COMMISSION OF THE EUROPEAN COMMUNITIES

Programme on Community action on the prevention of AIDS
and certain other communicable diseases.
DG SANCO Agreement No. VS/1999/3504 (99CVF4-031).

EU-IBIS
European Union Invasive Bacterial Infections Surveillance Network

INVASIVE *HAEMOPHILUS INFLUENZAE* IN EUROPE - 2001

Project leaders: ***Dr Mary Ramsay and Dr Mary Slack***

Scientific Co-ordinator: ***Sarah Handford***
Health Protection Agency
Communicable Disease Surveillance Centre
61 Colindale Ave, London NW9 5EQ, UK
Tel. +44-20-8200-6868 Fax. +44-20-8200-7868
Email : sarah.handford@hpa.org.uk

June 2003

CONTENTS

SUMMARY

Introduction

Decision No. 2119/98/EC for setting up a network for the epidemiological surveillance and control of communicable diseases in the Community stated as a priority "Diseases prevented by vaccination". *H. influenzae* infection comes within this priority. Using the framework already established in a BIOMED II Hib surveillance project (1996-1999), the DG SANCO surveillance network project for invasive *H.influenzae* disease was established in all 15 EU countries and 3 non-EU countries in 2000. Funding for continuation of the network is currently held for October 2001- October 2003

Aims

- To improve the epidemiological information on invasive *Haemophilus influenzae* disease within the European Union.
- To improve the laboratory capacity to accurately characterise the isolates *of H. influenzae*.
- To form a focus for wider collaboration with non European Union countries and candidate European Union countries.

Methods

Agreed usage of a minimum dataset and a standardised case definition for *H. influenzae* has enabled valid comparisons to be made of the disease epidemiology within Europe, and hence assist the monitoring of epidemiological changes. Information collected on the surveillance systems and the vaccination programme(s) in use by each participant country has also aided interpretation of the epidemiological analyses.

Improvements in the laboratory capacity within the EU to accurately identify *H. influenzae* have been achieved through gaining information on systems in use by participants, by running a laboratory workshop for new members to the network, and by undertaking an External Quality Assurance Scheme (EQAS) with the participant reference laboratories. The EQAS helped identify any existing problems in correctly serotyping *H. influenzae* isolates, and enabled corrections/assistance in laboratory methods to be made, hence improving comparability of data between countries. The laboratory workshop run for new members ensures standardised methods are being used, adding further to correct identification of isolates within the EU.

Results and Conclusions

Prior to introduction of Hib vaccination programmes the epidemiology of invasive Hib disease differed between the EU countries, with incidence rates in children under five varying between 12 and 60 per 100,000. The only country in this collaboration with no vaccination programme has demonstrated incidence rates in the same range (17/100,000). All EU countries now have national immunisation programmes, and therefore the incidence in children under five years, the age group with the highest incidence pre-vaccine, is now very low. Countries are at different stages of vaccine implementation, have different vaccines and schedules and have achieved different levels of coverage. Despite all of these considerations, the incidence of Hib infection in the EU is much lower than in the pre-vaccine era (between 0 and 3.0 per 100,000).

Surveillance systems varied slightly amongst the participating countries. As most countries include all invasive Hib disease in children under fifteen years, comparison of rates in under fives and under fifteens can be made. Differences may be explained by many factors, including different methods of surveillance and completeness of ascertainment. One of the most important factors is the microbiological practice in relation to the diagnosis of Hib disease. This practice can impact on the establishment of disease burden and on comparisons between countries. The importance of continued improvement of laboratory techniques and laboratory-based surveillance cannot be over-emphasised.

Although the incidence has fallen in countries using vaccine, the clinical presentation of Hib disease has not changed. Meningitis remains the predominant diagnosis, causing over 54% of disease in under two year olds, with epiglottitis being the second most common diagnosis in pre-school children. Pneumonia and bacteraemia are more common presentations in adults. Apparent differences between countries may be explained by different age distributions of cases and the small numbers of cases.

Amongst children under five in the EU countries, the highest incidence rates in 2001 were in Ireland (1.1), Italy (0.87), Netherlands (0.80) and the UK (2.66). The rate in Ireland decreased slightly from 2000, and that in Italy decreased considerably (from 1.57). These two countries were amongst those reporting the lowest coverage in the previous project (funded under DGXII).

However, the UK has seen a steady increase in the incidence rate in under 5 year olds from 1999 (0.94) to 2000 (1.80), and to 2001 (2.66). One of the major differences between the UK and Ireland and the remaining EU countries is the absence of a booster (third or fourth dose) in the second year of life. Although Ireland has a comparatively high rate, no obvious trend has been observed in 2001. Rates between years in each participant country vary due to small numbers, but the increase observed in the UK, one of the largest populations under surveillance, was responsible for an overall increase in incidence in the EU in 2001.

Changes in vaccination programmes have occurred over time and may be responsible for changes in incidence observed. It is unlikely that the increase observed so far can be explained by changes in the vaccine, and if a change in vaccine was implicated, we would expect to see increases in many EU countries. The importance of continued observation over the whole of the EU is therefore essential to ensure that such changes can be detected at the earliest possible stage.

Rates of non-b capsulated *H. influenzae* infection are low and no evidence of serotype replacement has been observed despite many years of vaccination in many of the EU countries. Rates of non-capsulate infection are now similar to those for type b and emphasises the importance of ensuring accurate identification of the organism in a national reference centre. The low rates observed in some countries, probably reflects the low proportion of strains that are referred and highlights the potential for improving ascertainment of such cases.

1. INTRODUCTION

Decision No. 2119/98/EC for setting up a network for the epidemiological surveillance and control of communicable diseases in the Community stated as a priority "Diseases prevented by vaccination". *H. influenzae* infection comes within this priority.

The BIOMED II Hib surveillance project in 9 EU countries and 2 non EU countries (1996-99) was established to describe the epidemiology of invasive *Haemophilus influenzae* and describe the risk factors associated with vaccine failure using different vaccines and schedules. Using the framework already established in the above project, a DG SANCO surveillance network project for invasive *H.influenzae* disease was established in all 15 EU countries and 5 non-EU countries in 2000 to improve epidemiological information and laboratory capacity to characterise isolates of these two invasive bacterial infections. This report is on the cases of invasive *Haemophilus influenzae* reported in 1999-2001

Aims

To improve the epidemiological information on invasive *Haemophilus influenzae* disease within the European Union.
To improve the laboratory capacity to accurately characterise the isolates *of H. influenzae*.
To evaluate the impact of vaccination with conjugate vaccines on the epidemiology of *H. influenzae*.
To compare the impact of vaccination with conjugate vaccines produced by different manufacturers and according to different schedules.
To form a focus for wider collaboration with non European Union countries and candidate European Union countries.

A European Union network for the surveillance of *Haemophilus influenzae* is important for the following aspects within the Community: pooling of case data; pooling of vaccine failure data; rapid alert of changes in the epidemiology of infection strains; setting standards. The collection of data at European level will be available to member states to inform policy development within each country. This may therefore contribute to the harmonisation of European Hib vaccine policy and schedules.

As *Haemophilus influenzae* disease in a vaccinated community is rare, this project allows pooling of such data to increase the power of any epidemiological analysis. Hib vaccine has been demonstrated to reduce nasopharyngeal carriage of Hib and it has been postulated that one consequence of reduced exposure to this organism could be the early waning of vaccine induced immunity. In addition, the potential emergence of non-vaccine preventable strains of *H. influenzae* has been suggested. European wide analysis should be able to detect an increase in cases of Hib in older children or adults, or an increase in the incidence of non-b *Haemophilus influenzae* at an earlier stage than analysis of a single country's data. In addition, by pooling data from all countries, the populations under surveillance will become sufficient to provide more precise estimates of vaccine efficacy and will be composed of a wide variety of ethnic groups. These estimates based on pooled data may be able to assess the potential decline in vaccine efficacy with age or in certain groups.

Hib disease in vaccinated children is extremely rare. Pooling of data on vaccine failures at European level is the only reliable means of describing potential risk factors specific to certain social situations or ethnic groups, and collection of data at a European level will also increase the ethnic and social diversity of the population under surveillance.

An established network is needed for the rapid dissemination of changes in the epidemiology of an infection which may have public health significance. In addition, it will facilitate the rapid exchange of information on imported strains *of H. influenzae* infections.

This project, which has included all 15 EU countries, Iceland and Norway, and 3 countries from outside the EU, will be able to set standards for the epidemiological surveillance of *H. influenzae* and for methods used in reference laboratories. Countries will be able to learn from models of good practice in other member states and these standards can also be applied in other countries, especially candidate EU and non-EU countries. In addition, establishment of this network may facilitate early dissemination of advances in therapy and in public health control measures and lead to the harmonisation of guidance on meningococcal disease. This project will also provide a model and focus for future research and public health collaborations, for example the evaluation of other new vaccines such as conjugate pneumococcal vaccines.

In this report a summary is given of the up-to-date epidemiological information gained by collecting and analysing *H. influenzae* disease case data from the network participants for 2001, with use of data from earlier years to make comparative comment. This displays the ability of the now established system to monitor changes in the epidemiology of the disease.

Finally, this project will provide substantial and up-to-date epidemiological information from which *H. influenzae* vaccination policy can be developed within individual countries introducing vaccination programmes, and help the development of guidance on prevention and control of meningococcal infection. It may also facilitate the eventual harmonisation of vaccine schedules in the European Union.

2. METHODS

Questionnaires on the surveillance system(s) and the laboratory diagnostic methods were sent to all new participant countries, and updates gained from countries already established as members of the network. The information from both these questionnaires is important for correct interpretation of the data which is gained from each individual country. A vaccination programme questionnaire was also administered to each new participating country, and updates obtained, where necessary, from existing members.

A minimum data set was received from the majority of countries for 2001. The minimum data set includes age, sex, date of onset, method of confirmation, site of identification, grouping, typing and subtyping results (as appropriate) (Refer Appendix 2). These datasets were in most cases electronically transferred to PHLS Communicable Disease Surveillance Centre, where they were entered onto the main Access database. In some instances paper listings of cases were received. The standardised case definitions developed as part of the DG XII project are used, and where surveillance is performed using other definitions, datasets are re-coded to provide comparable data for all participating countries.

Descriptive epidemiology is analysed using standard statistical packages on the minimum data set. Analysis of age-specific incidence rates, temporal trends and diversity of *H. influenzae* infections are compared. In countries with vaccination programmes, coverage data will also be requested and comparison of rates of infection in both vaccinated and unvaccinated cohorts will be interpreted in conjunction with coverage, schedule and vaccine used, since implementation and method of introduction

A central resource was provided in the UK to genotype *H. influenzae* strains from countries with established Hib vaccination programmes. Protocols for PCR genotyping were supplied by the PHLS Haemophilus Reference Unit, Oxford, UK, for laboratories wishing to establish their own system for genotyping strains of *H.influenzae*. For those countries not wishing to establish or use this method the Oxford laboratory offered to genotype any strains isolated from vaccine failure cases.

Dissemination of results from the surveillance of invasive *H. influenzae* disease in the EU occurs through annual reports to the network participants of the epidemiological data analyses, and presentation of results at meetings and scientific conferences. Feedback reports are given to microbiologist network participants when External Quality Assurance Schemes (EQAS) are undertaken.

A presentation on the epidemiology of *H. influenzae* type b in the EU countries was made at a international conference/workshop in Phoenix, Arizona in September, 2002.

3. RESULTS

Disaggregated data for 2001 was supplied by 15 countries in the network: Czech Republic, Denmark, England & Wales, Finland, Germany, Greece, Iceland Ireland, Italy, Netherlands, Norway, Portugal, Israel, and Australia. No disaggregated data was supplied by Austria, Belgium, France, Luxembourg, Spain, and Sweden.

3.1 Questionnaire surveys

3.1.1 Surveillance systems

3.1.1.1 Objectives

For countries with vaccination programmes, the objective of the surveillance was to monitor the impact of vaccination by universal case ascertainment of invasive Hib disease. In Portugal the additional objective was to monitor antibiotic resistance in cases of *Haemophilus influenzae* infection. In the Czech Republic, prior to the introduction of a vaccination programme in July 2001, the principal objective was the assesssment of the disease burden to inform decisions about the introduction of Hib vaccine.

3.1.1.2 Case definitions

The case definition used in each country, except Denmark and Finland, included all cases of invasive Hib disease with isolates from a sterile site. Denmark limited surveillance to meningitis. In Finland the case definition of 'invasive infection' for *H. influenzae* disease consists of blood and CSF isolations, but not isolations from other usually sterile sites.

