



## **Health Protection Scotland**

### **Scottish Bacterial Sexually Transmitted Infections Reference Laboratory (SBSTIRL)**

# **Guidance on the Introduction of molecular testing for *Neisseria gonorrhoeae* in Diagnostic Laboratories**

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On behalf of the *Molecular Testing for Gonorrhoea Working Group*  
(see Appendix)

August 2007

## **Background information**

### **Introduction**

Nucleic Acid Amplification techniques (NAATs) for identification of *Chlamydia trachomatis* have been available for a number of years and are used in the majority of diagnostic Microbiology laboratories providing a test for Chlamydia.

A number of commercially available platforms now provide combined testing for both *C. trachomatis* and *Neisseria gonorrhoeae* in a single test. Some laboratories can now offer in house NAATs for these organisms. Molecular testing for gonorrhoea can be done on less invasive specimens and specimens do not require stringent timely transport.

These new methods will thus lead to a shift away from culture for *Neisseria gonorrhoeae*. Implications of this include:

- The nature of the service provided by the SBSTIRL reference laboratory will need to change
- There may be less complete case reporting to the national surveillance programme for gonorrhoea (which currently achieves almost 100% coverage of cases.)
- Clear guidance is needed to interpret positive NAAT tests dependent on positive predictive values in different populations
- Antibiotic resistance surveillance may be less complete

This guidance document for laboratories introducing NAATs for *Neisseria gonorrhoeae* has been produced jointly by HPS and the SBSTIRL reference laboratory to address these concerns. Full membership of the advisory group is listed under appendix A.

### **Nucleic Acid Amplification tests (NAATs):**

The sensitivity and specificity of these tests varies between manufacturers but all have a proportion of false positive results. In populations with a low incidence of the infection this proportion becomes significant and therefore needs to be taken into account. The best way of confirming the result is to perform a second supplemental NAAT test on a different platform using a test that detects a different target.

It is possible to sequence strains of *N. gonorrhoeae* from NAAT samples. Typing of strains is important for contact tracing strategies and national surveillance.

### **Antimicrobial resistance surveillance:**

Resistance surveillance is an essential part of the national surveillance programme for *N. gonorrhoeae*. Changes in resistance profiles guide changes in first line management of cases. In GUM clinics patients presenting with a high probability of

gonorrhoeae are treated prior to a full antibiotic sensitivity result, based on guidance produced as a consequence of the national surveillance findings.

Once resistance to an antibiotic exceeds 5% of isolates, first line treatment needs to be changed. To better understand the extent of resistance it is necessary to stratify cases according to the origin of their infection e.g. acquired locally or overseas.

A high number of imported resistant strains in one area may skew the overall resistance data within Scotland leading to an inappropriate change in recommendations for first line prescribing. Given the sample size for Scotland (approximately 1000 isolates per annum) with almost 100% referral of culture confirmed cases, a significant drop off in numbers could alter the resistance profiles in subsets.

It is possible to give the probability of a resistance profile based on the sequence type of a strain. This does not allow identification of new phenotypic resistance patterns. It is not possible to test NAAT samples with probes for known resistance sequences because they will be contaminated with other confounding bacterial DNA. Only mutations known to confer resistance would be detected.

Given all of the above culture is still necessary for antibiotic sensitivity testing.

### **Sampling by anatomical site:**

Most Chlamydia testing for both men and women is performed using urine samples. In women, depending on the particular test, 8%-25% of cases of gonorrhoeae can be missed using urine rather than a cervical swab<sup>1</sup> As self-taken vulvovaginal swabs perform as well or better than clinician taken endo-cervical swabs in detecting gonococcal and chlamydial infection either of these specimens is acceptable.

Urine is the specimen of choice in men. The unsuitability of female urine as a specimen may have implications for the routine introduction of NAATs.

### **Dual testing and positive predictive values**

The availability of combined tests means that there is now in effect a STI screen available for general practise.

Prevalence of Chlamydia and gonorrhoea are very different and the different components of a dual test will therefore have markedly different positive predictive values.

Laboratories need to be clear about how they will interpret tests for *Neisseria gonorrhoeae* in this context given the potential for and implications of a false positive results in a population of low incidence and unsuspecting of the finding.

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<sup>1</sup> Gaydos CA and Quinn TC Urine nucleic acid amplification tests for the diagnosis of sexually transmitted infections in clinical practice Current Opinion in Infectious Diseases 2005; 18: 55-66.

## **Recommended protocol for introduction of GC NAATs**

### **1. Choice of method**

- Method may be determined by the platform already available in the Laboratory.
- The SBSTIRL is well placed to advise on the quality of commercial tests available and pointers for consideration when implementing a new test.

### **2. Introduction of Nucleic acid amplification tests, validation, confirmation and reporting of results.**

#### a) Introduction of testing:

- Introduction of GC NAATs should be done in discussion with a local genitourinary medicine consultant for services and the Lead Clinician for Sexual Health.
- During introduction of the test the SBSTIRL will provide advice and confirmation of results.

#### b) Samples

- Samples should be urine for men, self-taken vulvovaginal or clinician taken endo-cervical swabs for women. If urine samples are used for women it is recommended that a comment should be added to the report, e.g. “Urine is sub-optimal for detecting gonococcal infection in women and will miss up to 1 in 4 infected women – a self- taken vulvovaginal swab or an endo-cervical swab is more reliable for detecting or excluding gonococcal infection.”
- GC NAATs can be done on rectal, throat, and eye swabs although they are not licensed for these samples.

