Gonococcal infection (*Neisseria gonorrhoeae*)

Laboratory case definition

The Public Health Laboratory Network (PHLN) has developed standard case definitions for the diagnosis of key diseases in Australia. This document contains the laboratory case definition for *Neisseria gonorrhoeae*.

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1. Epidemiology of gonococcal disease

Gonorrhoea is a sexually transmitted disease caused by *Neisseria gonorrhoeae*, a Gram negative *diplococcus*. Global estimates of disease are difficult to obtain but the WHO suggests that worldwide, 65 million new cases of gonorrhoea occur annually.[1]

Gonorrhoea however is unevenly evenly distributed and is said to be a “disease of the marginalised”[2] In terms of economic marginalisation, the greatest burden of disease falls upon less developed countries so that the highest disease incidence and prevalence is found in Africa, South and East Asia and central and South America. Recent increases in disease rates have been noted in Eastern Europe.[1]

In more developed or Western-style industrialised countries those at the economic and/or social margins also bear the brunt of the disease. African and Hispanic Americans and those of Caribbean extraction in the United Kingdom, for example, have disproportionately high disease rates.[3] The other main concentration of disease in Western style countries is amongst homosexually active men (HAM). Rates of gonorrhoea in HAM have increased in a number of countries in recent years.[4]

The highest incidence of gonorrhoea occurs in those aged between 15 and 45 years. Particular subgroups of the population have high rates of disease – “core groups" or “high frequency transmitters”. Examples include long distance truck drivers in Africa and commercial sex workers. Gonorrhoea thus exists as a series of ’microepidemics’ concentrated in particular subgroups of the population.

In Australia slightly more than 6,000 cases of gonorrhoea were notified in 2000, this number representing an increase over the number notified in previous years (e.g. 4,700 in 1997). Gonorrhoea is notoriously under-notified so that the true incidence is not known with certainty. Increases in notifications may be attributable to improved diagnosis and reporting as well as representing a real increase in disease. All these factors may have operated in Australia in recent years.

The epidemiology of gonococcal disease in Australia is a combination of the less developed and Western-style country patterns described above. Extremely high rates of disease, sometimes in excess of 1,000 cases per 100,000 population per year, are present in indigenous populations in rural Australia. In urban populations gonorrhoea is found most frequently in HAM, clients of often illegal CSWs and travellers entering or returning to Australia. The gonococcal populations circulating in these groups, and their susceptibility to antibiotics used to treat gonorrhoea are usually quite distinct.

2. Clinical manifestations of gonorrhoea

Neisseria gonorrhoeae causes both symptomatic and asymptomatic genital and extragenital tract infections so that there is a wide spectrum of clinical presentations in infections caused by gonococci. There is also considerable overlap between these symptoms and those occurring in other STDs, most notably those due to *Chlamydia trachomatis*. It is thus difficult to establish an aetiological diagnosis of gonorrhoea on clinical grounds alone.

Uncomplicated gonococcal genital tract infections

The commonest infections are urethritis in men and endo-cervicitis in women. Men with urethritis usually complain of a urethral discharge in about 80% of cases and burning on micturition about half the time. Although about 10% of patients may be asymptomatic at the time of diagnosis, many of these are regarded as being in the pre-symptomatic stage of gonorrhoea. The incubation period of gonorrhoea in men was estimated to range between one and 57 days with a mean of 8.3 days and a median of 5.8 days since contact. Women with cervicitis may sometimes complain of an abnormal or increased vaginal discharge. When closely questioned many women acknowledge the presence of a discharge, which was however insufficiently discomforting for them to seek a remedy ("oligosymptomatic" cases). Asymptomatic genital infections are commoner in women than in men.

Extragenital gonococcal infections

These include those of the anorectum, oropharnyx and eyes. Proctitis, associated with anorectal pain, tenesmus and rectal discharge is one presentation complex of ano-rectal gonorrhoea although again many infections are asymptomatic. Pharyngeal gonococcal infection is rarely symptomatic. Ophthalmic infections in neonates present as conjunctivitis with discharge. Eye infections may also occur in adults. In both age groups there is risk of disease progression with corneal involvement and perforation. A non-progressive form of ophthalmic disease in children, seen in recurrent epidemics in Australia, has been repeatedly reported.

