UK Standards for Microbiology Investigations

Chlamydia trachomatis infection – testing by Nucleic Acid Amplification Tests (NAAT)
Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of Public Health England (PHE) working in partnership with the National Health Service (NHS), Public Health Wales and with the professional organisations whose logos are displayed below and listed on the website https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a steering committee (see https://www.gov.uk/government/groups/standards-for-microbiology-investigations-steering-committee).

The contributions of many individuals in clinical, specialist and reference laboratories who have provided information and comments during the development of this document are acknowledged. We are grateful to the medical editors for editing the medical content.

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Chlamydia trachomatis infection – testing by Nucleic Acid Amplification Tests (NAAT)

Contents

Acknowledgments ................................................................................................................. 2
Amendment table.................................................................................................................. 4
UK SMI: scope and purpose................................................................................................. 5
Scope of document............................................................................................................... 8
Introduction......................................................................................................................... 9
Technical information/limitations ...................................................................................... 12
Safety considerations.......................................................................................................... 13
Public health management................................................................................................. 13
Chlamydia trachomatis infection – testing by Nucleic Acid Amplification Tests (NAAT)14
Notification to PHE, or equivalent in the devolved administrations................................. 15
References ......................................................................................................................... 17
### Amendment table

Each UK SMI method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@phe.gov.uk](mailto:standards@phe.gov.uk).

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

<table>
<thead>
<tr>
<th>Amendment number/date</th>
<th>5/09.01.17</th>
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<tr>
<td>Issue number discarded</td>
<td>3.2</td>
</tr>
<tr>
<td>Insert issue number</td>
<td>4</td>
</tr>
<tr>
<td>Anticipated next review date*</td>
<td>09.01.20</td>
</tr>
<tr>
<td><strong>Section(s) involved</strong></td>
<td><strong>Amendment</strong></td>
</tr>
<tr>
<td>Whole document.</td>
<td>The whole document has been put into the more comprehensive V template. New sections include: Type of specimen Definitions Introduction Technical Information/Limitations Safety considerations Public Health Management Notification to the PHE or equivalent in devolved administrations</td>
</tr>
<tr>
<td>Algorithm.</td>
<td>An extra branch has been added to cover Medicolegal cases. Equivocal removed as an option.</td>
</tr>
<tr>
<td>Footnotes.</td>
<td>Information streamlined following the insertion of Medicolegal into the algorithm.</td>
</tr>
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</table>

*Reviews can be extended up to five years subject to resources available.
UK SMI#: scope and purpose

Users of UK SMIs
Primarily, UK SMIs are intended as a general resource for practising professionals operating in the field of laboratory medicine and infection specialties in the UK. UK SMIs also provide clinicians with information about the available test repertoire and the standard of laboratory services they should expect for the investigation of infection in their patients, as well as providing information that aids the electronic ordering of appropriate tests. The documents also provide commissioners of healthcare services with the appropriateness and standard of microbiology investigations they should be seeking as part of the clinical and public health care package for their population.

Background to UK SMIs
UK SMIs comprise a collection of recommended algorithms and procedures covering all stages of the investigative process in microbiology from the pre-analytical (clinical syndrome) stage to the analytical (laboratory testing) and post analytical (result interpretation and reporting) stages. Syndromic algorithms are supported by more detailed documents containing advice on the investigation of specific diseases and infections. Guidance notes cover the clinical background, differential diagnosis, and appropriate investigation of particular clinical conditions. Quality guidance notes describe laboratory processes which underpin quality, for example assay validation.

Standardisation of the diagnostic process through the application of UK SMIs helps to assure the equivalence of investigation strategies in different laboratories across the UK and is essential for public health surveillance, research and development activities.

Equal partnership working
UK SMIs are developed in equal partnership with PHE, NHS, Royal College of Pathologists and professional societies. The list of participating societies may be found at https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories. Inclusion of a logo in an UK SMI indicates participation of the society in equal partnership and support for the objectives and process of preparing UK SMIs. Nominees of professional societies are members of the Steering Committee and working groups which develop UK SMIs. The views of nominees cannot be rigorously representative of the members of their nominating organisations nor the corporate views of their organisations. Nominees act as a conduit for two way reporting and dialogue. Representative views are sought through the consultation process. UK SMIs are developed, reviewed and updated through a wide consultation process.

