Chlamydia trachomatis **Screening and Testing Guidelines**

Introduction/Scope and Purpose

This guideline makes recommendations regarding sampling and diagnostic testing for Chlamydia and does not discuss the utility or indications for Chlamydia population screening.

These guidelines are intended to be used in combination with the following guidelines:

The 2006 BASHH guidelines for the management of genital infection with *Chlamydia trachomatis* available at http://www.bashh.org/documents/61/61.pdf

The 2009 Scottish Intercollegiate Guideline Network (SIGN) guidelines for the management of genital *Chlamydia trachomatis* infection available at http://www.sign.ac.uk/pdf/sign109.pdf

Summary of Recommended Tests for *Chlamydia trachomatis*

Use of Tests with Appropriate Specimens

(NAAT: nucleic acid amplification test)

	Specimens					
Test	**first catch urine**	**cervix**	**urethra**	**pharynx**	**rectum**	**vulval-vaginal**
NAAT	1	1	1	2	2	1
tissue culture	4	3	3	3	3	4

Key:
1 Test of choice
2 Test of choice, not licensed
3 Medicolegal cases
4 Not recommended

Appropriate Specimens for men and women

	Specimen
Men	First catch urine or urethral swab
Men who have sex with men (MSM)	First catch urine or urethral swab and Pharyngeal and rectal swab
Women undergoing speculum examination	Endocervical swab
Women not requiring speculum examination	First catch urine or self taken lower vaginal swab

A variety of different tests is available to detect *Chlamydia trachomatis* in the genital tract. Their appropriate use depends on the characteristics of the test itself, the correct choice of sample and the clinical presentation of the patient. Currently there are no Enzyme Immuno-assays (EIAs), Point of care tests (POCT) or DNA probe technology that can be recommended for use in the diagnosis of *C. trachomatis* as they show inferior sensitivity and specificity to that of the recommended tests, the Nucleic Acid Amplification Tests (NAATs).

Nucleic acid amplification tests (NAATs)

The role of nucleic acid amplification technology in the routine diagnosis of *C. trachomatis* infections has evolved over the last decade. There are a number of commercial assays currently available for routine use. The four listed below are commonly used in clinical practice:

- COBAS Taqman, Polymerase chain reaction assay (Real-time PCR, Roche Diagnostics)
- BD ProbeTec ET, Strand displacement amplification (SDA, Becton Dickinson)
- GenProbe Aptima assay, Transcription mediated amplification assay (TMA, GenProbe)
- Abbott RealTime PCR assay (Abbott m2000, Abbott Diagnostics)

These commercial assays all detect both viable and non-viable organisms but differ in their target sequence and their method of amplification. These assays also offer dual detection of *C. trachomatis* and *Neisseria gonorrhoeae* from a single specimen. For further information regarding the detection of *N. gonorrhoeae* using NAATs please see BASHH gonorrhoea guidelines.

Recommendation

NAATs are the tests of choice for urethral, cervical, vaginal (self-taken and clinician-obtained) and first catch urine specimens[1] because of their superior sensitivity and high specificity (Ib, Grade A). All of the above commercial NAATs show adequate sensitivity and specificity. Only NAATs that detect all known variants can be recommended.

It is beyond the remit of these guidelines to recommend any one NAAT above another. The choice of testing platform will depend on a variety of factors including:

- The volume of samples to be processed
- Reproducibility
- Hands-on-time/automation
- Cost of reagents/equipment
- The relative sensitivity and specificity of the individual tests for different clinical specimens
- Whether the test is used to detect *C. trachomatis* alone or as a combined test for *C. trachomatis* and *N. gonorrhoeae*

No single test provides 100% sensitivity and specificity. Test problems include inhibitors[2], contamination[3], reproducibility[4] and hormonal factors[5], which can lower sensitivity.

Although not licensed, NAATs may be used and potentially give valid results from pharyngeal and rectal specimens. This should be validated locally for use on the individual platform.

Confirming positive NAATs by another technique.

Only another NAAT is sensitive enough to confirm a positive result [6]. Currently the HPA guidelines recommend that every positive Chlamydia result should be confirmed using a NAAT, preferably with an assay of equal sensitivity but with a different target. However recent data suggests that confirmatory testing may be unnecessary given that >90% of positive NAAT results will be confirmed[7]. A strategy of not confirming positive results appears more robust with some platforms although this data is limited to genital specimens only. Further work is required to validate this strategy for extra-genital specimens. Clinicians need to be aware of the potential for false positive results regardless of the site tested, particularly when using the test in a low prevalence population.

