PHLN
Public Health Laboratory Network

Invasive Meningococcal Disease|

Neisseria meningitidis

Laboratory case definition

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

Version	Status	Authorisation	Consensus Date
1.1	Update to new template and content to ensure gold standards of practice have been incorporated	PHLN	13 September 2024
1.0	Initial PHLN Laboratory Case Definition	PHLN	28 August 2008

1 PHLN summary laboratory definition

1.1. Condition

Invasive meningococcal disease

1.1.1. Definitive criteria

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

1.1.2. Suggestive criteria

- Detection of Gram-negative diplococci in Gram's stain of specimen from a normally sterile site or from suspicious skin lesion

2 Introduction

2.1. Epidemiology of meningococcal disease

Invasive meningococcal disease (IMD) is a rare but potentially life-threatening infection caused by the bacterium *Neisseria meningitidis*, a Gram-negative diplococcus. Disease peaks occur in infants and young children (0-4 years), and adolescents and young adults (15-24 years)(1). The epidemiology of IMD fluctuates, and additionally, variable *N. meningitidis* serogroup distribution is reported globally. Six *N. meningitidis* serogroups (A, B, C, W, X and Y) are responsible for most IMD cases. (2) In Europe, the Americas and Australia *N. meningitidis* B, W, Y, and C together account for a large majority of IMD. (3) Currently, meningococcal serogroups B and W cause most meningococcal disease in Australia. Meningococcal serogroup B (MenB) disease remains the most common cause of IMD in children, adolescents and young adults. Meningococcal disease caused by serogroups W and Y occurs over a more diverse age range (4) and may present with less typical clinical manifestations than disease due to other serogroups (5,6).

Several vaccines are available in Australia to reduce the risk of meningococcal disease. However, whilst no single vaccine protects against all serogroups, there are monovalent MenB and MenC vaccines and a quadrivalent MenACWY conjugate vaccine. Nationally vaccination recommendations include infants and adolescents, high risk groups and laboratory workers who frequently handle *N. meningitidis* and variably include MenACWY +/- MenB as detailed in the Australian Immunisation Handbook (7).

Globally, IMD 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. A seasonal peak in IMD incidence in temperate climates is usual with most cases occurring in late winter/early spring. The correlation between high rates IMD and crowded living and social conditions, smoking, and antecedent respiratory infections, particularly influenza is recognised, with clear correlation between influenza and IMD spikes reported in Australia and elsewhere. (8) Household contacts of meningococcal disease have a risk of acquiring infection approximately 600–1000 times the age-specific incidence in the general population. Important factors predisposing individuals to IMD include persistent complement component deficiencies (including the use of a complement component inhibitor e.g. eculizumab), functional or anatomic asplenia, persons with HIV, travel to regions where IMD is hyperendemic or epidemic, risk level exposure during an outbreak or exposure in a clinical or laboratory setting. Microbiologists who routinely work with isolates of *N. meningitidis* are also at risk.

2.2. Clinical manifestations of invasive meningococcal disease

N. meningitidis is an obligate commensal of humans normally colonising the mucosa of the upper respiratory tract. Carriage rates range from 1-40% varying with age, setting and population (9). Meningococci are transmitted via respiratory droplets or secretions from persons with asymptomatic colonisation or meningococcal disease. They attach to and multiply in the mucosal cells of the naso- and oropharynx and can penetrate the mucosal epithelial cells and enter the blood stream, where they can survive and multiply intravascularly (10).

The incubation period is typically 3 to 4 days, with a range of 1 to 10 days. Meningitis is the most common presentation of IMD (~50%) with symptoms typically including sudden onset of fever, headache, and stiff neck, often accompanied by nausea, vomiting, photophobia and altered mental status. *N. meningitidis* can be isolated from the blood in up to 75% of persons with meningitis (11.) Sepsis without meningitis occurs in about 30% of IMD, characterised by abrupt onset of fever; chills; cold hands and feet; severe aches or pain in the muscles, joints, chest; gastrointestinal symptoms including abdominal pain, vomiting and diarrhoea; and a petechial or purpuric rash, often associated with hypotension, shock, acute adrenal haemorrhage, and multiorgan failure. Meningococcal pneumonia is the most common presentation of IMD in adults over 65 years of age in the USA (11). Other IMD syndromes involving isolation of meningococci from normally sterile body sites, such as septic arthritis, are also reported. Meningococci also occasionally cause non-invasive infections such as

conjunctivitis or urethritis. Non-invasive illness due to meningococci is not nationally notifiable and the incidence is unclear, however the potential for progression to IMD is recognised.

The overall case-fatality ratio of IMD is 10% to 15%, even with appropriate antibiotic therapy and care, and can be higher in persons with meningococcal sepsis. As many as 20% of survivors have permanent sequelae, such as hearing loss, neurologic damage, or loss of a limb (11).

