

Haemophilus influenzae type b

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 *haemophilus influenzae type B*.

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1 PHLN Summary Laboratory Definition

1.1 Condition:

Haemophilus influenzae type b invasive disease.

1.1.1 Definitive criteria

- Isolation of *Haemophilus influenzae* type b by culture from a sterile site
- Detection of *Haemophilus influenzae* type b DNA by nucleic acid testing (NAT) from a sterile site.

1.1.2

- Detection of *Haemophilus influenzae* type b antigen in CSF by latex agglutination
- Detection of *Haemophilus influenzae* type b antigen in urine, in a child under 5 years old without another explanation for meningitis or epiglottitis.

2 Introduction

Haemophilus influenzae is a Gram negative coccobacillus that primarily colonises the upper respiratory tract. It may be further serotyped on the presence and nature of any extracellular polysaccharide capsule, with the six capsular types designated a through f, and those without a capsule being designated non-typeable (NTHi). *Haemophilus influenzae* type b (Hib) can cause a wide range of infections, including asymptomatic colonisation (i.e. carriage) of the upper respiratory tract or occasionally of genital tract, mucosal infections (e.g., otitis media, conjunctivitis, sinusitis) and invasive

infections, such as bacteraemia, meningitis, epiglottitis, septic arthritis.¹ Hib was the main cause of severe invasive disease, especially in young children, prior to the introduction of vaccination.

Humans are the only natural hosts for *H. influenzae*. The epidemiology of invasive Hib disease has been substantially altered in developed nations by the introduction of the Hib capsular polysaccharide vaccine in the early 1990s.^{2,3} In Australia, the rate of invasive Hib infection fell from 1.74 per 100,000 in 1993-94 to 0.08 per 100,000 in 2004-05 for all people and in New Zealand the rate fell from 41 per 100,000 to 1.65 per 100,000 in children under 5 years old between 1995 and 2009.^{2,3} However, Hib remains a serious problem in developing nations, causing an estimated worldwide 371,000 deaths in 2000.⁵

Hib is a frequent bacterial coloniser of the oropharynx (and more rarely the urogenital tract) in children and adults. Carriage rates for encapsulated strains have estimated between 3% and 10%, however, could reach 25% among household contacts of index cases. They are lowest in adults and infants and are highest in pre-school children. Vaccination has reduced but not eliminated the frequency of colonisation in both vaccinated and unvaccinated people.⁵ Hib is transmitted between people by respiratory droplets or contact with respiratory secretions. Although contagiousness of invasive Hib disease is limited, small outbreaks and direct secondary transmission of infection can occur, especially in young children. The progression from colonisation to invasive disease is not well understood and the incubation period is estimated at 2-4 days. Recent viral infection can facilitate colonisation and infection. Antibiotic prophylaxis of contacts and cases of invasive Hib reduces rates of colonisation and invasive disease.^{6,7}

2.1 Clinical presentation

2.1.1 Epiglottitis

Presentation is characterised by a progressively obstructed airway, frequently with significant drooling and effort to lean forward to maximise the airway aperture, along with fever and significant malaise.

2.1.2 Meningitis

Presentation is characterised by fever, headache and neck stiffness, frequently with systemic features. Altered consciousness is a late sign.

2.1.3 Other

Hib has been shown to cause pneumonia, primary bacteraemia, arthritis/osteomyelitis, buccal cellulitis and pericarditis.

2.2 Treatment

Hib remains susceptible to a variety of antibiotics including third generation cephalosporins and treatment reduces the morbidity and mortality of Hib disease.

2.3 Prevention

2.3.1 Prophylaxis

Administration of rifampicin is highly effective in eradicating the Hib carrier state and reducing invasive disease among contacts of Hib cases.^{6,7} Rifampicin should also be prescribed for primary cases, unless they were treated with ceftriaxone or cefotaxime, as other antibiotics are ineffective at eradicating the carrier state.⁸

2.3.2 Vaccine

A Hib-containing vaccine is recommended for all infants from 2 months of age. Polysaccharide polyribosylribitol phosphate (PrP) conjugated vaccines - require 3 primary doses, at 2, 4 and 6 months of age, followed by a booster dose at 12 months of age.⁹ Vaccination is also recommended for people with functional or anatomical asplenia and bone marrow transplant recipients.⁹ It has been suggested serotypes f and e are emerging after Hib vaccination as predominant *H. influenzae* clones.

3 Laboratory Diagnosis

3.1 Clinical specimens

Appropriate specimens for culture include blood and CSF, and rarely, joint aspirates, bone and pericardial fluid. Standard methods of processing these specimens will detect Hib. If processing is delayed, the specimen should be kept at 4°C. Appropriate specimens for nucleic acid detection include CSF and blood, while urine and CSF is appropriate for antigen detection.

