Meningococcal disease (*Neisseria meningitidis*)

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 meningitidis*.

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1 PHLN Summary Laboratory Definition

1.1 Condition:

Invasive meningococcal disease

1.1.1 Definitive Criteria

a Isolation of *Neisseria meningitidis* from a normally sterile site; OR
b Detection of specific meningococcal DNA sequences in a specimen from a normally sterile site by nucleic acid amplification testing.

1.1.2 Suggestive Criteria

a Detection of Gram-negative diplococci in Gram's stain of specimen from a normally sterile site or from suspicious skin lesion; OR
b High titre immunoglobulin class M (IgM) or significant rise in IgM or immunoglobulin class G (IgG) titres to outer membrane protein antigens of *N. meningitidis.*

2 Introduction

Epidemiology of meningococcal disease

Invasive meningococcal disease (IMD) is caused by the bacterium *N. meningitidis*, a Gram-negative diplococcus. The disease occurs sporadically and in clusters and epidemics. Endemic meningococcal

disease is found worldwide, sometimes with high disease incidence (hyperendemic disease). Some countries, many of which are in the sub-Saharan 'meningitis belt', experience epidemic waves of disease. It is estimated that about 500 000 cases of non-epidemic meningococcal meningitis occur globally each year with a mortality of about 50 000. These numbers do not take account of epidemic disease. No reliable global data are available for forms of invasive meningococcal disease other than meningitis. Even these are estimates as facilities are lacking to distinguish endemic meningococcal meningitis from other causes of cerebrospinal meningitis.

There are 13 serogroups of meningococci recognised. Explosive epidemic disease is mainly caused by serogroup A. The spread of epidemic 'clones' of serogroup A meningococci is associated with recurrent pandemics. In recent years epidemic disease with serogroup C and W135 has also been observed in the 'meningitis belt'. Disease due to serogroup W135 meningococci has also been spread internationally by Hajj pilgrims. In industrialised countries serogroups B and C predominate, with serogroups Y and W135 sometimes also involved. Australia conforms to this pattern in developed countries with about 400–500 cases per year giving an annual incidence of about 2–3 cases of IMD per 100 000 population. Most IMD in Australia is sporadic and due to group B and C meningococci, with some regional variation in the proportion of these two serogroups. Some clusters of serogroup C disease have occurred, but very few instances of serogroup A infections have been seen in recent years. Particular subtypes of serogroups B and C have been responsible for outbreaks and clusters of disease and for hyperendemic disease.

The highest incidence of IMD is in children aged less than 4 years. A secondary peak in incidence occurs in adolescents. Various associations with a higher incidence of IMD are recognised: a seasonal peak in incidence is usual with a majority of cases occurring in late winter/early spring; peak activity is also associated with periods of low absolute humidity; high attack rates are found in poor living conditions and overcrowded housing; nasopharyngeal shedding of respiratory pathogens is associated with an increase in incidence of meningococcal disease; household contacts of meningococcal disease have a risk of acquiring infection approximately 600–1000 times the age-specific incidence in the general population. Mortality is between 5 and 10% in industrialised countries.

Clinical manifestations of invasive meningococcal disease

IMD is most commonly manifested as septicaemia and/or meningitis. Organ specific disease (e.g. arthritis, pneumonia, pericarditis) may also occur. The meningococcus is carried in the throat from where it invades the bloodstream. The clinical spectrum of disease may range from clinically inapparent bacteraemia to fulminant and rapidly fatal septicaemia. Meningitis is common and is often the only manifestation of IMD and the meninges are seeded during the bacteraemic phase. Early in the disease clinical appearances are often non-specific. When clinical disease manifests, the presentation is determined by bacterial properties such as endotoxin release, and the host response and immune status. One well recognised feature of IMD is a rash, which however, may vary in type or be late or absent in a significant proportion of cases. Fevers and rashes due to other diseases are quite common in young children so that clinical diagnosis is often difficult when classical features of the disease are absent or muted. Thus IMD may be both under diagnosed or wrongly diagnosed on clinical grounds and laboratory confirmation of cases is required.

