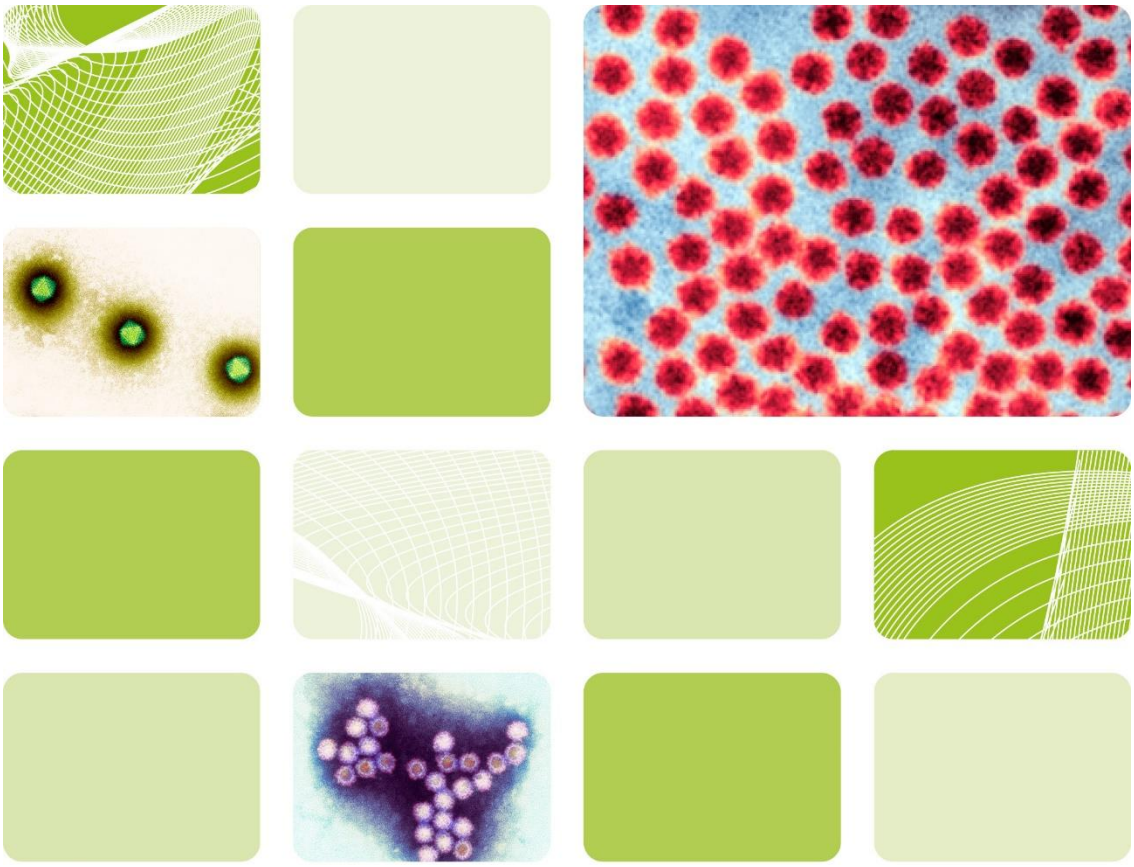
UK Health Security Agency

UK Standards for Microbiology Investigations

Chlamydia and Gonorrhoea infection – testing by Nucleic Acid Amplification Tests (NAATs)



Issued by the Standards Unit, UK Health Security Agency

Virology | V 37 | Issue number: 5 | Issue date: 12.12.24 | Page: 1 of 21

Acknowledgments

UK Standards for Microbiology Investigations (UK SMIs) are developed under the auspices of UKHSA working in partnership with the partner organisations whose logos are displayed below and listed on the UK SMI website. UK SMIs are developed, reviewed and revised by various working groups which are overseen by a 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.

UK SMIs are produced in association with:

Displayed logos correct as of December 2024

Contents

Amendment table

Each UK SMI document has an individual record of amendments. The amendments are listed on this page. The amendment history is available from standards@ukhsa.gov.uk.

Any alterations to this document should be controlled in accordance with the local document control process.

Amendment number/date	6/12.12.24
Issue number discarded	4
Insert issue number	5
Anticipated next review date*	12.12.27
Section(s) involved	**Amendment**
Whole document	The document has been transferred into a new template and headings have been reorganised. The hyperlinks in the document have been updated to direct the reader to UK SMI webpages hosted on the Royal College of Pathologists website. The information and references in the document have been updated. Title changed from "*Chlamydia trachomatis* infection – testing by Nucleic Acid Amplification Tests (NAAT)" to "*Chlamydia and Gonorrhoea* infection – testing by Nucleic Acid Amplification Tests (NAATs)"
Definitions	Addition of pooled and self-collection
Introduction	Addition of gonorrhoea and lymphogranuloma venereum
Screening	Updated to reflect current guidance
Public health management	Links updated
Specimen processing and procedure	Laboratory diagnosis updated. Table 1 Appropriate sample sites for *C. trachomatis* and *N. gonorrhoeae* NAAT dependent on sexual activity removed.
Laboratory tests **Test of cure**	*Neisseria gonorrhoeae* added.