3.2 Culture

Haemophilus influenzae is a relatively fastidious organism requiring either nutritious laboratory media (e.g., chocolate agar) or supplementation of agar with X (haemin or iron-containing pigment) and V (nicotinamide adenine dinucleotide, NAD) factors. On blood agar it may only grow as minute colonies or around colonies of another bacteria (satellite phenomenon), e.g. *Staphylococcus aureus* which produces haemolysin lysing the red blood cells, thereby liberating V factor. In the diagnosis of invasive disease, polymicrobial cultures are rare and generally differentiation on agar plates with bacitracin resistance, as is required in sputum culture, is unnecessary.

Identification of an isolate as *H. influenzae* is generally uncomplicated and in the majority of modern laboratories is accomplished via further work-up of individual colonies with typical morphology with use of matrix-assisted laser-desorption ionisation time-of-flight (MALDI-TOF) analysis and relatively few biochemical tests, such as X and V factor dependence. Identification of serotype b is reliant on further serological or nucleic acid based methods, outlined below.

3.2.1 Serotyping

Is performed on isolates of *H. influenzae* from normally sterile sites to determine if they are capsulated (a to f) or non-encapsulated (non-typeable).

3.2.1.1 Slide agglutination

Slide agglutination using sera that react against each of the six *H. influenzae* serotypes is the traditional method of determining serotype. The monoclonal antibodies will react only against those bacteria of a particular capsular subtype, generating an agglutination reaction that can be detected with the naked eye. In appropriate hands slide agglutination has a high specificity, however, the interpretation of serotyping can be sometimes subjective¹⁰ thus good quality assurance practices and the use of standardised antisera are important.

3.2.1.2 PCR based molecular serotyping

The PCR-based method for serotyping involves identification of the capsule transport gene *bexA* necessary for capsule formation, to differentiate encapsulated from non-encapsulated (non-typeable) strains, followed by a serotype-specific PCR, generally of region II, performed on pure culture. This method is highly sensitive and specific for the differentiation of Hib from other *H. influenzae* serotypes.¹⁰

3.2.1.3 Molecular subtyping

Multilocus sequence typing of seven house-keeping genes (*adk*, *atpG*, *frdB*, *fucK*, *mdh*, *pgi* and *recA*)¹¹ can be performed for molecular epidemiology and determination of clonal expansion, however, this method might not have sufficient discriminatory power to assist with outbreak investigations.

3.3 Nucleic acid detection from clinical specimens

Detection of specific nucleic acid in CSF and blood has been described for several targets including the capsule-specific *bexA*, the Hib-specific region II and the *H. influenzae* specific gene *fucK*. Assays have been developed in gel-based, real-time and loop-mediated isothermal amplification formats and in singleplex and multiplex formats.¹²⁻¹⁴ In general, the specificity of these assays approach 100% (if compared to other NAT formats), and the sensitivity is often greater than bacterial culture, probably due to detection of non-viable organisms/DNA fragments that can be present in CSF after antibiotic administration. Some assays targeting Hib-specific region II have the additional benefit of both detecting *H. influenzae* and serotyping Hib in the one assay. Recently recognised sequence diversity in *bexA* genes may lead to false-negative results. Appropriate negative and positive controls should be included in each run.¹⁵

3.4 Antigen detection

Commercial antigen detection using latex agglutination techniques have been applied successfully to CSF and urine. In urine, false positive tests for invasive disease can be caused by *H. influenzae* carriage and by recent vaccination, and the test should only be performed on patients with a high pre-test probability of invasive disease, i.e., children under 5 years with epiglottitis, meningitis or pneumonia. Urine should be boiled prior to testing as this increases specificity.¹⁶

In CSF, the sensitivity of antigen detection is over 90% and specificity approaches 100%.¹⁷ Antigen detection should only be performed in cases where meningitis is clinically suspected and supported by other biochemical tests.

4 Suitable External QC Programs

The RCPA QAP has modules within bacteriology and serotyping suitable for these tests. There is no suitable program for antigen and molecular detection.

