

Laboratory testing for *Chlamydia trachomatis* urogenital infections

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Introduction

This review focuses on the laboratory diagnosis of *Chlamydia trachomatis* urogenital infections in adult patients. Laboratory diagnoses of *C. trachomatis* have rapidly evolved over the last two decades. The evolution from culture to enzyme immunoassay to nucleic acid amplification assay was a big step in the advance in technology. This advance has helped to improve the diagnosis and prevention of the major public health problem that is chlamydia infection. The sensitivity and specificity data quoted in Table 1 are based on the new expanded gold standard from different studies, not just cell culture.

Table 1 Sensitivity and specificity of each assay type

	Culture	EIA	DFA	NAA
Sensitivity (%)	40–60	65–75	70–75	90–98
Specificity (%)	100	90–95	90–97	98–99

DFA; Direct fluorescent antibody assay; EIA, enzyme immunoassay; NAA nucleic acid amplification assays.

Specimen collection

The first critical requirement for accurate testing is the collection of a proper specimen. Chlamydia is an intracellular bacterium; the collection of a specimen containing columnar epithelial cells is paramount to the success of the test.

Swab specimens

The most common site for females is the endocervix. However, studies have shown that 10–23% of females will be infected in the urethra only.¹ The optimal specimen in a female patient will be the combination of endocervical and urethral swabs. The cervix should be first cleaned of mucus or discharge, then a swab should be inserted in the cervix 1–2 cm deep just pass the squamocolumnar junction and rotated several turns to obtain columnar cells. A second swab should then be inserted 1 cm into the female urethra and rotated once prior to removal. These two swabs can be pooled together into the same transport container to increase the sensitivity of the test and to reduce costs.

For the male patient, a swab should be inserted 2–4 cm into the urethra and rotated several turns to obtain columnar epithelial cells.

Non-invasive specimens

Both male and female first-voided urine (FVU) are excellent specimens for nucleic acid amplification assays (NAA). To obtain a first-voided urine sample the patient does not have to clean the urethra or perineum prior to

urination. It is preferable that the patient has not urinated in the last hour. The first 10–20 ml of urine should be collected into a sterile container. The first part of the urine sample contains epithelial cells from the urethra that may contain chlamydia. FVU will be a preferred specimen, especially in males. Research has shown that up to 30% of male swabs were inadequately obtained, which may lead to false-negative results.

Patient self-obtained vulval or vaginal swabs are alternative specimens for NAA testing.²

Assays performed in the clinical microbiology laboratory

Culture method

Cell culture remains the standard for medico-legal situations such as sexual assault or child abuse. Cotton, dacron, or rayon material on plastic or metal shafts should be used for collection of specimens. Individual batches of each of these swabs should be tested for cell culture toxicity before use. Wooden shafts should be avoided, as they may be toxic to cells. The swab should be inoculated into chlamydia transport media, such as 2-SP, M4 or FlexTrans, after collection. The specimen needs to be kept refrigerated, transported at 4°C and inoculated into cell culture within 24–48 hours in the laboratory. Keeping the specimen cold until arrival at the laboratory is critical for the viability of the organism. Urine is not suitable for culture. Even though cell culture has a specificity of 100% (no false-positives), its sensitivity is only around 55% for cervix and 37% for male urethra.³

Enzyme immunoassay (EIA) and DNA probes

These assays are grouped together because they have similar sensitivity and specificity. EIA involves the immunohistochemical detection of antigen. DNA probe is a DNA hybridisation assay that employs a chemiluminescent DNA probe that binds to chlamydial RNA and is detected with a luminometer. It is not a DNA amplification assay and should not be confused with nucleic acid amplification assays (NAA). False-positive or false-negative results are more common in these assays, especially for those specimens that have a tested value near the cut-off or the so-called ‘gray zone’ area. False-positive results can be due to cross-reaction with other bacteria from the genital tract. A false-negative result can result from poor specimen collection, or the presence of an inhibitory substance, such as spermicide that inhibits the EIA reaction. However, currently, there is no way to detect inhibition in an EIA or probe assay; such specimens would usually go out as a false-negative report. Laboratories that perform a

confirmatory assay as recommended by the Centers for Disease Control and Prevention (CDC)⁴ will improve the specificity of the test⁵ for specimens initially testing positive or falling within the 'gray zone' area. One advantage of these assays is that the specimen can be collected and stored at room temperature for several days until tested and is not required to be viable for detection as in the culture method. With NAA as the new gold standard, both EIA and DNA probe assays have sensitivities around 75% even with combined urethral and cervical swabs in females. The sensitivity is similar for the male urethral swab. The specificity is about 95% for both sexes when a confirmation assay is used. The sensitivity and specificity of EIA on urine specimens are lower than swabs, especially for female urine.

