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REVIEW ARTICLE

Guidelines for the laboratory diagnosis of *Chlamydia trachomatis* infections in East European countries

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Abstract

The present guidelines aim to provide comprehensive information regarding the laboratory diagnosis of infections caused by *Chlamydia trachomatis* in East European countries. These recommendations contain important information for laboratory staff working with sexually transmitted infections (STIs) and/or STI-related issues. Individual East European countries may be required to make minor national adjustments to these guidelines as a result of lack of accessibility to some reagents or equipment, or laws in a specific country.

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Keywords

Chlamydia trachomatis, Eastern Europe, guidelines, laboratory diagnosis

Conflicts of interest

None declared.

Introduction

The present guidelines aim to provide comprehensive informa tion regarding chlamydial sexually transmitted infection (STI) and the corresponding laboratory diagnosis for East European countries. In many East European countries, commercial kits, that are frequently available in the west, are often not affordable and often the tests used have not been validated against internationally accepted standards. These guidelines are primarily intended for professionals testing specimens from patients at a sexual health care clinic but may also be helpful for community based screening programmes. The guidelines represent the first attempt to introduce an evidence-based approach to the diagnosis of chlamydial infections in Eastern Europe It is recognized that, for different East European countries, minor national adjustments to these guidelines may be needed to meet local laws, health strategies and the availability of kits and reagents. The present guidelines were elaborated as a consensus document of the Eastern European Sexual and Reproductive Health Network[1] and comprise one element of a series of guidelines aimed at optimization, standard ization, and quality assurance of the laboratory diagnosis of the reproductive tract infections.[2–4]

Chlamydia trachomatis is a major cause of genital tract infections among sexually active adolescents and young persons.[5] It has been reported that pelvic inflammatory disease may occur in ≤ 40% of women with untreated *C. trachomatis* genital tract infection[6,7] with a significant but uncertain number developing tubal factor infertility, ectopic pregnancy or chronic pelvic pain.[8]

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Untreated *C. trachomatis* infection during pregnancy may lead to conjunctivitis or pneumonia in the newborn. In men, the sequel of protracted untreated *C. trachomatis* infection is epididymitis. Genital tract infection with *C. trachomatis* in either sex may also lead to reactive arthritis, while oro-genital contact may lead to pharyngitis and insertive anal contact, to proctitis. Accidental inoculation of the eye with genital discharge material may lead to adult inclusion conjunctivitis. The costs to national health services of treating the sequelae of chlamydial STI are clearly substantial. Cost-benefit studies in developed countries show that the best strategy is to diagnose and treat the initial uncomplicated infection.[9] However, the majority of chlamydial infections, particularly those in women, are asymptomatic. This has two implications. First, since the diagnosis of chlamydial genital tract infections based on signs and symptoms is unreliable, the use of effective laboratory tests to detect chlamydial infection is essential. Second, as infected persons often do not seek medical attention, screening strategies based on local criteria are required to ensure that chlamydial infections are not missed in high-risk population groups.[10]

Infections caused by the more invasive LGV biovars of *C. trachomatis* manifest as classical lymphogranuloma venereum and proctitis or proctocolitis in women and men who have sex with men (MSM). Recent outbreaks in Europe and North America have been particularly associated with MSM.[11,12]

Data on morbidity due to genital chlamydial infections in Eastern European countries are scarce and their reliability is doubtful owing to the comparatively low number of tests performed,[13] the low quality of the tests used and the methods chosen.[14–16]

Disease classification

The classification of infections caused by *C. trachomatis* is presented in Table 1.

C. trachomatis

The order Chlamydiales comprises Gram-negative, obligate intracellular bacteria with a characteristic, biphasic growth cycle. Infection is initiated by endocytosis of the environmentally resistant elementary body that, within a membrane bound inclusion, transforms into a larger fragile form, the reticulate body, which replicates by binary fission and ultimately gives rise to further infectious elementary bodies. Members of the order Chlamydiales cannot be grown on cell-free media. Within the order, there are several families. Of particular interest is the family Chlamydiaceae that contains the genera *Chlamydia* and *Chlamydophila*, members of which share a > 90% 16S rRNA sequence homology and a characteristic KDO-trisaccharide in their endotoxin. Within the genus *Chlamydia*, the species *C. trachomatis* is the main aetiological agent of chlamydial genital tract infection in humans. 16S and 23S rRNA sequences for the classification of the order Chlamydiales at family, genus and species levels have been described.[18] Furthermore, *C. trachomatis* can be typed serologically or genotypically into some 18 serovars (genovars) based on characteristic antigenic motifs on the major outer membrane protein. Serovars A to C cause the classic ocular disease trachoma, while serovars D to K primarily cause oculogenital infections and serovars L1, L2 and L3 cause the more invasive sexually transmitted infection, lymphogranuloma venereum, with its characteristic lymph node involvement or rectal infections.

