



# ***Chlamydia trachomatis* UK Testing Guidelines**

**Clinical Effectiveness Group**

**British Association for Sexual Health and HIV**

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## **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>



NHS Evidence has accredited the process used by the British Association for Sexual Health & HIV (BASHH) to produce UK national guidelines. Accreditation is valid for 3 years from January 2011 and is retrospectively applicable to guidance produced using the processes described in the BASHH Framework for Guideline Development and Assessment dated September 2010. More information on accreditation can be viewed at [www.evidence.nhs.uk](http://www.evidence.nhs.uk)

## Summary of Recommended Tests for *Chlamydia trachomatis*

### ***Use of Tests with Appropriate Specimens***

(NAAT: nucleic acid amplification test)

	<b>Specimens</b>							
	<b><i>first catch urine</i></b>	<b>cervix</b>	<b>vulval-vaginal</b>	<b>urethra</b>	<b>Glans penis</b>	<b>pharynx</b>	<b>rectum</b>	<b>Conjunctiva</b>
<b>NAAT</b>	1	1	1	1*	3	2#	2#	2
<b>Tissue culture</b>	3 (No longer recommended)							

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

\* men and women with persistent urethral symptoms where tests from other sites are negative or women who have undergone a hysterectomy

# men who have sex with men (MSM) and commercial sex workers (CSW)

### ***Appropriate Specimens for men and women***

	<b>Specimen</b>
<b>Men</b>	First catch urine (specimen of choice) or urethral swab
<b>Men who have sex with men (MSM)</b>	First catch urine (specimen of choice) or urethral swab and Pharyngeal and rectal swab
<b>Women undergoing speculum examination</b>	Endocervical swab
<b>Women not requiring speculum examination</b>	Self taken lower vaginal swab (specimen of choice) or first catch urine*

\* please note first catch urine samples may be less sensitive than endocervical or self taken lower vaginal swabs for the detection of *C. trachomatis*.

A variety of different tests are 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:

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

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 and BASHH/ Health Protection Agency guidance [www.hpa.org.uk](http://www.hpa.org.uk).

## Recommendation

NAATs are the tests of choice for urethral, cervical, vaginal (self-taken and clinician-obtained) and first catch urine specimens<sup>1</sup> because of their superior sensitivity and high specificity (Ib, Grade A). All of the above commercial NAATs show adequate sensitivity and specificity. The testing platform selected must have a positive predictive value (PPV) over 90% and detect all known variants (IV, Grade C).

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<sup>2</sup>, contamination<sup>3</sup>, reproducibility<sup>4</sup> and hormonal factors<sup>5</sup>, which can lower sensitivity.

Although not licensed for these sites, NAATs may be used and potentially give valid results from pharyngeal and rectal specimens (IIa, Grade B).<sup>12-13</sup> This should be validated locally for the individual platform used.

### Confirming positive NAATs by another technique.

Only another NAAT is sensitive enough to confirm a positive result.<sup>6</sup> (IIa, Grade B). 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.<sup>7</sup> (III, Grade B) The data are available for genital specimens and for some platforms only. Further work is required to validate this strategy for extra-genital specimens. Regardless of the site tested, clinicians need to be aware of the potential for false positive results, particularly when using the test in a low prevalence population.

When the test result is equivocal (unconfirmed reactive), 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. (IIa, Grade B)

### 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

Requirements for the transport, storage and handling of samples vary between commercial assays and the manufacturer's instructions should be followed. It is recommended that results should be available within seven working days of the specimen being taken. If supplementary testing of a sample is required then the results should be available within 14 working days of the specimen being taken.

### Quality assurance

All efforts need to be made to ensure all staff adhere to 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<sup>8</sup> (new-variant – nvCT). The deletion, 377bp in length, affected the target sequence of some 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 not all kits are capable of detecting the nvCT. Current NAATs are being modified and withdrawn so it is important that clinicians and microbiologists are aware of the status of the test they use. 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 who report rectal symptoms 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<sup>9</sup> (IV, Grade C). Further information regarding the LGV enhanced surveillance protocol is available at [http://www.hpa.org.uk/web/HPAwebFile/HPAweb\\_C/1194947374927](http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1194947374927) and the LGV guideline is available at <http://www.bashh.org/documents/92/92.pdf>

