

5. Laboratory diagnosis of gonorrhoea (*Neisseria gonorrhoeae*)

The main benefits of gonorrhoea testing are the timely diagnosis and appropriate treatment of a sexually transmitted infection in individuals, as well as the disruption of the chain of transmission within the population through case and partner management.

The major risk associated with gonorrhoea testing is the increased likelihood of false positive test results arising from low positive predictive value (PPV) of test results in low-prevalence populations as well as potential cross-reactions in the tests with non-gonococcal commensal *Neisseria* species. Misdiagnoses may lead to direct harm and distress to the individuals resulting from unnecessary treatment as well as inappropriate diagnoses and partner notification. At a population level, they can lead to unnecessary use of antimicrobial agents, selective pressure for antimicrobial resistance development, and avoidable financial costs.

The implementation of assays using NAATs for the detection of *Neisseria gonorrhoeae* is increasingly widespread among laboratories. While testing for gonorrhoea is clearly indicated within specialist clinical settings targeting higher risk populations or where clinically indicated, there is little evidence to support widespread opportunistic screening for gonorrhoea in community-based settings [61]. However, the increasing use of dual NAATs will result in the significant proportion of testing occurring where the prevalence of gonorrhoea is likely to be low. For gonorrhoea, localised interventions targeting high risk groups are more likely to be cost-effective than unselected screening in community-based settings [22].

The evolving development of antimicrobial resistance in *N. gonorrhoeae* is now increasingly recognised as an emerging public health threat globally. The Centers for Disease Control and Prevention (CDC) identified drug-resistant *N. gonorrhoeae* as one of the top three antibiotic resistance threats in the United States for 2013 [62]. The emergence of multidrug resistance in *N. gonorrhoeae* also underpins the importance of the continuation of culture services in microbiology laboratories for the purpose of antimicrobial resistance surveillance, which can inform future treatment strategies.

This chapter aims to inform decisions pertaining to the detection of gonorrhoea, and to recommend best practice for testing and the expected standard of care.

5.1 Laboratory diagnosis

5.1.1 Nucleic Acid Amplification Tests (NAATs)

NAATs are now widely accepted as the standard of care for gonorrhoea testing [23, 63, 64]. They are more sensitive and have less stringent transport requirements than culture, and they allow the testing of both invasively and non-invasively taken samples [65, 66]. Many commercially available NAATs offer dual testing for *Chlamydia trachomatis* and *N. gonorrhoeae* [66, 67, 68]. A suggested algorithm for NAAT testing in gonorrhoea cases is outlined in Figure 6.

5.1.1.1 Sample types for genital sites

Equivalent NAAT sensitivity for *N. gonorrhoeae* detection has been reported in urine and urethral swab specimens in men [66, 67]. For NAAT testing, first pass urine is the specimen of choice in men as it can be non-invasively sampled [57, 63]. Manufacturers' recommendations on sampling and transport conditions should be strictly adhered to. In women, urine is an inferior sample type to cervical swab in the detection of gonorrhoea [66, 68]. Acceptable samples in women for NAAT testing for *N. gonorrhoeae* include clinician-taken endocervical swabs and self-taken vulvovaginal swabs. 90 to 95% of gonorrhoea cases in women have been diagnosed using either sample types [69]. Users should follow manufacturers' instructions closely.

5.1.1.2 Positive predictive value (PPV) and dual testing for genital samples

PPV is the percentage likelihood that a positive result is a true positive, and is influenced by the specificity and sensitivity of the test, as well as the prevalence of the infection in the population. The PPV for *N. gonorrhoeae* NAAT in the primary care setting is likely to be lower than that in the sexual health clinic setting as the prevalence of gonorrhoea in the general population is likely to be lower than that in the population attending a sexual health clinic (Table 8).