When the test result is equivocal, arrangements should be made to re-test the original sample and request a further sample. Where possible this sample should be tested using a NAAT assay of equal sensitivity but with a different target.

Inhibition

Inhibitors can be identified in specimens from all sites, in particular first-void urine. An internal amplification control to identify inhibition should be used and is available using some commercial kits. Not all NAATs include an internal control (see individual manufacturer's instructions).

Transport, storage and handling of samples

Instructions regarding the transport, storage and handling of samples vary between commercial assays and should be in line with the manufacturer's instructions.

Quality assurance

All efforts need to be made to ensure all staff adhere the correct test procedures,

avoid sampling errors and environmental contamination. Participation in an External Quality Assurance (EQA) programme needs to be encouraged (essential in an accredited laboratory) to minimise common errors and ensure that reproducibility of testing is maintained.

New Variant *Chlamydia trachomatis*

In November 2006 a *C. trachomatis* strain with a deletion in the cryptic plasmid was discovered in Sweden[8] (new-variant – nvCT). The deletion, 377bp in length, affected the target sequence of some of the commercial tests resulting in false negative results. Isolated cases were found in Norway, Ireland, Denmark, France and Scotland. It is uncertain why this strain appears here and only enhanced surveillance will show whether it will be found elsewhere.

The target sequence of some commercially available kits has since been modified, but still not all kits are capable of detecting the nvCT. Current NAATs are being modified and withdrawn and therefore it is important that clinicians and their microbiologists are aware of this and understand the status of the test that they are currently using. Further variants could occur and may not be detected by current commercial assays.

Lymphogranuloma venereum

Lymphogranuloma venereum (LGV) is caused by the more invasive L serovars (L1, L2, L2a, and L3) of *C. trachomatis.* Since the end of 2003, an ongoing outbreak of LGV proctitis has been reported in Europe and North America among men who have sex with men (MSM), which has been strongly associated with HIV infection. It is recommended that all MSM with a positive rectal chlamydia NAAT and/or isolate

reporting rectal symptoms and/or who are a contact of someone with LGV should have a sample sent to the Sexually Transmitted Bacteria Reference Laboratory (STBRL), Health Protection Agency Centre for Infections, London, UK or the Scottish Bacterial Sexually Transmitted Infections Reference Laboratory (SBSTIRL). A real-time PCR assay is now available at STBRL and SBSTIRL which performs well for the detection of LGV, non-LGV or dual infections from rectal specimens[9] (IV, Grade C).

Tissue culture

The traditional method of diagnosing *C. trachomatis* was by cell culture. However, few laboratories in the UK still offer this service (except for medico-legal purposes). Cell culture procedures are expensive, labour intensive and time consuming.
Cell culture can be used in addition to NAAT testing for medico-legal purposes.

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 inter laboratory performance. Cell culture detects only viable organisms and hence, as with any other bacterial investigation, the specimen collection and transport to the laboratory has to be optimal, irrespective of which laboratory method is to be used. At best the sensitivity is probably no more than 75% [10], although specificity should be 100% if a *C. trachomatis* major outer membrane protein (MOMP) specific stain is used [11].

Appropriate Specimens for Testing for *Chlamydia trachomatis*

The performance of different tests for *Chlamydia trachomatis* can be influenced by the test specimen used.

First catch urine (FCU)

First catch urine comprises the first 15-50 mls of urine passed at anytime of the day (see individual pack inserts). The patient must not have urinated for at least one hour (or 2 hours for some kits). FCU is licensed in both men and women for most NAATs, FCU in women is less sensitive than using endocervical specimens. FCUs are ideal specimens for men. (IIa, Grade A)

Cervical specimens

Cervical samples are suitable for all tests. Specimens should be taken during a speculum examination with the swab inserted into the cervical os using the manufacturers swab collection packs and rotated for a few seconds. (IV, Grade C).

Urethral specimens

Both male and female urethral samples are suitable for all tests. For men either a urethral specimen or first catch urine are ideal specimens although a urethral specimen may cause discomfort. For a male urethral specimen the swab is inserted into the urethra 2-4 cm and rotated one or more times (Grade C). For women, introduce the swab 1 cm into the urethra and rotate one or more times. (IV, Grade C).

Pharyngeal specimens

A cotton tipped swab should be rubbed over the posterior pharynx and tonsillar crypts.

Pharyngeal samples are licensed for use with the tissue culture technique (IIa, Grade A).