Antigenic diagnosis was included in the case definitions used by Australia, Czech Republic, Finland and Italy (although some other countries reported such cases to the European data set). Australia was the only country to accept a clinical, non-microbiological diagnosis of epiglottitis (although these were not included in the study data set).

Data on other serotypes was also collected in Finland, Germany, Greece, Iceland, Ireland, Italy, Sweden, the Netherlands, Norway and the UK (England & Wales).

3.1.1.3 Population under surveillance

All participant countries, except Germany, Greece, Sweden, Israel and Austria, had a surveillance system across all ages. In Austria, Sweden, Germany, and Israel, cases were only reported in the paediatric population. In Greece (Attiki) surveillance was limited to to paediatric population (under 15 years) in a single region and in Italy enhanced surveillance was performed in seven regions.

3.1.2 Hib vaccination programmes

The details of the type of vaccines used and the immunisation schedules in the ongoing programmes are given below (Table 1). There is considerable variation between countries in the vaccines and schedules used. As well as countries concurrently using more than one vaccine type, the type(s) and schedules being used by a country has changed over time with the continual emergence of new Hib vaccines from the range of manufacturers. Also, a high proportion of the Hib vaccines used are now combination vaccines; possible components being DTaP, DTwP, IPV, or Hepatitis B.

Table 1 : Type of Hib conjugate vaccine and immunisation schedule used in the study participant countries

Country/region	Type of vaccine	Combined with	Immunisation schedule
Australia	**Pre 2001**		
	HbOC (95%)		2, 4, 6, 18 months
	PRP-OMP (5%)		2, 4, 12 months
	2001:		
5 states/territories	Pedvax Hib	DTPa, HepB, OPV	2, 4, 12 months
3 states	PRP-OMP (Comvax)	Hep B	2, 4, 12 months
Austria	**2000**		
	Infanrix + Hib (SKB)	DTaP	3, 4, 5 months & 2nd year of life
	Infanrix-IPV+Hib (SKB)	DTaP, IPV	3, 4, 5 months & 2nd life of life
	Procomvax (Aventis Pasteur MSD)	HBV	3, 4, 5 months & 2nd year of life
	2001		
	As in 2000,		
	plus Hexavac (DTaP-Hib-IPV-HBV)	DTaP, IPV, HBV	3, 4, 5, months and 2nd year of life
	2002		
	Infanrix + Hib (SKB)	DTaP, IPV	3, 4, 5, months and 2nd year of life
	Procomvax	HBV	3, 4, 5, months and 2nd year of life
	Hexavac (Aventis Pasteur MSD)	DTaP, IPV, HBV	3, 4, 5, months and 2nd year of life
	2003		
	As in 2002		
Belgium	**2003**		
	Hib-PRP-T (Hiberix & Act-Hib)	Not combined	2,3,4 months & 13-18 months
	Hib-HBOc	Not combined	2,3,4, months & 13-18 months
Czech Republic	**Jul 2001**		
	Hib-PRP-T (TETRACTHIB) – children <1 year	DTwP,	2,3,4 months & 18-20 months
Denmark	**1/6 1993 –1995**		
	PRP-T (Act-HIB Pasteu Merieux)	Not combined	5, 6, 16 months
	1996		
	PRP-T (Act-HIB Pasteur Merieux)	Not combined	5, 6, 15 months
	1997-2002		
	PRP-T (Act-HIB Pasteur Merieux)	Not combined	3, 5, 12 months
	1/7 2002		
	PRP-T (Act-HIB Pasteur Merieux)	DTaP-IPV/HIB	3, 5, 12 months

Table 1 : Type of Hib conjugate vaccine and immunisation schedule used in the study participant countries (continued)

Country/region	Type of vaccine	Combined with	Immunisation schedule
Finland	**1993 Act-Hib** (Pasteur Merieux)	Combined with Finnish DTwP	4, 6 & 14-18 months
	1994- Sep 2002 HibTITER (Wyeth-Lederle)	Not combined	
	Oct. 2002 Hiberix (GSK)	Not combined	
France	**1993**		
	Hib PRP-T	DTwP, IPV	Pentacoq • 2, 3, 4, 18 months Pent hibest • 2, 3, 4, 18 months
	1998		
	Hib PRP-T	DTwP, DTaP, IPV	Pentacoq • 2, 3, 4, 18 months Pent hibest • 2, 3, 4, 18 months Pentavac • 18 months Infanrix Polio Hib • 18 months
	2002 :		
	Hib-PRP-T	DTwP, DTaP, IPV	Pentacoq • 2, 3, 4, 18 months Pentavac • 2, 3, 4, 18 months Infanrix Polio Hib • 2, 3, 4, 18 months
	2003 : (not yet but should start this year) Hib-PRP-T	DTwP, DTaP, IPV, Hep B	Hexavac 2, 4, 18 months (vaccine with 5 antigens at 3 months of age)
Germany	**Since 1992/1993**		
	Hib-PRP-T-CRM$_{197}$		
(In 2001, all the vaccines listed were available and in use)	Hib-PRP-T	Not combined	2, 4 months, plus 11-14 months
	Since 1996		
	Hib-PRP OMPC	Not combined	2, 4 months, plus 11-14 months
	Hib-PRP-T	DTaP	2, 3, 4 months, plus 11-14 months*

	Since 1997/1998 Hib-PRP-T **Since 1999** Hib PRP-OMPC **Since 2000/2001**	DTaP-IPV Hep B DtaP-IPV-HBV	2, 3, 4 months, plus 11-14 months* 2, 4 months, plus 11-14 months 2, 3, 4 months, plus 11-14 months*
Country/region	**Type of vaccine**	**Combined with**	**Immunisation schedule**
Germany cont'd	Hib-PRP-T		* Given at least 4 weeks apart with a min. of 6 months between last dose (11-14 mth dose) and previous dose
Greece	**1999** PRP-T HbOC **2003** PRP-T (Act-Hib, Hiberix) HbOC (Hibtiter) HibOMP (Procomvax)	 DTaP, IPV DtaP, IPV, Hep B Not combined Hep B	 2, 4, 6, 18 months 2, 4, 6, 15-18 months 2, 4, 6, 15-18 months On special occassions, by case
Iceland	PRP-D ProHIBit **Jan 2000 onwards** PRP-T (Pentavac)	 DTaP, IPV	3, 4, 6, 14 months 3, 5, 12 months
Ireland	**Pre August 2001** PRP-T (ACTHib or HibTITRE(60%), Hiberix(30%) **Post August 2001** PRP-T (Pentavac) (100%) **2002** PRP-T (Infanrix) (70%) PRP-T (Pantavac) (30%)	 DTaP, IPV DTaP, IPV DTaP, IPV	 2, 4, 6 months 2, 4, 6 months 2, 4, 6 months 2, 4, 6 months
Israel	**1994-1997** PRP-OMP (90%) HbOC/PRP-T **Jul 1997 onwards** PRP-T 1999 PRP-T HboC	 DTwP DTwP	 2, 4, 12 months 2, 4, 6, 12 months 2, 4, 6, 12 months

Country/region	Type of vaccine	Combined with	Immunisation schedule
Italy	**1995-March 1999**		For all vaccines :
	PRP-T	Not combined	<6 months (3 doses + booster)
	HbOC	Not combined	6-12 months (2 doses + booster)
			>12 months (1 dose)
	April 1999 onwards		
	PRP-T	Not combined	For all vaccines :
	HbOC (not available in 1997)	Not combined	3, 5, 11-12 months
	PRP-T (since 1999)	DTaP, IPV	
	OMP (since 2000)	HepB	
	PRP-T (since 2001)	DTaP, IPV, HepB	
Netherlands	PRP-T	DTP, IPV (in other limb)	3, 4, 5, 11 months
	1999 PRP-T		2, 3, 4, 11 months
	Since 2003		
	Hib-PRP-T	DTwP, IPV	2, 3, 4, 11 months
Norway	**2001 onwards**		
	PRP-T (100%) (Infanrix-Polio+Hib)	DTaP, IPV	3, 5, 11-12 months
Portugal	*Not yet available*		
Spain	**2002**		
	Hib-PRP-T (Hiberix, ACT-Hib)	Not combined	2, 4, 6, 15-18 months
	Hib-PRP-T (Infanrix-Hib)	DTaP	2, 4, 6, 15-18 months
	Hib-PRP-T (TETRACT-Hib)	DTwP	2, 4, 6, 15-18 months
	Hib-PRP-T (PENTACT-Hib)	DTwP, IPV	2, 4, 6, 15-18 months
	CRM-197 (HibTitre)	Not combined	2, 4, 6, 15-18 months
	Note : Infanrix-Hib-IPV & Infanrix-Hexa - Hexavac are sold in pharmacies, but are not included in the official vaccination schedule.		

Country/region	Type of vaccine	Combined with	Immunisation schedule
Sweden	**1992-1993**		
	PRP-OMP (PedvaxHIB) or PRP-T (Act-Hib)	DT separately, IPV separately or mixed with PRP-T	3, 5, 12 months
	1993-1995		
	PRP-T (Act-Hib)	As above	3, 5 12 months
	1996-1997	DTaP separately,	3, 5, 12 months
	PRP-T (Act-Hib)	IPV separately or mixed with PRP-T	
	1998-1999		
	PRP-T (Act-Hib or Pentavac)	As above or in 5-valent combination vaccine	3, 5, 12 months
	1999		
	PRP-T (Pentavac or Infanrix-Polio+Hib)	In 5-valent combination vaccines	3, 5, 12 months
United Kingdom	**Pre 1996**		
	HBOC (Hib only)		2, 3, 4 months
	PRP-T (Hib only)		2, 3, 4 months
	Since 1996		
	DTwP/PRP-T (some DTaP used in 2000)	DTwP	2, 3, 4 months

3.1.2 Laboratory questionnaire

Information on laboratory methods was previously supplied by nineteen countries: Australia (Melbourne and Sydney) Austria, Belgium,Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Israel, Italy, Luxembourg, Netherlands, Portugal, Spain, Sweden and the UK).

3.1.2.1 Laboratory Hib identification and reference facilities.

All countries , except Greece, had reference laboratory facilities for *Haemophilus influenzae.* All countries had primary identification in over 80% of laboratories, except Greece where such facilities were available in only 50-80% of laboratories.The proportion of laboratories that referred isolates of *Haemophilus influenzae* to the reference laboratory ranged from <20% in Belgium and Sweden to 100% in Australia, Austria, Finland, Iceland, Ireland, Luxembourg and UK.

In most countries those hospital laboratories that could identify *H.influenzae* would normally test all specimens from cases of suspected bacterial meningitis and all blood cultures for *H.influenzae.* In Luxembourg 80-100% of hospital laboratories had facilities to identify *H.influenzae* and would only test specimens from cases of meningitis. In this country, only CSF isolates of *H.influenzae* were referred to the reference laboratories. In Sweden only strains from possible vaccine failure cases would be referred to the reference laboratory. Some hospital laboratories would look for *H.influenzae* in sterile site specimens such as joint aspirates.

3.1.2.2 Specimen transport, receipt and storage

All reference laboratories subcultured the strains immediately on receipt and either tested them immediately or in batches. All the media used to transport the strains to the reference laboratory and to subculture the strains were able to sustain the growth of *H. influenzae*. All but one of the laboratories (Greece) could store the strains long term at –80C.

3.1.2.3 Identification Methods, serotyping and genotyping

There were some minor differences in the identification methods used. The type of blood used in the preparation of blood and chocolate agar plates varied. In most countries that responded to the question either horse or sheep blood was used. In two countries (Austria and Greece) human blood was used. This is not to be recommended since human blood may be inhibitory to the growth of *H.influenzae.*

With the exception of Austria, all reference laboratories serotyped strains of *H.influenzae.* Austria stated that they planned to introduce serotyping shortly. The main difference in the identification methods used by the laboratories related to the ability to genotypically confirm vaccine failures as *H.influenzae* type b. Genotyping facilities were available in Australia, France,Italy and UK. The strains from vaccine failure cases in Ireland are sent to the PHLS Haemophilus Reference Unit in Oxford (UK).

3.1.2.4 Access to laboratory methods

A central resource was provided in the UK to genotype strains from countries with established Hib vaccination programmes. Protocols for PCR genotyping were supplied by the PHLS Haemophilus Reference Unit, Oxford, UK, for laboratories wishing to establish their own system for genotyping strains of *H.influenzae.* For those countries not wishing to establish or use this method the Oxford laboratory offered to genotype any strains isolated from vaccine failure cases.