Complicated gonococcal disease

The commoner complications of both genital and extragenital gonorrhoea have their greatest consequences in women and the newborn. Ascending reproductive tract infections in women (pelvic inflammatory disease - PID) again may have multiple presentations (endometritis, salpingitis, tubo-ovarian abscess or pelvic peritonitis) and be caused by a number of different agents alone or in combination. *Neisseria gonorrhoeae* is a prominent cause of this syndrome in many settings. Sterility

or damaged Fallopian tubes leading to ectopic pregnancy are well-recognised sequelae. First trimester abortion occurs in about 20% of those with gonorrhoea during this period of pregnancy.

About 1-2% of mucosal infections will give rise to disseminated gonococci infections (DGI) with women disproportionately affected. The percentage of DGI seen in a community is dependent on the subtype of gonococcus prevalent in that community. Subtypes of gonococci responsible for DGI are currently found more often in rural than in urban Australia. Presentation is usually with different combinations of mild systemic symptoms, an infective arthritis, rash and tenosynovitis.

To this list of 'traditional' and well-recognised complications may now be added the significant enhancement of sexual transmission of the human immunodeficiency virus (HIV) which occurs in the presence of gonorrhoea. This is thought to be as a result of both an increase in the HIV viral load in semen or cervico-vaginal fluids of those infected with gonorrhoea, and an increase in the number of target cells for HIV in the inflammatory exudate which accompanies symptomatic STDs.

From the above it can be seen that gonorrhoea has a wide variety of clinical presentations, none of them uniquely caused by the gonococcus. A significant proportion of gonococcal infections are asymptomatic or 'oligosymptomatic' or else incubating and 'presymptomatic' and are thus unrecognised.

Further, the clinical syndromes may represent simultaneous infection with more than a single agent. For example co-infection with both *N. gonorrhoeae* and *C. trachomatis* is commonly seen in some settings, although such overlap may not always be as common in others, e.g. homosexually active men.

This creates a number of dilemmas for diagnosis and management of the individual patient and also for establishing public health measures for control of gonorrhoea.

3. Relevant features of *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is a Gram negative diplococcus and a human specific pathogen, highly adapted to existence in the genital tract. Unlike *Neisseria meningitidis*, the gonococcus lacks a capsule and humoral immunity is poorly developed, although the outer membrane protein component of the cell wall includes immunogenic porin proteins. Another relevant feature of gonococci is their genetic diversity, explained in part at least by horizontal intra-species recombination and acquisition of genetic material from closely related *Neisseria* species by processes of transformation and conjugation. These features are relevant to both diagnostic and typing methods for gonococci.

4. Laboratory diagnosis

The laboratory diagnosis of gonorrhoea depends on the demonstration of *N. gonorrhoeae*, or detection of its DNA in samples.

The demonstration of the presence of *Neisseria gonorrhoea* from any site constitutes a case of gonococcal disease for public health purposes.

4.1 Diagnostic microbiological studies and specimens

General remarks

The ideal diagnostic test for gonorrhoea is one which is rapid (so that results are available while the patient waits), easy to perform, cheap, uses samples obtained by non-invasive methods, is reliable, reproducible, robust and with high sensitivity and specificity and requires no special equipment. Additionally, an essential requisite for a test for gonorrhoea is that it should also provide a means of assessing the antibiotic susceptibility of gonococcal isolates for public health purposes.

While such a test does not yet exist, there are a variety of both culture based and non-culture methods available for the diagnosis of gonorrhoea. These can be used singly or in various combinations according to needs and resources and for different purposes.

These purposes include:

- confirmation of diagnosis in symptomatic patients;
- screening for infection in asymptomatic patients (case finding);
- for epidemiological purposes e.g. data on gonococcal prevalence and/or incidence to support syndromic management algorithms.
- Choice of test(s) may also be influenced by logistic factors such as access to facilities, and available transport.

As for all diagnostic tests, the collection and transport of samples are often critical determinants of success or failure in demonstrating the presence of an infecting agent. For example, gonococci in the female genital tract reside in the endocervical glands and not the vaginal squamous epithelium, so that a proper sample for culture requires that an endocervical swab be taken under direct vision, in preference to a vaginal swab. This presumes the availability of privacy, proper lighting and a vaginal speculum that can be reliably resterilised. Such facilities are not present in many settings. Other reasons (related to cultural practices and beliefs, or even the personal reluctance to undergo a pelvic examination) also may preclude the collection of genital tract specimens from females.