Relevant features of *Neisseria meningitidis*

The bacterium *N. meningitidis* is a Gram-negative diplococcus and a human specific pathogen. Strains from invasive disease almost always possess a polysaccharide capsule which defines the serogroup. Humoral immunity to the capsule is an essential factor in prevention of meningococcal disease. Meningococci have a typical Gram-negative cell wall and the lipopolysaccharide components (endotoxin) of the outer membrane are continually shed as blebs during multiplication in vivo

provoking the host response and clinical features of the disease. The outer membrane protein component of the cell wall includes immunogenic porin proteins involved in adhesion and attachment of meningococci to epithelial surfaces. Most Neisseria species express one porin protein but meningococci possess and express both porA and porB genes which are relevant to in vitro pheno- or genotyping systems and contribute to virulence. Another relevant feature of meningococci is their genetic diversity, explained in part at least by horizontal inter and intraspecies recombination and acquisition of genetic material from closely related Neisseria species and other genera located in the respiratory tract. These features are relevant to both diagnostic and typing methods for meningococci.

3 Laboratory diagnosis/tests

The laboratory diagnosis of IMD depends on the demonstration of *N. meningitidis*, or specific meningococcal DNA sequences in samples from normally sterile sites, or positive serology. In situations where there is a strong clinical suspicion of IMD, antibiotic therapy must not be delayed while initiating or awaiting results of diagnostic tests. Latex particle agglutination tests from any site are no longer recommended for the diagnosis of IMD and will not be discussed further. Samples available for this type of testing are best submitted for assay by the more sensitive PCR methodologies now widely available. A trial of an ultrasound enhanced latex agglutination test was undertaken in Australia but results were unsatisfactory[12].

3.1 Microscopy

Microscopy, if positive for Gram-negative diplococci from sites such as CSF or skin smears, provides a highly specific confirmatory test. The sensitivity and specificity is affected by the adequacy of specimen collection, stage of the disease, intercurrent use of antibiotics and experience of the microscopist.

3.1.1 CSF specimens

Classically CSF from a case of meningococcal meningitis reveals a high neutrophil count, low glucose and high protein content. Gram-negative diplococci, if observed within neutrophils, provide evidence of meningococcal meningitis. There are numerous exceptions to this classical picture so that low or absent white cells do not exclude meningitis. In meningococcal disease with high white cell counts the number of organisms may be so low as to be undetectable. Prior administration of antibiotics will remove or else distort the appearance of the diplococci.

3.1.1.1 Test sensitivity

The sensitivity of the Gram's stain in CSF is estimated to be of the order of 65%. This is affected by the stage of disease, number of organisms present (which may vary considerably between patients) and timing of lumbar puncture in relation to antibiotic administration.

3.1.1.2 Predictive value

A negative CSF examination does not exclude the possibility of invasive meningococcal disease and is of no relevance in the diagnosis of meningococcal septicaemia without meningeal invasion.

3.1.2 Aspirates of skin lesions and joint fluid specimens

Gram's stains of aspirates from sterile sites obtained on admission provide confirmation of IMD in the presence of a clinically compatible illness, i.e., they are highly specific, but are not sufficiently sensitive, so that a negative result does not exclude IMD.

3.1.2.1 Test sensitivity

Gram's stains of skin lesion aspirate or biopsy specimens have a reported sensitivity of 30 to 70% at presentation but this varies with the form of meningococcal disease and type of skin lesion, being highest in haemorrhagic lesions of meningococcal septicaemia[1]. Skin lesions occur in 50–75% of cases of IMD so that overall sensitivity in all cases of IMD is correspondingly lower. Gram's stains of skin biopsy may remain positive for long periods (about 48 hours) after antibiotic administration (said to be due to poor penetrability of antibiotics into poorly perfused lesions) but the test sensitivity at this time is not known.

3.1.2.2 Predictive value

Positive predictive value is unknown. False positive tests may occur but the frequency is undefined. Negative predictive value is unknown. A negative result does not exclude IMD.

3.2 Culture of *Neisseria meningitidis*

Culture of *N. meningitidis* from blood, CSF or other normally sterile site provides unequivocal confirmation of IMD[2]. Additionally, culture provides isolates for strain differentiation and susceptibility testing. For these reasons all isolates of meningococci from suspected cases of IMD should be sent to the appropriate National Neisseria Network (NNN) laboratory. In cases where meningococcal disease is suspected clinically, it is STRONGLY RECOMMENDED by the NH&MRC that antibiotics be given before transfer to hospital and not withheld pending collection of diagnostic specimens. This decreases the likelihood of a positive culture but not to the same extent from all sampling sites. Collection of diagnostic samples should nevertheless still proceed even after administration of antibiotics as these samples may still yield a positive culture, and also may be used for non-culture diagnostics such as PCR.

3.2.1 Media

Meningococci are not overly fastidious in their growth requirements and grow well on good quality media such as blood or chocolate agar. These media are suitable for culture from sterile sites. For culture from non-sterile sites such as the throat, selective media such as Modified New York City or Modified Thayer Martin medium are required.
Culture plates should be incubated for a minimum of 48 hours with a source of 5% CO_2.

