilitates infections by other serovars. LGV strains are capable of serial growth in cell culture without centrifugation. McCoy, HEp-2 and HeLa cells are most commonly used for C *trachomatis*. Clinical specimens should be inoculated onto cycloheximide-treated monolayer cultures of McCoy cells or other appropriate cells. Inoculation involves centrifugation of the specimen onto the cell monolayer followed by incubation for 48 h to 72 h and staining for intracytoplasmic inclusions. For the shell

vial method, McCoy cells are plated onto 12 mm glass cover slips in 15 mm diameter 3.697 mL disposable glass vials. The cell concentration (approximately $1\times10^5$ cells/mL to $2\times10^5$ cells/mL) is selected to give a light, confluent monolayer after 24 h to 48 h of incubation at 35°C to 37°C in 5% $CO_2$. For optimal results, the cells should be used within 24 h after reaching confluency.

Clinical specimens are shaken with sterile 5 mm glass beads to lyse the epithelial cells and release the chlamydiae before being used for inoculation. This procedure is safer and more convenient than sonication. For inoculation, the medium is removed from the cell monolayer and 0.1 mL to 1 mL of inoculum is added to the cells. The specimen is centrifuged onto the cell monolayer at approximately 3000 *g* at room temperature for 1 h. Where passaging is intended or likely to be needed, specimens are inoculated in duplicate. Shell vials are incubated at 35°C in 5% $CO_2$ for 2 h to allow for the uptake of chlamydiae. The medium is then discarded and replaced with medium containing 1 µg of cycloheximide/mL. The cells are incubated at 35°C in 5% $CO_2$ for 48 h to 72 h, and one cover slip is examined for inclusions by immunofluorescence, iodine staining or Giemsa staining. Although a fluorescent microscope is required, immunofluorescence is the preferred method because it is more specific than iodine or Giemsa staining and can give a positive result as early as 24 h postinoculation. For trachoma, inclusion conjunctivitis and genital tract infections, culture is performed as described above. For LGV, the aspirated bubo pus or rectal swab must be diluted (1:10 and 1:100) with cell culture medium before inoculation. Second passages should always be made because detritus from the inoculum may make it difficult to read the slides.

**Antigen detection**

A number of commercial EIAs are available for the detection of chlamydial antigens in clinical specimens (13). These products use either monoclonal or polyclonal antibodies to detect chlamydial lipopolysaccharide (LPS), which is more soluble than the major outer membrane protein (MOMP). Most EIAs take several hours to perform and are suitable for batch processing. The sensitivity profiles of the commercially available *C trachomatis* EIAs range from 65% to 75% compared with NAA assays. Without confirmation, the tests have a specificity of 97%. Therefore, they are not amenable to screening low-prevalence populations because of the low predictive value of a positive result in such groups. To address this problem, confirmatory tests have been developed in which all tests giving positive results are repeated in the presence of a monoclonal antibody directed against the group-specific epitope on the LPS. This blocks the specific reactions but not the false-positive reactions (14). The appropriate application of confirmatory tests increases the specificity to approximately 99.5%. Another approach to confirmation involves testing the specimen by a second test based on a different principle (eg, a DFA test based on MOMP detection to confirm an LPS-based EIA).

Diagnostic tests involving monoclonal antibodies are based on detecting elementary bodies (EBs) in smears (15). The DFA test has approximately 75% to 85% sensitivity and 98% to 99% specificity compared with culture, and lower sensitivity (approximately 70%) compared with NAA tests. Several DFA assays are commercially available and use monoclonal antibodies directed against MOMP (Syva Microtrak, USA; Trinity Biotech, Ireland) or LPS (Kallestad, USA). Monoclonal antibodies to the LPS will stain all chlamydiae, but the specimen may be more difficult to read because of the uneven distribution of LPS on the chlamydial particle. The anti-MOMP monoclonal antibodies are prepared against *C trachomatis*; therefore, they are species-specific. The quality of fluorescence is better because MOMP is evenly distributed on the chlamydial particle. This procedure offers rapid diagnosis, taking only 30 min to perform. A variation on the DFA procedure involves centrifugation of the transport medium being used for other tests (cell culture, NAA or EIA), preparation of a slide from the sediment, and staining with the fluorescent antibody reagents. This is often used as a confirmatory test for positives in other tests. DFA requires the expertise of an experienced microscopist, and the procedure is presently not amenable to automation. It is therefore not suitable for very high volume laboratories.