Medico-legal cases	Addition of BASHH National Guideline on the management of STI and related conditions in children and young people
Algorithms	Algorithm 1 *Chlamydia trachomatis* infection – testing by Nucleic Acid Amplification Tests (NAATs) updated. Addition of Algorithm 2: Testing for *Neisseria gonorrhoeae.* Addition of Algorithm 3: Testing for Medico-legal cases.
Interpreting and reporting laboratory results	New section added
References	Updated

*Reviews can be extended up to 5 years where appropriate

1 General information

View general information related to UK SMIs.

2 Scientific information

View scientific information related to UK SMIs.

3 Scope of document

This UK SMI covers the testing of clinical samples for the investigation of rectal, pharyngeal and urogenital *Chlamydia trachomatis,* lymphogranuloma venereum (LGV) and *Neisseria gonorrhoeae* infection by Nucleic Acid Amplification Tests (NAATs).

This UK SMI does not cover gonococcal eye infection, gonococcal arthritis, ocular trachoma, or neonatal infections (including pneumonia and conjunctivitis). The use of point of care tests (POCTs), where the test result can be delivered to the patient without the sample being sent to the laboratory, is not covered in this UK SMI. NAATs which are not approved for extra-genital and pooled specimens should be validated locally. Enzyme immunoassays (EIA) tests are not recommended (1).

Refer to UK SMI Q 4 Good practice when performing molecular amplification assays and UK SMI S 11 red or painful eye.

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

Definitions

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.

Self-collected – the individual collects their own specimens by following instructions and sends them back to the laboratory for testing.

Pooled samples - individual specimens (swabs or urine) are combined into a pooled specimen to reduce the cost of screening.

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 amplification. A further specimen is required. The term used may be different for various platforms, for example “invalid”.

Reporting of invalid/inhibitory results should be based on manufacturer’s interpretation.

4 Introduction

Chlamydia

Chlamydia is caused by the bacterium *Chlamydia trachomatis* and is the most common sexually transmitted infection (STI) in the UK. It is found most frequently in people aged 15-24 years. Spread is primarily via sexual transmission; however babies can acquire chlamydia infection from the birth canal during delivery and subsequently develop conjunctivitis or pneumonia in the first few weeks of life.

Most sexually-acquired cases are asymptomatic. However, the patient can have the following signs and symptoms:

- Women/persons with a vagina: vaginal discharge, intermenstrual bleeding, dysuria, lower abdominal pain, dyspareunia, mucopurulent cervicitis, pelvic tenderness and cervical motion tenderness.
- Men/persons with a penis: urethral discharge and dysuria.

Sexually-acquired infections can also occur at extra-genital sites, such as the rectum and pharynx.

If chlamydia is not treated, it can lead to complications such as pelvic inflammatory disease (PID), ectopic pregnancy, infertility, endometritis, salpingitis, reactive arthritis, perihepatitis (Fitz-Hugh-Curtis syndrome) and long term pelvic or abdominal pain (1).

Gonorrhoea

Gonorrhoea is caused by the Gram negative diplococcus *Neisseria gonorrhoeae.* The key communities that have a disproportionate burden of gonorrhoea in the UK are people aged 15-24 years, gay, bisexual and other men who have sex with men (GBMSM), and people living in the most deprived areas. It is spread by sexual contact through the vagina, anus and by oral sex (2). Refer to UK SMI ID 6: Identification of Neisseria species for more information.

Gonorrhoea rates in England increased 7.5% from 79,268 diagnoses in 2022 to 85,223 diagnoses in 2023 (3). Antimicrobial resistance to first and second line therapeutics is also increasing (4). Currently 1g ceftriaxone is the recommended first-line therapy (5). Ceftriaxone resistance is most common in the Asia-Pacific region and is occasionally detected in the UK in people who have travelled to or moved from this region (6).

Lymphogranuloma venereum

LGV is a STI caused by 3 genovars of the bacterium *C. trachomatis:* L1, L2 and L3. LGV is almost exclusively detected in GBMSM in the UK, though may affect all patient groups. Symptoms can be complex, severe and may involve multiple sites in the body such as the genitals, anus, rectum and lymph nodes (7). The incubation period can range from 3 – 30 days from the time of contact with an infected individual. Patients typically present with proctitis however asymptomatic infection may occur (7). There have been recent increases in LGV cases in England from 1,173 cases in 2022 to 2,069 in 2023 (3).