Table 1 International Classification of Diseases 10, 2007 revision[17] caused by *C. trachomatis*

A55	Chlamydial lymphogranuloma (venereum)
A56	Other sexually transmitted diseases
A56.0	Chlamydial infection of lower genitourinary tract
A56.1	Chlamydial infection of pelviperitoneum and other genitourinary organs
A56.2	Chlamydial infection of genitourinary tract, unspecified
A56.3	Chlamydial infection of anus and rectum
A56.4	Chlamydial infection of pharynx
A56.8	Sexually transmitted chlamydial infection of other sites

C. trachomatis is mainly transmitted by intimate contact between the mucosal membranes of the urogenital tract, anal canal, or oropharynx during sexual activity. However, neonates can be infected during passage through an infected birth canal, giving rise to conjunctivitis or to an atypical pneumonia.

In men, the infection most frequently manifests as a urethritis, while in women it most often causes an endocervicitis. Asymptomatic infection occurs in men in approximately 40–50% of cases, and in women in 70–80%. If left untreated, ascending *C. trachomatis* genital tract infection in women may lead to a transient endometritis and subsequent inflammation and blockage/dysfunction of the fallopian tubes resulting in pelvic inflammatory disease, ectopic pregnancy and/or infertility. The main symptoms, clinical manifestations and complications of chlamydial infection are summarized in Table 2.

Risk factors for chlamydial infection:

- sexually active young people less than 25 years of age;
- multiple sexual partners;
- unprotected sex with a new partner;
- recent history of STIs;
- sexual contact with a person diagnosed with chlamydial infection or urethritis/cervicitis;
- persons engaged in commercial sex; and
- sexual abuse/rape.

Main indications for testing for *C. trachomatis* infections are presented in Table 3.

Sampling, sample storage and transportation

General

For direct immunofluorescence (DIF): The swab is rolled against the surface of the slide. There should be a thin layer of specimen on the slide.

Table 2 Main symptoms, clinical manifestations and complications of chlamydial infection

Patients	Clinical symptoms	Clinical manifestations	Complications
Women	• Cervical and/or vaginal mucopurulent discharge • Contact bleeding • Dysuria • Lower abdominal pain • Irregular vaginal and/or blood stained cervical discharge • Dyspareunia	• Cervicitis • Urethritis • Proctitis; pharyngitis asymptomatic infection	• Pelvic inflammatory disease • Perihepatitis • Infertility • Ectopic pregnancy • Inclusion conjunctivitis • Reactive arthritis
Men	• Mucopurulent urethral discharge • Dysuria • Pain during micturition • Scrotal pain	• Urethritis • Proctitis • Pharyngitis • Proctocolitis • Inguinal bubos • Asymptomatic Infection	• Epididymo-Orchitis • Conjunctivitis • Reactive arthritis
Neonates and babies	• Conjunctivitis • Atypical pneumonia in the first 6 months of life	• Conjunctivitis • Vulvovaginitis • Otitis media[19] • Pneumonia during the first 6 months of life	
Children	• Mucopurulent discharge from urethra (boys) and vagina (girls) • Dysuria • Lower abdominal pain (boys and girls) • Rectal pain and discharge	• Urethritis • Vulvovaginitis • Proctitis • Pharyngitis • Conjunctivitis	

Table 3 Main clinical indications for testing for chlamydial infection

Patients	Indications
Men	• Purulent or mucopurulent urethral discharge • Dysuria • Proctitis/proctocolitis • Scrotal pain/swelling
Women	• Cervical and/or vaginal discharge • Acute pelvic pain
Neonates	• Neonatal conjunctivitis • Atypical pneumonia
Other indications	• Sexual contact(s) with persons with diagnosed chlamydial infection • Extragenital specimens (pharyngeal, rectal) should be taken when history indicates exposure at that site • Examination for other STIs • Termination of pregnancy • Any intrauterine interventions or manipulations

***For culture*:** The specimen is collected into special tubes with transport medium [e.g. sucrose phosphate (2SP) containing antibiotics such as vancomycin, gentamicin, and amphotericin B included].[20] The swab, if non-toxic, is placed into the tube, broken off against the edge of the tube and left inside.

***For nucleic acid amplification based tests (NAAT)*:** The swab is placed into the transport medium provided by the manufacturer. If transport medium is not provided by the manufacturer, 2SP medium (with antibiotics such as vancomycin, gentamicin, and amphotericin B included) can be used. The swab is placed into the tube, broken off against the edge of the tube and left inside. Should specialized transport medium not be available, dry swabs may perform equally well.[21,22] For further information, see Tables 5 and 6.