For culture based systems where direct inoculation and incubation of culture media is not possible, transport systems are needed to maintain the viability of gonococci. Such systems are also more suited to the diagnosis of male urethritis than female endocervicitis in that there is a much higher number of gonococci in urethral discharges than in endocervical material. The former specimens also have only a small amount of normal flora whereas the female genital tract flora is extensive and present in larger amounts. Thus both inoculum reduction over time and overgrowth by normal flora adversely affect gonococcal survival for culture from endocervical material.

4.2 Microscopic techniques

Examination of a Gram stained smear requires availability and maintenance of a good quality microscope and the services of a competent microscopist.

In symptomatic male urethritis, it is a sensitive (95%) and specific (98%) test that is cheap and rapid and can be performed as a near patient test. The sensitivity of the Gram stain is greatly reduced in asymptomatic male urethritis.

In (female) endocervicitis, the sensitivity of the test is less than 50% when compared with properly performed culture.

Gram-stained smears are less useful in diagnosis of rectal gonorrhoea and best results are obtained when a sample is taken under direct vision via a proctoscope. Pharyngeal specimens are not suitable for examination by Gram-stained smears. Gram stain diagnosis of gonorrhoea from other sites such as conjunctiva and skin is sub-optimal.

Lower detection rates with Gram-stained smears are obtained when they are examined by inexperienced microscopists or when skills are not maintained.

4.3 Culture based methods

(See also remarks on collection and transport, above, which are critical to sensitivity of culture based methods).

Culture based systems provide high specificity (virtually 100% if definitive identification procedures are applied – see below), but are expensive and require personnel trained in the handling of the fastidious gonococcus. Culture media vary widely in quality, but optimally specified, prepared and quality controlled GC agars are critical to the success of this diagnostic method.

There are two basic requirements for reliable gonococcal culture media, namely, an enriched medium to support the growth of the fastidious gonococcus, and a selective medium to allow the isolation of the organism from specimens with a wide and numerous normal flora. The wide variety of media available and the constant refinements to these media suggest that none combine these two aspects perfectly. However both the nutrient and selective properties of gonococcal culture media have undergone significant developments so that the sensitivity of culture as a diagnostic tool has significantly increased where these improved media are available and used to best advantage. Not all comparisons of culture and non-culture based methods have used optimised culture based techniques.

In addition to using isolation media of demonstrable quality, the conditions needed for reliable growth also impinge on the success or otherwise of culture based techniques. The presence of CO_2, is required to initiate gonococcal growth, and an incubation temperature of 35 - 37°C and a humid atmosphere is also necessary. These requirements can be met reliably by modern incubators and carbon dioxide can be supplied by a variety of means.

4.3.1 Identification of *N. gonorrhoeae*

This is also not without problems and again a number of techniques are available, and their application and use depends on resource availability, expertise and circumstance. For a review of relevant aspects see Knapp, 1988 (5).

Presumptive identification, based usually on growth on selective GC agar, Gram stain morphology of this growth and simple and inexpensive tests for ‘oxidase’ and ‘superoxol’ positivity, is regarded as sufficient for diagnosis in many settings, especially for gonococci grown from genital tract sites.

In Australia, *definitive* identification is more usual especially for isolates from extragenital sites. This identification relies on the above criteria plus one or more techniques that demonstrate the

carbohydrate utilisation patterns, immunological characteristics or enzyme profiles of the organisms, either alone or in combination.

Carbohydrate utilisation tests are most widely used. These may be growth dependent tests for the acidification of media containing glucose but not those supplemented with other sugars, or non-growth dependent tests in which preformed enzymes utilise the carbohydrate substrate. The latter test is considered to be less demanding, cheaper and more reliable. Nucleic acid based techniques for identification of gonococci are also available but have little practical application because of cost.

Choice of tests for definitive diagnosis may also depend on the role of the testing laboratory. For STD-clinic-based laboratories, distinction between gonococci and non-gonococcal *Neisseriae* is often all that is required and test methods are selected accordingly. In this circumstance immunological methods such as co-agglutination, or in the past fluorescent antibody tests, are often used. For laboratories with a wider role, and where identification of related organisms from other clinically relevant sites is necessary, full speciation procedures for all isolates are usual.