3.2.2 Suitable specimens

Blood for culture should always be obtained, CSF, skin sterile site, rash aspirate, skin biopsy. Throat swabs in cases of suspected IMD only.

3.2.3 Test sensitivity

Several variables affect the sensitivity of blood cultures in IMD: the number of blood cultures collected, the volume of the sample (it is difficult to collect anything other than small volumes in infants), and the prior use of antibiotics. Cultivation of meningococci from liquid media is compromised by anticoagulants usually present in blood culture media. The sensitivity of blood culture is reported to be only 50% in untreated cases of IMD falling to 5% or less if antibiotics have been used[3].The sensitivity of CSF culture is about 95% in cases of untreated meningococcal meningitis. This percentage falls rapidly after treatment as viable meningococci are quickly cleared from CSF. It is again emphasised that a negative CSF culture does not exclude non-meningeal IMD. Culture of skin aspirates/biopsies is similar in sensitivity to Gram's stain of the same lesion. Combined Gram's stain/culture of skin lesions has a sensitivity of about 60–65%, but is higher for haemorrhagic lesions

of meningococcal septicaemia[1]. Throat swabs (post nasal or per nasal) may yield meningococci in about 50% of cases of IMD and are less affected by prior antibiotic therapy[4]. In sporadic cases, a positive culture provides corroborative but not definitive evidence of IMD. Routine swabbing of close contacts is not recommended nor are throat swabs for meningococci suggested where meningococcal disease is not suspected clinically or in outbreaks.

3.2.4 Test specificity

Approaches 100% if isolation of confirmed *N. meningitidis* from sterile site. In sporadic cases, a positive throat swab culture provides corroborative but not definitive evidence of IMD. Routine swabbing of close contacts is not recommended nor are throat swabs for meningococci suggested where meningococcal disease is not suspected clinically or in outbreaks.

3.2.5 Predictive values

Positive predictive value approaches 100% for sterile site specimens. A negative culture does not exclude IMD and depends on the adequacy of collection, specimen transport and storage conditions prior to culture, stage of disease and prior antibiotic treatment.

3.2.6 Suitable acceptance criteria

Isolation of a *N. meningitidis* confirmed by biochemical and phenotypic parameters.

3.2.7 Suitable internal controls

A properly documented, relevant, quality control program for each type and batch of medium used.

3.2.8 Suitable external QC programmes

Difficulties arise with QA/QC for *N. meningitidis* insofar as it is an organism with a lethal potential and has been associated with laboratory-acquired infection. For these reasons the RCPA/NATA programme rarely if ever includes cultures of meningococci in its surveys.

3.3 Identification of *Neisseria meningitidis*

Presumptive identification can be based on Gram's stain and oxidase reaction, but definitive diagnosis requires confirmation by a combination of tests including those for detection of acid production, and if necessary, enzyme substrate tests (meningococci are gamma-glutamyl aminopeptidase positive—this is a rapid test and a suitable substrate is readily obtainable from clinical chemistry departments), nitrate reduction and superoxol tests, polysaccharide/sucrose production tests meningococci are negative but N. polysaccharea positive on 1% sucrose agars); and susceptibility to colistin[18] (meningococci are colistin resistant and will grow on selective media containing VCN inhibitor).

3.3.1 Presumptive identification

Growth of Gram negative diplococci which are oxidase positive.

3.3.2 Definitive identification

Tests for production of acid from glucose and maltose supplemented, if necessary, by enzyme substrate tests, nitrate reduction and superoxol tests, polysaccharide/sucrose production tests, and susceptibility to colistin.[18]

3.3.3 Predictive values

Other *Neisseria* such as *N. polysaccharea,* some *N. subflava* and, rarely, lactose-negative *N. lactamica* may produce similar acid production reactions. Only a small amount of acid is produced by the *Neisseria,* and that this is not always detected. For a number of reasons the traditional cystine trypticase agar test (CTA) is no longer recommended for this purpose by the CDC and rapid non-growth dependent tests for carbohydrate production are now preferred by them[19].

3.3.4 Suitable test criteria

Refer to instruction leaflet for commercial kits and published data for in house tests [20].

3.3.5 Suitable internal controls

The NNN provides appropriate type culture *N. meningitidis* controls.

3.3.6 Suitable validation criteria

Defined by the manufacturer of commercial kits or published method for in house tests[20]
Aberrant results should be further investigated by molecular techniques[21].