Note: The laboratory must be involved in any consideration of sampling sites and devices and specimen transportation.

Summary of methods for the diagnosis of chlamydial infections:

- culture;
- antigen detection methods (DIF; enzyme immunoassay, EIA);
- nucleic acid detection methods: detection of *C. trachomatis*–specific nucleic acid sequences;
- rapid, or point of care (POC) tests;
- serology.

The success of laboratory testing results depends partly on patient factors at the time of sampling. Ideally, samples should be collected under the following conditions:

- antibiotic treatment should not have been provided during the previous 48–72 h;
- if possible, women should not be sampled during menstruation;
- in men and women, urethral and urine samples should be taken at least 2 h after urination.

Sampling devices:

- sterile cotton/dacron swabs;
- sterile special brushes;
- sterile cotton swab for removal of discharge;
- sterile container for urine.

Table 4 Samples and methods recommended for diagnosis of chlamydial infections

Sampling site or category	Method of diagnostics	Comments
• Endocervix (in women) • Urethra (in men)	• Nucleic acid detection	• Preferred diagnostic method
	• Antigen detection (DIF, EIA)	• Should be applied when molecular-biological methods are not available
	• Culture	• Not recommended for routine use**
• First catch urine*** and vaginal samples	• Nucleic acid detection	• Preferred diagnostic method
• Urethra (in women)	• Nucleic acid detection	• Preferred diagnostic method*
• Pharynx/conjunctiva/rectum	• Nucleic acid detection****	• Only where specifically indicated by clinical history. Most commercial assays are not licensed for these sites, though they may still be useful
• Screening asymptomatic women	• Nucleic acid detection	• Self-taken vaginal sample recommended
• Screening asymptomatic men	• Nucleic acid detection	• First-catch urine recommended

*Additional testing of urethral specimens from women may increase the yield of positive specimens when compared to endocervical specimens alone.
**Should be restricted to reference laboratory for the purpose of forensic medicine or for the determination of sensitivities to antibiotics.
***First 5–10 ml of voided urine.
****DIF test based on monoclonal antibody may be used for conjunctival swabs, particularly from new born babies.

Sampling swabs may contain fibres, shaft material or glue that may be toxic for cells and inhibit the growth of *C. trachomatis*. Plastic or metallic shafts are preferred to wooden shafts. Dacron, artificial silk, cotton fibres are preferred to calcium alginate. Cytobrushes on wire or plastic shafts are excellent for collecting the samples from the endocervical canal.

Note: Use of brushes is contraindicated in pregnancy.

Culture-based diagnosis of *C. trachomatis*

General

Until the early 1980s, the main method of confirming a provisional diagnosis of chlamydial infection was by the centrifuge-assisted inoculation of clinical material onto susceptible living cells in tissue culture followed by the demonstration of characteristic chlamydial inclusions after a suitable incubation period.[27] It was considered that this method was 100% specific, as there is little ambiguity about the presence of a characteristic *C. trachomatis* inclusion. Consequently, this was the initial gold standard against which the newer, non-viability dependent tests of chlamydial infection were evaluated. Isolation of chlamydiae by cell culture is now seldom performed as a diagnostic procedure in North America or Western Europe and is not recommended as a routine diagnostic procedure for Eastern Europe except, for medico-legal reasons and in cases of child sexual abuse, or when viable chlamydiae are necessary for research purposes, such as the *in vitro* measurement of chlamydial antibiotic resistance or as a test of cure. The problem with cell culture is that it necessitates a rapid cold transport system to preserve the viability of the small numbers of chlamydiae likely to be present in a clinical sample. Furthermore, it is a demanding and time-consuming procedure for which there are neither rigorously standardized methods nor materials. Factors such as the calf serum batch, the differential susceptibility of different variants of the same cell lines and the quality of the microscopy are critical variables which are difficult to standardize.

However, maintaining the ability to perform chlamydial cell culture in reference laboratories is very important. The maintenance of culture was one reason the so-called 'Swedish' mutant was detected because organisms could be cultured, but not detected when applying certain NAATs based on the detection of chlamydial cryptic plasmid DNA sequences.[28,29]

Sensitivity and specificity

There is now general agreement that chlamydial isolation in cell culture is a substantially less sensitive diagnostic procedure than the newer methods based on nucleic acid amplification. Moreover, it is no longer assumed that cell culture is 100% specific; it requires only a moment's inattention to cross contaminate cell cultures and it is also possible for inexperienced microscopist to misidentify artifacts as chlamydial inclusion bodies.

Toxic factors

Culture of chlamydiae can be affected by various factors. For example, local application of gynaecological lubricants, irrigation, or spermicidal agents may negatively influence chlamydial isolation. Cotton, calcium alginate, and dacron swabs can all be toxic for cell cultures and chlamydiae. This is due to the fact that unsaturated fatty acids in cotton fibres, chlorine-based bleaching agents, resin in wooden shafts, and glue used to connect swabs to the shaft may be toxic.

