

Referral of pneumococcal PCR positive samples to Australian Pneumococcal Reference Laboratories for molecular serotyping

Public Health Laboratory Network (PHLN)

Enhanced Invasive Pneumococcal Disease Surveillance Working Group (EIPDSWG)

Purpose

This document has been developed to increase the overall serotype completeness for cases of invasive pneumococcal disease (IPD) nationally through the promotion and standardisation of referral practices for PCR positive samples in instances where isolate serotyping is not available. The targeted audiences are clinicians and laboratory professionals.

Background

The Public Health Laboratory Network (PHLN) and the Enhanced IPD Surveillance Working Group (EIPDSWG), a subcommittee of the Communicable Diseases Network Australia (CDNA) are collaborative groups that support the overarching Australian Health Protection Principal Committee (AHPPC) in its goal to provide national strategic direction, coordination and advice on health protection issues associated with communicable disease.

IPD has been a nationally notifiable disease since 2001. The IPD case definition includes the culture of *Streptococcus pneumoniae* (Spn) or detection by nucleic acid test (NAT) in blood, CSF or from another sterile site. In addition to the information provided by the diagnosing laboratory report as notified to the individual State and Territory public health units, other demographic, clinical, risk factor, vaccine and serotype information is obtained and collated nationally. Serotyping of isolates is performed by 4 reference laboratories using the conventional Quellung reaction and in most years around 90% Spn serotype completeness overall has been achieved in the national dataset. Serotype information is an important component for IPD control in regards to vaccine policy, impact of vaccination programs and future vaccine requirements.

Culture remains the preferred diagnostic procedure for serotyping and antibiotic susceptibility testing however in recent years there has been a gradual increase in IPD cases diagnosed by NAT only. While it is recognised that NAT detection is beneficial in improving overall case ascertainment, the reliance on direct PCR-based testing for Spn can reduce the proportion of culture-confirmed IPD notifications and potentially reduce overall serotyping and antimicrobial susceptibility information.

This potential reduction in serotype identification for those PCR-positive samples not cultured can now be largely overcome as 3 Australian pneumococcal reference laboratories have the capacity to perform culture-independent molecular serotyping. To ensure the most comprehensive serotyping data possible for cases of IPD, the PHLN and EIPDSWG have developed the following guideline for diagnostic laboratories covering the preferred methods of collection, storage and transportation of pneumococcal PCR positive samples.

Specimen collection for molecular serotyping

Specimens from any sterile body sites can be tested for presence of Spn using NAT. The most common specimen types are blood, CSF, pleural fluid and joint fluid but in practice Spn can be recovered from any sterile site of the body relevant to the clinical presentation. Clinical samples and/or DNA extracts tested positive by Spn specific PCR can be serotyped

using molecular methods. Molecular serotyping is recommended in all instances where conventional serotyping of an isolate by Quellung is not possible.

Storage and transportation of samples

Primary specimens should be refrigerated and forwarded to a reference laboratory as soon as possible with a cold pack in an ice box (regardless of specimen type) to be processed for DNA extraction.

Fresh DNA extracts can be sent at ambient temperature by mail. If transportation time is expected to exceed 48 hours, then the sample should be frozen at the collection site and sent with a cold pack when transport can be facilitated. DNA extracts initially stored by the diagnostic laboratory at -80°C or -20°C should be transported to a reference laboratory on dry ice or with an ice pack.

Referral

The submission form should include a laboratory name with its contact details as well as patient identifier, date of birth, sex, specimen reference number, source, date collected and clinical history, where available.

The order of preference for referred samples for molecular serotyping is:

- 1) extracted DNA,
- 2) primary specimen which is positive by PCR, antigen testing* or by Spn culture when viable bacteria are no longer available for typing.

*Antigen testing is not part of the current case definition but may be referred for typing where Spn has been isolated or detected by PCR in addition to antigen detection but the culture or sample is no longer available for referral.

Details of reference laboratories currently performing molecular serotyping:

Diagnostic laboratory origin	Recommended reference laboratory	Contact Details
NSW, ACT, WA(via Pathwest)	Centre for Infectious Diseases and Microbiology Laboratory Services, Westmead Hospital, Sydney, NSW	NSW Pneumococcal Reference Laboratory, CIDMLS, Level 3, ICPMR Building, Westmead Hospital, Westmead, NSW 2145 (02 9845 6255)
Vic, SA, Tas	Microbiological Diagnostic Unit Public Health Laboratory (MDU), University of Melbourne, Melbourne, Victoria	MDU Public Health Laboratory Department of Microbiology and Immunology at the Peter Doherty Institute; The University of Melbourne 792 Elizabeth Street Parkville VIC 3010 (03) 8344 5713
Qld, NT	Public Health Microbiology, Forensic and Scientific Services, Queensland Health, Brisbane, Queensland	Public Health Microbiology, Forensic and Scientific Services, 39 Kessels Road, Coopers Plains Queensland, 4018 (Ph 07 32749096)