NAATs are not licensed for use with pharyngeal specimens but accumulating evidence suggests they perform well (IIa, Grade C).[12-13]

There is limited data regarding self taken pharyngeal specimens among MSM but this suggests similar sensitivity and specificity compared to samples obtained by healthcare workers.[13]

Rectal specimens

A cotton tipped swab should be rubbed against the rectal wall. This should ideally be taken at proctoscopy but data suggests that rectal swabs taken without proctoscopy have similar sensitivity.[13]

Tissue culture is validated for detecting *C. trachomatis* from rectal specimens. (IIa, Grade A). There are no licensed NAATs for the detection of *C. trachomatis* in rectal specimens but data is available supporting the validity of these tests for use here. Routinely available NAATs for *C. trachomatis* will detect all serovars including LGV (III, Grade B).[11]

There is limited data regarding self taken rectal specimens among MSM but this suggests similar sensitivity and specificity compared to samples obtained by healthcare workers via proctoscopy.[13]

Vulval-vaginal specimens (VV)

Insert the swab into the vagina, about two inches and gently rotate the swab for 10 to 30 seconds.

Some commercially available NAATs are licensed for use with VV samples, either clinician obtained or self-taken (further information is available from each manufacturer's kit insert). VV specimens have been demonstrated by a number of workers to produce similar sensitivity to cervical testing.[14]

Factors which may alter recommended tests or test sites

Recommendations for testing are unaltered for:

- sexual contacts of known chlamydia infection
- sex workers
- pregnant women
- presence or absence of symptoms

however testing in women who have undergone a hysterectomy should be undertaken using either a FCU or VV specimen

Sexual Assault Victims

Culture was previously the recommended method for detecting *C. trachomatis* at all exposed sites following sexual assault in adults because of it provides 100% specificity. It is now recommended that a NAAT be taken from all exposed sites in addition to a chlamydial culture (if culture is available) because of the low sensitivity of culture (IIa, Grade C). Samples for confirmation for medico-legal purposes should be discussed with the Sexually Transmitted Bacterial Reference Laboratory or the Scottish Bacterial Sexually Transmitted Infections Reference Laboratory (SBSTIRL).

Frequency of repeat testing in an asymptomatic patient

Re-exposure to a possible source of chlamydia should lead to re-screening if the patient re-presents. However there is no evidence currently to guide the frequency of repeat testing in those without a clear history of re-exposure to chlamydia. The DoH Chlamydia Screening Programme recommends repeat testing annually or every time someone has a new sexual partner.

When to test following potential exposure to infection

Individuals should be advised to have a test for chlamydia with a NAAT when they first present and, if potential exposure occurred within the last two weeks, they should also be asked to return for a repeat NAAT two weeks after the exposure. (IV, Grade C). (http://www.bashh.org/documents/1743/1743.pdf)

Test of Cure (TOC) following *C. trachomatis* treatment

Test of cure (repeat testing to confirm clearance of infection) is not routinely recommended if:

- standard treatment has been given
- there is confirmation that the patient has adhered to therapy
- 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 and sample type as used for the initial testing. Ideally, a minimum of 5 weeks post treatment (6 weeks after Azithromycin) is required [15] as NAATs will detect residual DNA/RNA even after successful treatment of the organism (IIb, Grade A).

Stakeholder Involvement

Clinicians and scientists from the Bacterial Special Interest Group of BASHH have been involved in the development of this guideline. These guidelines have been posted for comment on the BASHH website for 3 months for consultation. Patient

involvement was not attempted but the authors drew on their experience as practising clinicians.

Search criteria

A search using the terms *Chlamydia trachomatis*, diagnosis and genital was undertaken using Embase from January 2004 until the end of 2008. The journals Sexually Transmitted Infections and Sexually Transmitted Diseases were handsearched for relevant articles. A total of 98, 201 and 100 references were identified respectively. The most relevant references (those providing additional or new evidence) were included after reviewing titles and abstracts.

Applicability

This guideline has been designed for use in sexual health clinics in the UK but its principles are also applicable to other healthcare settings where screening or testing for *C. trachomatis* is undertaken.

Auditable Outcome Measures

95% of testing for chlamydia performed using a test of choice (Table 1).

Authors

Caroline Carder, Biomedical Scientist, University College London Hospitals NHS Foundation Trust

Danielle Mercey, Consultant in GUM, University College London

Paul Benn, Consultant in GUM, Mortimer Market Centre, Camden PCT

Editor

Jonathan Ross, Whittall Street Clinic, Birmingham

Conflict of Interest

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