#### c) Reporting results

- Because the specificity of NAATs for gonorrhoea is not absolute, some tests are prone to cross-reaction with other *Neisseria* species, a positive test should also be positive with a second assay before a positive result is reported. (See caveat on GUM results below)
- Because of the high test sensitivity a negative test can reliably exclude gonorrhoea on an appropriate sample.
- Local agreement should be reached with GUM services on reporting of preliminary results given that the higher prevalence in this population increases the positive predictive value.
- No sample from primary care should be reported prior to a supplemental positive test because of the low prevalence of gonorrhoea in this population

d) Confirmation of results

Option 1

- Diagnostic laboratory performs two separate NAATs each targeting different regions of DNA or RNA. This could be in a multiplex reaction or as two separate tests (ideally on two different platforms).
- Aliquots of the original sample from all positive or equivocal results sent to the SBSTIRL for sequence typing by NG-MAST (see culture below)

Option 2

- Diagnostic laboratory performs local NAAT.
- All positive and equivocal results are sent to SBSTIRL for a second NAAT targeting a different region of DNA or RNA as outlined above and subsequent sequence typing by NG-MAST if confirmed positive.
- Commercial platforms recommend use of their own swab collection kits (swabs and transport medium) for sampling. Some manufacturers also provide specific urine transport tubes containing preservation fluid.
- The SBSTIRL can provide confirmatory results regardless of the commercial system used locally. The Aptima GC (genprobe) and Aptima Combo assays will be used at the reference laboratory as the supplemental test. The table below indicates which specimen collection systems are compatible with Aptima assays. Research was performed with specific reference to the Aptima combo 2 test and the detection of *C. trachomatis*, (see Scragg et al Sex Trans Infect.2006 82:295-297) but the swab collection kits are the same in each case.

Specimen type	Compatibility with Aptima as the supplemental test
Urine	Yes
Becton Dickinson BD Probetec ET swab	Yes
Roche Cobas Amplicor swab	Yes
Abbott Real Time CT/NG urine or swab	No – a nucleic acid preparation may be necessary before retesting.

Further validation of specimens and storage conditions will be performed at the reference laboratory.

### 3. Turn around times

- The requirement for confirmation of findings may impact on local clinical practise due to a lengthening of turn around times. This needs to be discussed and agreed locally
- The SBSTIRL will offer a three working day turn around for a result from receipt of the sample in the department
- Where the SBSTIRL confirms a positive result it will perform sequence typing (NG-MAST) of the sample unless a culture is available (see culture below).

### 4. Minimum Criteria for culture: (Note: a NAAT test should also be taken)

- Patients attending genito-urinary medicine clinics should have swabs taken for culture in the following circumstances: -
  - Any patient **symptomatic** for gonorrhoea
  - Any patient with **gram negative diplococci** in the gram film
  - Any cases of gonococcal **treatment failure**
  - Any **contact** of gonorrhoea
  - All return patients who are NAAT positive not covered above.
- Patients attending general practice:
  - Where the NAAT is **confirmed** positive, follow up and culture will depend on local arrangements
  - Blind antibiotic treatment should be chosen according to national guidelines to treat >95% effectively
  - Those clinically **failing therapy** must be referred to a GUM service with ability to take samples for genital culture
- In the presence of a positive NAAT from a genital specimen, local laboratory identification of isolates could be restricted to oxidase positive gram negative diplococci if they so wish.
- All isolates should be sent to the SBSTIRL for antibiotic sensitivity testing and sequencing.
- The SBSTIRL will hold NAAT samples for up to 2 weeks prior to sequencing to link with cultures.

## **5. Role of SBSTIRL**

SBSTIRL will:

- Advise on commercial systems available, their strengths and drawbacks
- Provide supplemental testing during the period of introduction of NAATs until an acceptable level of concordance between laboratory results is established.
- Provide ongoing supplemental testing for labs that detect only one target (see implications for turnaround times above).
- Sequence type all strains either from NAAT or culture to support local contact tracing and National surveillance.
- Perform sensitivity testing on all isolates received to advise on individual treatment and support national surveillance programme.

## **6. Interpretation of Results**

See Table on Page 9

## **Appendix**

### **Membership of advisory group**

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**Table: Interpretation of Results from SBSTIRL**

Primary result	Supplemental results		Interpretation/Report
	Aptima Combo	Aptima GC	
Pos	Pos	Pos	<i>N. gonorrhoeae</i> nucleic acid present. This result confirms the original NAAT test.
Pos	Pos	Neg	<i>N. gonorrhoeae</i> nucleic acid present. This result confirms the original NAAT test
Pos	Neg	Pos	<i>N. gonorrhoeae</i> nucleic acid present. This result confirms the original NAAT test
Pos*	Neg	Neg	This result does not confirm the original NAAT test. Consider repeat if clinically indicated
Equivocal	Pos	Pos	<i>N. gonorrhoeae</i> nucleic acid present. This result confirms the original NAAT test.
Equivocal	Pos	Neg	<i>N. gonorrhoeae</i> nucleic acid present. This result confirms the original NAAT test.
Equivocal	Neg	Pos	<i>N. gonorrhoeae</i> nucleic acid present. This result confirms the original NAAT test.
Equivocal*	Neg	Neg	This result does not confirm the original NAAT test. Consider repeat if clinically indicated.
Neg/invalid			<u><i>Not routinely tested. Please discuss</i></u>

\* This pattern reflects either a false positive first test or a low level of nucleic acid