For LGV testing refer to BASHH guidance (7).

4.1 Screening

The National Chlamydia Screening Programme (NCSP) has been developed to focus on reducing the harms from untreated chlamydia infection. The harmful effects of chlamydia occur predominantly in women/persons with a vagina (this includes transgender men, non-binary people assigned female at birth and intersex people with a womb or ovaries) under the age of 25. The NCSP recommends that they are offered a chlamydia test after sexual intercourse with a new partner, or annually if aged under 25 years (8,9).

Chlamydia screening in community settings, such as GPs and pharmacies, will only be proactively offered to young women/persons with a vagina. All people can request testing if needed, but men/persons with a penis will not be proactively offered a test unless an indication has been identified, such as being a partner of someone with chlamydia or having symptoms (9). Services provided by sexual health services remain unchanged.

Testing for gonorrhoea is recommended in any setting where clinically indicated, such as for symptomatic patients, contacts of those infected or attendees of sexual health clinics (10).

Where chlamydia testing or screening is performed, gonorrhoea testing is often offered simultaneously due to the widespread use of dual chlamydia/gonorrhoea NAAT.

5 Safety considerations

The section covers specific safety considerations (11-30) related to this UK SMI, and should be read in conjunction with the general safety considerations.

C. trachomatis and *N. gonorrhoeae* are hazard group 2 organisms. 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.

6 Public health management

For further information on public health management refer: https://www.gov.uk/government/collections/chlamydia-surveillance-data-screening-and-management.

For information regarding the National Chlamydia Screening programme refer to:

https://www.gov.uk/government/collections/national-chlamydia-screening-programme-ncsp

Also refer to the BASHH guidelines and NICE guidelines for the management of *Chlamydia trachomatis* infection, *Neisseria gonorrhoeae* infection and lymphogranuloma venereum (7):

https://www.bashh.org/current-guidelines/all-guidelines/

https://cks.nice.org.uk/topics/chlamydia-uncomplicated-genital/

Partner notification should be discussed at the time of diagnosis. All sexual partners of patients with a positive *C. trachomatis* or *N. gonorrhoeae* NAAT should be offered full STI screening (1,31).

7 Specimen processing and procedure

7.1 Specimen type

Specimen types: First-void urine, vulvovaginal swabs, urethral swabs, endocervical swabs, rectal swabs, oropharyngeal swabs (self-collected or clinician-collected).

Note: Urine in women/ persons with a vagina is not a suitable sample type (32).

7.1.1 Laboratory diagnosis

Compared to culture, NAATs have shown to be more sensitive and specific for the detection of *C. trachomatis* and *N. gonorrhoeae* are therefore recommended in this UK SMI for diagnosis and screening of urogenital samples. NAATs may also be used for the investigation of extra-genital infections, where validated locally. Most commercially available testing platforms are capable of detecting *C. trachomatis* and *N. gonorrhoeae* from a single specimen. In medico-legal cases a positive NAAT for either *C. trachomatis* or *N. gonorrhoeae* should be confirmed using a second NAAT with a different genomic target (1) and for specific patient groups/specimen types a second NAAT should be used to confirm *N. gonorrhoeae* positives (see section 8). Detection of LGV genovars of *C. trachomatis* is carried out via a separate test but in most cases will be able to use the original specimen which tested positive by chlamydia NAAT (33).

The recommended sample type for women/persons with a vagina is a vulvovaginal swab (VVS), which may be self-collected. Testing of self-collected swabs may need to be locally validated if not included in manufacturer's intended use.

Endocervical swabs must be taken by a healthcare worker as they have been shown to be less sensitive than self-collected vulvovaginal swabs (1). The testing of first catch urine specimens from women/ persons with a vagina should only be used if

other specimens are not available, for example when testing vulnerable patients (1,33).

In men/ persons with a penis, first void urine has been shown to be more sensitive than urethral sampling and is the sample type of choice (1). Urine should be held for a minimum of one hour and the first 20 mL sampled upon subsequent urination.

Rectal samples may be taken during proctoscopy, or directly by the patient or healthcare worker. Throat swabs may also be taken by clinician or self-collected. When required, local validation should be carried out for testing extra-genital specimens. Refer to UK SMI Q 1: Evaluations, validations and verifications of diagnostic tests for further information.

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

7.2 Specimen transport and storage conditions

Samples should be stored in an appropriate transport medium and transported to the laboratory within 24 hours of collection or within the timescales detailed in the manufacturers' instructions.

Please refer to the Guidance for the design of self-sampling packs and associated support for self-sampling processes within Sexually Transmitted Infection and Blood Borne Virus testing for more information.