Direct fluorescent antibodies (DFA)

DFA is similar to EIA; however this is the only assay that simultaneously assesses the adequacy of the specimen based on the number and types of cell observed under the microscope. DFA works by directly visualising the chlamydial organism by staining with fluorescein-labelled specific antibodies. The sensitivity and specificity of the assay both depend on the experience of the laboratory technician in detecting small numbers of elementary bodies (EBs) and discriminating between specific and non-specific staining. The sensitivity of DFA is about 75%, i.e. similar to EIA. The specificity is between 90% and 97% depending on the technician's experience.

Nucleic acid amplification assays (NAA)

Polymerase chain reaction (PCR), ligase chain reaction (LCR), transcription-mediated amplification (TMA) and strand displacement amplification (SDA) are commercially available NAA assays that are exquisitely sensitive and highly specific, and capable of detecting a single copy of the target gene. Some NAA assays require that the specimen be kept refrigerated until it reaches the laboratory, thus necessitating the laboratory to check their specimen storage facilities. This technology provides tests with the highest sensitivity and specificity available at the present time. Some of these assays also include an internal control, which indicates whether there is an inhibitor, such as blood, present in the specimen that inhibits the reaction; this prevents a false-negative report. This type of internal control is not available with any of the other assays mentioned previously. Often the laboratory may be able to remove the inhibition. If that is not possible, a second specimen may be required. Because of its extreme sensitivity, it can be used in specimen types such as urine, vagina or vulva that are not possible with other technologies. This is an excellent tool for public health screening, especially in an asymptomatic population. Most NAA assays have a sensitivity in the 90–98% range and a specificity close to 98%. Because of such high specificity, confirmation tests for positive results are not required. For male patients, FVU provides a more reproducible specimen than do swabs and it is more sensitive.⁶ For female patients, a combined cervical and urethral specimen is preferred. However FVU is also an excellent alternative. In one of our studies, female FVU gave a sensitivity of 93% as compared with combined cervical and FUV which gave 98%, while combined cervical and urethral swabs using EIA only gave a sensitivity of 75%.⁷ This demonstrates that female FVU alone using NAA assay has a much higher sensitivity than EIA assay even with combined specimens. Our current laboratory protocol using NAA assay for chlamydia testing

is to submit a FVU combined with a cervical swab if a vaginal examination is done. However, a FVU alone is an acceptable alternative. For male patients, FVU is used.

Serology

Serological diagnosis of *C. trachomatis* urogenital infection is not recommended. This is due to persistent antibody presence in patients who have had chlamydia infection in the past, and patient antibody response that may be modest or non-existent. Also the cross-reactivity with other chlamydia species, i.e. *C. pneumoniae*, renders most serology assays unsuitable.

Cost-effectiveness

Chlamydial infections are a highly prevalent treatable disease, with many asymptomatic cases that could result in complications. NAA assays are the most sensitive and specific assays currently available in the laboratory and can be used in both invasive and non-invasive specimens. For the reasons mentioned above, several papers based on NAA assays have shown that screening populations with a prevalent rate as low as 2% will be cost-effective,^{8,9} provided the laboratory has sufficient volume. Thus NAA assays are a cost-effective way of screening low prevalence and asymptomatic populations. Another advantage of the NAA assays in screening is that a urine sample can be easily obtained, thus more male patients are willing to be tested. Also, in jail or juvenile detection centres there are significant savings in having the female patient provide a FVU sample rather than having to transfer the patient to an off-site facility to access an examination room and the medical staff required to obtain a cervical specimen. In our sexually transmitted disease (STD) clinics, some patients well known to the clinic may not have time to wait to be seen, but are willing to provide a urine sample and return to the clinic another day for the results.

Future direction for STD testing

The future of STD testing will be the use of NAA assays expanded to detect other STD pathogens. Currently, *Neisseria gonorrhoeae* can be tested using a NAA similar to chlamydia with the same specimens.¹⁰ Other studies have shown that herpes simplex and human papillomavirus can be detected using NAA with urine specimens. When these assays are validated and become commercially available, future testing will include all these pathogens and employ a NAA assay in non-invasive specimens.

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