Several commercial point-of-care EIA tests have been developed and have shown variable performance traits. These tests are usually performed within 30 min and facilitate immediate treatment of infected patients. Currently available point-of-care tests are 60% to 70% sensitive compared with NAA tests, and specificity may also be less than 100%. These tests are not inexpensive and are not intended to be used in laboratory settings. If their performance can be improved and their costs can be decreased, commercial point-of-care EIA tests would offer the ability to test populations that are difficult to access and that almost inevitably fail to return for follow-up care; this would allow for the treatment of more cases (16).

**NAH**

Commercially available NAH tests for *C trachomatis* in some parts of the world have been used as extensively as EIAs in laboratories handling large numbers of specimens on a daily basis. One commercially available probe test (PACE 2, Gen-Probe Inc, USA) uses DNA-RNA hybridization in an effort to increase sensitivity by detecting chlamydial RNA. Available data suggest that this probe test is relatively specific and provides sensitivity similar to that of the better antigen detection and cell culture methods (17). Another NAH test, the Hybrid Capture II for *C trachomatis* (Digene Corporation, USA) uses a signal amplification component to increase sensitivity to approximately 90% of NAA assays (18).

**NAA**

Several NAA methods are currently licensed in Canada for the detection of *C trachomatis* in clinical specimens: polymerase chain reaction [PCR] Amplicor (Roche Molecular Systems, USA), ligase chain reaction LCx assay (Abbott Laboratories, USA), transcription-mediated amplification AMP-CT and APTIMA Combo 2 (Gen-Probe Inc, USA), and strand displacement amplification ProbeTec (BD Diagnostic Systems, USA). Only approved specimens as outlined in each package insert should be tested in these assays. The PCR, ligase chain reaction and strand displacement amplification assays amplify nucleotide sequences of the cryptic plasmid, which is present in multiple copies in each *C trachomatis* EB. The transcription-mediated amplification reaction is directed against ribosomal RNA, which is also present in multiple copies. Theoretically, given the multiplicity of target sites for the amplification procedures being used, these techniques should be able to detect less than one EB, and can do so in purified suspensions of chlamydial particles. However, the actual sensitivity with clinical specimens is lower (90% to 96%) because of sampling variability and inhibition of

the amplification reactions by factors in the specimens (19). All of the assays appear to be highly specific if problems with crosscontamination of reactions are avoided. Clinical evaluations of these amplification methods have demonstrated higher sensitivity than culture and the other nonculture methods (microscopy, immunoassays and NAH assays) (20,21). The NAA assays currently used have low specimen throughput, but laboratories can use cost-effective FVU or swab specimen pooling procedures while maintaining test accuracy (22). The NAA assays have become the tests of choice for the diagnosis of C *trachomatis* infection in routine clinical laboratories. Although the commercial NAA tests have not been cleared for rectal samples, most will detect all C *trachomatis* serovars, including L strains 1-3 (LGV). Identification of these strains requires confirmatory testing with restriction fragment length polymorphism or sequencing techniques because treatment regimens are different. In 2002 (23), the United States Centers for Disease Control and Prevention recommended that all positive NAA test results should be repeated in the same test or an additional different NAA assay. Repeat testing samples near the cutoff of an assay may also clarify whether it is positive or negative. Because these manoeuvres are costly, time consuming and require validation, the authors do not at this time recommend adoption of these manoeuvres on a routine basis.

A number of manufacturers now supply nucleic acid-based systems designed to detect both C *trachomatis* and *Neisseria gonorrhoeae* in the same assay using a single specimen. This enhances the value of the laboratory in patient management because a significant number of patients infected with C *trachomatis* have been shown to have concurrent *N gonorrhoeae* infection. The improvement of partial automation has allowed these systems to support a throughput of several hundred specimens per day.

### Serology

Serology is not recommended for diagnosing chlamydial infections, with the exception of infection in neonates, patients with tubal factor infertility and occasionally for LGV infections when bubo aspirates are not available. Serology is often not available except in reference laboratories such as the National Microbiology Laboratory (Winnipeg, Manitoba). The classical complement fixation test is rarely performed today. The microimmunofluorescence test, developed by Wang and Grayston (24), is the current method of choice for the serodiagnosis of chlamydial infection. Microimmunofluorescence testing is useful in diagnosing chlamydial infection in neonates. High levels of IgM antibody are regularly associated with disease. IgG antibodies are less useful because infants present clinically when they still have a high level of maternal IgG. Because six to nine months are required for maternal antichlamydial antibodies to disappear, infants older than nine months may be tested for IgG. Infants with inclusion conjunctivitis or respiratory tract carriage of chlamydiae without pneumonia usually have very low levels of IgM antibodies. Thus, a single IgM titre of 32 or greater may support the diagnosis of chlamydial pneumonia in these cases.