Source of mistakes:

- incorrect sampling of biological material for testing;
- incorrect transportation of samples;
- incorrect culture performance;
- conditions of preparation and storage of culture media;

Table 5 Methods of collection of specimens for *C. trachomatis* testing

Specimen type (from)	Collection methods	Comments
Male urethra	• Introduce a sampling swab into the urethral meatus (1–2 cm) • Slightly rotate swab in the urethra for few seconds, then take it out	• It is not recommended to take urethral samples in prepubertal children. Exudates are collected solely from the urethral meatus with a small swab or urine is obtained for testing with DNA/RNA-based methods
Endocervix	• Thoroughly clean the external cervical os to remove contaminating vaginal discharge by using large cotton swab • Introduce a sampling swab into the endocervical canal for 1–2 cm, by rotating it several times inside the endocervical canal for 15 s, then take it out	• Endocervical specimens are not taken in girls of prepubertal age. Should chlamydial infection be suspected in this age, specimens should be sampled from the vestibule of the vagina, and a urine sample should also be tested
Female urethra	• In case of abundant discharge clean the external opening with a cotton swab • Introduce a sampling swab into the urethral meatus (1–2 cm) • Rotate swab in the urethra for few seconds, then take it out	• It is not recommended to take urethral samples from prepubertal children. Exudate from urethra is collected from external opening using small swabs
agina (e.g. self-sampling*, and in girls of prepubertal age)	• A speculum should not be used • A sampling swab is carefully introduced into the vagina	• Pregnant women can be tested for chlamydia at any time during pregnancy. Samples are taken from the urethra and endocervical canal. In puerperant women, the samples are taken from urethra, and on the 3rd day after birth from the endocervical canal. In women, after hysterectomy samples for *C. trachomatis* are taken from the urethra and vagina
Conjunctiva	• The inferior eyelid is retracted and a swab is moved across the surface of the inferior palpebral conjunctiva towards the median corner of the eye • If purulent discharge is observed, it should be removed with a cotton swab	• Sometimes, the procedure is painful; therefore, local anaesthesia may be used
Rectum	• A sampling swab is introduced 2–3 cm into the anal canal and material is obtained from all walls of the rectum with the movement from inside to outside and then circular movements	• In routine screening where there are no signs of proctitis but anal infection is suspected a 'blind' rectal swab (proctoscopy not essential) should be collected • In patients with symptomatic proctitis, especially but not exclusively MSM, proctoscopy should be performed to identify any regions of ulceration and to direct areas for sampling by rectal swab • If faecal contamination is observed on the swab, it should be discarded and a fresh swab taken. With a proctoscope the risk of faecal contamination is reduced since sampling is done under visual control
Pharynx	• A sampling swab is moved across the posterior pharyngeal wall above the inferior edge of the soft palate and also across the tonsillar surface	• Pharyngeal specimens ought to be sampled in patients who have had oro-genital contact, or if this is suspected
First catch urine	• Ask patient to collect the first 10–15 ml of the freely voided urine	• A patient should not have urinated for at least 2 h prior to sampling
Ocular	• Remove purulent exudate if present • Rotate sampling swab tip over conjunctival surface	

*Self-obtained vaginal swabs for the diagnosis of chlamydial infections using NAATs are sensitive, specific and well accepted by patients.[23–26]

- the quality of the cell lines (insensitive or mycoplasma-infected cell line);
- availability of quality control of prepared culture media;
- availability of control strains of *C. trachomatis*;
- CO_2 concentration;
- quality of monoclonal antibodies;
- quality of microscope;
- quality of transport media;
- inexperienced observer or specialist.

Evaluation of the results

Results should be available in 2–3 days. The result is considered positive if at least one characteristic chlamydial inclusion body is detected in cell culture using DIF with fluorescein-labelled monoclonal antibodies, and there are no reasons to suspect contamination from another positive specimen. If there is a suspicion, the test should be repeated using residual clinical specimen material and ideally, also some other diagnostic methods should be applied.