### **Tissue culture**

The traditional method of diagnosing *C. trachomatis* was by cell culture. Although chlamydiae are bacteria, they cannot be cultivated in non-living or cell free media. However, few laboratories in the UK still offer this service. Cell culture procedures are expensive, labour intensive and time consuming. Cell culture allows viable isolates to be obtained from cases where therapeutic failure is suspected, to test for antimicrobial resistance. At best the sensitivity of cell culture is probably no more than 75%<sup>10</sup>, although specificity should be 100% if a *C. trachomatis* major outer membrane protein (MOMP) specific stain is used<sup>11</sup>. Cell culture is no longer required for medico-legal purposes. (IV, Grade C)

### **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 or self taken lower vaginal specimens.<sup>14</sup> FCUs are the preferred specimens for men. (IIa, Grade B)

#### ***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. (IIa, Grade B)  
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). A urethral sample may be required in women with persistent urethral symptoms in whom specimens for *C. trachomatis* at other sites are negative or in those who have undergone a hysterectomy. (IV, Grade C) For a female urethral specimen 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 B).<sup>12-13</sup> Pharyngeal specimens should be taken and tested for *C. trachomatis* in MSM (IIa, Grade B) and commercial sex workers (CSW) reporting sexual behaviours which may result in pharyngeal infection. (IV, Grade C) There is insufficient evidence to recommend testing for pharyngeal *C. trachomatis* using NAATs in heterosexual men and women. (IV, Grade C)

There is limited data regarding self taken pharyngeal specimens among MSM but what there is suggests similar sensitivity and specificity to samples obtained by healthcare workers.<sup>13</sup>

### ***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.<sup>13</sup>

Tissue culture is validated for detecting *C. trachomatis* from rectal specimens. (IIa, Grade B). 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 (IIa, Grade B).<sup>12</sup> Rectal specimens should be taken and tested for *C. trachomatis* in MSM (IIa, Grade B) and CSWs (IV, Grade C) reporting sexual behaviours which may result in rectal infection. There is insufficient evidence to recommend testing for rectal *C. trachomatis* using NAATs in heterosexual men and women. (IV, Grade C)

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.<sup>13</sup>

### ***Vaginal specimens (VS)***

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 vaginal samples, either clinician obtained or self-taken (further information is available from each manufacturer's kit insert). Vaginal specimens have been demonstrated by a number of workers to produce similar sensitivity to cervical testing.<sup>14</sup> (IIa, Grade B)

Vulval specimens are not recommended for testing for *C. trachomatis*.

### ***Conjunctival specimens***

A cotton tipped swab should be used to for *C. trachomatis* from the conjunctiva. NAATs have a higher sensitivity to detect infection from the conjunctiva than other methodologies.<sup>15</sup>

### ***Specimens from the glans penis***

NAATs have poor sensitivity to detect *C. trachomatis* from clinician and self-taken specimens from the glans penis and cannot be recommended.<sup>16-17</sup> (IIa, Grade B)

### **Factors which may alter recommended tests or test sites**

Recommendations for testing are unaltered for:

- sexual contacts of known chlamydia infection
- sex workers – pharyngeal and rectal specimens should be taken and tested for *C. trachomatis* in CSWs (IV, Grade C) reporting sexual behaviours which may result in infection at these sites.
- pregnant women
- presence or absence of symptoms

however testing in women who have undergone a hysterectomy should be undertaken using either a FCU, VV specimen or a urethral swab. (IV, Grade C)

### ***Sexual Assault Victims***

Culture is no longer recommended for detecting *C. trachomatis* at all exposed sites following sexual assault in adults because of its low sensitivity. It is recommended that a NAAT be taken from all exposed sites. (IIa, Grade C). Confirmation of a positive NAAT for *C. trachomatis* should be undertaken using a NAAT with a different target in medico-legal cases. This is available in some laboratories and 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 for under 25's 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. Few data are however available<sup>18</sup> regarding the optimal time to undertake a TOC. It is recognised that NAATs will detect residual DNA/RNA even after successful treatment of the organism four to six weeks after treatment (IIb, Grade B).

### **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, Sexually Transmitted Diseases and the International Journal of STDs and AIDS were hand searched 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)
- 95% of results available within seven working days of specimen collection
- 95% of results available within 14 working days of specimen collection if further testing is required.

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### **Conflict of Interest**

Each of the authors has declared that they have no conflicts of interest

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