2.3. Relevant features of ***Neisseria meningitidis***

N. meningitidis is a human specific pathogen. Strains causing invasive disease almost always possess a polysaccharide capsule that 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

The laboratory diagnosis of IMD depends on the demonstration of *N. meningitidis*, or specific meningococcal DNA sequences in samples from normally sterile sites.

3.1. Microscopy

Visualisation of Gram-negative diplococci (GNDC) is sufficient for the presumptive diagnosis in sterile sites. The sensitivity of microscopy is dependent on the anatomical site, stage of the disease, intercurrent use of antibiotics and experience of the microscopist.

3.1.1. Cerebrospinal fluid specimens

Cytocentrifugation of cerebrospinal fluid (CSF) increases the sensitivity of microscopy. Classically CSF from meningococcal meningitis reveals a high neutrophil count, low glucose

and high protein content. On Gram-stained CSF meningococci can be observed both outside and within polymorphonuclear leukocytes. *N. meningitidis* may resist decolourisation on Gram-staining.

3.1.2. Test sensitivity

The sensitivity of the Gram's stain in CSF is estimated to be of the order of 65%.

3.1.3. Predictive value

A negative CSF examination does not exclude IMD, particularly if collected following administration of antibiotics.

3.1.4. Aspirates of skin lesions and joint fluid specimens

Gram-stains of aspirates from sterile sites provide supportive evidence of IMD in the presence of a clinically compatible illness.

3.1.5. Test sensitivity

Gram's stains of skin lesion aspirate or joint fluid 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 (12). Skin lesions occur in 50–75% of cases of IMD so that overall sensitivity in all cases of IMD is correspondingly lower. Gram-stains of skin biopsy may remain positive for long periods (about 48 hours) after antibiotic administration (due to reduced penetration of antibiotics into poorly perfused sites). This test is not commonly performed since the widespread availability of nucleic acid amplification tests such as polymerase chain reaction (PCR) assays.

3.1.6. Predictive value

Positive and negative predictive value 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 confirmation of IMD (13). Additionally, positive cultures allow for antimicrobial susceptibility testing (AST) to be performed. All isolates of meningococci from suspected cases of IMD should be referred to the appropriate jurisdictional National Neisseria Network (NNN) laboratory for confirmation of identification and AST. In cases where IMD is suspected clinically in an out of hospital setting, antibiotics may be given before transfer to hospital decreasing the likelihood of a positive culture, however diagnostic samples should still be collected for culture and PCR.

3.2.1. Media

Meningococci grow well on blood and chocolate agar. These media are suitable for culture from sterile sites. For culture from mucosal surfaces (e.g. pharyngeal sites) selective media such as Modified New York City or Modified Thayer Martin medium is required. Culture plates should be incubated at 35-37C in 3-7% CO2 in a moist atmosphere, and examined at 24, 48 and 72 hours. If no growth after 72 hours a negative result can be reported.

3.2.2. Suitable specimens

- Blood cultures, CSF
- +/- sterile site, rash aspirate, skin biopsy where appropriate

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, and pre-treatment with antibiotics. 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 given prior to collection (14).

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.

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 (12).

3.2.4. Test specificity

Approaches 100% if isolation of confirmed *N. meningitidis* from sterile site.

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 test acceptance criteria

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

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 QA programmes

For the Neisseria Reference Laboratories that form the NNN and provide data for the Australian Meningococcal Surveillance Programme (AMSP), a programme specific Quality Assurance (QA) panel is provided annually by the NNN Coordinating Laboratory in Sydney. A QA panel for *N. meningitidis* is not provided by the RCPA. Internationally the National External Quality Assessment Service of the United Kingdom has a *N. meningitidis* programme.

3.3. Identification of *Neisseria meningitidis*

After 48 hours colonies of *N. meningitidis* appear as smooth, entire edges, are ~1mm in diameter. They are grey, convex, glistening and occasionally mucoid. Grey-green discolouration of the blood agar may be evident. Presumptive identification can be based on Gram's stain and positive oxidase reaction. Identification of *Neisseria meningitidis* by matrix assisted laser desorption ionisation time of flight (MALDI TOF) mass spectrometry should be confirmed by an additional identification method. Commercial kits (e.g., API-NH) assess differential ability to oxidise glucose, maltose, sucrose, lactose (*N. meningitidis* catabolises glucose and maltose).

3.3.1. Presumptive identification

Growth of Gram-negative diplococci which are oxidase positive. Identification as *N. meningitidis* by MALDI-TOF.

3.3.2. Definitive identification

Acid production by oxidation of glucose and maltose (rapid carbohydrate utilisation test)

3.3.3. Predictive values

MALDI-TOF *N*. *meningitidis* 92% (95% CI, 75–98) specificity

3.3.4. Suitable test criteria

Refer to Manufacturer's instructions

3.3.5. Suitable internal controls

The NNN provides appropriate type culture *N. meningitidis* control strains.