Table 6 Recommendations for sample transportation and storage using different diagnostic methods

Test method	Conditions	Comments
DIF	• Specimens for DIF should, ideally, be prepared at the 'bedside', and be transported to the laboratory on the day of collection • If necessary, specimens should be fixed for 1–2 min with cold acetone and may subsequently be stored at +6 ± 2 °C for 3 days, at –20 °C for 1 month	• Where the rules for sampling and transportation of biological material have not been followed (eg broken slides, slides stuck together or improperly labelled, absence of sufficient epithelial cells) specimens should be discarded and the clinician advised accordingly
Enzyme immunoassay (EIA)	• Specimen must be collected and transported in the appropriate transport medium	• Follow the manufacturer's recommendations precisely
Cell culture	• Collect specimens into suitable transport medium (for example 2SP including antibiotics), maintain in a refrigerator at +6 ± 2 °C and transport to the laboratory in a refrigerated bag within 24 h • Testing requires viable chlamydiae and any change of temperature during transportation can compromise obtaining a reliable result • At the laboratory, samples can be stored at +6 ± 2 °C for 24 h. If case of need for longer storage, samples are frozen at –70 °C	• It is difficult to maintain chlamydial viability, so transportation conditions, including the maintenance of a cold chain, must be strictly followed • Difficult to ensure quality control in the absence of agreed standards • Long-term storage must be at –70 °C or below
Nucleic acid tests (NAAT)	• Samples may be transported and kept in media provided by the manufacturer, otherwise (for most tests) in 2SP media (including antibiotics) or dry[21,22] • During transportation of the specimen to the laboratory, the specimen should be kept at 6 ± 2 °C. The urine samples can be stored at 6 ± 2 °C for 1 week and do not need to be placed in the freezer	Follow the manufacturer's recommendations precisely

Storage of *C. trachomatis* isolates

Isolates of *C. trachomatis* may be stored in 2SP medium in an ultra low temperature freezer (–70 ºC). Storage at temperatures higher than this should not be attempted.

Laboratory conclusions based on results using culture:

C. trachomatis was detected.

C. trachomatis was not detected.

Antigen detection methods

General

As with nucleic acid detection, chlamydial viability is not an issue so specimen transport and sample stability is less of a problem than is the case for chlamydial culture. *C. trachomatis* antigen in clinical material is detected either by direct microscopy using DIF assay or, indirectly, by an EIA. The specificity of these methods has been improved substantially by the introduction of antigen-specific monoclonal antibodies. Note that assays based on polyclonal antibodies are generally less specific. Sensitivity has also been improved by careful assay optimization and by the introduction of signal amplification methods. Nevertheless, the sensitivity is substantially less than that offered by methods based on nucleic acid amplification.[30] Furthermore, many of the DIF tests used in East European countries have a suboptimal sensitivity as well as specificity.[31,32] EIA can be automated permitting much greater specimen throughput. The microscopy involved in DIF is relatively tedious, but it has the important advantage over EIA and many other methods that the adequacy of the clinical sample can be assessed. With modern commercial assays, the sensitivity of EIAs is similar to, or a little better than, cell culture and specificity is adequate for use in populations with a medium to high prevalence of infection (e.g. ≥ 5%).[33]

DIF

Quality control of sampling on microscopic slides

Slides are evaluated by examining at least 10 fields using a ultraviolet (UV) fluorescence microscope under the magnification of ×400. One microscopic field should contain more than 5 columnar epithelium cells. Criteria used for evaluation include the size of elementary bodies (EB) – 200–300 nm, apple-green colour, correct round form. If the preparation contains enough epithelial cells and specific fluorescing elementary bodies are found (more than 5 EB in preparation), the result is considered positive.

Sources of error:

- incorrect and inadequate sampling of clinical material may lead to wrong interpretation of the smear;
- use of a marker not designed specifically for marking the slides may pollute the preparation during fixation and staining;
- incorrect application of the clinical material on the slide (it is necessary to roll the swab against the slide) may cause damage to the cells and distort cell morphology;

- quality of monoclonal antibodies;
- availability of appropriate controls;
- quality of microscope;
- prolonged usage of UV bulbs.

Slides should not be evaluated if:

- There are less than 5 cells columnar epithelial cells per high power field;
- There are only squamous epithelial cells in the specimen;
- There are only erythrocytes in the specimen;
- The specimen contains a significant amount of mucus, artefacts or exhibits nonspecific fluorescence (colour and shape are not consistent with elementary bodies).

Antigen detection using EIA

There are a number of commercially available EIAs. The test methods range from direct antigen detection to antigen capture with or without enzymatic amplification to enhance the sensitivity of detection. EIA systems are more suited to batch processing of large numbers of specimens. The overall sensitivity and specificity of different EIAs and DIF methods is similar. The sensitivity, however, is lower than that of NAATs.[34]

Sources of error

The EIA test is based on detection of chlamydial lipopoly saccharide (LPS), which is structurally related to the LPS of other microorganisms. Theoretically, this may result in false-positive results but this has largely been overcome by the use of highly specific monoclonal antibodies.

POC tests

The Unipath Clearview and other rapid, immunochromatographic, POC tests are also based on antigen detection technologies. Such tests should only be used when laboratory facilities are not available[35] since they lack sufficient sensitivity when compared to nucleic acid amplification tests.[36] However, use of POC tests for chlamydia testing outside clinical settings are not recommended because of their poor performance.