Figure 6 Algorithm for gonorrhoea testing of specimens from genital and extra-genital sites by NAAT

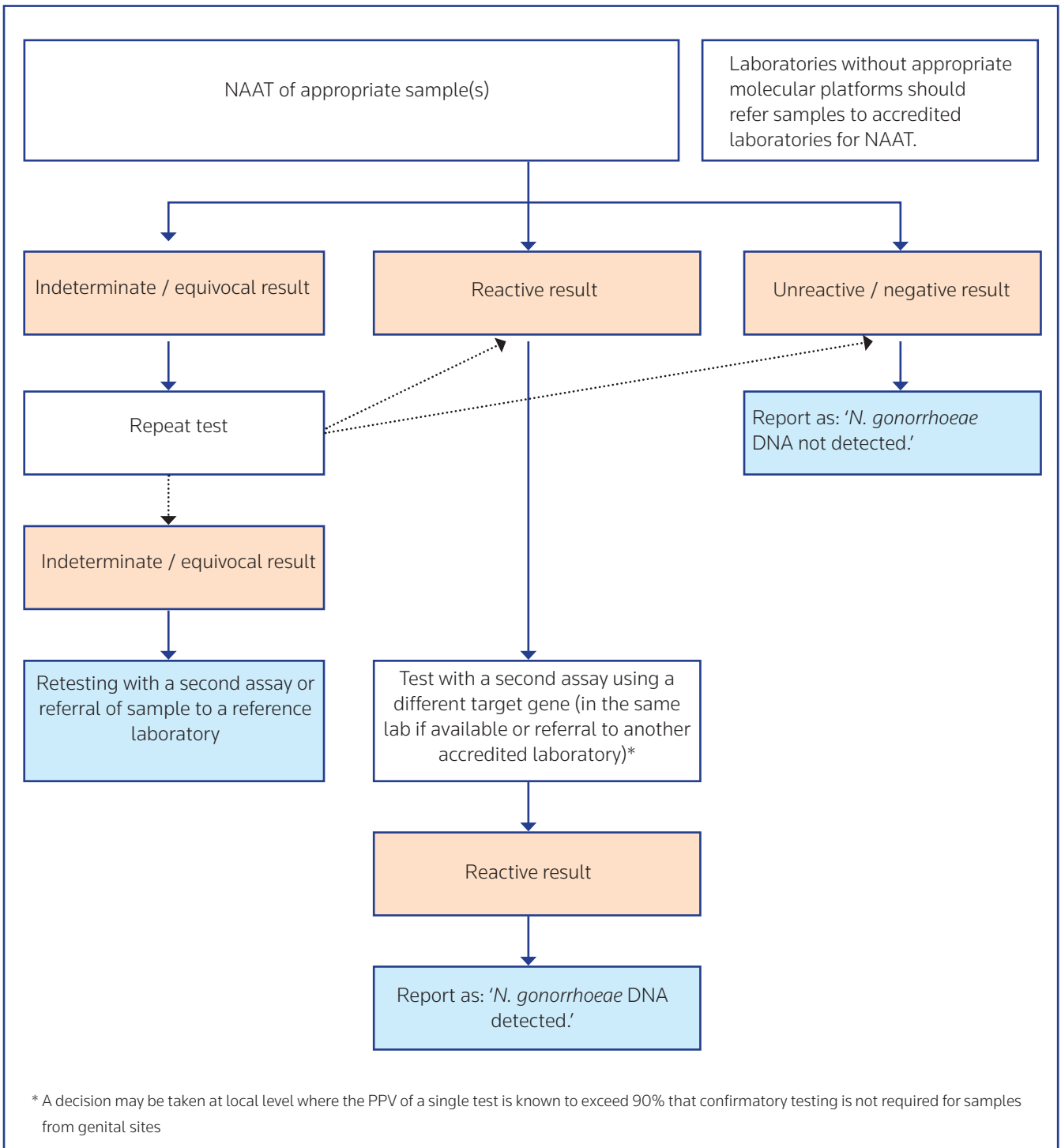


Table 8 PPV of single NAAT test with sensitivity of 99% and specificity of 99% for different population prevalence of gonorrhoea

Prevalence	PPV
10%	92%
1%	50%

The formula for calculation of PPV is the following (PHE(c), 2014):

$$\text{PPV} = \frac{(\text{sensitivity} \times \text{prevalence})}{(\text{sensitivity} \times \text{prevalence}) + ((1 - \text{specificity}) \times (1 - \text{prevalence}))}$$

In view of the potential for false-positive results and possible consequences, it is recommended that in populations and specimen types where the calculated PPV of the NAAT is below 90%, a second NAAT (with a different target and with little or no cross-reactivity with other *Neisseria* species) should be used for the confirmation of the initial positive result [23, 63, 64]. In many cases, even in settings where the prevalence of gonorrhoea exceeds 1%, it will still be necessary to use a second gene target to achieve an acceptable PPV. In such settings, results should only be reported following confirmation by supplementary testing [23, 63, 64].