3.2 Laboratory External Quality Assurance Scheme (EQAS) for *Haemophilus influenzae*

Strain Number	Intended Result	Lab Number 1	Lab Number 2	Lab Number 3	LAB NUMBER 4	Lab Number 5
19	Hib biotype I ß-lactamase +ve AMP R CHLOR,CRO,TRIM,RIF, TET S	Hib biotype I	Hib biotype I ß-lactamase +ve AMP R CTX,TET,CHLOR S	Hib biotype I ß-lactamase +ve AMP R	Hib biotype I ß-lactamase +ve AMP R CTX,CHLOR,TET, CMX S	Hib biotype I ßlactamase –ve P , AMP R
25	Hi non-typable Biotype IV ß-lactamase –ve AMP,CHLOR,CRO,TRIM, RIF,TET S	Hi non-typable Biotype IV Weak agglutination with type d Would check with PCR	Hid ß-lactamase –ve AMP,CTX,TET,CHLOR S	Hi ? a,b,d biotype IV AMP S a+ b+ d+ unusual agglutination in 3 antisera	Hi non-typable Biotype IV ß- lactamase –ve AMP,CTX,CHLOR,TET, CMX S	Hia (?result tippexed over?) Biotype I ß- lactamase –ve P I, AMP S
28	Hi non-typable Biotype II ß-lactamase –ve AMP.CHLOR,CRO,RIF, TET S TRIM R	Hi non-typable biotype II	Hif ß-lactamase –ve **AMP R therefore also CO-AM, cephalosporin R** TET,CHLOR S	Hi non-typable Biotype II AMP S	Hi non-typable Biotype II ß-lactamase –ve AMP,CTX,CHLOR, TET S CMX R	Hic Biotype II ? -lactamase –ve P R, AMP S
32	Hie Biotype IV ß-lactamase –ve AMP,CHLOR,CRO,TRIM, RIF, TET S	Hie Biotype IV	Hi non-typable ß-lactamase –ve PV+ve a, b, e+ve determine serotype by PCR AMP,CTX,CHLOR,TET S	Hie Biotype IV AMP S	Hie biotype IV ß-lactamase –ve AMP,CTX,CHLOR,TET, CMX S	Hie Biotype IV ? -lactamase –ve P I, AMP S
33	Hi non-typable biotype III weak PV +ve, a,b,c,d,e,f - ve, ß-lactamase –ve AMP,CRO,CHLOR,TET S RIF R, TRIM R	Hi non-typable Biotype III	Hi non-typable ß-lactamase –ve, PV+ve, d e +/- Determine serotype by PCR AMP,CTX,TET,CHLOR S	Hib Biotype III SXT R, AMP S Discrete autoagglutination	Hi non-typable Biotype III ß-lactamase –ve AMP,CTX,CHLOR, TET S CMX R	Hib III ? -lactamase –ve P R, AMP I

Strain Number	Intended Result	Lab Number 1	Lab Number 2	Lab Number 3	Lab Number 4	Lab number 5
34	H.paraphrophilus AMP,CHLOR,CRO,TRIM, RIF, TET S	H.paraphrophilis	H ? autoagglutination PV+ve ß-lactamase _ve determine serotype by PCR AMP,CTX,CHLOR, TET S	H.parainfluenzae ?atypical biotype ODC ++, urease ++, indole ++	Non H.influenzae ß-lactamase –ve needs RapIDNH AMP,CTX,CHLOR,TET CMX S	H.aphrophilus P S, AMP S
Strain Number	**Intended Result**	**Lab Number 6**	**Lab Number 7**	**Lab Number 8**	**Lab Number 9**	**Lab number 10**
19	Hib biotype I ß-lactamase +ve AMP R CHLOR,CRO,TRIM,RIF, TET S	Hib	Hib Biotype I ß-lactamase +ve	Hib biotype I	Hib ß-lactamase +ve AMP R CRO S CMX S	Hib ß-lactamase +ve AMP R
25	Hi non-typable Biotype IV ß-lactamase –ve AMP,CHLOR,CRO,TRIM , RIF,TET S	Hi not capsulated	Hi non-typable Biotype IV Antibiotic susceptible strain	Hid (by serum agglutination) Biotype IV Negative by PCR	Hi non-typable ß-lactamase –ve AMP ,CRO, CMX S	Hi Non-encapsulated AMP S
28	Hi non-typable Biotype II ß-lactamase –ve AMP.CHLOR,CRO,RIF,T ET S TRIM R	Hi not capsulated	Hi non-typable Biotype II AMP less susceptible- needs MIC	Hi non-typable biotype I	Hi non-typable ß-lactamase –ve AMP ,CRO S CMX I	Hi non-encapsulated AMP S
32	Hie Biotype IV ß-lactamase –ve AMP,CHLOR,CRO,TRIM ,RIF, TET S	Hie	Hie Biotype IV Antibiotic susceptible	Hie Biotype IV	Hie ß-lactamase –ve AMP ,CRO, CMX S	Hi Non-encapsulated Autoagglutinating ß-lactamase +ve AMP R
33	Hi non-typable biotype III weak PV +ve, a,b,c,d,e,f - ve, ß-lactamase –ve	Hi not capsulated	Hi non-typable Biotype III RIF,CMX R AMP less susceptible- needs MICI	Hi non-typable Biotype II	Hi non-typable ß-lactamase –ve AMP ,CRO S CMX R	Hib ? -lactamase +ve AMP R

	AMP,CRO,CHLOR,TET S RIF, TRIM R					
34	H.paraphrophilus AMP,CHLOR,CRO,TRIM ,RIF, TET S	Not Hi	H.parainfluenzae ONPG +	H.parainfluenzae	H.aphrophilus ß-lactamase –ve AMP,CRO,CMX S	H.parainfluenzae AMP S
Strain Number	**Intended Result**	**Lab Number 11**	**Lab Number 12**	**Lab Number 13**	**Lab Number 14**	
19	Hib biotype I ß-lactamase +ve AMP R CHLOR,CRO,TRIM,RIF, TET S	Hib biotypeI ß-lactamase +ve AMP R , CO-AM , CRO, CXM R AZT,IM,CHLOR,RIF, TET,CLAR,CIP, CMX S	Hib Biotype I ß-lactamase +ve	Hib Biotype I ß-lactamase +ve AMP R COAM,CTX,CEC,CXM,TET,CHLOR, CMX, RIF,CIP,AZT S	Hib Biotype I ß-lactamase +ve AMP R COAM,CXM,CEF,CTX CMX,CHLOR,RIF,CIP S	
25	Hi non-typable Biotype IV ß-lactamase –ve AMP,CHLOR,CRO,TRIM, RIF,TET S	Hid Biotype IV AMP,COAM,CRO,CXM,AZT,IM,CHLOR,RIF, TET,CLAR,CIP,CMX S	Hi non-typable Biotype IV	Hi non-typable Biotype IV ß-lactamase –ve AMP COAM,CTX,CEC,CXM,TET,CHLOR, CMX, RIF,CIP,AZT S	Hi non-typable Biotype IV ß-lactamse –ve AMP , COAM,CXM,CEF,CTX CMX,CHLOR,RIF,CIP S	
28	Hi non-typable Biotype II ß-lactamase –ve AMP.CHLOR,CRO,RIF, TET S TRIM R	Hi non-typable Biotype II AMP,COAM,CRO,CXM,AZT,IM,CHLOR,RIF, TET S ,CLAR I ,CIP S ,CMX R	Hi non-typable Biotype II	Hi non-typable Biotype II ? -lactamase –ve AMP COAM,CTX,CEC,CXM,TET,CHLOR, RIF,CIP,AZT S CMX R	Hi non-typable Biotype II ß-lactamase –ve AMP , COAM,CXM,CEF,CTX CMX,CHLOR,RIF,CIP S	
32	Hie Biotype IV ß-lactamase –ve AMP,CHLOR,CRO,TRIM,RIF, TET S	Hie Biotype IV AMP,COAM,CRO,CXM,AZT,IM,CHLOR,RIF, TET,CLAR,CIP,CMX S	Hie Biotype IV	Hie Biotype IV ß-lactamase –ve AMP, COAM,CTX,CEC,CXM,TET,CHLOR, CMX, RIF,CIP,AZT S	Hie Biotype IV ß-lactamase –ve AMP , CO-AM,CXM,CEF,CTX CMX,CHLOR,RIF,CIP S	

33	Hi non-typable biotype III weak PV +ve, a,b,c,d,e,f -ve, ß-lactamase –ve AMP,CRO,CHLOR,T ET S RIF, TRIM R	Hi non-typable Biotype III AMP,COAM,CRO,CX M,AZT,IM,CHLOR TET,CLAR,CIP S,CMX R RIF R	**HI NON-TYPABLE** **BIOTYPE III**	Hi non-typable Biotype III ß-lactamase –ve AMP, COAM,CTX, ,CXM,TET,CHLOR,CIP, AZT S CEC R RIF,CMX R possible BLNAR	Hi non-typable biotype III ß-lactamase –ve AMP , CO-AM,CXM,CEF,CTX CMX,CHLOR,CIP S RIF R
34	H.paraphrophilus AMP,CHLOR,CRO,T RIM,RIF, TET S	H.paraphrophilus AMP,COAM,CRO,CX M,AZT,IM,CHLOR,RI F, TET,CLAR,CIP,CMX S	H.paraphrophilus	H.paraphrophilus AMP, COAM,CTX,CEC,CXM,T ET,CHLOR, CMX, RIF,CIP,AZT S	*H.aphrophilus/* *H.paraphrophilus* *16s RNA fraction sequence*

Antibiotic Code:

AMP	**= Ampicillin**	**CMX**	**= Co-Trimoxazole**
COAM	**= Co-Amoxyclav**	**CLAR**	**= Clarithromycin**
CHLOR	**= Chloramphenicol**	**CIP**	**= Ciprofloxacin**
CTX	**= Cefotaxime**	**RIF**	**= Rifampicin**
CRO	**= Ceftriaxone**	**TET**	**= Tetracycline**
CXM	**= Cefuroxime**	**P**	**= Penicillin**
CEC	**= Cefaclor**	**AZT**	**= Aztreonam**
CEF	**= Cefixime**	**IM**	**= Imipenem**

Concordant antimicrobial susceptibilities shown in red
Discrepant typing and antimicrobial susceptibility results shown in blue

Laboratory Number	1.1.1.1. Methods Used
1.1.1.2. Oxford	
1	Phadebact “Hi”, Difco-Bacto polyvalent a-f, Murex monovalent, ALA(remel), Biotyping, X&V on NA, Satellitism, Urease, Indole, ODC (+ Cysteine tryptase agar, G/lactose/maltose/sucrose, CO_2 requirement, ALA weak+ve, no 34), PCR available, bex A + b cap, no routine antibiotic sens)
2	Slide aggs, E-test, NCCLS
3	Rosco tabs, Difco AS, NCCLS
4	Porphyrin, I Urease ODC, H_2S production, Glucose, lactose, mannose, sucrose, Murex monovalent AS, PCR, RapID NH, Nitrocefin, E-test
5	Rosco tabs, E-test, Cefinase
6	X&V, latex agglutination + CIE, PCR, OMP2 VK a-f
7	X&V, API 10S
8	API NH, serum agglutination, PCR
9	NCCLS
10	X&V, API NH, E-test, Cefinase, PCR, Serotyping
11	Murex Antisera, API 10S, API NH
12	X&V, Coagglutination, ALA, I Ure ODC, Haemolysis
13	API NH, Porphyrin, PCR, Slide agglutination, Nitrocefin, Oxidsae, Microdilution MICs (Dade Behring)
14	PCR

3.3 Data on invasive *Haemophilus influenzae* infection 1999-2000

3.3.1 Overall incidence of invasive Hib disease

Data on cases in all age groups was provided by 9 European countries (Czech Republic, Finland, Iceland, Ireland, Italy, Netherlands, Norway, Portugal and the UK) and by Australia, for 2001(Table 2). Data on meningitis in all age groups was supplied by Denmark. The crude incidence was low in the European Union countries in 2001 (0.16 per 100,000 population), but increased from the 1999 and 2000 rates (0.12 and 0.13, respectively). All these EU countries have vaccination programmes established. Of those countries with a vaccination programme, the UK had the highest incidence rate (0.28) in 2001. This higher rate in the UK was the major contributor to the increased incidence rate seen in the combined European Union countries. The number of confirmed adult cases in Australia is greater than the number for which case details are held by EU-IBIS (personal communication). When these cases are added to the totals in Table 2, the rate in Australia becomes the second highest in countries with a routine Hib vaccination programme in 2001(0.20). However, only cases for which case details are provided are included in tables in this report.