Quality assurance
NICE has accredited the process used by the UK SMI working groups to produce UK SMIs. The accreditation is applicable to all guidance produced since October 2009. The process for the development of UK SMIs is certified to ISO 9001:2008. UK SMIs

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Microbiology is used as a generic term to include the two GMC-recognised specialties of Medical Microbiology (which includes Bacteriology, Mycology and Parasitology) and Medical Virology.
Chlamydia trachomatis infection – testing by Nucleic Acid Amplification Tests (NAAT)

represent a good standard of practice to which all clinical and public health microbiology laboratories in the UK are expected to work. UK SMIs are NICE accredited and represent neither minimum standards of practice nor the highest level of complex laboratory investigation possible. In using UK SMIs, laboratories should take account of local requirements and undertake additional investigations where appropriate. UK SMIs help laboratories to meet accreditation requirements by promoting high quality practices which are auditable. UK SMIs also provide a reference point for method development. The performance of UK SMIs depends on competent staff and appropriate quality reagents and equipment. Laboratories should ensure that all commercial and in-house tests have been validated and shown to be fit for purpose. Laboratories should participate in external quality assessment schemes and undertake relevant internal quality control procedures.

Patient and public involvement

The UK SMI working groups are committed to patient and public involvement in the development of UK SMIs. By involving the public, health professionals, scientists and voluntary organisations the resulting UK SMI will be robust and meet the needs of the user. An opportunity is given to members of the public to contribute to consultations through our open access website.

Information governance and equality

PHE is a Caldicott compliant organisation. It seeks to take every possible precaution to prevent unauthorised disclosure of patient details and to ensure that patient-related records are kept under secure conditions. The development of UK SMIs is subject to PHE Equality objectives https://www.gov.uk/government/organisations/public-health-england/about/equality-and-diversity.

The UK SMI working groups are committed to achieving the equality objectives by effective consultation with members of the public, partners, stakeholders and specialist interest groups.

Legal statement

While every care has been taken in the preparation of UK SMIs, PHE and the partner organisations, shall, to the greatest extent possible under any applicable law, exclude liability for all losses, costs, claims, damages or expenses arising out of or connected with the use of an UK SMI or any information contained therein. If alterations are made by an end user to an UK SMI for local use, it must be made clear where in the document the alterations have been made and by whom such alterations have been made and also acknowledged that PHE and the partner organisations shall bear no liability for such alterations. For the further avoidance of doubt, as UK SMIs have been developed for application within the UK, any application outside the UK shall be at the user’s risk.

The evidence base and microbial taxonomy for the UK SMI is as complete as possible at the date of issue. Any omissions and new material will be considered at the next review. These standards can only be superseded by revisions of the standard, legislative action, or by NICE accredited guidance.

UK SMIs are Crown copyright which should be acknowledged where appropriate.
Scope of document

Type of specimen

Urine, vulvo-vaginal swabs, urethral swabs, endocervical swabs, rectal swabs, oropharyngeal swabs

Note: First-void urine in women should be avoided.

This UK SMI covers the testing of clinical samples for the investigation of urogenital Chlamydia trachomatis infection (including rectal and oropharyngeal infection) by NAAT.

This UK SMI does not distinguish between lymphogranuloma venereum (LGV) serovars and non-LGV serovars of C. trachomatis and does not include ocular trachoma or neonatal infections (including pneumonia and conjunctivitis).

This UK SMI does not cover the testing of pooled samples or the use of point of care tests (POCT, where the test result can be delivered to the patient without the sample being sent to the laboratory or the use of dual NAATs testing. Nucleic amplification tests (NAAT) for extra-genital and pooled specimens should be validated locally. EIA tests are not recommended.

Refer to UK SMI Q 4 - Good laboratory practice when performing molecular amplification assays.

This UK SMI should be used in conjunction with other UK SMIs.