For settings and specimen types where the locally-calculated PPV of the NAAT exceeds 90%, supplementary testing may not be required [64]. Should supplementary testing be deemed necessary in such settings, local agreement should be reached with stakeholders on the reporting of preliminary results by the laboratory [64].

5.1.1.3 Extragenital samples

At present no commercial NAATs that are CE-marked for use with extragenital samples such as throat/pharyngeal swabs, rectal swabs, or eye swabs are available. However, NAATs have a superior sensitivity to culture for the detection of *N. gonorrhoeae* in extragenital samples [65, 70]. A local validation procedure should be carried out in the laboratory prior to the introduction of NAAT testing of extragenital samples. Due to the risk of false-positive results produced by cross-reactivity with commensal *Neisseria* species present in the throat or rectum, all positive NAAT results from extragenital samples should be confirmed by a second NAAT, targeting an alternative gene [57, 63, 71, 72].

5.1.1.4 Handling of samples

NAATs are highly sensitive in detecting even small amounts of nucleic acids in samples. Care should be taken by healthcare workers in the laboratory and clinic to prevent cross-contamination of samples. Laboratories and clinics should have decontamination protocols in place to minimise the risk of cross-contamination.

5.1.2 Culture and identification of *Neisseria gonorrhoeae*

Culture is a specific and inexpensive method for the isolation of *N. gonorrhoeae*, but can be technically and logistically demanding. It has lower test sensitivity when compared to NAATs, particularly at extragenital sites [65, 70]. Due to the high test sensitivity of NAATs, a negative NAAT result can reliably exclude gonorrhoea if an appropriate sample is taken at an appropriate time following sexual exposure. Consideration should be given for the development of a laboratory policy of selective culture, such as culturing of specimens from high risk patients (e.g. attendees of STI clinics) and/or culturing of specimens taken from optimal sites (e.g. endocervical swabs in females and urethral swabs in males). Adoption of such a policy may be cost-effective through reduction in culture workload while still obtaining isolates from the majority of gonorrhoea cases [63].

It is essential to obtain as many isolates from gonorrhoea episodes as possible for the purpose of antimicrobial susceptibility testing. Susceptibility test results can inform individual patient management, while resistance surveillance is crucial since evolving trends in resistance profiles can guide changes in the management of cases and public health policies. Isolates may also be utilised for molecular typing in outbreak investigations. Isolation rates of 70% and 40% for genital and rectal samples respectively may be achieved [63]. Where possible, samples from relevant sites should be taken for culture prior to the commencement of treatment in cases of suspected gonorrhoea and/or

with confirmed NAAT-positive results [23, 63, 64]. Samples should also be taken for cases of treatment failure (see Section 5.5). Even where culture yield is expected to be low, such as samples from pharyngeal infections, it should still be attempted as treatment failure has been more frequently reported in pharyngeal infections [73, 74].

For culture of *N. gonorrhoeae* from genital sites, endocervical swab remains the sample of choice in women, while urethral swab is the specimen of choice in men [23, 57]. As *N. gonorrhoeae* is a fastidious organism, direct plating after sampling should be considered. Rapid transport of samples/plates to the laboratory is also required to maximise the viability and recovery of the organism. Charcoal medium is the recommended specimen transport medium for swabs taken for culture. Selective culture media such as New York City agar are also recommended to minimise the level of contaminants on culture.

5.1.3 Microscopy

N. gonorrhoeae can be visualised microscopically in genital specimens using Gram stain. They appear as Gram-negative diplococci predominantly within polymorphonuclear leukocytes. Sensitivity of 90-95% has been reported for this method in symptomatic men with urethral discharge, and microscopy, if available, is recommended to facilitate immediate diagnosis in symptomatic men [23, 57, 75]. The test sensitivity of microscopy is lower for urethral swab in asymptomatic men (50-75%), endocervical smear in women (37-50%), and urethral swab in women (20%) [75, 76]. Microscopy is not recommended for pharyngeal specimens due to poor specificity and sensitivity [23].