In July 2001 the Czech Republic introduced a routine Hib vaccination programme to children under one year of age, and a small decrease of 1.02 to 0.92 cases per 100,000 population can already be seen in this country between 2000 and 2001.

3.3.2 Age disribution of cases

Amongst those EU countries with surveillance in all age groups, the overall percentage of cases in children under 5 years of age was 57%. Comparative figures for 1999 and 2000 were 58% and 63%, respectively. (Table 3) This percentage ranged widely between all the reporting countries (0-100%) over 2001. Eight countries showed a decrease in the percentage of cases in the under five population, while one showed an increase, and two remained the same. However, account must be taken of the very low number of cases some countries are experiencing now they have had vaccination programmes running for a substantial period of time. The Czech Republic, which did not have a vaccination programme instituted until mid 2001, had an age distribution similar to all the other countries in the network prior to vaccination introduction: over 75% of the cases in children under 5 years of age.

The overall percentage of cases in children under one year of age in EU countries reporting Hib cases in all age groups was 16% in 2001, a decrease from the percentages for this age group in 1999 and 2000 (27% and 20%, respectively).

3.3.3 Incidence of invasive Hib disease in childhood

Data on all cases in children under 15 years was provided by 11 European Union countries (Ireland, Finland, Germany, Greece, Iceland, Italy, Netherlands, Norway, Portugal, Sweden, UK), and by three countries outside the EU (Australia, Czech Republic, Israel). (Table 4) Denmark provided data on meningitis only. The annual incidence in the EU was 0.43 per 100,000 population. This value has seen a steady increase since 1999. In 1999 the rate was 0.30, while in 2000 it was 0.39 per 100,000 population. Of the EU countries, the UK has the highest rate (1.01 per 100,000 population), and as a result of being a large population country, has impacted on the rate seen in the combined EU countries. The incidence in the EU was higher in children under five than in those under fifteen, and increased over 1999 (0.84), 2000 (1.08), and 2001 (1.17). (Table 5) The highest rate in 2001 in the under fives was in the United Kingdom (2.66), with rates above one per 100,000 observed in Ireland, Iceland and Israel. In contrast to all other participating countries, the UK showed a steady increase in incidence rate in the under five year olds between 1999 and 2001. This formed the major contribution to the overall increase.

Overall incidence in the EU for Hib meningitis in children under 5 years of age saw a decrease from 2000 (0.61) to 2001 (0.54) (Table 6) The major contributors to this decrease were Italy, and the Netherlands, with lesser additions being made by Denmark, Finland, Greece, Ireland and Portugal. In Australia, Hib meningitis incidence in under fives displayed a marked decrease over the three years, 1999-2001 (0.92 to 0.23). The Czech Republic, having only introduced the vaccination programme half way through 2001, has a high rate (9 per 100,000), but a decrease has been seen since 2000(11 per 100,000).

Table 2 : Numbers of cases and crude incidence (per 100,000 population) of invasive Hib disease for all age groups, by country : 1999, 2000 & 2001

Country	Year	<1 y	1 yr	2 yrs	3 yrs	4 yrs	5-9 yrs	10-14 yrs	15+yrs	NK	Total cases	Population	Rate
Denmark*	1999	1	0	0	0	0	0	0	2	0	3	5,313,577	0.06
	2000	0	0	0	0	0	0	0	0	0	0	5,330,020	0.00
	2001	0	0	0	0	0	1	0	0	0	1	5,349,212	0.02
Finland	1999	2	0	0	0	0	0	1	4	0	7	5,116,826	0.14
	2000	1	0	0	0	1	0	0	0	0	2	5,116,826	0.04
	2001	0	0	0	0	0	0	0	3	0	3	5,116,826	0.06
Iceland	1999	0	0	0	0	0	0	0	0	0	0	278,702	0.00
	2000	0	0	0	0	0	0	0	0	0	0	278,702	0.00
	2001	1	0	0	0	0	0	0	0	0	1	278,702	0.36
Ireland	1999	1	2	0	0	0	1	0	3	0	7	3,744,700	0.19
	2000	2	1	0	1	0	1	0	2	0	7	3,787,100	0.18
	2001	1	1	0	1	0	2	0	3	0	8	3,839,000	0.21
Italy (enhanced)	1999	17(7)	6(3)	9(2)	2(0)	0(0)	2(0)	0(0)	8(5)	0(0)	44(17)	27,880,793	0.16
	2000	9(8)	2(0)	4(2)	1(1)	2(2)	0(0)	1(1)	2(2)	0(0)	21(16)	27,880,793	0.08
	2001	2(0)	3(2)	1(1)	3(3)	1(1)	1(0)	0(0)	6(3)	0(0)	17(10)	27,880,793	0.06
Netherlands	1999	5	0	1	1	0	1	0	4	0	12	15,760,225	0.08
	2000	3	3	2	0	0	0	0	7	0	15	15,863,950	0.09
	2001	3	1	2	1	1	0	1	8	0	17	15,987,075	0.11
Norway	1999	0	2	0	0	0	0	0	3	0	5	4,445,329	0.11
	2000	0	0	1	0	0	0	0	6	0	7	4,478,497	0.16
	2001	0	0	0	0	0	0	0	2	0	2	4,503,436	0.04
Portugal	1999	2	0	0	0	0	0	0	0	2	4	9,920,762	0.04
	2000	0	0	1	1	0	0	0	0	1	3	9,920,762	0.03
	2001	1	0	0	0	0	0	0	1	0	2	9,920,762	0.02
UK	1999	12	6	5	4	5	4	0	30	0	66	51,820,200	0.13
	2000	15	10	12	15	9	5	2	31	2	101	51,820,200	0.19
	2001	23	30	19	14	4	9	2	44	0	145	51,820,200	0.28
EU TOTAL*	**1999**	**39**	**16**	**15**	**7**	**5**	**8**	**1**	**52**	**2**	**145**	**118,967,537**	**0.12**
	2000	**30**	**16**	**20**	**18**	**12**	**6**	**3**	**48**	**3**	**156**	**119,146,830**	**0.13**
	2001	**31**	**35**	**22**	**19**	**6**	**12**	**3**	**67**	**0**	**195**	**119,346,794**	**0.16**

Country	Year	<1 y	1 yr	2 yrs	3 yrs	4 yrs	5-9 yrs	10-14 yrs	15+yrs	NK	Total cases	Population	Rate
Australia	1999	10	8	2	0	0	1	1	5	0	27	18,925,855	0.14
	2000	6(4)	2	1(0)	1	0	3	1	0	0	14(11)	19,153,380	0.07
	2001	5	3	2	1	2	3	1	0	0	17	19,413,240	0.09
Czech Rep.	1999	17	18	16	13	14	9	0	5	0	92	10,282,784	0.89
	2000	14	29(27)	10	16(15)	15(13)	13	1	7	0	105(100)	10,272,503	1.02
	2001	14(13)	18(17)	12(12)	15(14)	18(17)	9(6)	1(1)	7(4)	0	94(84)	10,272,503	0.92

* Denmark reports only meningitis and is therefore excluded from the EU totals
* Numbers in parentheses indicate cases confirmed by isolation in countries where antigen detection is included

Table 3 : Age distribution of cases of invasive Hib disease by country for 1999, 2000 and 2001

Country	Year	Under 1		1-4 years		0-4 years		5-14 years		15+ years		Total
		No	%	No	%	No	%	No	%	No	%	
Denmark*	1999	1	33%	0	0%	1	33%	0	0%	2	67%	3
	2000	0	0%	0	0%	0	0%	0	0%	0	0%	0
	2001	0	0%	0	0%	0	0%	1	100%	0	100%	1
Finland	1999	2	29%	0	0%	2	29%	1	14%	4	57%	7
	2000	1	50%	1	50%	2	100%	0	0%	0	0%	2
	2001	0	0%	0	0%	0	0%	0	0%	3	100%	3
Iceland	1999	0	-	0	-	0	-	0	-	0	-	0
	2000	0	-	0	-	0	-	0	-	0	-	0
	2001	1	100%	0	0	1	100%	0	0	0	0	1
Ireland	1999	1	14%	2	28%	3	43%	1	14%	3	43%	7
	2000	2	29%	2	29%	4	57%	1	14%	2	29%	7
	2001	1	13%	2	25%	3	38%	2	25%	3	38%	8
Italy (enhanced)	1999	17	39%	17	39%	34	77%	2	5%	8	18%	44
	2000	9	43%	9	43%	18	86%	1	4.8%	2	10%	21
	2001	2	12%	8	47%	10	59%	1	6%	6	35%	17
Netherlands	1999	5	42%	2	17%	7	58%	1	8.3%	4	33%	12
	2000	3	20%	5	33%	8	53%	0	0%	7	47%	15
	2001	3	18%	5	29%	8	47%	1	6%	8	47%	17
Norway	1999	0	0%	2	40%	2	40%	0	0%	3	60%	5
	2000	0	0%	1	14%	1	14%	0	0%	6	86%	7
	2001	0	0%	0	0%	0	0%	0	0%	2	100%	2
Portugal	1999	2	100%	0	0%	2	100%	0	0%	0	0%	2
	2000	0	0%	2	100	2	100%	0	0%	0	0%	2
	2001	1	50%	0	0%	0	50%	0	0%	1	50%	2
UK	1999	12	18%	20	30%	32	48%	4	6.1%	30	45%	66
	2000	15	15%	46	46%	61	62%	7	7.0%	31	31%	99
	2001	23	16%	67	46%	90	62%	13	9%	44	30%	147
EU TOTAL*	**1999**	**39**	**27%**	**43**	**30%**	**82**	**58%**	**9**	**6.3%**	**51**	**36%**	**143**
	2000	**30**	**20%**	**66**	**43%**	**96**	**63%**	**9**	**6%**	**48**	**31%**	**153**
	2001	**31**	**16%**	**82**	**42%**	**112**	**57%**	**17**	**9%**	**67**	**34%**	**196**
Australia	1999	10	37%	10	37%	20	74%	2	7.4%	5	19%	27
	2000	6	43%	4	29%	10	71%	4	29%	0	0%	14
	2001	5	29%	8	47%	13	76%	4	24%	0	0%	17
Czech Rep.	1999	17	18%	61	66%	78	85%	9	9.8%	5	5.4%	92
	2000	14	13%	70	67%	85	81%	14	13%	7	7.0%	105
	2001	14	15%	63	67%	77	82%	10	11%	7	7%	94

*** Denmark reports only meningitis and is therefore excluded from the EU totals**

Table 4 : Numbers of cases and crude incidence (per 100,000 population) of invasive Hib disease in children under 15 years of age, by country : 1999, 2000 and 2001