Definitions

For all antigen, antibody and NAAT testing the following definitions apply:

During testing process

Reactive – Initial internal-stage positive result pending confirmation.

Not reactive – Initial internal-stage negative result.

Equivocal or Indeterminate – Result is not clearly positive or negative. Further testing is required.

Reporting stage

These terms are used for final or preliminary reports.

Detected – Report-stage confirmed reactive result.

Not detected – Report-stage not reactive result.

Indeterminate – Reactive result that cannot be confirmed.

Inhibitory – The presence of inhibitors within the sample has prevented PCR amplification. A further specimen is required. The term used may be different for various platforms, for example “invalid”. See also page 12 'NAAT inhibition'.
Chlamydia trachomatis infection – testing by Nucleic Acid Amplification Tests (NAAT)

**Introduction**

*Chlamydia trachomatis* infection is the most prevalent bacterial, sexually transmitted infection in the UK, particularly in young adults. There are three biovars of *C. trachomatis* and fifteen serovars; trachoma biovars (serovars A-C), urogenital biovars (serovars D-K) and lymphogranuloma venereum (LGV) biovars (serovars L1-L3).

Risk factors for non-LGV *C. trachomatis* infection include being <25 years of age, having a new sexual partner or more than one sexual partner in the past year and inconsistent use of condoms.

Diagnostic testing should be offered for: patients who have symptoms or signs suggestive of chlamydial infection; sexual partners of those with suspected or proven chlamydial infection; all men or women with another sexually transmitted infection; patients with reactive arthritis who are sexually active; parents of infants with chlamydial conjunctivitis/pneumonia; egg and semen donors; or for any other patient on request.

Several outbreaks of LGV have occurred in men who have sex with men (MSM) since the early 2000s and high rates of infection have been observed in MSM in the UK. However, increased rates may in part be due to increased use of NAAT for extra-genital samples in this risk group. Samples for LGV identification should be sent to the Sexually Transmitted Reference Unit (STBRU) (or other laboratory with validated test) for diagnosis.

**Screening**

In England, routine screening for sexually active young people under the age of 25 is recommended annually or more frequently if there has been a change of partner. Those aged 15 and 16 may be screened if they meet the Fraser criteria for consent to testing. Those over 25 years of age may be screened if they have had a new sexual partner, or more than one sexual partner in the last 12 months. Repeat testing should be carried out 3-6 months following the completion of treatment in those diagnosed with chlamydial infection who are under 25 years old.

Routine screening is not recommended in pregnant women unless they are from high prevalence populations or within the national screening programme age range above; however, screening is recommended for those seeking termination of a pregnancy.

Screening is also important in patients undergoing IVF.

**Laboratory diagnosis**

Several diagnostic strategies are available for the identification of *C. trachomatis* infection. These include NAAT, cell culture, enzyme immunoassays and direct fluorescence assay. NAAT has been shown to be more sensitive and specific than other tests and therefore NAAT is recommended in this UK SMI for all diagnostic and screening scenarios outlined above. NAAT may also be used for the investigation of extra-genital infections where validated locally. In medico-legal cases a positive NAAT should be confirmed using a second NAAT with a different genomic target.

The sampling sites should relate to the type of sexual activity reported and the patient group (see Table 1). Chlamydia organism load varies by specimen type and site of
**Chlamydia trachomatis** infection – testing by Nucleic Acid Amplification Tests (NAAT)

sampling[12]. The recommended sample type for women is a vulvo-vaginal (V/V) swab which may be self-collected and submitted by post. Endocervical swabs have been shown to be less sensitive than vulvo-vaginal swabs and must be taken by a healthcare worker[2]. The testing of first catch urine specimens from women may result in lower sensitivity than V/V swabs and is not recommended by European guidelines[2,4].

In men, first void urine has been shown to be more sensitive than urethral sampling and is the sample type of choice[2]. Urine should be held for a minimum of one hour and the first 20mL sampled upon subsequent urination.

Rectal samples may be taken during proctoscopy, or directly by the patient or healthcare worker. Local validation should be carried out for testing extra-genital specimens (see Q 1 – Commercial and in-house diagnostic tests: evaluations and validations for further information).