5.2 Antimicrobial susceptibility testing

Various scientific organisations provide guidelines on susceptibility test methodologies and interpretive criteria for *N. gonorrhoeae*. The European Committee on Antimicrobial Susceptibility Testing (EUCAST), the Clinical and Laboratory Standards Institute (CLSI), and the British Society of Antimicrobial Chemotherapy (BSAC) have regular updates on their respective methodologies and interpretive breakpoints. For further technical details, please refer to the organisations' respective websites and relevant documents [77, 78, 79]. Many laboratories in Ireland are currently using EUCAST guidelines for antimicrobial susceptibility testing in *N. gonorrhoeae*. BSAC is also currently supporting the EUCAST method for antimicrobial susceptibility testing in preference to the current BSAC method. While CLSI and BSAC have interpretive criteria for disc diffusion as well as MIC methods, EUCAST has recommended that an MIC method be used for susceptibility testing in *N. gonorrhoeae* as disc diffusion interpretive criteria have not yet been defined by them [77]. Where a commercial MIC method is employed, such as the gradient MIC method (e.g. Etest, BioMerieux), EUCAST has recommended that users follow the manufacturer's instructions closely.

The numbers of antimicrobial agents with available interpretive criteria for *N. gonorrhoeae* vary according to the recommendations of the respective scientific bodies. However, interpretive criteria are available from the above committees for the following classes or agents: penicillin, cephalosporins (cefixime, cefotaxime, ceftriaxone), quinolones (ofloxacin, ciprofloxacin), tetracycline, and spectinomycin. EUCAST and BSAC also have interpretive criteria for azithromycin [77, 79].

Testing for beta-lactamase in *N. gonorrhoeae*, as recommended by EUCAST and BSAC, detects a commonly occurring mechanism of plasmid-mediated penicillin resistance. A positive beta-lactamase test result predicts resistance to penicillin, ampicillin, and amoxicillin. However the beta-lactamase test will not detect other mechanisms of penicillin resistance such as chromosomal mutations of genes encoding penicillin-binding proteins.

5.3 Molecular typing

Typing of *N. gonorrhoeae* isolates for epidemiological studies using traditional non-DNA-based methods have now largely been replaced by DNA-based methods such as *Neisseria gonorrhoeae* multi-antigen sequence typing (NG-MAST). Compared to traditional typing methods such as auxotyping and serotyping, DNA-based methods (especially sequence-based analysis) are more discriminatory and reproducible, and potentially more cost-effective [80]. Ireland currently does not have a designated reference laboratory for purposes such as the typing of *N. gonorrhoeae* isolates. St James's Hospital microbiology laboratory currently performs molecular typing such as NG-MAST and whole genome sequencing for research purposes. Irish laboratories and users requiring such typing services would usually have to send isolates/specimens to reference laboratories in neighbouring countries such as England and Scotland, or alternatively, can discuss with St James's Hospital microbiology laboratory. The National Sexual Health Strategy [5]

calls for the designation of a reference laboratory for STIs. Once this designation has been made, such work should be referred to the reference laboratory.

5.3.1 NG-MAST

NG-MAST is a sequence-based typing method that examines the variable segments of two highly polymorphic loci of the *N. gonorrhoeae* genome: *porB* (a 490-bp segment) and *tbpB* (a 390-bp segment) [81]. This typing method is relatively fast and easy to perform and is highly discriminatory and reproducible. A public database (<http://www.ng-mast.net>) is also available, and can be accessed for analysis of the results and for assignment of allele numbers and sequence types. It is a recommended typing method when analysing strains collected over relatively short periods (days to a few years), and hence is an ideal typing method for analysing isolates from settings such as outbreaks, core groups or sexual networks, examination of the transmission of individual strains, partner notification, investigations of suspected clusters, and characterisation of clones [80]. NG-MAST is performed in many laboratories offering reference services for *N. gonorrhoeae*.