The PHLN recommendation is for definitive identification by a recognised method.

Antibiotic susceptibility testing should be part of the diagnostic and public health process if at all possible.

4.4 Enzyme immunoassay

Tests using antigen detection and enzyme immunoassay as the signalling mechanism were developed and extensively trialed. However after evaluation in low and high prevalence settings and in symptomatic and asymptomatic men and women it was concluded in a meta-analysis that “there are few to no situations for which this assay is recommended”[6]

4.5 Molecular techniques

General remarks

Difficulties in specimen collection and transport and culture based diagnosis have accelerated the development and application of nucleic acid detection tests, with or without amplification, for the diagnosis of gonorrhoea. Molecular technology has been used to develop tests with a reported high sensitivity and specificity. For the diagnosis of urethritis and endocervicitis, a first voided urine sample i.e. a non-invasive sample usually suffices. Thus a test which provides a highly reliable result, which minimises collection difficulties (and is therefore more acceptable to patients) and which is less demanding in terms of transport requirements is not only available but is now increasingly being introduced.

In Australia, the application of NAA technology has significantly advanced the accuracy of data on disease incidence.

The second, and significant, consideration is that these non-culture tests at present do not allow surveillance of antimicrobial susceptibility, a factor regarded as essential for disease control.

Nucleic acid hybridisation tests ('probe' tests)

In tests of this type a specific probe binds to any complementary nucleic acid present in the sample. The nucleic acid is not amplified by cycling probe technology, but measuring an attached chemiluminescent label enhances estimation of bound probe. A commercial version is available that provides specimen collection kits and a lytic transport medium that stabilises the preparation for one month. The test is now combined with a hybridisation test for *C. trachomatis*. The sensitivity of the test is about 85% and the specificity 99%. These data were derived from a review of a number of studies[6] where the sensitivity was as low as 54% in some evaluations.[7]

This technology can also be used for confirmation of cultures of *N. gonorrhoea,* but is expensive for this purpose.

Nucleic-acid-based amplification assays (NAA)

A number of technologies are available for the amplification of any gonococcal DNA present in samples by cycling probe technology including PCR, LCR and TMA. Theoretically even a single-gene copy of live or dead organisms can be amplified to detectable levels.

A systematic review estimated a *sensitivity* of about 95% for all anatomical sites.[6]

This varies between sites, sample types and technologies.

Suitable samples

Commercial NAA tests currently licensed for gonococcal testing are restricted for use to genital sites.

Specificity also varies with the technique used and is such that a confirmatory process is recommended for all tests positive in initial assays.

False positive results may also arise from specimen to specimen contamination (e.g. leaking urine containers transported over long distances) and environmental contamination of samples.[8] Although it is claimed that new laboratory procedures minimise this risk, Shapiro[9] has suggested additional use of probability theory is advantageous in detecting the possibility of contamination.

False negative results may arise from the presence of a wide range of inhibitors of NAA naturally present in body fluids. Additionally some self collected patient specimens may be inadequately sampled or even deliberately not collected. Only some of these aspects have been addressed in the test protocols in commercial systems.[10]

Further experience since the above review[6] was published indicates that the specificity of some commercial NAA tests is also not as high as previously thought. When one commercial test was applied in high prevalence populations not previously tested with these products, 16% of test positives were negative on a confirmatory assay and 82% of equivocal results could not be confirmed. The diversity of the Neisseriaceae in different geographic areas was thought to be the basis for this finding.[11]

NAA assays are particularly useful in outreach situations to allow access to tests to those who fail to utilise existing services for a variety of reasons.[12] These groups include alienated urban youth groups who do not attend clinics and populations in remote areas where culture based testing is impractical. The utility of samples other than urine in females - such as self collected tampons or swabs - has also been demonstrated.[10] It has been suggested that NAA tests can be used initially to better define risk

factors or markers where tests of this type can be best deployed. Continuous monitoring of the utility of these tests in target populations is suggested.[8]

4.6 Serological tests are not reliable for the diagnosis of gonorrhoea

4.7 Summary of laboratory diagnostic methods

A table of available test protocols, modified from the WHO Western Pacific document on detection of reproductive tract infections follows.[13]