7.3 Specific technical limitations

NAAT inhibition

It has been recognised that samples may contain lubricants or other substances that can inhibit amplification, potentially causing false negative results (33). It is recommended to use an inhibition control in NAAT testing (1). Internal and cellular controls exist within most commercial kits. Many NAATs are able to remove inhibitory substances during the nucleic acid extraction process. Rectal specimens, urine from pregnant people, and urine collected in the third week after menstrual bleeding may contain higher levels of inhibitors (it is likely that hormones have a role to play in this inhibition) (33). In duplex or multiplex assays, where several targets may be detected, competitive inhibition may be observed. The test manufacturers' instructions should be followed as they may contain a list of substances which have been identified as inhibitory through verification and validation. The laboratory may consider regular monitoring of inhibition levels and positive rates.

Contamination

The risk of contamination should always be considered when using NAATs. Refer to UK SMI Q 4 Good laboratory practice when performing molecular amplification assays.

8 Investigation

8.1 Laboratory tests

Chlamydia trachomatis

Chlamydia NAATs are the assays of choice for both genital and extra-genital samples, though the sensitivity and specificity may vary.

If the sample is associated with a medico-legal case, then confirming positive results with a different genomic target is required even in a high-risk population (1).

Rectal samples can be sent to the UKHSA Sexually Transmitted Infections Reference laboratory (STIRL) for LGV testing, or a local laboratory with validated test (1). Acceptable sample types include residual clinical specimens in which *C. trachomatis* has been detected by the local laboratory (by NAATs) or extracted DNA samples.

Neisseria gonorrhoeae

Microscopy of Gram stained specimens enables the direct visualisation of *N. gonorrhoeae* as Gram negative diplococci. Microscopy sensitivity is increased in men/ persons with a penis who have discharge and people with rectal symptoms. Microscopy is not recommended in specimens from people without symptoms, pharyngeal specimens and urethral/cervical/vaginal specimens due to reduced sensitivity (6).

Culture remains primarily to detect antimicrobial, resistant *N. gonorrhoeae* by antimicrobial susceptibility testing. Resistant *N. gonorrhoeae* is a global concern as it continues to evolve and spread. A culture specimen should be taken from people with suspected or confirmed gonorrhoea infection prior to treatment. Culture sensitivity is increased when time from sample collection to plating and incubation is minimised. Thus, direct plating in the clinic is advised or timely transfer to laboratory in transport media for immediate plating. Molecular methods to assess genomic sequences/ regions which may confer resistance to antibiotics are becoming available (6).

Gonorrhoea NAATs have high sensitivity (>95%) in samples from patients with and without symptoms. NAATS are also recommended for pharyngeal and rectal specimen testing (if locally validated). However, it should be noted commercial NAATs may cross react with commensal *Neisseria* species, especially in the pharynx. It is recommended to confirm positive results with a separate molecular target in samples from a population with a test positive predictive value of <90%, especially extra-genital specimens (6).

Refer to UK SMI ID 6: Identification of Neisseria species and Guidance for detection of gonorrhoea in England.

8.2 Window period and test of cure (TOC)

Neisseria gonorrhoeae

Following a positive test result, treatment is recommended for patients presenting after 14 days of exposure. For patients presenting more than 14 days after exposure, patients should be offered testing, with treatment recommended for those with a positive result.

For patients presenting within 14 days of exposure, empirical treatment is recommended based on a risk assessment and discussion with patient.

In asymptomatic patients, it may be appropriate to repeat testing 2 weeks after exposure.

All patients diagnosed with gonorrhoea should be advised to return for TOC. Refer to BASHH guidelines for further information (6).

Chlamydia trachomatis

TOC is not routinely recommended for uncomplicated genital chlamydia infection or following completion of treatment but should be performed in the following circumstances:

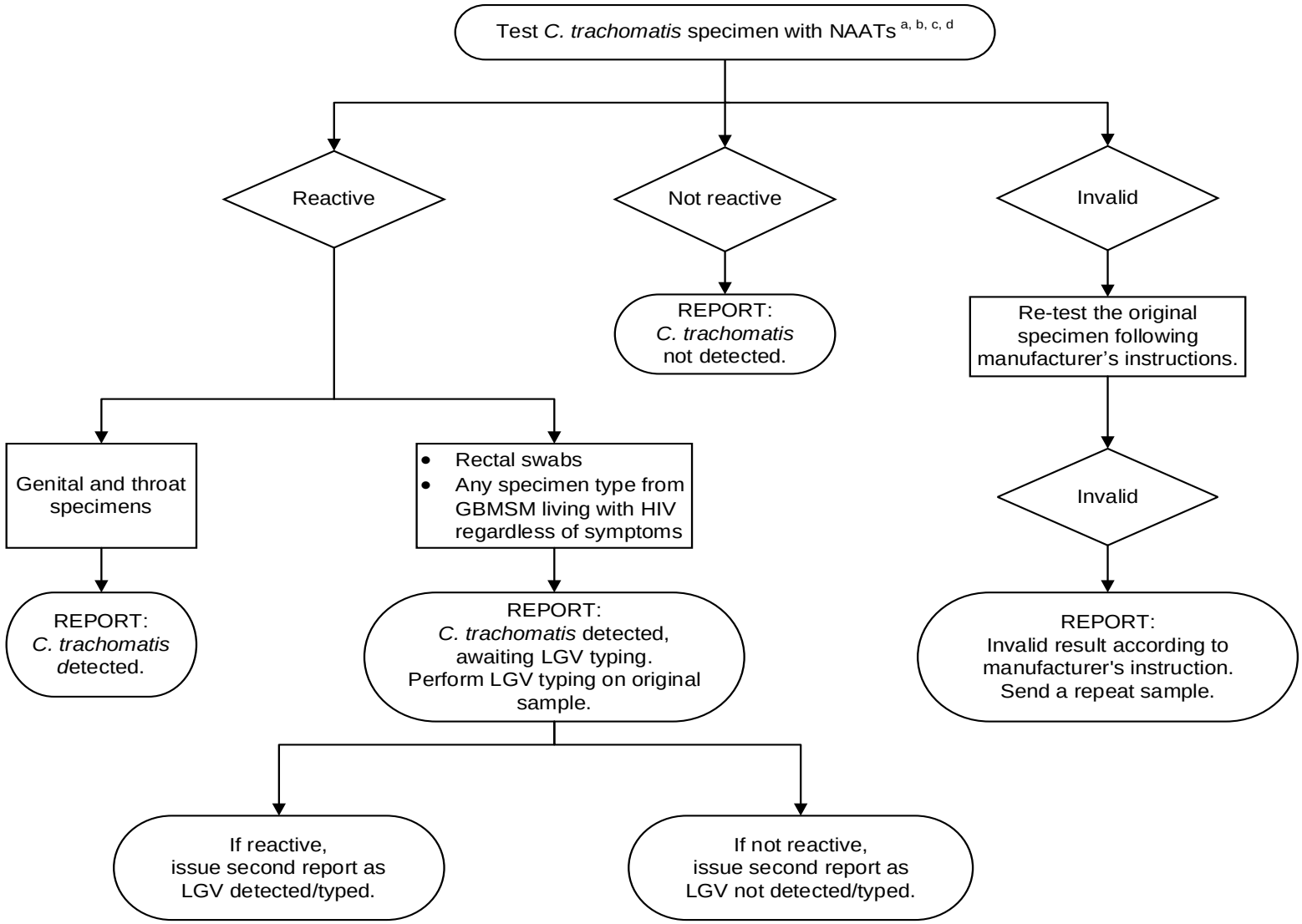
- in pregnancy
- where LGV (in the absence of a definite negative result) is suspected
- where poor compliance is suspected
- where symptoms persist
- in rectal infection when single-dose azithromycin or one week of doxycycline have been used (these are inadequate for treatment of LGV).

TOC should be performed no earlier than three weeks after completion of treatment. Repeat testing (to monitor for re-infection) should be performed 3–6 months after treatment in under 25-years olds diagnosed with chlamydia. Refer to BASHH guidelines for further information (1).

8.3 Medico-legal cases

Where results are likely to have medico-legal significance, specimens should be handled in accordance with Royal College of Pathologists Guidance for handling medicolegal samples and preserving the chain of evidence and BASHH National Guideline on the management of STI and related conditions in children and young people. Refer to manufacturer's instruction when using testing kits.

9 Algorithm 1: Testing for *Chlamydia trachomatis* infection



9. Footnotes

a) Recommended specimen types are first-catch urine (preferred) or urethral swab for men/person with a penis and vulvovaginal swab (which may be self-collected) for women/ persons with a vagina.

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.