Conclusions based on results using antigen detection tests:

Antigen specific for *C. trachomatis* was detected.

Antigen specific for *C. trachomatis* was not detected.

Nucleic acid detection tests

Nucleic acid hybridization tests

All nucleic acid–based chlamydial diagnostic assays depend on hybridization in some way, but here, we refer to those methods not involving the preceding amplification of target nucleic acid. The best example of such a test is the GenProbe PACE 2 test, first introduced in 1988 and still widely used. Its sensitivity is based on careful optimization and on the fact that it is targeted against high copy number chlamydial rRNA, which effectively serves as natural pre-amplification. As rRNA is mostly single stranded, no thermal denaturation for strand separation is necessary prior to hybridization, which takes 60 min at 60 °C. An acridinium ester-labelled probe generates a light signal readable in a simple luminometer.

Advantages of DNA hybridization

The test is popular because it is robust, simple to perform, relatively rapid (2 h), and can be up-scaled for large specimen numbers. The combination of simplicity and lower cost make its use attractive in many settings.

Disadvantages of DNA hybridization

Special hardware is required. The sensitivity of non-amplified probe tests is significantly lower than that of the leading nucleic acid amplification based assays.

NAATs

NAATs employ the principle of identifying specific nucleic acid (DNA or RNA) sequences in the specimen under test and, using specific enzymes, creating numerous copies of that sequence which can be detected using a variety of techniques.

The major target for amplification-based tests against *C. trachomatis* is also a multiple-copy gene product, such as the cryptic chlamydial plasmid or rRNA.

The clinic or laboratory contemplating the adoption of such tests needs to consider not just sensitivity, specificity and the clinical requirement, but also the suitability of the test for the facilities, workflow within the laboratory, human resources and number of specimens to be processed.

Advantages of NAATs

The main advantage of the nucleic acid amplification-based diagnostics for *C. trachomatis* is that such methods combine state of the art sensitivity with excellent specificity. Such tests enable a high detection rate for *C. trachomatis* in symptomatic individuals and an adequate detection rate in asymptomatic individuals (where there are often fewer chlamydial particles present). They also permit the use of non invasive clinical samples such as first-catch urine specimens or self-collected vaginal swabs.[22,23,37] NAATs capable of detecting multiple infections (most commonly chlamydial and gonococcal infection) are available but may be associated with different problems (e.g. cross-reactions with other *Neisseria* species). This is beyond the scope of this guideline.

Disadvantages of NAATs

A disadvantage of NAATs is that they are generally more expensive than other assays. The great sensitivity of these assays means that accidental cross-contamination of specimens with amplified product (amplicon) is a problem of major importance for kit design, laboratory workflow and personnel. False-negative results may occur as a result of components in the clinical specimen inhibiting nucleic acid amplification. They may also be

Table 7 The effect of sensitivity, specificity and predictive values on the performance of an NAAT. The example here from the package insert shows the performance of the Roche Amplicor CT for cervical swabs at various levels of prevalence of the infection

Prevalence (%)	Sensitivity (%)	Specificity (%)	Positive Predictive Value (PPV; %)	Negative Predictive Value (NPV; %)
1	93.4	96.7	22.5	99.9
5	93.4	96.7	60.2	99.6
10	93.4	96.7	76.1	99.2
20	93.4	96.7	87.8	98.3

a result of suboptimal nucleic acid extraction. These tests require well-trained laboratory technicians, which mean that they are usually laboratory based, with the results not available while the patient is still in the clinic.

Usefulness for screening

A number of studies have demonstrated that NAAT screening programmes targeting at risk populations for chlamydial infection can be cost effective at preventing disease sequelae. Unfortunately, few such studies have been undertaken in Eastern Europe. However, for population screening, in Eastern Europe too, it has been shown that costs may be substantially decreased by pooling samples[25,38,39] as only those pools containing a positive specimen require individual components of the pool to be screened separately. However, the cost savings are highly dependent on the prevalence of infection.[25,38–40]

Qualitative polymerase chain reaction (*e.g. Roche Amplicor*)

General

The Roche Amplicor CT polymerase chain reaction (PCR) for *C. trachomatis* was the first commercial nucleic acid amplification test to be introduced for the diagnosis of chlamydial infection. Subsequently a multiplex assay was introduced permitting simultaneous PCR amplification of *C. trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) target DNA as well as an internal control DNA, which identify inhibited samples. The test has two main formulations: the Roche Amplicor CT/NG MWP (multi-well plate) PCR and the fully automated Roche COBAS Amplicor PCR.

