PART 2B

Chlamydia trachomatis

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AVAILABLE TESTS

Nucleic acid amplification tests

The role of the nucleic acid amplification technology in the routine diagnosis of *Chlamydia trachomatis* infections is evolving rapidly. Three commercial assays are now available for routine use:

- Polymerase chain reaction (PCR; Roche Diagnostics)
- Strand displacement amplification (SDA; Becton Dickinson)
- Transcription mediated amplification (TMA; GenProbe).

Although these commercial assays differ in their target sequence and their method of amplification, it is their ability to produce a positive signal from theoretically a single copy of the target DNA or RNA (see pack inserts from the kit manufacturers) that has led to the reported increased sensitivity of nucleic acid amplification tests (NAATs).[1] Similar to other non-culture tests, NAATs do not require viable organisms.

With the advent of molecular diagnostic technology, it is now appreciated that no single test provides 100% sensitivity and specificity. Currently, NAATs are proving to be the best tests on the market. There is no room for complacency, however, as further work is required to eliminate test problems, such as inhibitors, contamination,[2] reproducibility,[3] and hormonal factors[4] that have played a part in lowering sensitivity.

Confirming positive NAATs by another technique

Only another NAAT is sensitive enough to confirm a positive result.[5] This approach needs further evaluation, as it is rare that individual laboratories will be able to offer more than one NAAT platform.

Equivocal results

Retest the original sample (according to manufacturer's instructions).

Inhibition

Inhibitors can be identified from all sites, in particular first void urine. An internal amplification control to identify inhibition should be used and is available using some of the commercial kits. The Gen-Probe TMA test has a stage in the extraction process that the manufacturer claims removes the majority of inhibitors and therefore no inhibitory control is needed (see individual manufacturer's instructions).

Pooling samples

This is possible and improves cost efficiency but is not licensed. Optimal pool sizes will vary according to the prevalence in the population being tested.

Tissue culture (TC)

The traditional method of diagnosing *C trachomatis* was by cell culture. However, few laboratories in the United Kingdom still offer this service. Cell culture procedures are expensive, labour intensive, and time consuming.

Although chlamydiae are bacteria, they cannot be cultivated in non-living or cell free media. Tissue culture techniques vary among laboratories. With no standardised protocol it is difficult to compare interlaboratory performance. Cell culture detects only viable organisms, and, hence, as with any other bacterial investigation the specimen collection and transport to the laboratory have to be optimal, irrespective of which laboratory method is to be used. Even under ideal conditions the sensitivity is probably no more than 75%,[6] although specificity should be 100% if a *C trachomatis*-MOMP specific stain is used.[7]

Direct fluorescent antibody (DFA)

Specimen material is obtained with a swab or brush, which is then rolled over the specimen well of a slide. Once air dried and fixed the specimen can be stained using either a MOMP or lipopolysaccharide (LPS) fluorescein labelled monoclonal antibody that binds to *C trachomatis* elementary bodies. Stained elementary bodies can then be identified using a fluorescence microscope. This technique is ideally suited for small numbers. It can give a quick turnaround time but its sensitivity and specificity are dependent on the expertise of the laboratory. DFA detects both viable and non-viable organisms.

This is the only test allowing simultaneous assessment of specimen adequacy.

Enzyme immunoassay (EIA)

There are many commercially available EIA tests on the market for detecting *C trachomatis* infection. They detect chlamydial lipopolysaccharide (LPS) with a monoclonal or polyclonal antibody that has been labelled with an enzyme. The enzyme converts a colourless substrate into a coloured product, which is detected by a spectrophotometer.

As the EIA detects LPS, there is a potential that cross reaction occurs with other micro-organisms causing a false positive reaction, hence it is vital that confirmation either by DFA or blocking antibody test is performed.

Sensitivity has been shown to be lower than for NAATs.[6]

''Point of care''/serological tests/leucocyte esterase tests

As they stand at present, they are not advised for diagnosis of genital *C trachomatis* in the GUM setting (recommendation grade C).

RECOMMENDATIONS

Because of the superior sensitivity and good specificity of NAATS these are the tests of choice for urethral, cervical, and first catch urine specimens (recommendation grade A).

Abbreviations: DFA, direct fluorescent antibody; EIA, enzyme immunoassay; LPS, lipopolysaccharide; MOMP, major outer membrane protein; NAATs, nucleic acid amplification tests; PCR, polymerase chain reaction; SDA, strand displacement amplification; TC, tissue culture; TMA, transcription mediated amplification

SITES FOR TESTING

Guidance on how to take samples can be made by following the pack inserts from the different manufacturer's kits.