Country	Year	<1 yr	1-4 yrs	5-9 yrs	10-14 yrs	Total cases	Population	Rate
Austria	1999					12	1,356,807	0.88
	2000&2001	N/A	N/A	N/A	N/A	N/A		N/A
Denmark*	1999	1	0	0	0	1	967,643	0.10
	2000	0	0	0	0	0	981,148	0.00
	2001	0	0	1	0	1	998,305	0.10
Ireland	1999	1	2	1	0	4	829,300	0.49
	2000	2	2	1	0	5	824,400	0.61
	2001	1	2	2	0	5	821,700	0.61
Finland	1999	2	0	0	1	3	971,770	0.31
	2000	1	1	0	0	2	971,770	0.21
	2001	0	0	0	0	0	971,770	0.00
Germany	1999	2	8	3	0	13	12,897,014	0.10
	2000	10	11	2	2	25	12,897,014	0.19
	2001	9	7	1	3	20	12,897,014	0.16
Greece	1999	1	0	0	0	1	558,558	0.18
	2000	2	1	0	0	3	558,558	0.54
	2001	1	0	0	0	1	558,558	0.18
Iceland	1999	0	0	0	0	0	64,711	0.0
	2000	0	0	0	0	0	64,711	0.0
	2001	1	0	0	0	1	64,711	1.55
Italy (enhanced)	1999	17(7)	17(5)	2(0)	0(0)	36(12)	3,595,194	1.00
	2000	9(8)	9(5)	0(0)	1(1)	19(14)	3,595,194	0.53
	2001	2(0)	8(7)	1(0)	0(0)	11(7)	3,595,194	0.31
Netherlands	1999	5	2	1	0	8	2,915,911	0.27
	2000	3	5	0	0	8	2,945,543	0.27
	2001	3	5	0	1	9	2,977,428	0.30
Norway	1999	0	2	0	0	2	882,408	0.23
	2000	0	1	0	0	1	894,717	0.11
	2001	0	0	0	0	0	902,431	0.00
Portugal	1999	2	0	0	0	2	1,744,600	0.11
	2000	0	2	0	0	2	1,744,600	0.11
	2001	1	0	0	0	1	1,744,600	0.06
Sweden	1999	1	4	0	1	6	1,654,452	0.36
	2000&2001	N/A	N/A	N/A	N/A	N/A		N/A
UK	1999	12	20	4	0	36	10,001,300	0.36
	2000	15	46	5	2	68	10,001,300	0.68
	2001	23	67	9	2	101	10,001,300	1.01
EU TOTAL*	**1999**	**43**	**55**	**11**	**2**	**111**	**37,472,025**	**0.30**
	2000	**42**	**78**	**8**	**5**	**133**	**34,497,807**	**0.39**
	2001	**41**	**89**	**13**	**6**	**149**	**34,534,706**	**0.43**
Australia	1999	10(10)	10(10)	1(1)	1(1)	22(22)	3,950,872	0.56
	2000	6(4)	4(3)	3(3)	1(1)	14(11)	3,966,067	0.35
	2001	5(5)	8(7)	3(3)	1(1)	17(16)	3,987,198	0.43
Czech Republic	1999	17(17)	61(61)	9(9)	0(0)	87(87)	1,728,678	5.03
	2000	14(14)	70(65)	13(13)	1(1)	98(93)	1,685,398	5.81
	2001	14(13)	63(59)	9(6)	1(1)	87(79)	1,685,398	5.16
Israel	1999	3	3	0	0	6	1,638,400	0.37
	2000	6	3	2	0	11	1,638,400	0.67
	2001	4	2	1	0	7	1,638,400	0.43

* Denmark reports only meningitis and is therefore excluded from the EU totals

* Numbers in parentheses indicate cases confirmed by isolation in countries where antigen detection is included

Table 5 : Numbers of cases and crude incidence rate (per 100,000 population) in children under 5 years of age, by country : 1999, 2000 & 2001

Country	Year	<1 yr	1 yrs	2 yrs	3 yrs	4 yrs	Total cases	Population	Rate
Denmark*	1999	1	0	0	0	0	1	344,685	0.29
	2000	0	0	0	0	0	0	340,593	0.00
	2001	0	0	0	0	0	0	341,381	0.00
Ireland	1999	1	2	0	0	0	3	259,400	1.16
	2000	2	1	0	1	0	4	265,100	1.51
	2001	1	1	0	1	0	3	270,800	1.11
Finland	1999	2	0	0	0	0	2	324,870	0.62
	2000	1	0	0	0	1	2	324,870	0.62
	2001	0	0	0	0	0	0	324,870	0.00
Germany	1999	2	2	5	1	0	10	3,947,634	0.25
	2000	10	6	3	2	0	21	3,947,634	0.53
	2001	9	3	2	2	0	16	3,947,634	0.41
Greece	1999	1	0	0	0	0	1	169,648	0.59
	2000	2	1	0	0	0	3	169,648	1.77
	2001	1	0	0	0	0	1	169,648	0.59
Iceland	1999	0	0	0	0	0	0	20,981	0.00
	2000	0	0	0	0	0	0	20,981	0.00
	2001	1	0	0	0	0	1	20,981	4.77
Italy (enhanced)	1999	17(7)	6(3)	9(2)	2(0)	0(0)	34(12)	1,147,352	2.96
	2000	9(8)	2(0)	4(2)	1(1)	2(2)	18(13)	1,147,352	1.57
	2001	2(0)	3(2)	1(1)	3(3)	1(1)	10(7)	1,147,352	0.87
Netherlands	1999	5	0	1	1	0	7	976,175	0.72
	2000	3	3	2	0	0	8	983,491	0.81
	2001	3	1	2	1	1	8	1,001,085	0.80
Norway	1999	0	2	0	0	0	2	301,963	0.66
	2000	0	0	1	0	0	1	302,387	0.33
	2001	0	0	0	0	0	0	300,954	0.00
Portugal	1999	2	0	0	0	0	2	555,730	0.36
	2000	0	0	1	1	0	2	555,730	0.36
	2001	1	0	0	0	0	1	555,730	0.18
Sweden	1999	1	3	1	0	0	5	518,532	0.96
	2000	N/A	N/A	N/A	N/A	N/A	N/A		N/A
	2001	N/A	N/A	N/A	N/A	N/A	N/A		N/A
UK	1999	12	6	5	4	5	32	3,387,800	0.94
	2000	15	10	12	15	9	61	3,387,800	1.80
	2001	23	30	19	14	4	90	3,387,800	2.66
EU TOTAL*	**1999**	**43**	**21**	**21**	**8**	**5**	**98**	**11,610,085**	**0.84**
	2000	**42**	**23**	**23**	**20**	**12**	**120**	**11,104,993**	**1.08**
	2001	**41**	**38**	**24**	**21**	**6**	**130**	**11,126,854**	**1.17**
Australia	1999	10(10)	8(8)	2(2)	0(0)	0(0)	20(20)	1,284,153	1.56
	2000	6(4)	2(2)	1(0)	1(1)	0(0)	10(7)	1,282,357	1.01
	2001	5(5)	3(2)	2(2)	1(1)	2(2)	13(12)	1,297,534	1.00
Czech Republic	1999	17(17)	18(18)	16(16)	13(13)	14(14)	78(78)	463,569	16.83
	2000	14(14)	29(27)	10(10)	16(15)	15(13)	84(79)	452,761	18.55
	2001	14(13)	18(16)	12(12)	15(14)	18(17)	77(72)	452,761	17.01
Israel	1999	3	1	2	0	0	6	567,000	1.06
	2000	6	0	3	0	0	9	567,000	1.59
	2001	4	1	1	0	0	6	567,000	1.06

* Denmark reports only meningitis and is therefore excluded from the EU totals

- Numbers in parentheses indicate cases confirmed by isolation in countries where antigen detection is included

Table 6 : Numbers of cases and incidence (per 100,000 population) of i nvasive Hib meningitis in children under 5 years by country : 1999, 2000 & 2001

Country	Year	< 1 yr	1 yr	2 yrs	3 yrs	4 yrs	Total cases <5 years	Population	Rate
Denmark	1999	1	0	0	0	0	1	344,685	0.29
	2000	0	0	0	0	0	0	340,593	0.00
	2001	0	0	0	0	0	0	341,381	0.00
Ireland	1999	0	1	0	0	0	1	259,400	0.39
	2000	1	1	0	1	0	3	265,100	1.13
	2001	1	1	0	0	0	2	270,800	0.74
Finland	1999	0	0	0	0	0	0	324,870	0.00
	2000	1	0	0	0	1	2	324,870	0.62
	2001	0	0	0	0	0	0	324,870	0.00
Germany	1999	1	1	4	1	0	7	3,947,634	0.18
	2000	7	3	2	1	0	13	3,47,634	0.33
	2001	7	2	1	2	0	12	3,947,634	0.30
Greece	1999	0	0	0	0	0	0	169,648	0.00
	2000	1	1	0	0	0	2	169,648	1.18
	2001	1	0	0	0	0	1	169,648	0.59
Iceland	1999	0	0	0	0	0	0	20,981	0.00
	2000	0	0	0	0	0	0	20,981	0.00
	2001	0	0	0	0	0	0	20,981	0.00
Italy (enhanced)	1999	16	6	9	2	0	33	1,147,352	2.88
	2000	7	2	4	1	2	16	1,147,352	1.39
	2001	1	3	1	2	1	8	1,147,352	0.70
Netherlands	1999	3	0	1	1	0	5	976,175	0.51
	2000	3	3	2	0	0	8	983,491	0.81
	2001	3	0	1	0	0	4	1,001,085	0.40
Norway	1999	0	1	0	0	0	1	301,963	0.33
	2000	0	0	0	0	0	0	302,387	0.00
	2001	0	0	0	0	0	0	300,954	0.0
Portugal	1999	1	0	0	0	0	1	555,730	0.18
	2000	0	0	1	0	0	1	555,730	0.18
	2001	0	0	0	0	0	0	555,730	0.00
Sweden	1999	0	2	0	0	0	2	518,532	0.39
	2000	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	2001	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
United Kingdom	1999	6	3	2	2	2	15	3,387,800	0.44
	2000	7	4	5	5	2	23	3,387,800	0.68
	2001	10	11	6	5	1	33	3,387,800	0.97
TOTAL	**1999**	**27**	**14**	**16**	**6**	**2**	**65**	**11,610,085**	**0.56**
TOTAL	**2000**	**27**	**14**	**14**	**8**	**5**	**68**	**11,104,993**	**0.61**
	2001	**23**	**17**	**9**	**9**	**2**	**60**	**11,126,854**	**0.54**
Australia	1999	6	5	1	0	0	12	1,284,153	0.93
	2000	5	0	0	0	0	5	1,278,970	0.39
	2001	2	1	0	0	0	3	1,282,357	0.23
Czech Republic	1999	13	14	6	6	8	47	463,569	10.14
	2000	11	23	5	8	4	51	452,761	11.26
	2001	11	12	9	5	5	42	452,761	9.28
Israel	1999	2	0	0	0	0	2	567,000	0.35
	2000	4	0	0	0	0	4	567,000	0.71
	2001	1	0	0	0	0	1	567,000	0.18

3.3.4 Clinical diagnosis

Meningitis remains the dominant clinical diagnosis amongst cases in children. However, the distribution of cases between the clinical diagnoses has changed between 1999 and 2001. (Table 7) The percentage of cases reported as meningitis has decreased from 60% to 39%, while the percentage for septicaemia/bacteraemia and epiglottitis have each increased to 20% of reported cases with known clinical diagnosis. The actual increase was, however, considerably smaller for epiglottitis than for septicaemia (1.9% and 6.6%, respectively, over 1999-2001). Another noticeable increase was seen in the percentage of pneumonia cases reported; 3.9% to 7.2% between 1999 and 2001. These changes in the clinical diagnosis distribution reflect the reduced incidence of invasive Hib disease in children since introduction of vaccination programmes.

The proportion of meningitis was highest in all countries except Australia, Iceland, Israel and the Netherlands, where (except for Iceland) septicaemia/bacteraemia represented a higher proportion. (Table 8) Caution has to be taken with these proportions, however, as the number of cases with known clinical diagnosis are low in some countries.

In 2001, the proportion of cases with meningitis was much lower amongst adult cases than in children.(Table 9) Epiglottitis was more common in older children (aged 2-14), than in infants, one year olds and adults. Pneumonia and septicaemia/bacteraemia were more common among adult cases.