The performance characteristics of the Roche Amplicor test (Table 7) indicate that, at low prevalence of infection, the positive predictive value of the test may be unacceptably low. This is of course true of other NAATs as well.

Advantages

Advantages of the test include the various flexible formats, the ability to use the test with cervical specimens collected for cytological screening, and the provision of an internal control for amplification.

Disadvantages

There are two main disadvantages:

- First, especially in female urines, human beta-chorionic gonadotropin, crystals and other urine components may inhibit amplification giving rise to false-negative results, a problem that will be detected by use of the supplied internal control;
- Second, *C. trachomatis* variants lacking the NAAT target on the cryptic chlamydial plasmid due to a deletion have recently emerged in Scandinavia. These strains are not detected by the conventional Amplicor CT. This could become a major concern if these or similar variants become more widespread.[28,29] At present, only a few variants have been detected outside Scandinavia.[41] Furthermore, these variants have not yet been detected in Eastern Europe, although there have been only limited studies.[42,43]

Strand displacement amplification: the Becton Dickinson BD ProbeTec ET

General

This system uses the strand displacement isothermal DNA amplification process which, unlike conventional PCR, means that time is not wasted in repeated thermal cycling. The target gene is the cryptic chlamydial plasmid. A multiplex assay that simultaneously identifies *C. trachomatis* and *N. gonorrhoeae* target DNA can be used. Clinical swabs are expressed into a lysis diluent and heated to release nucleic acid. Urine samples are collected in a plastic sterile container together with special reagents provided by the manufacturer to remove amplification inhibitors. Furthermore, each of the examined samples has a corresponding amplification control well in order to determine if any of the specimens contain amplification inhibitors. Amplification and detection takes one hour, during which a fluorochrome-labelled probe binds to the amplified product as it is produced, a process detected in real time by fluorescence energy transfer (ET) measurement using a specialized plate reader. In practical terms, this assay has similar performance to the other NAATs but is particularly subject to inhibitors present in female urine.

Transcription-mediated amplification: The GenProbe Aptima

General

The problem of amplification inhibitors in the patient specimen can be overcome if the target nucleic acid is first removed from the rest of the specimen. This process of target capture is used in

the second-generation GenProbe Aptima-Combo 2 test. A multiplex assay that simultaneously identifies *C. trachomatis* and *N. gonorrhoeae* target DNA can be used. The patient sample is solubilized in a special transport medium. Specific capture oligonucleotides are added and bind to the target multi copy chlamydial rRNA at elevated temperature. On returning to room temperature, the capture hybrid oligonucleotide with its poly dA tail binds to poly dT-labelled magnetic particles retained in the reaction vessel with an external magnet while the remaining clinical specimen and inhibitors are washed off. The captured target is then ready for transcription-mediated amplification (TMA). The TMA process is complex and will not be detailed here, but essentially reverse transcriptase is used to create complementary DNA template which, with RNA polymerase, is used to create 100–1000 copies of RNA amplicon. From each of these, reverse transcriptase plus primer generates a double stranded DNA copy. The net result is that after 60 min of amplification, more than a billion transcripts are produced from a starting target of just 1000 molecules of the multi-copy RNA. A sophisticated dual-kinetic detection assay is then used which involves dual detection probes with different kinetics of light emission; a fast 'flasher' probe for *C. trachomatis* and a slow 'glower' probe for *N. gonorrhoeae*. A programmed luminometer calculates the relative contribution of each of these probes to total light output.

Advantages

Advantages of the TMA assay are as follows: (i) clinical specimens in transport medium are stable at room temperature for up to a month; (ii) target capture removes specimen inhibitors; (iii) rRNA provides an initial multi-copy target; (iv) TMA generates massive amplification; (v) flexible format, with a fully automated high-throughput version available for large reference centres.

Real-time (quantitative) NAATs

General

In a real-time NAAT, the development of specific amplification product is continuously monitored. The hardware for performing this kind of reaction, such as the Cobas Taqman48, LightCycler, Abbott m2000, NucliSens EasyQ Analyser and others, are becoming increasingly available. Development has been further driven by many new types of sensor probes, e.g. the so called molecular beacons, etc.[44]

Advantages

Real-time NAATs can be used in research applications as quantitative assays. This has given rise to a new generation of research papers which attempt to relate chlamydial load to disease. At present, there is no obvious clinical necessity for the use of quantitative, as opposed to qualitative, NAATs for chlamydial diagnosis. However, a significant advantage of real-time systems is that the whole procedure can be carried out in a sealed tube, thereby minimizing the possibility of cross contamination of samples with amplified products. The Becton Dickinson BD ProbeTec ET is an example of a commercially available real-time NAAT.