3.3.7 External QC programmes

None widely available owing to the dangerous nature of this organism. The NNN laboratories conduct a QA programme for their participating laboratories.

3.4 Strain differentiation of *Neisseria meningitidis*

Differentiation of meningococci from cases of invasive meningococcal disease is undertaken for public health reasons, e.g., to confirm or to exclude a suspected outbreak of cases. A true epidemiological link between cases can only be established by public health investigations. Laboratory typing results can confirm or exclude such a link, but do not establish one in the absence of these public health data.

3.5 Serotyping

Currently isolates are phenotyped by NNN laboratories by determining the serogroup as soon as practicable after receipt and then the serotype and serosubtype using standard monoclonal reagents. Serotyping and serosubtyping is performed by batching of isolates and testing at regular intervals – less frequently in low incidence periods and more frequently in the winter/spring. Serotyping and subserotyping is NOT *routinely* performed on an emerging basis as it is wasteful of reagents which are no longer produced. These techniques can however be rapidly employed if an epidemiological link between cases is established or suspected and can quickly exclude the presence of clustering of cases. However, many serogroup B strains are non-typable and reagent stocks are finite.

3.6 Molecular Typing

Genotyping (molecular) procedures are now supplanting phenotyping (serotyping) methods. Those available include pulsed field gel electrophoresis (PFGE), *porA/porB* sequencing and MLST.

These techniques are used for different purposes, e.g. PFGE and *porA* sequencing are used for short-term studies of strain relatedness and MLST for longer term population studies of meningococci. PFGE methods are not uniform between laboratories. Further, PFGE patterns are usually considered of short-term use for differentiating suspected outbreaks under local conditions. Thus inter-laboratory

comparisons of PFGE patterns are not suitable for distinguishing invasive meningococci separated temporally and/or geographically across Australia.

Similarly *porA/porB* typing can be applied for short term examination of possible outbreaks but is less also suited to long term longitudinal genotyping studies. A global standard nomenclature for *porA* sequencing is being developed, meaning that greater comparability of strains can be achieved by this means. A world wide web database can be accessed via the internet at http://neisseria.org/perl/agdbnet/agdbnet.pl?file=poravr.xml for classifying *por*A genotypes. Other genotyping systems are also being developed such as *fet*A which are accessible via the same website.

MLST is currently a technique more appropriately used for long-term population studies of meningococcal populations as it examines more stable parts of the genome. Its use defines clonal complexes of invasive meningococci and related sequence types within these clonal complexes.

It should also be remembered that the presence of isolates with an indistinguishable phenotype (serogroup, serotype and serosubtype) or genotype does not of itself establish a true epidemiological link which should *first* be properly established by clinical public health procedures.

The application and development of these techniques in Australia is under constant review by the NNN.

3.7 Serological Diagnosis

Serological testing, based on an enzyme immunoassay using outer membrane proteins (OMP) as the antigens, was developed by the UK PHLS Meningococcal Reference Centre[9]. The assay has value in retrospective diagnosis of IMD when culture has been negative due to early antibiotic treatment or CSF has not been available for PCR. This laboratory kindly supplied methods, suitable isolates for antigen preparation reagents and controls for use in Australia and the OMP test has now been evaluated under local conditions[10]. A second assay has been described for the detection of antibodies to the polysaccharide component of serogroup C meningococci and this too has been evaluated and used in Australia[11]. No assay is available for serogroup B antibody.

3.7.1 Specimens

Serum is preferred but assays can be performed on plasma.

3.7.2 Test sensitivity

The sensitivity of the OMP assay has been shown to approach 100% in adults and older children (4 years or older) in culture proven culture positive cases of IMD from 5–21 days after presentation. A single high IgM titre is usually sufficient for diagnosis in these age groups. However, the assay is slightly less sensitive in children less than 4 years of age owing to different kinetics of their antibody production and paired sera are currently required for diagnosis in this age group. Sensitivity of the Serogroup C assay for disease due to serogroup C meningococci is slightly lower than the OMP assay.

3.7.3 Test specificity

The OMP assay has a specificity of approximately 94%. OMP antibody may be formed by exposure to commensal Neisseria that share identical or closely related OMPs and in some cases of disseminated

gonococcal infection. Serogroup C assay is more specific but becomes positive after immunisation with vaccines containing serogroup C polysaccharide as well as after IMD.