In all cases laboratories should follow manufacturers’ instructions regarding individual specimen types.

**Table 1.** Appropriate sample sites for **Chlamydia trachomatis** NAAT dependent on sexual activity[2,11]

<table>
<thead>
<tr>
<th>Type of sex:</th>
<th>Oral</th>
<th>Vaginal</th>
<th>Anal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Insertive: 1st void urine</td>
<td></td>
<td>Receptive peno-anal: rectal swab</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Receptive oro-anal: rectal swab</td>
</tr>
<tr>
<td>Heterosexual male[<strong>a,</strong> b]</td>
<td>Fellatio: 1st void urine</td>
<td>1st void urine</td>
<td>Peno-anal: 1st void urine</td>
</tr>
<tr>
<td>Female</td>
<td>Fellatio: consider oropharyngeal swab</td>
<td>Self-taken V/V swab</td>
<td>Receptive: consider rectal swab</td>
</tr>
</tbody>
</table>

**Footnotes to Table 1**

a) In MSM, where there is also sexual activity with women, refer to heterosexual male for appropriate sample type following vaginal sexual activity.

b) Cunnilingus does not require a pharyngeal swab from the male.

**Laboratory tests**

NAATs which can detect the Swedish new variant *C. trachomatis* (nvCT) should be used. Varying sensitivities and specificities for diagnostic tests in urogenital specimens have been demonstrated in clinical trial data, manufacturers’ validation data and published papers.
Postal test-kits (PTK) have also been trialled as a form of sample collection for home-based screening and are widely used\(^{14-16}\). Self-collected specimens (swabs or preserved urine) were posted to the laboratory for NAAT and results were comparable to traditional collection methods\(^{14-16}\). Validation should be carried out locally prior to use.

**Confirmation**

**C. trachomatis**

In laboratories where confirmatory testing results are found to be consistently concordant following audit, confirmatory testing may be deemed unnecessary. Any decision to retest with a second platform depends on the sample type (for example samples from extra-genital sites such as rectal swabs), the platform that has been used for initial screening and the prevalence of *C. trachomatis* in the population tested. However, if the sample is associated with a medicolegal case then testing with a different genomic target is required even in a high risk population\(^2\).

Samples can be sent to the sexually transmitted bacteria reference unit (STBRU), or a local laboratory with validated test, for *C. trachomatis* confirmation and LGV diagnosis. Acceptable sample types include residual clinical specimens in which *C. trachomatis* has been detected by the local laboratory (by NAAT) or extracted DNA samples.

**Lymphogranuloma venereum**

LGV testing is recommended for men or women with proctitis and also for HIV positive MSM, with or without symptoms, with *C. trachomatis* infection at any site\(^2,5\). Send *C. trachomatis* positive samples from rectal sites for LGV testing if the infection is suspected\(^17\).

**Window period and test of cure\(^2\)**

Patients should be offered testing when they first present. If there has been possible sexual exposure within the previous two weeks, patients should return for a repeat test two weeks following exposure.

Test of cure (TOC) is not recommended after treating uncomplicated genital chlamydia infection. TOC is recommended if the patient is pregnant, if poor compliance is suspected and if symptoms persist. In addition, TOC is recommended in some cases of rectal infection depending on treatment type. Samples for TOC of cure should be taken at least three weeks after the completion of treatment\(^2\).

**Persistent Infection**

Persistent chlamydial infection has been demonstrated in patients who are at low risk of re-infection and who have tested positive for *C. trachomatis*, at least twice (using NAAT), despite having completed appropriate courses of antimicrobial therapy\(^18,19\). Asymptomatic patients with persistent infection may not be aware and may not be detected as test of cure is not routinely recommended.

**Medicolegal cases**

Where results are likely to have medicolegal significance, specimens should be handled in accordance with Royal College of Pathologists’ guidance\(^20,21\). Legal
precedent is limited but, for best practice, laboratories should confirm a reactive NAAT result by using a different target to ensure accurate results2.