5.4 Test following sexual exposure

Incubation period for gonorrhoea can range from 2 to 10 days, and testing by culture within 48 hours following sexual exposure may yield false-negative results [82]. In the UK, it had been recommended that to confidently exclude gonorrhoea in patients presenting early (within 3 days) following sexual contact, repeat testing 14 days after contact should be considered if effective therapy had not been given [57]. It is unclear if this recommendation was influenced by considerations pertaining to culture detection of *N. gonorrhoeae*. In the European guidelines, no recommendation was made with respect to the minimum incubation period necessary before testing can be performed as the data was lacking, although the authors noted that clinical experience suggested the possibility of positive NAAT results within 1 to 2 days of infection [23]. Furthermore, based on expert opinion, the Public Health Agency of Canada has recommended that NAAT may be performed at the time of presentation, without having to wait for at least 48 hours following sexual contact [82]. The committee recommends that, regardless of time since exposure, a NAAT test be performed at presentation. When tested at less than 48 hours since exposure and test negative, if high risk exposure and symptomatic repeat NAAT after a week, if high risk exposure and asymptomatic retest more than 48 hours after exposure.

5.5 Test of Cure (ToC)

ToC is recommended for all cases of gonorrhoea to identify cases of treatment failure. Testing is particularly important in pharyngeal infections, where effective eradication can be more difficult to achieve than in genital or anorectal infections [73, 74].

ToC can be performed by either culture or NAAT depending on clinical circumstances. UK and European guidelines have recommended that if symptoms or signs persist after therapy, ToC with culture method should be performed at least 72 hours (between three to seven days) after completion of therapy [23, 57]. Supplementary testing with a NAAT for increased sensitivity can be considered one week after the ToC if the culture is negative.

Asymptomatic patients should be tested with a NAAT two weeks after completion of therapy. In the event of a NAAT-positive ToC result, culture should be performed for the purpose of antimicrobial susceptibility testing and resistance surveillance [23, 63].

5.6 Notification of gonorrhoea cases for public health surveillance

Public health surveillance data are essential in monitoring trends in STI diagnoses, to determine specific groups at risk of infection and to take action where there is a need for additional control measures, such as in the case of an outbreak. The data can be used to inform public health response by improving the planning and management of services, developing and refining interventions, and monitoring the effectiveness of sexual health policies.

Gonorrhoea is a notifiable disease in Ireland, Appendix 2 [24]. Both laboratory and clinical notifications are required, and are made to the MOH. All notifications sent electronically should be encrypted. Laboratory notifications are made electronically via CIDR. For more information on infectious disease notification, please refer to the HPSC website at www.hpsc.ie.

HPSC currently defines a confirmed case of gonorrhoea as any person who meets at least one of the following four laboratory criteria [25]:

- Isolation of *N. gonorrhoeae* from a clinical specimen;
- Detection of *N. gonorrhoeae* nucleic acid in a clinical specimen;
- Demonstration of *N. gonorrhoeae* by a non amplified nucleic acid probe test in a clinical specimen;
- Microscopic detection of intracellular gram negative diplococci in a urethral male specimen.

Recommendations

- A reference laboratory service for *N. gonorrhoeae* should be established in Ireland.
- Laboratory capacity for STI testing should be established in a way that provides timely and accessible testing for patients and health care providers.
- NAATs accredited to ISO 15189 standard should be the standard of care in the laboratory detection of *Neisseria gonorrhoeae*.
- Where possible, PPV of test results should be critically appraised.
- Supplementary testing with a second gene target is recommended in most clinical settings (following consideration of the sample types as well as disease prevalence in the population being investigated).
- Microscopy, if available, is recommended to facilitate immediate diagnosis of gonorrhoea in symptomatic men.
- Culture is still an essential laboratory investigation as isolates are required for antimicrobial susceptibility testing and molecular typing. Results can inform individual case management as well as public health policies and strategies for control of gonorrhoea.
- Consideration should be given to the development of a laboratory policy of selective culture, such as culturing of specimens from high-risk patients (e.g. attendees of STI clinics) and/or culturing of specimens taken from optimal sites (e.g. endocervical swabs in females and urethral swabs in males).
- Laboratories should be adequately resourced to perform the recommended tests such as NAATs, culture and antimicrobial susceptibility testing. A survey of Irish laboratories' capacity and resources to provide the expected diagnostic standard of care for gonorrhoea should be carried out. A mapping exercise and needs assessment of STI diagnostics is one of the priority actions currently being undertaken by the HSE Sexual Health and Crisis Pregnancy Programme, through implementation of the Sexual Health Strategy.
- ToC is recommended for all cases of gonorrhoea, to identify cases of treatment failure.