3.7.4 Predictive values

The negative predictive value of both assays is high and can be used to exclude IMD in clinically notified but unconfirmed cases of IMD. Positive predictive values: High if used to confirm IMD in cases of clinically compatible disease. The OMP assay should not be used as a screening test because of cross-reactions with other Neisseria. Antibodies develop after natural exposure to serogroup C meningococci and also after vaccination. Its usefulness for diagnostic purposes is thus limited to those who are not immunised with serogroup C vaccines (either conjugated or polyvalent unconjugated types). This assay is useful for the detection of antibody post vaccination, that is, it can detect the success or failure of the vaccination process.

3.7.5 Suitable test criteria

Positive IgM test in a single sample or seroconversion if paired sera are available from a patient with a recent infection compatible with meningococcal infection.

3.7.6 Suitable internal controls

The concentrations and dilutions of antigen conjugate and serum are determined using chequerboard titrations of known positive (culture confirmed) and negative serum. The cut off value was established using samples from blood donors as well as serum from patients with throat carriage without evidence of IMD. International standard anti C capsule serum from CDC used to calibrate the C capsule antibody assay.

3.7.7 Suitable validation criteria

Specificity calculated using serum from a variety of other bacterial and viral diseases. Sensitivity calculated using serum collected at known times after culture confirmed IMD.

3.7.8 External QC programmes

None available.

3.8 Nucleic Acid Diagnosis

More rapid treatment of suspected cases of meningococcal disease with effective antimicrobials and reluctance to lumbar puncture mean that NAA—essentially PCR in this disease—are becoming more important in the laboratory diagnosis of IMD. PCR assays may increase the laboratory diagnosis of cases of meningococcal disease by more than 30%[5] and meningococcal DNA in CSF samples[5] has been detected up to 72 hours after commencement of antimicrobial treatment[6]. The target sequence most used for PCR based assays is the ctrA gene. PCR tests for serogroup determination should be performed both from a confirmatory and epidemiological point of view. Primers for various regions in the siaD gene specific for serogroups B, C, W135 and Y have been published[8]. Serogroup determination for B and C meningococci by PCR is widely performed in Australia. Positive meningococcal DNA preparations should be stored (preferably at –70 °C) for subsequent porA/porB sequencing and multi-locus sequence typing (MLST) analysis if required.

PCR-based diagnosis provides confirmation of IMD from blood, CSF or other normally sterile sites with a validity comparable to that of culture-based diagnosis. Additionally, nucleic acid methods can provide diagnostic information pertinent to patient care and public health management. For these

reasons it is recommended that (i) samples such as CSF and EDTA blood from which DNA was extracted for PCR based diagnosis as well as (ii) the residual DNA remaining after PCR testing, both be sent to the appropriate NNN laboratory. (The original samples of CSF and EDTA blood should be forwarded to ensure adequate amounts of DNA and uncontaminated DNA preparations are available as required.)

3.8.1 Suitable specimens

Primarily applied to CSF and blood specimens

3.8.2 Test sensitivity

>95% for CSF using *ctr*A gene sequence amplification[7] and approx. 87% when testing blood samples [6,27] . Data is not available for skin lesions.

3.8.3 Test Specificity

> 95% or more when using *ctr*A gene sequence amplification[7].

3.8.4 Predictive values

Positive Predictive value of 98% and negative predictive value of 86% when testing CSF samples to the serogroup level[8]. A negative NAA test does not totally exclude IMD in a patient with compatible symptoms and signs.

3.8.5 Suitable test criteria

N. meningitidis NAA can be performed on blood or CSF samples when invasive meningococcal infection is suspected.

3.8.6 Suitable internal controls

The exact number of controls required for PCR-based systems depends on the number of samples in each run, although in general, two types of negative control should be included: a sample that is negative for the abnormality or pathogen and a 'no nucleic acid' sample (i.e., all reagents except the DNA/RNA). Negative controls should be placed after the last patient samples. Where test runs are expected to contain a large proportion of positive results, then it is recommended that additional 'no template' controls be interspersed among the patient samples at an appropriate frequency as validated by the particular laboratory. This should also be done if contamination problems have occurred.

Positive controls should preferably be just above the limit of sensitivity of the test. Negative controls should be dispensed last so they reflect the state of the reagents added. These controls should be subject to the whole test process, including the extraction Inhibition Controls the use of an inhibition control for each test sample should also be used to ensure the absence of any inhibitory substances within the nucleic acid extract. Refer See the relevant NPAAC standards.[28,29]

3.8.7 Suitable validation criteria

Assays should be validated in accordance with appropriate guidelines and NPAAC standards [28, 29]

3.8.8 Suitable quality assurance programmes

There is currently no external quality assurance programme offered in Australia for NAA of *N. meningitidis.*

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