Technical information/limitations

Limitations of UK SMIs

The recommendations made in UK SMIs are based on evidence (for example, sensitivity and specificity) where available, expert opinion and pragmatism, with consideration also being given to available resources. Laboratories should take account of local requirements and undertake additional investigations where appropriate. Prior to use, laboratories should ensure that all commercial and in-house tests have been validated and are fit for purpose.

Specimen containers

UK SMIs use the term “CE marked leak proof container” to describe containers bearing the CE marking used for the collection and transport of clinical specimens. The requirements for specimen containers are given in the EU in vitro Diagnostic Medical Devices Directive (98/79/EC Annex 1 B 2.1) which states: “The design must allow easy handling and, where necessary, reduce as far as possible contamination of and leakage from, the device during use and, in the case of specimen receptacles, the risk of contamination of the specimen. The manufacturing processes must be appropriate for these purposes”.

NAAT inhibition

All sample types may contain substances that inhibit the process of nucleic acid amplification, potentially causing false negative results4. Use of an inhibition control in NAAT testing is therefore recommended2. Many modern NAATs are able to remove inhibitory substances during the nucleic acid extraction process. Rectal specimens, urine from pregnant women and urine from women in the third week after menstrual bleeding may contain high levels of inhibitors (it is likely that hormones have a role to play in this inhibition)4. In duplex or multiplex assays, where several targets may be detected, competitive inhibition may be observed. The test manufacturers’ instructions should be followed when interpreting inhibited test results.

PCR

The risk of contamination should always be considered when using NAATs22,23. See also Q 4 - Good laboratory practice when performing molecular amplification assays. Laboratories should ensure that their assay is capable of detecting the Swedish new variant C. trachomatis (nvCT).
Safety considerations

*Chlamydia trachomatis* is a hazard group 2 organism.

Refer to current guidance on the safe handling of all sample types and organisms documented in this UK SMI.

The above guidance should be supplemented with local COSHH and risk assessments.

Public health management

For information regarding notification to PHE (or equivalent in the devolved administrations) refer to page 15.


For information regarding the national Chlamydia screening programme refer to: [http://www.chlamydiacscreening.nhs.uk/ps/resources.asp](http://www.chlamydiacscreening.nhs.uk/ps/resources.asp)

Also refer to the British Association for Sexual Health and HIV guidelines and NICE guidelines for the management of *Chlamydia trachomatis* infection and lymphogranuloma venereum⁵:


[http://cks.nice.org.uk/chlamydia-uncomplicated-genital](http://cks.nice.org.uk/chlamydia-uncomplicated-genital)

Partner notification should be discussed at the time of diagnosis. All sexual partners of patients with a positive *C. trachomatis* NAAT should be offered full STI screening²,⁸.
**Chlamydia trachomatis** infection – testing by Nucleic Acid Amplification Tests (NAAT)

**Test specimen with NAAT**

- **Reactive**
  - **Medicolegal cases**
    - Test using a different genomic target. Send to reference laboratory if test unavailable locally.
  - **All other cases**
  - **Reactive**
    - **REPORT:** Chlamydia trachomatis detected
  - **Inhibitory**
    - **REPORT:** Chlamydia trachomatis not detected
  - **Negative**

- **Inhibitory**
  - Re-extract nucleic acid from the original sample and retest using the same assay, following manufacturer’s instructions.
  - **REPORT:** Inhibitory result, please send another sample
  - **Negative**

**REPORT:** Chlamydia trachomatis not detected
Chlamydia trachomatis infection – testing by Nucleic Acid Amplification Tests (NAAT)

Footnotes

a) Recommended specimen types are first catch urine (preferred) or urethral swab for men and vulvo-vaginal swab (which may be self-collected) for women.

b) Laboratories using dual NAAT capable of detecting both C. trachomatis and N. gonorrhoeae should follow nationally agreed algorithms and confirmatory strategy for the N. gonorrhoeae component of the test.

c) Laboratories should follow good practice when undertaking molecular testing. For C. trachomatis this should include environmental sampling. See Q 2 – Quality assurance in diagnostic virology and serology laboratory and Q 4 – Good laboratory practice when performing molecular amplification assays for further information.

d) It is recommended to use an inhibition control for each specimen\(^2\). Failure to do so may lead to false negative results.

e) Many authorities no longer recommend testing with a second target unless testing is associated with a medico-legal case\(^2\). The decision on whether to retest with a second platform depends on the sample type (for example samples from extragenital sites such as rectal swabs), the platform that has been used for screening and the prevalence of C. trachomatis in the population tested.

f) If appropriate, send samples for C. trachomatis confirmation/LGV diagnosis to the STBRU or other local laboratory with validated tests.