Table 7 : Cases of invasive Hib disease by clinical diagnosis and year in children under 15 years of age, 1999-2001 inclusive

	1999		**2000**		**2001**	
Meningitis	138	59.5%	145	55.3%	118	38.9%
Epiglottitis	42	18.1%	46	17.6%	59	20.2%
Cellulitis	3	1.3%	7	2.7%	7	2.1%
Osteomyelitis / septic arthritis	2	0.9%	7	2.7%	8	2.4%
Pneumonia	9	3.9%	8	3.1%	6	7.2%
Septicaemia / bacteraemia	29	12.5%	33	12.6%	40	19.9%
Other	5	2.2%	13	5.0%	27	8.1%
Not known	4	1.7%	3	1.1%	2	1.2%
TOTAL	**232**	**100%**	**262**	**100%**	**267**	**100%**

Table 8a : Cases of invasive Hib disease in children under 15 years of age by clinical diagnosis and country : 1999 & 2000 combined

Country	**Meningitis**		**Epiglottitis**		**Cellulitis**		**Osteomyelitis/ septic arthritis**		**Pneumonia**		**Septicaemia/ bacteraemia**		**Other**		**Not known**	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%%	No.	%
Australia	18	51%	2	6%	2	6%	0	0%	2	6%	9	26%	1	3%	1	3%
Czech Republic	119	58%	63	31%	0	0%	4	2%	4	2%	14	7%	0	0%	2	1%
Ireland	4	44%	0	0%	0	0%	1	11%	0	0%	3	33%	0	0%	1	11%
Finland	3	60%	0	0%	0	0%	0	0%	0	0%	2	40%	0	0%	0	0%
Germany	23	61%	7	18%	0	0%	0	0%	0	0%	6	16%	2	5%	0	0%
Greece	2	50%	0	0%	0	0%	0	0%	0	0%	2	50%	0	0%	0	0%
Israel	6	35%	0	0%	2	12%	0	0%	6	35%	3	18%	0	0%	0	0%
Italy(enhanced)	52	95%	0	0%	0	0%	0	0%	0	0%	1	2%	0	0%	2	4%
Netherlands	14	89%	0	0%	0	0%	0	0%	0	0%	2	13%	0	0%	0	0%
Norway	1	33%	0	0%	0	0%	0	0%	1	33%	0	0%	1	33%	0	0%
Portugal	1	25%	1	25%	0	0%	0	0%	0	0%	0	0%	0	0%	2	50%
Sweden	2	33%	1	17%	0	0%	1	17%	0	0%	2	33%	0	0%	0	0%
UK	43	41%	14	13%	5	5%	3	3%	4	4%	21	20%	14	13%	0	0%
TOTAL	**288**	**57%**	**88**	**18%**	**9**	**2%**	**9**	**2%**	**17**	**3%**	**64**	**13%**	**18**	**4%**	**8**	**2%**

Table 8b : Cases of invasive Hib disease in children under 15 years of age by clinical diagnosis and country : 2001

Country	Meningitis		Epiglottitis		Cellulitis		Osteomyelitis/ septic arthritis		Pneumonia		Septicaemia/ bacteraemia		Other		Not known	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Australia	3	18%	3	18%	1	6%	0	0%	0	0%	6	35%	4	24%	0	0%
Czech Republic	48	55%	29	33%	0	0%	2	2%	3	3%	5	6%	0	0%	0	0%
Ireland	2	40%	1	20%	0	0%	0	0%	0	0%	1	20%	0	0%	1	20%
Finland	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Germany	14	74%	2	11%	0	0%	0	0%	1	5%	2	11%	1	0%	0	0%
Greece	1	100%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Iceland	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	1	100%	0	0%
Israel	2	29%	0	0%	0	0%	0	0%	1	14%	4	57%	0	0%	0	0%
Italy(enhanced)	9	81%	1	9%	0	0%	0	0%	0	0%	1	9%	0	0%	0	0%
Netherlands	4	44%	0	0%	0	0%	0	0%	0	0%	5	56%	0	0%	0	0%
Norway	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%	0	0%
Portugal	0	0%	0	0%	0	0%	0	0%	0	0%	1	100%	0	0%	0	0%
Sweden	N/A		N/A		N/A		N/A		N/A		N/A		N/A		N/A	
UK	35	35%	23	23%	5	5%	6	6%	1	1%	15	15%	15	15%	1	1%
TOTAL	**118**	**45%**	**59**	**23%**	**6**	**2%**	**8**	**3%**	**6**	**2%**	**40**	**15%**	**21**	**8%**	**2**	**1%**

Table 9a : Cases of invasive Hib disease by clinical diagnosis and age group : 1999 & 2000 combined

Diagnosis	< 1 yr	1 yr	2 yrs	3 yrs	4 yrs	5-9 yrs	10-14 yrs	15+ yrs	NK
Meningitis	93 (67%%)	66 (66%)	42 (55%)	29 (45%)	19 (41%)	21 (42%)	4 (44%)	14 (12%)	1 (14%)
Epiglottitis	2 (1%)	12 (12%)	18 (23%)	23 (36%)	16 (35%)	18 (36%)	1 (11%)_	12 (10%)	1 (14%)
Cellulitis	5 (4%)	1 (1%)	1 (1%)	1 (2%)	0 (0%)	1 (2%)	1 (11%)	1 (1%)	0 (0%)
Osteo/SA	3 (2%)	2 (2%)	2 (3%)	1 (2%)	0 (0%)	0 (0%)	0 (0%)	4 (3%)	0 (0%)
Pneumonia	5 (4%)	3 (3%)	2 (3%)	0 (0%)	3 (7%)	1 (2%)	1 (11%)	26 (22%)	1 (14%)
Septicaemia	25 (18%)	10 (10%)	8 (10%)	7 (11%)	5 (11%)	6 (12%)	2 (22%)	32 (28%)	1 (14%)
Other	2 (1%)	4 (4%)	4 (5%)	3 (5%)	2 (4%)	3 (6%)	0 (0%)	17 (15%)	0 (0%)
Not known	4 (3%)	2 (2%)	0 (0%)	0 (0%)	1(2%)	0 (0%)	0 (0%)	10 (9%)	3 (43%)
All diagnoses	**139**	**100**	**77**	**64**	**46**	**50**	**9**	**116**	**7**

Table 9b : Cases of invasive Hib disease by clinical diagnosis and age group : 2001

Diagnosis	< 1 yr	1 yr	2 yrs	3 yrs	4 yrs	5-9 yrs	10-14 yrs	15+ yrs
Meningitis	37 (58%)	30 (50%)	18 (46%)	14 (38%)	7 (27%)	11 (44%)	2 (25%)	10 (14%)
Epiglottitis	3 (5%)	10 (17%)	12 (31%)	17 (46%)	13 (50%)	4 (16%)	0	8 (11%)
Cellulitis	3 (5%)	1 (2%)	1 (3%)	1 (3%)	0	0	0	1 (1%)
Osteo/SA	1 (2%)	4 (7%)	0	2 (5%)	1 (4%)	0	0	
Pneumonia	1 (2%)	0	4 (10%)	0	0	1 (4%)	0	18 (25%)
Septicaemia	13 (20%)	7 (12%)	1 (3%)	3 (8%)	4 (15%)	7 (28%	4 (50%)	26 (37%)
Other	5 (8%)	8 (13%)	3 (8%)	0	1 (4%)	2 (8%)	2 (25%)	6 (8%)
Not known	1 (2%)	0	0	0	0	0	0	2 (3%)
All diagnoses	**64**	**60**	**39**	**37**	**26**	**25**	**8**	**71**

3.3.5 Non capsulated H. influenzae infection

The incidence of non-capsulated invasive *H. influenzae* disease in children under fifteen was similar to that of type b infection in 2001. Like type b infection, the incidence of non-capsulated invasive *H.influenzae* has shown an increase from 1999 to 2001.(Table 10) This emphasises the importance of accurate identification of strains of *H. influenzae* in children. The range of incidence observed, however, ranged widely between countries, suggestin g that ascertainment may be more variable than for type b infections. In view of the technical expertise required to identify non-capsulate infections and the varying use of national reference centres described in the laboratory questionnaire, this is not surprising.

3.3.6 Other capsulated serotypes of H.influenzae

Compared to both type b and non-capsulate infections, invasive disease due to other capsulated organisms was rare. (Table 11) Type f infections were the most common serotype observed and little change occurred between years of the study.

Table 10 : Incidence of non-capsulated and type b *H. influenzae* in children under 15 years of age–1999, 2000 & 2001

Country	Year	Non capsulated	Incidence	Type b	Incidence	Population
Denmark*	1999	1	0.10	1	0.1	967,643
	2000	1	0.10	0	0.0	981,148
	2001	0	0.00	1	0.10	998,305
Finland	1999	1	0.11	3	0.31	971,770
	2000	1	0.10	2	0.21	971,770
	2001	0	0.00	5	0.51	971,770
Germany	1999	12	0.09	13	0.10	12,897,014
	2000	28	0.22	25	0.19	12,897,014
	2001	20	0.16	20	0.16	12,897,014
Iceland	1999	2	3.09	0	0	64,711
	2000	0	0.0	0	0	64,711
	2001	1	1.55	0	0.00	647,11
Ireland	1999	0	0.0	4	0.48	829,300
	2000	1	0.12	5	0.61	824,400
	2001	1	0.12	5	0.61	821,700
Italy (enhanced)	1999	1	0.03	36	1.00	3,595,194
	2000	0	0.0	19	0.53	3,595,194
	2001	1	0.03	2	0.06	3,595,194
Netherlands	1999	19	0.65	8	0.27	2,915,911
	2000	7	0.24	8	0.27	2,945,543
	2001	9	0.30	12	0.40	2,977,428
Norway	1999	7	0.79	2	0.23	882,408
	2000	6	0.67	1	0.11	894,717
	2001	0	0.00	4	0.44	902,431
Portugal	1999	1	0.06	2	0.11	1,744,602
	2000	2	0.11	2	0.11	1,744,602
	2001	6	0.34	1	0.06	1,744,602
UK	1999	39	0.39	36	0.36	10,033,595
	2000	55	0.55	68	0.68	10,033,595
	2001	101	1.01	101	1.01	10,033,595
EU TOTAL*	**1999**	**82**	**0.24**	**104**	**0.31**	**33,934,505**
	2000	**100**	**0.29**	**130**	**0.38**	**33,971,546**
	2001	**139**	**0.40**	**150**	**0.43**	**35,006,750**
Israel	2000	1	0.06	11	0.67	1,638,400
	2001	7	0.43	16	0.98	1,638,400

***Denmark reports only meningitis and is therefore excluded from the EU totals**

Table 11 : Other *H. influenzae* serotypes in childre n under 15 years: all countries combined : 1999, 2000 & 2001

Year	Type a	Type c	Type e	Type f	Non-b
1999	1	0	3	10	1
2000	4	2	1	13	4
2001	1	0	3	14	2

4. CONCLUSIONS

Prior to introduction of Hib vaccination programmes the epidemiology of invasive Hib disease differed between the EU countries, with incidence rates in children under five varying between 12 and 60 per 100,000. The Czech Republic only introduced a vaccination programme in mid 2001, and has demonstrated an incidence rate in the same range for 2001 (17/100,000). All EU countries now have national immunisation programmes, and therefore the incidence in children under five years, the age group with the highest incidence pre-vaccine, is now very low. Countries are at different stages of vaccine implementation, have different vaccines and schedules and have acheived different levels of coverage. Despite all of these considerations, the incidence of Hib infection in the EU is much lower than in the pre-vaccine era (between 0 and 3.0 per 100,000) in all participating countries.

With the falling incidence of Hib disease , the clinical presentation of Hib disease has also shown changes. Meningitis still remains the predominant diagnosis, but the proportion of cases presenting with meningitis in each year age group under five has decreased . Pneumonia and bacteraemia are more common presentations in adults, and an increase is seen in the overall proportion of cases diagnosed with pneumonia. Apparent differences between countries may be explained by the different age distribution of cases and the small numbers of cases.

Amongst children under five in the EU countries, the highest incidence rates in 2001 were in the UK, Ireland and Iceland. In 2001, the highest incidence was observed in the UK, which experienced a trebling of the number of cases in children under five years of age. One of the major differences between the UK and Ireland and the remaining EU countries is the absence of a booster (third or fourth dose) in the second year of life. Although Ireland has a comparatively high rate, no obvious trend has been observed over 1999-2001. Rates between years in each participant country vary due to small numbers but the increase observed in the UK, one of the largest populations under surveillance, was responsible for an overall increase in incidence in the EU in 2001. Between 1999 and 2000, Germany had seen an doubling in the incidence rate in the under fives, but then saw a reduction in 2001.

Changes in vaccination programmes have occurred over time, and, in particular, the change from using Hib alone or in combination with DTwP to using combinations with DTaP has occurred in many countries.). As this combined vaccine is associated with lower post-vaccination antibody levels to Hib, it has been important to continue monitoring Hib incidence with this new vaccine. In the UK, DTwP vaccine combinations are recommended but during 2000 and 2001, DTaP combinations were used because of a supply problem. Preliminary studies in the UK suggest that change has been contributing to the increase in incidence rate observed. Although this phenomenon has not yet been observed in other countries, possibly due to different schedules in use, the importance of continued observation over the whole of the EU is therefore essential. For smaller countries, pooling data at an EU level may help to ensure that such changes can be detected at the earliest possible stage.

Surveillance systems varied slightly amongst the participating countries. As most countries include all invasive Hib disease in children under fifteen years, comparison of rates in under fives and under fifteens can be made. Differences may be explained by many factors, including different methods of surveillance and completeness of ascertainment. One of the most important factors is the microbiological practice in relation to the diagnosis of Hib disease. This practice can impact on the establishment of disease burden and on comparisons between countries. If laboratories in some countries do not routinely test blood cultures or specimens from other sterile sites for *H. influenzae* in cases with clinical disease compatible with Hib infection then *H. influenzae* and Hib disease will not be diagnosed. The importance of continued improvement of laboratory techniques and laboratory based surveillance cannot be over-emphasised.