Use of non-approved NAATs

National regulatory processes should provide safeguards on the quality and performance of diagnostic tests. However, less than half the countries in the world have a regulatory framework for *in vitro* diagnostics for infectious diseases and, of these, even fewer require the submission of clinical trial data. There are no approved international guidelines for the evaluation of diagnostic tests. Product inserts make claims of high sensitivity and specificity, but there is no requirement to report the sample size or confidence intervals.[45] At the present date, the Roche Amplicor, Becton Dickinson BD ProbeTec and GenProbe Aptima tests have all received official regulatory approval in some western countries, but for a limited number of sample types only. Subsequent research studies may indicate these tests are appropriate for other samples (e.g. self-collected vaginal swabs, pharyngeal, rectal or penile swabs). It is **strongly recommended** that, wherever possible, internationally approved tests should be used for the diagnosis of *C. trachomatis* genital tract infection. Where this is not possible, it may be necessary to consider a locally produced test. In this situation, it is strongly **recommended** that the suitability of the proposed test for local requirements is strictly validated. Criteria for validation are given below.

Quality control

In each DNA extraction and subsequent analysis of samples, an internal positive control, allowing detection of substances in samples that inhibit the amplification reaction and controls the quality of sample preparation, and a negative control are necessary.

Certified and registered reference panels comprising coded control specimens should ideally be used for intra- and inter-laboratory quality control for PCR diagnostics. The use of specimen panels is standard for test system operation. These act as indicators of sensitivity, specificity and reproducibility independently of the test systems used.

Laboratory conclusions based on results using NAATs:
Nucleic acid specific for *C. trachomatis* was detected.
Nucleic acid specific for *C. trachomatis* was not detected.

Validation of diagnostic tests*

General criteria for the validation of diagnostic tests have been published by the WHO/TDR diagnostics expert panel.[46] These criteria are demanding and beyond the means of most individual groups. Guidelines for the minimum requirements for the validation of a new or modified test have been published.[47] At the

*A separate guideline is being prepared in this series on quality control procedures for STI diagnostics. Evaluations of the procedures that can be used in Eastern Europe are ongoing.

present time, only one validation study has been conducted in Eastern Europe, following these principles.[43]

Based on these principles, for validation of a new test for chlamydial infection, we recommend:

- Validation of the proposed test should be performed in comparison with either the Roche Amplicor, the Becton Dickinson BD ProbeTec ET, or the GenProbe Aptima CT test;
- A minimum of 50 positive clinical specimens (as shown by the reference test) and 100 negative specimens should be tested;
- Specimens that are weakly positive should be included. Replicate dilutions of a strong positive specimen should also be included to assess the reproducibility of detection at low copy numbers;
- The sensitivity and specificity of the proposed test must not be more than 5% below that of the chosen reference test.

Serology

Numerous studies have shown an association between chlamydial disease (e.g. pelvic inflammatory disease/tubal infertility) and the presence of high titres of antichlamydial antibody. This has lead to the suggestion that the detection and quantification of chlamydial antibody is useful for the diagnosis of chlamydial infection, even though there have been few attempts to explain the presence of high levels of antibody in apparently disease free individuals. Chlamydial antibody can be used as a useful marker in epidemiological studies for the cumulative history of exposure of a sample population to chlamydial infection. However, the measurement of chlamydial antibody is fraught with problems. The reproducibility of methods such as the micro-immunofluorescence test is poor, and there are no generally agreed standards. In some individuals the chlamydial antibody response may be delayed or even absent following chlamydial infection. Where there is an antibody response, it generally persists long after the infection has been cleared. For these reasons, we **do not recommend** serological testing for the diagnosis of individual cases. A diagnosis must be based on the direct demonstration of the causative organism.

Safety of personnel

For all methods, the safety of laboratory personnel should be ensured by compliance with safety procedures for working with potentially infected material (use of gloves, laminar flow hood, disinfectants, UV radiation, etc.).

Summary

- Approved NAATs are **recommended**, but controls that are able to detect inhibition of amplification should be included where appropriate. They offer adequate sensitivity and specificity for routine testing. The choice between these tests will depend on availability, cost and adaptability to local work flows and specimen throughput. High-throughput, automated versions of all these tests are available;
- It is **recommended that** any non-validated NAAT test must be validated against a recommended approved NAAT;
- Chlamydial culture is **not recommended** as a routine diagnostic procedure in Eastern Europe except where: (a) it is necessary for research purposes; (b) it is required (rarely) for medico legal cases; or (c) where no better methods are available;
- Measurement of chlamydial antibody is **not** useful for the routine diagnosis of chlamydial genital tract infections;
- POC tests lack sensitivity when compared to NAATs. Their use is therefore **not recommended** in situations where adequate laboratory facilities exist.

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The web site http://www.chlamydiae.com contains extensive reviews of chlamydial infections and their diagnosis, hyperlinked to the original literature.