3.3.6. Suitable validation criteria

Defined by the manufacturer of commercial kits or published method for in house tests (15).

3.3.7. External QC programmes

See above

3.4. Strain differentiation of *Neisseria meningitidis*

Once the identity of *N. meningitidis* is confirmed the serogroup (based on capsular polysaccharides) is determined. This is done by PCR based-methods (also known as genogrouping). Alternative serogrouping methods are less reliable.

3.5. Molecular Typing

Whole genome sequencing (WGS) of IMD isolates may be undertaken for the following reasons: to confirm or exclude a suspected outbreak based on epidemiological links established through public health investigations or to determine mechanisms of resistance and possible vaccine coverage for meningococcal serogroup B isolates.

3.5.1. Directed amplicon-based sequencing.

These methods are mostly implemented in the setting when an isolate is unavailable for whole genome sequencing.

Various gene targets have been identified for short term examination of possible outbreaks including the P*orA/porB* gene. Similarly, multilocus sequence typing (MLST) and corresponding clonal complex can assist in long-term invasive meningococci population studies.

It should be noted that 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.

3.5.2. External QC programmes

Local and international QAP programs are available.

3.6. Nucleic Acid Diagnosis

Nucleic acid amplification tests (NAAT), particularly PCR are important in the laboratory diagnosis of IMD. NAAT may increase the laboratory diagnosis of IMD by more than 30% and meningococcal DNA in CSF samples (16) has been detected up to 72 hours after

commencement of antimicrobial treatment (17). The target sequence most used for PCR based assays is the ctrA gene. Rapid diagnostic assays that identify *N. meningitidis* may not determine serogroup.

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, W and Y have been published (18) and these assays are widely performed in Australia.

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.

3.6.1. Suitable specimens

- CSF
- Blood, preferably in EDTA

3.6.2. Test sensitivity

>95% for CSF using *ctr*A gene sequence amplification (19) and approx. 87% when testing blood samples (17)(20).

3.6.3. Test Specificity

> 95% or more when using *ctr*A gene sequence amplification (14).

3.6.4. Predictive values

Positive predictive value of 98% and negative predictive value of 86% when testing CSF samples to the serogroup level (18). A negative test does not totally exclude IMD in a patient with compatible symptoms and signs.

3.6.5. Suitable test criteria

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

3.6.6. Suitable internal controls

Suitable controls are required for PCR based systems and may include positive, negative, internal and sample adequacy controls. Refer See the relevant NPAAC standards. (21)(22)

3.6.7. Suitable validation criteria

Assays should be validated in accordance with appropriate guidelines and NPAAC standards (21)(22).

3.6.8. Suitable quality assurance programmes

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

3.7. Antimicrobial Susceptibility testing

Antimicrobial susceptibility testing is performed in NNN laboratories for antibiotics used for treatment and chemoprophylaxis. The AMSP has reported longitudinal AMR data for IMD isolates since 1984 and currently reports ceftriaxone, penicillin, rifampicin and ciprofloxacin AST on IMD isolates for surveillance. Historically, resistance to the antibiotics used for meningococcal treatment and prophylaxis is uncommon in Australia. However, AMR surveillance is important as AMR is increasingly reported. The USA has recently reported that the number of IMD cases caused by penicillin and ciprofloxacin resistant *Neisseria meningitidis* strains has increased and, in some States, these account for over half of all reported IMD (23).

Disk testing is not recommended for *Neisseria species* and the NNN Laboratories determine the MIC values for meningococcal isolates using agar dilution or gradient strip diffusion tests (BioMerieux Etest) and report either CLSI or EUCAST guidelines (24).

4 Laboratory nomenclature for national data dictionary

SNOMED CT code	Concept name	Description
707225006	Invasive Meningococcal Disease	*Disorder*

5 References

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6 Glossary

AMR – Antimicrobial resistance

CLSI – Clinical and Laboratory Standards Institute

CSF – Cerebrospinal fluid

DNA – Deoxyribonucleic acid

EDTA – Ethylenediaminetetraacetic acid

EUCAST – European Committee on Antimicrobial Susceptibility Testing

In vitro – performed in a test tube, culture dish, or elsewhere outside a living organism

In vivo – performed or taking place in a living organism

MALDI-TOF – Matrix-assisted laser desorption ionization-time of flight

MLST – Multilocus sequence type/ing

NAAT – Nucleic acid amplification test/ing

NPAAC – National Pathology Accreditation Advisory Council

PCR – Polymerase chain reaction

QAP – Quality assurance program

QC – Quality control

RCPA – Royal College of Pathologists of Australasia

Serotype – Pathogens of the same species that are antigenically different

Strain – Variant that possesses unique and stable phenotypic characteristics

Test sensitivity – Ability of a test to correctly identify patients with a disease

Test specificity – Ability of a test to correctly identify people without the disease

WGS – Whole genome sequencing

WHO – World Health Organization

WHO CC – WHO Collaborating Centre