Other assays include the whole-inclusion immunofluorescence test, EIA using EBs, reticulate bodies or infected cells and a recombinant enzyme-linked immunosorbent assay to LPS. Other serological tests include indirect hemagglutination, neutralization, precipitation, gel diffusion, enzyme-linked fluorescence, immunoperoxidase and immunoelectrophoresis. Most of these assays employ in-house methods, although a few have been commercialized and are being used by clinical laboratories. Total antibody determination by complement fixation or whole-inclusion immunofluorescence has been useful in identifying patients with tubal factor infertility (25,26).

## ANTIMICROBIAL SUSCEPTIBILITY TESTING

Susceptibility testing has had little clinical utility to date, but concerns about antimicrobial resistance in chlamydiae persist (27-29). Antibiotic-resistant C *trachomatis* can be induced in the laboratory by subinhibitory concentrations of antimicrobials. Chlamydial species are susceptible to the tetracycline, macrolide and fluoroquinolone classes of antibiotics. The most active of these – doxycycline, erythromycin, azithromycin, rifampin, ofloxacin and clindamycin – are usually used to treat chlamydial infections. Antimicrobial susceptibility testing in chlamydiae is typically performed in cell culture with increasing concentrations of antibiotics (30). Drug efficacy is then determined by staining cells with fluorescently labelled antichlamydial antibodies and microscopically enumerating the intracellular chlamydial inclusions. Susceptibility testing of chlamydiae is problematic due to the lack of standardized techniques and because of the variability introduced by the type of cell culture system used, different cell types, inoculum size and the timing and duration of antibiotic application to the cell culture. It is unclear whether the end points measured by in vitro susceptibility testing are relevant when applied to a naturally occurring infection with dividing and nondividing bacteria that infect multiple cell types in vivo. Thus, the results of in vitro susceptibility testing may not predict the microbiological efficacy in vivo. For these reasons, susceptibility testing is not routinely performed by clinical laboratories, and such requests are forwarded to a reference laboratory. Molecular methods using reverse transcription PCR have been described (31) and may simplify C *trachomatis* antibiotic susceptibility testing.

## CONTINUOUS QUALITY IMPROVEMENT

Continuous quality improvement involves the administration of a program for proficiency testing, quality control and quality assurance. These are particularly important to minimize reporting of false-positive and false-negative results. Because nucleic amplification assays are very sensitive, each laboratory must use procedures to avoid contamination that could lead to incorrectly reporting negative specimens as positive ones (32,33). The specimen processing area should be separate from the amplification room, and barrier micropipette tips should be used. Dedicated pipettes and similar equipment should be used in each area. Gloves and gowns should also be kept separate for each area. Contamination checks should be performed on a regular basis.

Proficiency testing for the detection of chlamydial antigens and nucleic acids is provided by the Quality Management Program – Laboratory Services in Ontario. Analyte challenges are sent to licensed Ontario laboratories at least twice a year. The program requires both correct test performance and interpretation accuracy. Mistakes or inaccuracies are identified and corrective action is taken to improve proficiency. Laboratories in Ontario and other provinces may participate in sample challenges sent by the College of American Pathologists. Results are collated and laboratories can compare their performances with others.

Day-to-day quality control monitoring of the procedures used for the isolation or detection of C *trachomatis* should be built into laboratory protocols, with overall objectives focused on detecting errors in the performance of the procedures and problems with the reagents or kits used. Specimen collection, specimen type and transportation, culture, microscopy, antigen detection, serology and nucleic acid detection procedures need to be monitored on a regular basis. Each laboratory, through a consensus format, needs to document ongoing procedures for monitoring quality. For example, to monitor the quality of specimens for nonmicroscopic C *trachomatis* testing, one could prepare a smear and stain it to determine whether epithelial cells are in the specimen as a measure of adequacy of specimen collection. Although most commercial kits have positive and negative controls, it is a good idea to insert known high, medium or low positive clinical specimens into a run on a periodic basis.

Quality assurance monitoring is usually preceded by the clear communication of expectations to laboratory users concerning submission of specimens, turn around time, reporting and utilization of results, susceptibility testing, infection control, patient benefit and user perception. Programs to monitor these criteria throughout the laboratory investigation process would provide continuous quality improvement.

The incidence of chlamydia infection in the populations and communities being served should be monitored. Any sudden variation from this expected rate should initiate investigation into any variation in laboratory practice, specimen collection and transport procedures, or changes in the population served.

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