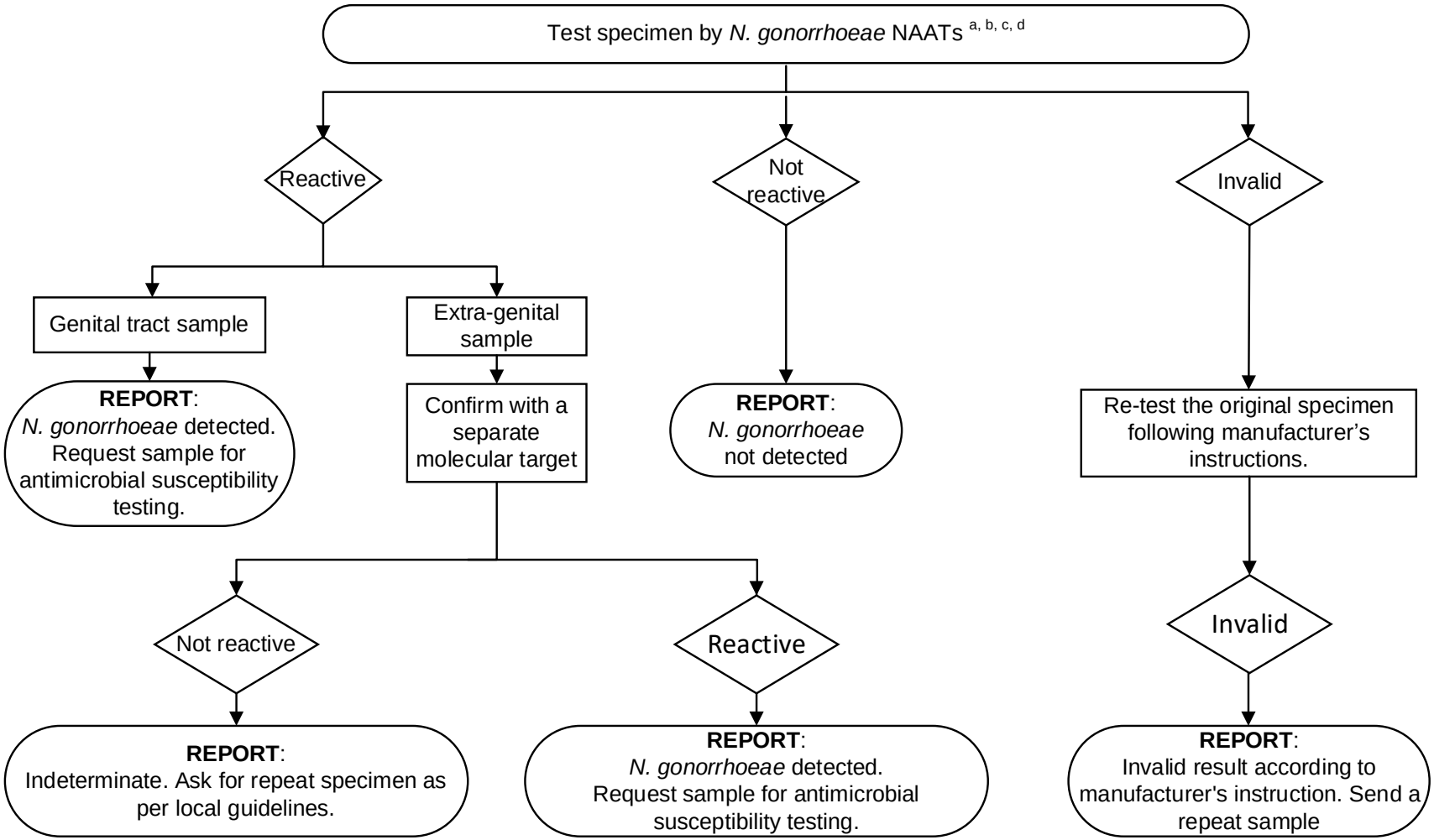
c) Laboratories should follow good practice when undertaking molecular testing. For *C. trachomatis* this should include environmental sampling. See UK SMI Q 2 Quality assurance in diagnostic infection sciences laboratory and UK SMI Q 4 Good laboratory practice when performing molecular amplification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

d) It is recommended to use an inhibition control for each specimen (1). Failure to do so may lead to false negative results.

For LGV testing, refer to BASHH guidance (7).

9 Algorithm 2: Testing for *Neisseria gonorrhoeae*

PPV – positive predictive value is the probability that a person with a positive test result actually has the tested infection