Rates of non-b capsulated *H. influenzae* infection are low and no evidence of serotype replacement has been observed despite many years of vaccination in many of the EU countries. Rates of non-capsulate infection are now similar to those for type b and emphasises the importance of ensuring accurate identification of the organism in a national reference centre. The low rates observed in some countries,

probably reflects the low proportion of strains that are referred and highlights the potential for improving ascertainment of such cases. Information on the underlying variability in rates of non-capsulate infection are not known, but the ability to detect such infections may be a useful indicator of the quality of microbiological services in that country. This indicator may help to validate observations of changes in Hib incidence.

5. APPENDICES

5.1 Appendix 1 : *H. influenzae* surveillance network collaborators

AUSTRIA

Reference laboratory

Dr Sigrid Heuberger
BBSUA Graz
Beethovenstr 6
A-8010 Graz, AUSTRIA
Tel. -43-316-32-16-43 Fax. -43-316-38-84-70
Email sigrid.heuberger@sime.com

Epidemiology

Dr Reinhild Strauss
FM for Social Security & Generations
Div VIII/D/2
Radetzkystr 2
A-1031 Vienna, AUSTRIA
Tel: -43-71174367 Fax: -43-1-718-71-83
Email: reinhild.strauss@bmsg.gv.at OR reinhild.strauss@uibk.ac.at

BELGIUM

Reference laboratory

Dr Francoise Crokaert
Institut Jules bordet
Laboratoire
rue Heger-Bordet, 1
B-1000 Bruxelles, BELGIUM
Tel: -32-2-541-3700 OR -32-2-541-3706 Fax: -32-2-541-3295
Email: fcrokaert@usa.net

Epidemiology

Dr Germaine Hanquet
Epidemiology Section
Scientific Institute of public Health – Louis Pasteur
14, J. Wytsmansstreet, B-1050 Brussels
Tel : 32-2-642-5781 Fax: -32-2-642-5410
Email: germaine.hanquet@iph.fgv.be

Reference laboratory

Dr Helle Bossen Konradsen
Head of Department of Respiratory Infections, Meningitis and STIs
Statens Serum Institut
Artillerivej 54
2300 Copenhagen S, DENMARK
Tel: -45-3268-3277 Fax: -45-3268-3862
Email: HBK@ssi.dk

Epidemiology

Dr Susanne Samuelsson
Dept of Epidemiology
Statens Serum Institut
Artillerivej 5
2300 Copenhagen S
Tel: -45-3268-8414/-45-32-683-356 Fax: -45-3268-3874 / -45-32-683-874
Email: STG@ssi.dk & SSM@ssi.dk

FRANCE

Reference laboratory

Pr Henri Dabernat
Centre National de Reference des HI
Laboratoire de Microbiologie
CHU de Purpan
31059 Toulouse
FRANCE
Tel: -33-5-61-77-21-22 Fax: -33-5-61-77-23-33
Email: DABERNAT.H@chu-toulouse.fr

Epidemiology

Dr Anne Perrocheau
Department des Maladies Infectieuses
Institut de Veille Sanitaire
12 rue du val d'Osne
94415 Saint-Maurice Cedex, FRANCE
Tel: -33-1-41-79-67-20 Fax: -33-1-41-79-67-69
Email: a.perrocheau@invs.sante.fr

FINLAND

Reference laboratory

Dr Elja Herva & Prof Maija Leinonen
National Public Health Institute
Department in Oulu
Box 310
FIN-90101, Oulu, FINLAND
Tel (Elja): -358-8-537-6210
Tel (Maija): -358-8-537-6235 Fax: -358-8-537-6251
Email: Elja.Herva@ktl.fi maija.leinonen@ktl.fi

Epidemiology

Dr Petri Ruutu
Department of Infectious Disease Epidemiology
National Public Health Institute
Mannerheimintie 166
FIN-00300 Helsinki
FINLAND
Tel: -358-9-4744-8670 Fax: -358-9-4744-8468
Email: petri.ruutu@ktl.fi

GERMANY

Reference laboratory

Prof H J Schmitt
University of Mainz
Children's Hospital
Microbiol. Laboratory
Langenbeckstr. 1
D-55101 Mainz
Email: Schmittj@kinder.klinik.uni-mainz.de

Epidemiology

Dr Anette Siedler
Robert Koch-Institute
FG 33 / Respiratory and Vaccine preventable diseases
Seestraße 10
D-13353 BERLIN
Tel: -49-30-4547-3452 Fax: -49-30-4547-3514
Email: SiedlerA@rki.de

GREECE

Reference laboratory

Dr Anastasia Pangalis
Dept of Clinical Microbiology
Aghia Sophia Children's Hospital
Athens 11527
Tel: -30-1-7467-669 Fax: -30-1-7797-649

Epidemiology

Professor Marie Theodoridou
Paediatric Clinic of the University of Athens
Aghia Sophia Children's Hospital
Tel: -30-1-17770-152 Fax: -30-1-17770-152
Email: mecha23@otenet.gr

ICELAND

Reference laboratory

Dr Hjordis Hardartottir
Department of Microbiology
National University Hospital
PO Box 1465
121 Reykjavik
ICELAND
Tel: -354-560-1900 Fax: -354-560-1957
Email: hjordish@landspitali.is

Epidemiology

Dr Haraldur Briem
State Epidemiologist
Division of Infectious Disease Control
Directorate of Health
Lauavegar 116
150 Reykjavik
ICELAND
Tel: -354-510-1900 Fax: -354-510-1920
Email: hbriem@landlaeknir.is

IRELAND

Reference laboratory

Dr Mary Cafferkey
Consultant Microbiologist
Meningococcal Reference Laboratory
Children's Hospital
Temple Street, Dublin
Email: mcafferkey@rotunda.ie

Epidemiology

Dr Joan O'Donnell
National Disease Surveillance Centre
Sir Patrick Dunne's Hospital
Lower Grand Canal Street
Dublin 2
IRELAND
Tel : +353-1-876-5374 Fax: -353-1-876-5484 Email : joan.odonnell@ndsc.ie

ITALY

Reference laboratory

Dr Marina Cerquetti
Laboratorio di Bacteriologia e Micologia
Instituto Superiore di Sanita
Viale Regina Elena, 299
ROME 00161
Tel: -39-06-4990-2343 Fax: -39-06-4638-7112
Email: mcerquet@iss.it

Epidemiology

Dr Marta Ciofi degli Atti
Reparto Mallatie Infettive
Laboratorio di Epidemiologia e Biostatistitica
Instituto Superiore di Sanita
Viale Regina Elena, 299
ROME 00161
Tel: -39-06-4938-7215; -4938-7212 Fax: -39-06-4938-7292
Email: ciofi@iss.it

LUXEMBOURG

Reference laboratory

Dr Francois Schneider, Director
Laboratoire National de Sante
42 ru du laboratoire
L-1911 LUXEMBOURG
Tel: -352-494-939 Fax: -352-404-238
Email: francois.schneider@crp-sante.lu AND fschneid@pop2.restena.lu

Epidemiology

Dr Pierette Huberty-Krau
Direction de la Sante
Medecin Chef de l'Imspection Sanitaire
5A, rue de Prague
L-2348 LUXEMBOURG
Tel: -352-478-5650 Fax: -352-480-323
Email: Pierette.Huberty-Krau@ms.etat.lu

NETHERLANDS

Reference laboratory

Dr Lodewijk Spanjaard
Academia Medical Centre
Dept of Medical Microbiology & Reference Laboratory for Bacterial Meningitis
PO Box 22660
1100 DD AMSTERDAM
Tel: -31-20-566-9111 tracer 63126 Fax: -31-20-697-9271
Email: L.Spanjaard@amc.uva.nl

Epidemiology

Dr Hester de Melker
Dept of Infectious Diseases Epidemiology
RIVM
P O Box 1
3720 BA Bilthoven
The Netherlands
Tel: -31-30-274-3958 Fax: -31-30-274-4409 Email: h.de.melker@rivm.nl

NORWAY

Reference laboratory

Dr Arne E Hoiby
Norwegian Institute of Public Health
Division of Infectious Disease Control
Postboks 4404 Nydalen
N-0403 OSLO
NORWAY
Tel: -47-22-04-2400 OR -2200 Fax: -47-22-04-25-13
Email: arne.hoiby@folkehelsa.no

Epidemiology

Dr Oistein Lovoll
Senior Medical Officer
Norwegian Institute of Public Health
Division for Infectious Disease Control
Postboks 4404 Nydalen
0403 Oslo
NORWAY
Tel: -47-22-04-24-59 Fax: -47-22-04-25-13
Email: oistein.lovoll@fhi.no

PORTUGAL

Reference laboratory & Epidemiology

Dr Manuela Canica
Research Co-ordinator
Head of Antibiotic Resistance Unit
Nationa Institute of Health Dr Ricardo Jorge
Avenida Padre Cruz
1649-016 Lisboa
PORTUGAL
Tel: -351-21-752-9246 Fax: -351-21-759-0441
Email: mcanica@yahoo.com

Dr Paula Lavado
Research Assitant
National Institute of Health Dr Ricardo Jorge
Avenida Padre Cruz
1649-016 Lisboa
PORTUGAL
Tel: -351-21-751-9246 Fax: -351-21-759-0441
Email: paulalavado@yahoo.com

SPAIN

Reference laboratory & Epidemiology

Dr Jose Campos
National Haemophilus Reference Laboratory
Centro Nacional de Microbiologia
Majadahonda
MADRID 28200
Tel: +349-1509-7901 ext. 3643 Fax: -349-1-509-7966
Email: jcampos@isciii.es

SWEDEN

Reference laboratory

Dr Brigitta Henrigues
SMI
Email: birgitta.henriques@smi.ki.se

Epidemiology

Margareta Lofdhal.
Department of Epidemiology,
Swedish Institute for Infectious Disease Control, SMI
SE-171 82 Solna, Sweden
Phone + 46 8 457 2387 Fax: + 46 8 30 06 26

UNITED KINGDOM

Reference laboratory

Dr Mary Slack
Consultant Microbiologist
PHLS Haemophilus Reference Laboratory
John Radcliffe Hospital
OXFORD OX3 9DU
Tel: -44-1865-220859 Fax: -44-1865-220890
Email: mary.slack@ndp.oxac.uk

Epidemiology

Dr Mary Ramsay
PHLS CDSC
61 Colindale Avenue
LONDON NW9 5EQ
Tel: -44-208-200-6868 xt 4085 Fax: -44-208-200-7868
Email: mramsay@phls.nhs.uk

NON-EU COUNTRIES

AUSTRALIA

Reference laboratory

Prof Geoff Hogg
Microbial Diagnostic Unit
Dept of Microbiology
University of Melbourne
Parkville
VICTORIA 3052
Email: g.hogg@mdu.unimelb.edu.au

Prof Lyn Gilbert
Microbial Diagnostic Unit
ICPMR & new Children's Hospital
Level 3, ICPMR
Westmead Hospital
WESTMEA D
NSW 2145
Email: lyng@cidm.wh.su.edu.au

Epidemiology

Dr Peter McIntyre
National Centre for Immunisation & Surveillance
P O Box 3515
Paramatta
NSW 2124
Email: peterm@nch.edu.au

ISRAEL

Reference & Epidemiology

Professor Ron Dagan
The Paediatric Infectious Disease Unit
Soroko University Medical Centre
Beer Sheva 84101
P O Box 151
ISRAEL
Tel: -972-8-640-0547/-972-8-640-3412 Fax: -972-8-623-2334
Email: rdagan@bgumail.bgu.ac.il

CZECH REPUBLIC

Reference laboratory

Dr. Vera Lebedova
Head of NRL for Haemophilus Infections
Center of Epidemiology and Microbiology
National Institute of Public Health
Srobarova 48
100 42 Prague 10
Czech Republic
Tel.: +420-2-6708-2241
Fax: +420-2-6731-1454
E-mail: lebedova@szu.cz

Epidemiology

Dr. Paula Kriz
Head of Department of Bacterial Airborn Infections
Center of Epidemiology and Microbiology
National Institute of Public Health
Srobarova 48
100 42 Prague 10
Czech Republic
Tel.: +420-2-6708-2259
Fax: +420-2-6731-1454
E-mail: pavla.krizova@szu.cz **also** krizova@szu.cz