Table. Characteristics of *N. gonorrhoeae* detection tests (a)

	Microscopy	**Culture**	**DNA Detection**	**Amplification & Detection**	
			PACE 2C	**PCR**	**LCR**
Sensitivity[1]	95%	85%-100%	85%	89%-97%	90%-95%
Specificity[1]	98%-100%	100%	98%	94%-100%	98%-100%
Advantages	rapid, inexpensive	gold standard, * susceptibility testing available	rapid, viable organisms not required	viable organisms not required, extremely sensitive, allow noninvasive sampling can detect *C. trachomatis* in same sample	
Disadvantages	insensitive for females, rectal samples; ineffective for pharyngeal samples	stringent handling, usually requires 48hrs plus identification	Expensive *susceptibility testing not possible approved for male urethral	expensive, requires expertise no test for sample inhibitors *susceptibility testing not possible	

	Microscopy	Culture	DNA Detection	Amplification & Detection	
			PACE 2C	PCR	LCR
		multi staged QA necessary	and female endocervix		
Level of use	on-site lab	on-site lab, intermediate	intermediate, referral lab	intermediate, referral lab	intermediate, referral lab
Training	moderate	moderate	moderate	moderate to extensive	moderate
Equipment	light microscope	incubator, light microscope, candle jar	water bath, luminometer	microfuge, thermal cycler, incubator, microwell reader	heat block, thermal cycler, microfuge, Imx processor
Ease of performance	easy	moderate	moderate	moderate to difficult, automated	moderate, automated

1. Sensitivity and specificity are for detection of *N. gonorrhoeae* in urethral, endocervical and urine samples by culture except for microscopy, which is for detection in urethral samples from symptomatic men.

(a) Modified from "Laboratory tests for the detection of reproductive tract infections"[13]

5. Strain differentiation of *N. gonorrhoeae*

Typing of gonococci is used for a number of epidemiological purposes including population studies of gonococci , assessment of prevalent subtypes of gonococci, transmission chains, assessment of possible treatment failure, enhanced contact tracing in low prevalence settings and antibiotic resistance profiling.

The *phenotype* is best determined by a combination of the auxotype and serovar (A/S typing). This is time consuming and reagents currently available are expensive. There are a variety of *genotyping methods* described (PFGE, AFLP, por sequenecing, opa typing) which have particular rather than general applications. Current opinion (J.S Knapp, C. Ison personal communication) favours first the use of phenotypic separation followed, if still necessary, by the use of a validated genotyping application suited to the purpose of the investigation.

Antibiotic susceptibility surveillance

Antibiotic treatment is an essential control measure for gonorrhoea. Antibiotic treatment is usually administered as a “standard” treatment of a single dose of antibiotic. The standard treatment regimen should cure more than 95% of cases. Antibiotic resistance in *N. gonorrhoeae* to agents used for treatment has been a continuing problem so that surveillance of antibiotic resistance with a change in treatment regimens when resistance occurs in >5% of isolates is recommended.

Antibiotic resistance in *N. gonorrhoeae* in Australia varies by region, being most diverse and pronounced in larger centres such as Sydney and Melbourne and least developed in rural areas of North Australia. However resistance to individual antibiotics may arise and spread rapidly through spread of a resistant subtype or by dissemination of resistance mechanisms into multiple subtypes. In either circumstance change in antibiotic treatment may be required. The need for such a change may be anticipated through appropriate surveillance. This in turn requires the availability of a sufficiently large and representative sample of gonococcal isolates for susceptibility testing and analysis. These functions are currently undertaken through the Australian Gonococcal Surveillance Programme[14] which has member laboratories in each jurisdiction.

7. Quality control/Quality assurance

The RCPA/QAP currently provides external QA procedures for culture of gonococci. An external QA programme has recently been introduced for NAA. The AGSP has a programme specific QA for susceptibility testing.

8. References

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9. PHLN Laboratory Definition

Definitive Criteria:

i. Isolation of *N. gonorrhoeae* by culture; *OR*

ii. Confirmed detection of gonococcal DNA in genital specimens, urine or normally sterile sites by NAA.

Suggestive Criteria:

i. Detection of Gram negative intracellular diplococci in smears of urethral exudates from symptomatic men or endocervical secretions from women; OR

ii. Unconfirmed detection of gonococcal DNA