9. Footnotes

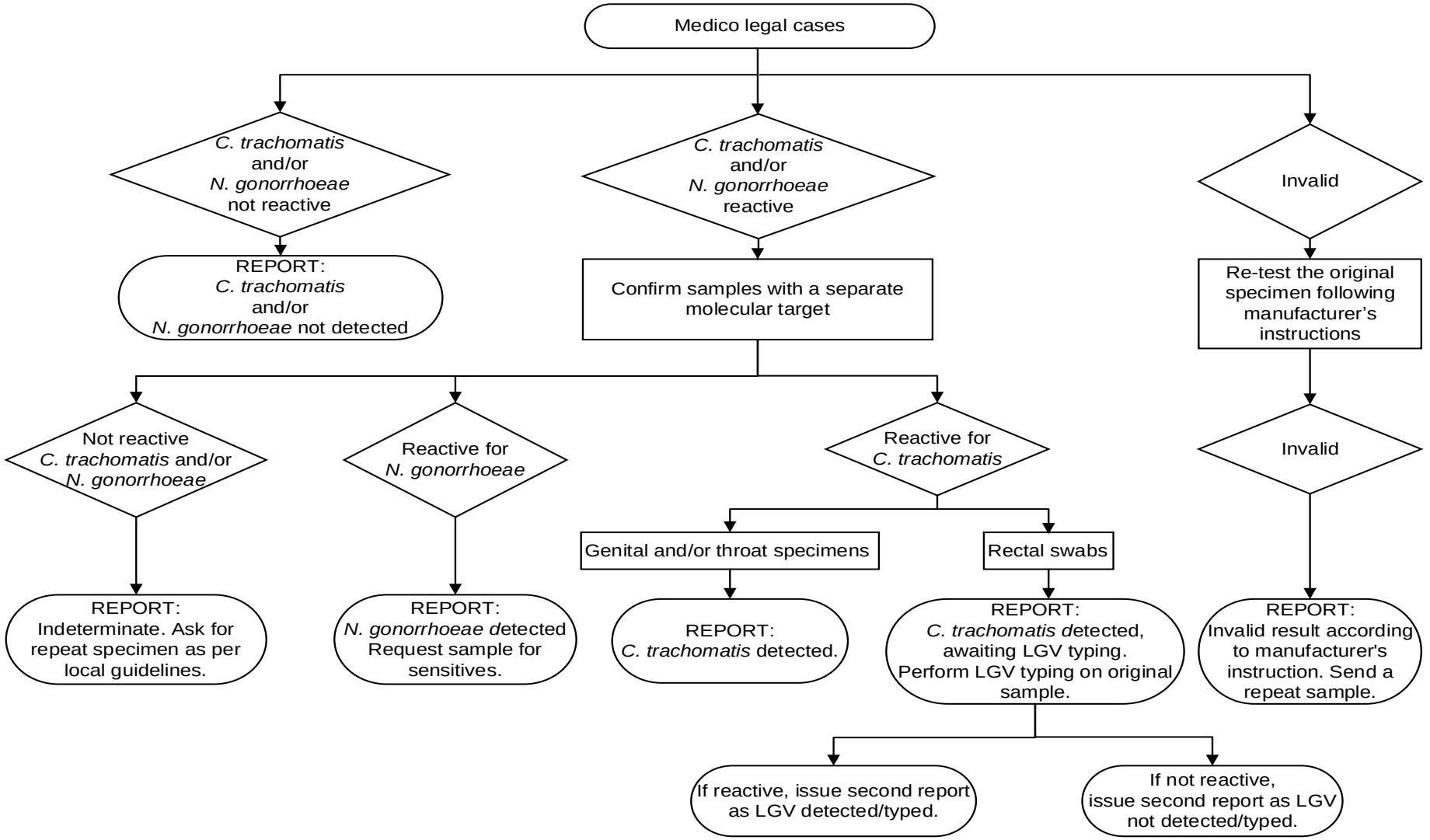
a) Recommended specimen types are first-catch urine (preferred) or urethral swab for men/person with a penis and vulvovaginal swab (which may be self-collected) for women/ persons with a vagina.

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 UK SMI Q 2 Quality assurance in diagnostic infection sciences laboratory and UK SMI 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 (1). Failure to do so may lead to false negative results.

9 Algorithm 3: Testing for Medico-legal cases



10 Interpreting and reporting laboratory results

***C. trachomatis* screening assay**	***C. trachomatis* confirmatory assay**	**Reported result**	**Interpretative comment**	**Notes**
Reactive	N/A	Detected	Consistent with *C. trachomatis* infection	Refer relevant samples for LGV typing
Reactive	Reactive	Detected	Consistent with *C. trachomatis* infection	Refer relevant samples for LGV typing
Not Reactive	N/A	Not detected	C. trachomatis not detected	
Reactive	Not Reactive	Indeterminate	Initial reactivity has not confirmed. Consider sending repeat specimen.	

***N. gonorrhoeae* screening assay**	***N. gonorrhoeae* confirmatory assay**	**Reported result**	**Interpretative comment**
Reactive	N/A	Detected	Consistent with *N. gonorrhoeae* infection
Reactive	Reactive	Detected	Consistent with *N. gonorrhoeae* infection
Not Reactive	N/A	Not detected	*N. gonorrhoeae* not detected
Reactive	Not Reactive	Indeterminate	Initial reactivity has not confirmed. Consider sending repeat specimen.

References

An explanation of the reference assessment used is available in the scientific information.