5.2 Appendix 2 : Minimum dataset

Variable name	**Further description**	Field type	Coding
Country		Text	
Year		Number	
IDNO	Identification numbers/letters	Text	
INIT	Initials	Text	
Firstname		Text	
DOB	Date of birth	DD/MM/YY	
DOO	Date of onset	DD/MM/YY	
AgeYr1	Age in years	Number	
Agemth	Age in months in months if <1 year	Number	
Sex		Number	1=male 2=female 3=not known
Geog	Geographical area/region	Text	
Clin	Clinical diagnosis	Number	1=meningitis 2=epiglottitis 3=cellulitis 4=osteomyelitis/septic arthritis 5=pneumonia 6=septicaemia 7=other (specify in 'OthClin') 9=not known
OthClin	Other clinical diagnosis, if specified	Text	
Method of confirmation		Number	1=culture 2=antigen 3=clinical diagnosis 9=not known
Antigen	*H. influenzae* antigen test positive for type b	Number	
Othisol	Other method of confirmation, if specified	Text	
Site	Site of specimen	Number	1=blood 2=CSF 3=blood & CSF 4=other invasive 5=not relevant 6=other (non invasive) 7=other (not known) 8=other (Ag)
OthSite	Other site, if specified	Text	
Serotype	Serotype if known	Text	B = *H. influenzae* type b A = *H. influenzae* type a C = *H. influenzae* type c E = *H. influenzae* type e F = *H. influenzae* type f NC = *H. influenzae* non-capsulated/not typeable NT = *H. influenzae* un-typed NK = not known

Vacc	Vaccination status	Number	1= vaccinated 2=not vaccinated 3=not applicable 4=not known
Doses	No. of doses of vaccine given pre-onset	Text	99=not known
VF	Vaccine failure	Text	TVF = True Vaccine Failure AVF = Apparent Vaccine Failure PVF = Possible Vaccine Failure
Dose1	Vaccine type	Text	
Date1	Date given	DD/MM/YY	
Dose2	Vaccine type	Text	
Date2	Date given	DD/MM/YY	
Dose3	Vaccine type	Text	
Date3	Date given	DD/MM/YY	
Boost	Booster vaccine type	Text	
Bdate	Date booster given	DD/MM/YY	
Outcome		Number	1=alive 2=died 3=not known

5.3 Appendix 3 : H. influenzae Surveillance systems questionnaire

Hib Vaccination in Europe - Invasive *Haemophilus influenzae* infections

Surveillance systems questionnaire

Country: .....
Name of respondent: .....
Position: .....
Centre: .....
Address: .....
.....
.....

The purpose of this questionnaire is to describe the current surveillance systems for *Haemophilus influenzae* in your country and to provide comparative information for each participating country.

Notes for completion of questionnaire
Please complete Part A **once** for overall *H. influenzae* surveillance.
Please complete Part B for **each** surveillance system.
Please attach any additional information/reports.

Part A

1 Surveillance methods

1.1 Methods

What methods of surveillance of *Haemophilus influenzae* are used in your country?
(please list the methods used and complete Part B of the questionnaire once for each system)

1.2 Data collation

If more than one system: How is the data collated at a national or regional from each system?

- ☐ Individual case reconciliation*
- ☐ Comparison of aggregate data only
- ☐ No collation of systems
- ☐ Not relevant

* "reconciliation" - cases in one system merged with cases in another system and duplicates removed.

For each method of surveillance please complete one questionnaire Part B.

Part B

1 Surveillance system

1.1 Objectives

What are the objective(s) of this *Haemophilus influenzae* surveillance system method? (please specify if the system aims for sentinel or universal case ascertainment)

1.2 Case definitions

What is the case definition or case category of the health event under surveillance?

H. influenzae type b ☐ *H.influenzae* ☐ Other ☐
Please specify "Other" .....
.....

Meningitis ☐ All invasive ☐ Other ☐
Please specify "Other" .....
.....

1.3 Population

What is the population under surveillance?

Whole country ☐ Region ☐ Please specify which region(s)
.....
Total population ☐
Under 15 years of age ☐
Under 10 years of age ☐
Under 5 years of age ☐
Other (specify) .....

1.4 Type of surveillance system

What type of surveillance system is this?

Type of system
Active ☐
Passive ☐

Characteristics of system
Stimulated ☐ Not stimulated ☐
Statutory reporting ☐ Voluntary reporting ☐

Zero-reporting ☐ / No zero reporting ☐

1.5 Start of surveillance system

Which year did this surveillance system start? .....
Years for which data is available .....

2 Data collection

2.1 Information collected

What information/data is collected?
(please specify the variables routinely collected)

Age ☐
Sex ☐
Date of onset ☐
Geographic location ☐
Clinical condition ☐
Organism ☐
Method of confirmation ☐
Vaccination status ☐
Other ☐ Please specify "Other" .....
.....
.....

2.2 Reporting sources

Who provides the data? (please specify who reports the data used)
Clinicians ☐
Paediatricians ☐
Microbiologists ☐
Epidemiologists ☐
Scientific staff ☐
Administrative staff ☐
Other, please specify .....

Where is the data received from?
Hospitals ☐
Clinics ☐
Reference laboratory ☐
Local laboratories ☐
Other, please specify .....
.....

2.3 Time period

How frequently is the data reported locally?
Weekly ☐ Monthly ☐ Quarterly ☐
Six-monthly ☐ Annually ☐ Other .....

How frequently is the data aggregated nationally?
Weekly ☐ Monthly ☐ Quarterly ☐
Six-monthly ☐ Annually ☐ Other .....

2.4 Duplicate reports

Are duplicates routinely detected and eliminated?

3 Data analysis

3.1 Analysis

Who analyses the data at a national level?

Clinicians ☐
Paediatricians ☐
Microbiologists ☐
Epidemiologists ☐
Scientific staff ☐
Adminstrative staff ☐
Other, please specify .....
.....

4 Data dissemination

4.1 Regular reports

4.1a Frequency

How often are reports of the surveillance system produced?
(please state this for all regular reports)

Weekly ☐
Monthly ☐
Quarterly ☐

Six-monthly ☐
Annually ☐
Other .....

4.1b Method of reporting

How are the reports disseminated?
(please state if this is by bulletin, website, newsletter, etc)

4.1c Audience

Who are reports disseminated to?

4.2 Recent publications

Are there any recent or relevant publications demonstrating application(s) of the surveillance system? **And** Are there any recent or relevant publications about evaluation(s) of the system and/or changes in the system?
(please list any recent or relevant publications)

5.4 Appendix 4 : Laboratory diagnostic methods questionnaire

Hib Vaccination in Europe - Invasive *Haemophilus influenzae* infections

Laboratory Diagnostic Methods Questionnaire

Country :.....

Name of respondent .....

Position .....

Centre .....

Address .....
.....
.....
.....

The first section aims to describe the facilities which are available in the hospitals which refer strains to you.

The purpose of the second section is to describe the methods used to identify H.influenzae by laboratories collaborating in this study.

Please return both sections of completed questionnaire to:-

Dr. Mary P.E. Slack
Haemophilus Reference Laboratory
Public Health Laboratory,
Level 6/7, John Radcliffe Hospital,
Headington,
Oxford, OX3 9DU
U.K.

(Tel: +44-1865-220879/220884 Fax: +44-1865-220890)

SURVEY OF LABORATORY FACILITIES FOR THE IDENTIFICATION OF HAEMOPHILUS INFLUENZAE IN.....

I)What proportion of hospitals in your country/area have the facilities to do the primary identification of H.influenzae strains?

100%	☐
80-100%	☐
50-80%	☐
20-50%	☐
<20%	☐

II) For those hospitals which can identify H.influenzae, what type of cases/specimens would they look for/try to grow the organism from?

All CSFs from suspected bacterial meningitis	☐
All CSFs from suspected bacterial meningitis in children	☐
All blood cultures	☐
All blood cultures in children	☐
Blood cultures from cases of epiglottitis	☐
Blood cultures from cases of epiglottitis in children	☐
Other conditions, please describe	☐
(e.g. osteomyelitis, septic arthritis, pneumonia)	_____

III) What proportion of hospitals would be able to perform serotyping on isolates of :

H.influenzae type b

100%	☐
80-100%	☐
50-80%	☐
20-50%	☐
<20%	☐

Other H.influenzae

100%	☐
80-100%	☐
50-80%	☐
20-50%	☐
<20%	☐

IV) What proportion of hospitals refer isolates to the reference lab (i.e. your lab)?

100%	☐
80-100%	☐
50-80%	☐
20-50%	☐
<20%	☐

V) For those hospitals which do refer isolates to your lab, what type of cases are they referred for?

All invasive H.flu	☐
All invasive H.flu in children	☐
H.flu meningitis	☐
H.flu meningitis in children	☐
H.flu epiglottitis in children	☐
Other, please describe	☐

REFERENCE LABORATORY METHODS

1.1 Receipt of strains

		yes	no
1.11	Are the strains subbed immediately on receipt?	☐	☐
1.12	Are the strains tested on receipt, or batched?	☐	☐
1.13	Are the strains stored and tested in batches?	☐	☐

2.1 Media

2.11 What media is used to transport strains to the laboratory?
.....……

2.12 What media is used to subculture the strains?
.....

2.13 What media is used to test growth factor requirement?
.....

2.14 What media is used for susceptibility testing?
.....

2.15 What media is used for long term storage of strains?
.....

2.16 Please state atmosphere of incubation.
.....

2.17 Please state duration of incubation.
.....

2.2 Identification Methods

Are the following tests performed? (Please tick the appropriate box)

	yes	no
Catalase	☐	☐
Oxidase	☐	☐
Dependence on growth factors		
i) by disc method	☐	☐
ii) by plate incorporation method	☐	☐
Porphyrin	☐	☐

Satellitism on blood agar yes ☐ no ☐
(please state origin of blood used i.e. horse, sheep) .....
.....

Haemolysis yes ☐ no ☐
(please state origin of blood used).....
.....

Nitrate yes ☐ no ☐
If Yes, please state method
.....
.....

O.N.P.G. yes ☐ no ☐

Commercially available identification kit yes ☐ no ☐
(Please give details).....
.....
.....

Other, please specify yes ☐ no ☐
.....
.....

2.3 Are the strains biotyped using the following tests?

Indole	yes	☐	no	☐
Urease	yes	☐	no	☐
Ornithine decarboxylase	yes	☐	no	☐

2.4 Are the strains serotyped?

If so, which of the following methods are used:

Slide agglutination with polyvalent antisera yes ☐ no ☐
If yes, give details of antisera used
.....
.....

Slide agglutination with type specific antisera yes ☐ no ☐
If yes, give details of antisera used
.....
Counter current immunoelectrophoresis yes ☐ no ☐
PCR yes ☐ no ☐
If yes, give details of primers used
.....
.....
Other yes ☐ no ☐
If yes, give details
.....

2.5 Are the strains further subtyped? yes ☐ no ☐

If yes, which typing method is used?

OMP ☐
Ribotyping ☐
LPS ☐
PFGE ☐
Other, please specify ☐

2.6 Susceptibility testing.

2.6.1 Please list antimicrobial chemotherapeutic agents tested, and concentrations (e.g. disc content, breakpoint values, etc.)
.....
.....
.....
.....
.....
.....

2.6.2 With method of susceptibility testing is used?

	yes	no
Disc diffusion - please state method e.g.	☐	☐
Control organism on the same agar plate	☐	☐
Control organism on a separate agar plate	☐	☐
Break points	☐	☐
Other, please specify	☐	☐

2.6.3 If MICs are required, which method is used?

	yes	no
Broth dilution	☐	☐
Agar incorporation	☐	☐
E-test (AB BIODISK)	☐	☐
Commercially prepared MIC microtitre trays	☐	☐
If so, please give details of kit used		
.....		
Other	☐	☐

Please specify
.....
.....

2.7 Do you test for beta-lactamase production? yes ☐ no ☐
If yes, please state method used
.....
.....
.....

2.8 Do you test for chloramphenicol acetyltransferase (CAT) production? yes ☐ no ☐
If yes, please state method used
.....
.....
.....

2.9 Long term storage
How do you store strains long term?

	yes	no
Agar slopes	☐	☐
Frozen at -80oC	☐	☐
Other	☐	☐

Please specify .....
.....

Please give any other information regarding your laboratory methods not covered above.
(Please attach additional sheets if necessary, or include your laboratory standard operating procedures)