
Preface

The species *Haemophilus influenzae* belongs to the family *Pasteurellaceae* that contains two other genera, *Actinobacillus* and *Pasteurella*. *H. influenzae* is a small Gram-negative pleomorphic rod that ranges in shape from coccobacillus to long filament. It has fastidious growth requirements, including X and V factors (hemin and NAD, respectively) for aerobic growth on artificial media. *H. influenzae* is classified as nontypeable (NTHi) or typeable, with six antigenically distinct serotypes (a–f) based on capsular polysaccharide type. Strain Egan is the archetype pathogenic strain and has a genome of approximately 2.1 Mb, whereas strain Rd (KW20) is avirulent with a 1.83 Mb genome. The genome of KW20 has been completely sequenced and contains 1738 genes.

Meningitis and epiglottitis are life-threatening manifestations of *H. influenzae* serotype b (Hib) infection, except in countries that have introduced Hib immunization programs. Capsular types a and c–f only occasionally cause invasive disease, and NTHi does so rarely. NTHi generally causes localized disease, including otitis media, sinusitis, conjunctivitis, and community-acquired pneumonia, and is implicated in exacerbations of chronic lung disease, as in chronic bronchitis, bronchiectasis, and cystic fibrosis. The species may also cause a variety of rare infections, including parapneumonic effusions and empyema, genitourinary sepsis, endocarditis, osteomyelitis, septic arthritis, obstetric and neonatal infections, and Brazilian Purpuric Fever. The burden of disease is difficult to define, but *H. influenzae* is the second leading bacterial cause of community-acquired pneumonia, possibly accounting for many thousands of deaths per year worldwide, especially of children in tropical countries. Future work on pathogenesis may lead to the identification of targets for novel vaccine and antibiotic development, ultimately culminating in the development of agents to prevent all *H. influenzae* disease rather than only that caused by Hib.

Historically, research on *H. influenzae* has produced groundbreaking contributions in the fields of bacterial genetics, molecular biology, pathogenesis, and vaccine development. In the 1950s, *H. influenzae* became the second example of a naturally transformable organism. In the 1960s, Hamilton Smith discovered type II restriction enzymes in *H. influenzae* while working on homologous recombination, and later received the 1988 Nobel Prize for

this work. In the 1980s, work on pathogenesis led to the development of the first highly successful conjugate vaccine, the Hib vaccine, which was introduced into many countries between 1989 and the early 1990s and led to the virtual eradication of invasive *H. influenzae* disease. Finally in 1995, *H. influenzae* strain KW20 (Rd) was the first free-living organism to have its genome completely sequenced (www.tigr.com). *H. influenzae* is a model organism with which to study pathogenesis because it is genetically malleable, even more so than *E. coli*.

Haemophilus influenzae Protocols is divided into 19 chapters, starting with two introductory chapters reviewing the pathogenesis of NTHi and Hib. The book brings together a critical mass of chapters covering the major molecular and immunological techniques relevant to pathogenesis research. Chapters 3 and 4 give basic methodology necessary for working with the organism, including culture and storage conditions, DNA extraction, and transformation. The reader is also referred to Barcak GJ et al. "Genetic systems in *Haemophilus influenzae*." (*Methods Enzymology*. 1991; 204: 321-342). Chapter 5 covers molecular diagnosis. For epidemiological typing, the reader is referred to: Herbert MA, Crook D, Moxon ER. Molecular methods for *Haemophilus influenzae*. In: Woodford N, Johnson A, eds. *Molecular Bacteriology: Protocols and Clinical Applications*. New Jersey: Humana Press. 1988. Chapter 15: 243-263. Chapters 6-13 cover the major molecular techniques that have been applied to *H. influenzae*, including plasmid analysis, proteomics, genomics, DNA array technology, gene expression techniques, mutagenesis, and structural analysis of macromolecules. Mutagenesis is divided into two chapters, a review covering transposon and non-transposon methods, and a chapter on Tn10 mutagenesis, one of a few transposons now available for near-random mutagenesis of *H. influenzae*. Chapters 14-16 cover antibody techniques, including IVIAT, ELISA, and opsonophagocytosis assays. In vivo expression systems, such as IVET and STM, are reviewed in the IVIAT chapter. Chapters 17-19 describe systems for exploring pathogenesis, both in vitro and in animal models. *Haemophilus influenzae* Protocols will be an invaluable laboratory resource for all those working with this pathogen, including both PhD students and those established researchers who are exploring a technique with which they are unfamiliar. The book may also be applicable to molecular and immunological studies of such human pathogens as *H. ducreyi*, and to such veterinary pathogens as *H. somnus*, *H. paragallinarum*, *H. parasuis*, and *Actinobacillus pleuropneumoniae*.

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