1. Nwokolo NC, Dragovic B, Patel S, Tong CY, Barker G, Radcliffe K. 2015 UK national guideline for the management of infection with Chlamydia trachomatis. International Journal of STD and AIDS 2016;27:251-67. **++** 10.1177/0956462415615443

2. Gonorrhea – CDC Basic Fact Sheet. 2023 ed.vol 26.04.2023. 2022. **++**

3. Sexually transmitted infections and screening for chlamydia in England: 2023 report vol 17.06.2024. GOV.UK; 2024. **++**

4. UK Health Security Agency. GRASP report: data to June 2023 GOVUK 2023. **++**

5. UK Health Security Agency. Case-series of ceftriaxone-resistant Neisseria gonorrhoeae infections detected in England GOVUK 2024. **++**

6. Fifer H, Saunders J, Soni S, Sadiq ST, FitzGerald M. 2018 UK national guideline for the management of infection with Neisseria gonorrhoeae. Int J STD AIDS 2020;31:4-15. **++** 10.1177/0956462419886775

7. 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 Health and HIV (CEG/BASHH) Guideline development group. Int J STD AIDS 2013;24:593-601. **++** 10.1177/0956462413482811

8. UK Health Security Agency. Standards English National Chlamydia screening programme 8th edition 2022. **++**

9. National Chlamydia Screening Programme (NCSP) GOV.UK; 2023. **++**

10. Guidance for the detection of gonorrhoea in England; 2021. **++**

11. Advisory Committee on Dangerous Pathogens. The Approved List of Biological Agents. Health and Safety Executive 2023. 1-39. **++**

12. UK Health Security Agency (UKHSA). Laboratory reporting to UKHSA: a guide for diagnostic laboratories. UKHSA 2023. 1-31. **++**

13. British Standards Institution (BSI). BS EN12469 - Biotechnology - performance criteria for microbiological safety cabinets 2000. **++**

14. British Standards Institution (BSI). BS 5726:2005 - Microbiological safety cabinets. Information to be supplied by the purchaser and to the vendor and to the installer, and siting and use of cabinets. Recommendations and guidance. 2005. 1-14. **++**

15. Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. MMWR Surveill Summ 2012;61:1-102. **+**

16. Department for Transport, Maritime and Coastguard Agency, HSENI, Civil Aviation Authority. Transport of infectious substances UN2814, UN2900 and UN3373 Guidance note number 17/2012 (revision 7). 2013. **++**

17. Department of Health. Health Protection Legislation (England) Guidance. 1-112. 2010. **++**

18. Health and Safety Executive. Managing risks and risk assessment at work (accessed 28/07/2021). https://www.hse.gov.uk/simple-health-safety/risk/index.htm. **++**

19. Health and Safety Executive. Blood-borne viruses in the workplace. Guidance for employers and employees. HSE. 2001. **++**

20. Health and Safety Executive. Control of Substances Hazardous to Health Regulations. The Control of Substances Hazardous to Health Regulations 2002 (as amended). Approved Code of Practice and guidance L5 (sixth edition). HSE Books. 2013. **++**

21. Health and Safety Executive. Risk assessment: A brief guide to controlling risks in the workplace. HSE. 2014. **++**

22. Health and Safety Executive, Advisory Committee on Dangerous Pathogens. Management and operation of microbiological containment laboratories. HSE. 2019. **++**

23. Health Services Advisory Committee. Safe working and the prevention of infection in clinical laboratories and similar facilities. Books. H 2003. **++**

24. Home Office. Public Health Act (Northern Ireland) 1967 Chapter 36. 1967. **++**

25. Home Office. Anti-terrorism, Crime and Security Act. 2001. **++**

26. Official Journal of the European Communities. Directive 98/79/EC of the European Parliament and of the Council of 27 October 1998 on *in vitro* diagnostic medical devices 1998. 1-37. **++**

27. Scottish Government. Public Health (Scotland) Act. 2008. **++**

28. The Royal College of Pathologists. The retention and storage of pathological records and specimens (5th edition). 1-59. 2015. **++**

29. The Welsh Assembly Government. Health Protection Legislation (Wales) Guidance. 2010. **++**

30. World Health Organization. Guidance on regulations for the transport of infectious substances 2021-2022. WHO. 2021. **++**

31. National Institute for Health and Care Excellence. Chlamydia - uncomplicated genital 2016. **++**

32. Aaron KJ, Griner S, Footman A, Boutwell A, Van Der Pol B. Vaginal Swab vs Urine for Detection of Chlamydia trachomatis, Neisseria gonorrhoeae, and Trichomonas vaginalis: A Meta-Analysis. Ann Fam Med 2023;21:172-9. **1+** 10.1370/afm.2942

33. Lanjouw E, Ouburg S, de Vries HJ, Stary A, Radcliffe K, Unemo M. 2015 European guideline on the management of Chlamydia trachomatis infections. Int J STD AIDS 2016;27:333-48. **++** 10.1177/0956462415618837