5 Snomed-CT Terminology

SNOMED CT concept	Code
<i>Haemophilus influenzae</i> (organism)	44470000
<i>Haemophilus influenzae</i> type B (organism)	103442008
<i>Haemophilus influenzae</i> infection (disorder)	91428005
<i>Haemophilus influenzae</i> meningitis (disorder)	5900006
Sepsis due to <i>Haemophilus influenzae</i> (disorder)	447685007
Invasive <i>Haemophilus influenzae</i> disease (disorder)	406583002
<i>Haemophilus influenzae</i> antigen assay (procedure)	122340004

6 References

1. Gilbert GL, Johnson PDR, Clements DA (1995) Clinical manifestations and outcome of *Haemophilus influenzae* type b disease. *J Paediatr Child Health*, 31: 99-104.
2. Wang H, Deeks S, Glasswell A, McIntyre P (2008) Trends in invasive *Haemophilus influenzae* type b disease in Australia, 1995-2005. *Comm Dis Intell*, 32(3): 316-25.
3. Leung B, Taylor S, Drinkovic D, Roberts S, Carter P, Best E (2012) *Haemophilus influenzae* type b disease in Auckland children during the Hib vaccination era, 1995-2009. *New Zealand Med J*, 125(1365).

4. Jackson ML, Rose CE, Cohn A, Coronado F, Clark TA, Wenger JD, Bulkow L, Bruce MG, Messonnier NE, Hennessy TW (2012) Modeling insights into *Haemophilus influenzae* type b disease, transmission and vaccine programs. *Emerg Infect Dis*, 18(1): 13-20.
5. Watt JP, Wolfson LJ, O'Brien KL, Henkle E, Deloria-Knoll M, McCall N, Lee E, Levine OS, Hajjeh R, Mulholland K, Cherian T (2009) Burden of disease caused by *Haemophilus influenzae* type b in children younger than 5 years: global estimates. *The Lancet*, 374: 903-911.
6. Makintubee S, Istre GR, Ward JI (1987) Transmission of invasive *Haemophilus influenzae* type b disease in day care settings. *J Paed* 111(2): 180-6.
7. Gilbert GL, MacInnes S, Guise IA (1991) Rifampicin prophylaxis for throat carriage of *Haemophilus influenzae* type b in patients with invasive disease and their contacts. *BMJ*, 302: 1432-5.
8. [Ladhani S](#), [Neely F](#), [Heath PT](#), [Nazareth B](#), et al (2009) Recommendations for the prevention of secondary *Haemophilus influenzae* type b (Hib) disease. *J Infect* 58(1): 3-14.
9. Australian Immunisation Handbook, 10th Edition, accessed online September 18, 2015.
10. LaClaire LL, Tondella MLC, Beall DS, et al (2003) Identification of *Haemophilus influenzae* serotypes by standard slide agglutination serotyping and PCR-based capsule typing. *J Clinical Microbiol*, 41(1): 393-6.
11. Meats F, Feil EJ, Stringer S, et al. Characterization of encapsulated and non-encapsulated *Haemophilus influenzae* and determination of phylogenetic relationships by multilocus sequence typing. *J Clin Microbiol* 2003;41:1623-36.
12. Kim DW, Kilgore PE, Kim EJ, Kim SA, Anh DD, Seki M (2011) Loop-mediated isothermal amplification assay for detection of *Haemophilus influenzae* type b in cerebrospinal fluid. *J Clin Microbiol* 49(10): 3621-6.
13. Corless CE, Guiver M, Borrow R, Edwards-Jones V, Fox AJ, Kaczmarek EB (2001) Simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicaemia using real-time PCR. *J Clin Microbiol* 39(4): 1553-8.
14. Wang X, Theodore MJ, Mair R, Rujillo-Lopez E, et al (2011) Clinical validation of multiplex real-time PCR assays for detection of bacterial meningitis pathogens. *J Clin Microbiol*, 50(3): 702-8.
15. Zhou J, Law DK, Sill ML, Tsang RS. Nucleotide sequence diversity of the *bexA* gene in serotypable *Haemophilus influenzae* strains recovered from invasive disease patients in Canada. *J Clin Microbiol* 2007;45(6):1996-9.
16. [Saha SK](#), [Baqui AH](#), [El Areefin S](#), [Qazi S](#), [Billal DS](#), [Islam M](#), [Roy E](#), [Ruhulamin M](#), [Black RE](#), [Santosham M](#) (2006) Detection of antigenuria for diagnosis of invasive *Haemophilus influenzae* type b disease. *Ann Trop Paediatr*, 26(4): 329-36.
17. [Camargos PA](#), [Almeida MS](#), [Cardoso I](#), [Filho GL](#), [Filho DM](#), [Martins JI](#), [Batista KW](#), [Silva RC](#), [Antunes CM](#) (1995) Latex particle agglutination test in the diagnosis of *Haemophilus influenzae* type B, *Streptococcus pneumoniae* and *Neisseria meningitidis* A and C meningitis in infants and children. *J Clin Epidemiol*, 48(10): 1245-50.