First catch urine (recommendation grade C)

- The first 15–50 ml of urine passed any time of the day. The patient must not have urinated for at least 1 hour (maybe 2 hours for some kits). Follow manufacturer's instructions.
- First catch urine (FCU) of both males and females licensed for most NAATs, although less sensitive than from urethral or endocervical specimens.
- Male urine licensed for some EIAs, shown to be sensitive with symptomatic, relatively insensitive for asymptomatic males.
- Female urine unsuitable for EIAs.
- Urine suitable but not ideal for DFA, needs expertise.
- Urine unsuitable for tissue culture techniques.

Cervical

- Cervical samples are suitable for all tests. Taken under speculum examination, the swab inserted into the os using the manufacturer's swab collection packs and rotated two or more times for 15–30 seconds (recommendation grade C).

Urethral

- Both male and female urethral samples are suitable for all tests.
- For men the swab is inserted into the urethra 2–4 cm and rotated one or more times (recommendation grade C).

Pharynx

- Pharyngeal samples licensed for tissue culture technique (recommendation grade A).
- DFA is licensed for pharyngeal swab specimens but not suitable for large throughput use (recommendation grade C).
- Not licensed for most EIAs.
- NAAT not licensed but increasing work on validation means that for any centre without access to culture this is the test of choice (recommendation grade C).

Rectal (obtained via proctoscopy)

- Rectal samples validated for tissue culture technique (recommendation grade A).
- DFA is licensed for rectal swab specimens but not suitable for large throughput use (recommendation grade C).
- Not licensed for EIA testing owing to the cross reaction with other organisms leading to false positive EIA results.
- Routinely available NAATs for *C trachomatis* will detect all serovars including LGV serovars and are licensed for genital specimens. There are no licensed NAATs for the detection of *C trachomatis* in rectal specimens but data are available supporting the validity of these tests for use with rectal specimens and therefore for centres without access to culture this is the test of choice (evidence level III, recommendation grade B).

Vulvo-vaginal

- Not licensed for use with NAATs, but demonstrated by a number of workers to produce equivalent sensitivity to cervical testing.[8]

SCREENING IN THE FOLLOWING PATIENT GROUPS

Owing to the frequently asymptomatic nature of genital *C trachomatis* there is no difference in the screening guidelines for those showing symptoms from those who do not.

Frequency of repeat testing in an asymptomatic patient

This is in part being addressed by the DoH Chlamydia Screening Programme. Re-exposure to a possible source of chlamydia should lead to rescreening if the patient re-presents.

Heterosexual women

Cervical or vulvo-vaginal (clinician or self taken) or FCU (recommendation grade A).

Heterosexual men

Urethral or FCU (recommendation grade A).

Homosexual men

Urethral or FCU (recommendation grade A).

Young women

Offer non-invasive tests if speculum examination is declined
Vulvo-vaginal (clinician or self taken) or FCU (recommendation grade A).

Young men

Offer non-invasive testing if urethral specimen is declined.
FCU (recommendation grade A).

Pregnant women

As for heterosexual women. See notes below on test of cure.

Contacts

No different advice.

Sex workers

No different advice.

Sexual assault victims

Culture was the recommended method for detecting *C trachomatis* at all exposed sites following sexual assault in adults because of 100% specificity (recommendation grade C). This guideline recommends that a NAAT be taken from all exposed sites in addition to a chlamydial culture (if culture is available) owing to the low sensitivity of culture and lack of availablilty.

TEST OF CURE

Test of cure (TOC) is not routinely recommended if standard treatment has been given, there is confirmation that the patient has adhered to therapy, and there is no risk of re-infection. However, if these criteria cannot be met or if the patient is pregnant a TOC is advised. This should be taken using the same technique as was used for the initial testing. Ideally, a minimum of 3–5 weeks post-treatment is required[9] as NAATs will demonstrate residual DNA/RNA even after successful treatment of the organism (recommendation grade A).

Table 1 Summary of recommended tests for use with different sites of samples

Test	Sites FCU	Cervix	Urethra	Pharynx	Rectum	Vulvo-vaginal
NAAT	1	1	1	3	3	3
EIA	4	2	2	5	5	5
DFA	2	2	2	2	2	5
TC	5	2	2	1	1	5

NAAT, nucleic acid amplification test; EIA, enzyme immunoassay; DFA, direct fluorescent antibody; TC, tissue culture.
1, test of choice; 2, acceptable, but not first choice; 3, not licensed, although encouraging work being performed; 4, only for use in asymptomatic males; 5, not recommended.
All recommendations are at grade B unless stated otherwise.

APPLICABILITY/RESOURCE REQUIREMENTS

The availability of different microbiology tests may vary and use of optimal tests as outlined in this guideline may have resource implications.

AUDIT STANDARD

Ninety five per cent of testing for chlamydia performed using a test of choice or acceptable test (table 1).

SEARCH CRITERIA

A Medline search using the terms ''Chlamydia trachomatis'', ''diagnosis'', and ''genital'', from 1996 to January 2004 was conducted and the most relevant references are included.

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