Notification to PHE\(^{24,25}\), or equivalent in the devolved administrations\(^{26-29}\)

The Health Protection (Notification) regulations 2010 require diagnostic laboratories to notify Public Health England (PHE) when they identify the causative agents that are listed in Schedule 2 of the Regulations. Notifications must be provided in writing, on paper or electronically, within seven days. Urgent cases should be notified orally and as soon as possible, recommended within 24 hours. These should be followed up by written notification within seven days.

For the purposes of the Notification Regulations, the recipient of laboratory notifications is the local PHE Health Protection Team. If a case has already been notified by a registered medical practitioner, the diagnostic laboratory is still required to notify the case if they identify any evidence of an infection caused by a notifiable causative agent.

Notification under the Health Protection (Notification) Regulations 2010 does not replace voluntary reporting to PHE. The vast majority of NHS laboratories voluntarily report a wide range of laboratory diagnoses of causative agents to PHE and many PHE Health Protection Teams have agreements with local laboratories for urgent reporting of some infections. This should continue.

Note: The Health Protection Legislation Guidance (2010) includes reporting of Human Immunodeficiency Virus (HIV) & Sexually Transmitted Infections (STIs), Healthcare Associated Infections (HCAIs) and Creutzfeldt–Jakob disease (CJD) under
'Notification Duties of Registered Medical Practitioners': it is not noted under 'Notification Duties of Diagnostic Laboratories'.

https://www.gov.uk/government/organisations/public-health-england/about/our-governance#health-protection-regulations-2010

Other arrangements exist in Scotland\textsuperscript{26,27}, Wales\textsuperscript{28} and Northern Ireland\textsuperscript{29}.
Modified GRADE table used by UK SMIs when assessing references

Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) is a systematic approach to assessing references. A modified GRADE method is used in UK SMIs for appraising references for inclusion. Each reference is assessed and allocated a grade for strength of recommendation (A-D) and quality of the underlying evidence (I-VI). A summary table which defines the grade is listed below and should be used in conjunction with the reference list.

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<tr>
<td></td>
<td>Evidence from randomised controlled trials, meta-analysis and systematic reviews</td>
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<tr>
<td>B</td>
<td>Recommended but other alternatives may be acceptable</td>
</tr>
<tr>
<td></td>
<td>Evidence from non-randomised studies</td>
</tr>
<tr>
<td>C</td>
<td>Weakly recommended: seek alternatives</td>
</tr>
<tr>
<td></td>
<td>Non-analytical studies, for example, case reports, reviews, case series</td>
</tr>
<tr>
<td>D</td>
<td>Never recommended</td>
</tr>
<tr>
<td></td>
<td>Expert opinion and wide acceptance as good practice but with no study evidence</td>
</tr>
<tr>
<td></td>
<td>Required by legislation, code of practice or national standard</td>
</tr>
<tr>
<td></td>
<td>Letter or other</td>
</tr>
</tbody>
</table>


5. White J, O'Farrell N, Daniels D. 2013 UK National Guideline for the management of lymphogranuloma venereum: Clinical Effectiveness Group of the British Association for Sexual
Chlamydia trachomatis infection – testing by Nucleic Acid Amplification Tests (NAAT)


17. Van Liere GAFS, Hoebe CJP, Wolffs PFG, Dukers-Muijers NHTM. High co-occurrence of anorectal chlamydia with urogenital chlamydia in women visiting an STI clinic revealed by routine universal testing in an observational study; a recommendation towards a better anorectal chlamydia control in women. BMC Infectious Diseases 2014;14. B, II


   B, V


