Generic protocol for population-based surveillance of *Haemophilus influenzae* type B

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Preface

The WHO Global Programme for Vaccines and Immunization (GPV) has had a long-standing interest in vaccines against *Haemophilus influenzae* type b (Hib). The Expanded Programme on Immunization (part of GPV) reviewed information on Hib vaccines jointly with the WHO Programme for Acute Respiratory Infections (ARI) in 1988 and again in 1992. Further studies of clinical schedules of Hib vaccine and Hib vaccine efficacy in developing countries were encouraged. A review paper, prepared by Dr P. Wright on behalf of EPI, broadly outlined types of studies that would be useful for Hib and other types of meningitis in developing countries. It emphasized that the mortality from meningitis appeared to be much higher in developing countries than in industrialized countries and that the assessment of new vaccines would therefore need to include meningitis as an outcome[1].

As of November 1995, several WHO-sponsored Hib vaccine studies are in progress in developing countries. In The Gambia, a large-scale field trial of Hib vaccines, sponsored by WHO/ARI, is expected to be completed soon. In Niger, a WHO/GPV-sponsored field trial is examining several schedules of Hib vaccine given simultaneously with other EPI-vaccines.

The GPV Steering Committee on Epidemiology and Field Research has identified the need to develop a practical method for immunization programme managers to assess the local burden of severe disease due to Hib. At the request of the Steering Committee, this generic protocol was prepared by staff of the Child & Respiratory Diseases Branch, Division of Bacterial & Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, USA.

WHO/GPV provides this generic protocol free-of-charge. In return, the programme would appreciate being informed about studies conducted using this protocol. The generic protocol may be freely adapted, but this WHO document should be referenced in any publication resulting from its use. Comments or suggestions for improving this protocol are welcome and should be sent to: Vaccine Research and Development Unit, Global Programme for Vaccines and Immunization, WHO, 1211 Geneva 27, Switzerland.

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[1]   Wright PF. Approaches to prevent acute bacterial meningitis in developing countries. *Bull WHO* 1989;5:479-486.

1. Introduction

Severe disease due to *Haemophilus influenzae* type b (Hib) is rapidly disappearing from many industrialized countries where infants are routinely immunized with Hib conjugate vaccines [1-4]. Among the factors critical to the acceptance of these vaccines were: (i) the ability to deliver Hib conjugate vaccines at the same time, and even in the same syringe, as other routine immunizations; and (ii) studies demonstrating that the benefits of vaccination greatly exceed the cost of including Hib conjugate vaccine in the immunization programme.

Despite the success of Hib conjugate vaccines in industrialized countries, these vaccines have not yet been incorporated into the routine immunization schedules in developing countries. The use of Hib conjugate vaccines in developing countries has been limited, in part, because not enough data on the local burden of severe Hib disease were available to assess the potential impact of integrating Hib conjugate vaccines into routine childhood immunization programmes [5].

The purpose of this generic protocol is to describe a surveillance method that will allow estimation of the local burden of Hib disease and thereby assist individual immunization programme managers who are considering whether to add Hib conjugate vaccine to their immunization programme. In addition, if Hib conjugate vaccine becomes part of the routine infant immunization programme, the data generated by this generic protocol will provide a baseline that can be used to demonstrate the impact of immunization.

1.1 *Haemophilus influenzae* serotypes

Although other serotypes of *H. influenzae*, and non-typable (unencapsulated) *H. influenzae* do cause some disease, type b is responsible for the overwhelming majority of severe *H. influenzae* cases in children less than five years old. Nearly all population-based studies indicate that >97% of all *H. influenzae* meningitis is due to Hib [6,7]. Two exceptions are reports from Papua New Guinea [8] and the White Mountain Apaches [9] where 78-82% of *H. influenzae* meningitis was due to Hib. Non-typable *H. influenzae* and other *H. influenzae* serotypes may be responsible for the majority of non-bacteremic *H. influenzae* pneumonia among children <5 years old; however, Hib accounts for 60-80% of bacteremic pneumonia due to *H. influenzae* in this age group [6]. More importantly, the currently available conjugate vaccines provide protection against Hib only [3]. For these reasons, surveillance focusing on disease due to *H. influenzae* serotype b infections will identify the potentially preventable cases of severe disease.

1.2 Studies of Hib disease in industrialized countries

In industrialized countries, most studies of Hib disease have focused on invasive infections, which include meningitis, sepsis, bacteremic pneumonia, and other syndromes (e.g., epiglottitis) accompanied by the presence of the organism in a normally sterile site (e.g., blood, cerebro spinal fluid (CSF), pleural fluid, joint fluid, etc.). Population-based studies have provided accurate estimates of the invasive Hib disease burden in several industrialized countries [10-14] .

Table 1. Reported annual incidence of Hib meningitis and all invasive Hib disease among children less than five years old in industrialized countries before the widespread use of Hib conjugate vaccines, selected studies.

Country	Years	Reference	Annual incidence per 100 000 children < 5 years of age	
			Hib meningitis	All invasive Hib disease
Australia	1985-1987	[10]	25	39-59
Finland	1985-1986	[11]	26	52
Israel	1988-1990	[12]	18	34
USA	1959-1984	[13]	40-69	67-130
Wales	1989-1990	[14]	22-30	35-44

These studies served as the basis for widespread use of Hib conjugate vaccines in industrialized countries, where the costs of caring for patients with invasive bacterial disease and their sequelae are substantial [15-20].

1.3 Studies of Hib disease in developing countries

In developing countries and less-developed areas of industrialized countries, population-based estimates of the incidence of Hib meningitis and invasive Hib disease are limited [9,10,21-26] (Table 2). As a consequence, cost-benefit and cost-effectiveness analyses are even less common [27,28]. The calculation of incidence rates requires that surveillance be conducted in a defined population of known size. Although there have been many reports of the proportion of meningitis due to various bacteria, these data are typically derived from evaluation of patients at a single hospital or treatment center. Because the population served by a health facility cannot be determined and because facility-based studies are influenced by referral patterns, disease incidence or pathogen-specific disease burden cannot be estimated from these studies.

1.4 Types of severe Hib disease

Characterizing the burden of Hib disease requires attention to several key issues. The common syndromes caused by Hib -- meningitis, sepsis, and pneumonia -- cannot be distinguished clinically from these illnesses caused by other encapsulated bacteria (e.g., *Neisseria meningitidis, Streptococcus pneumoniae*), or by a number of other bacterial or viral agents. Thus, establishing the rate of disease due to a particular bacterium requires laboratory identification of the pathogen from clinical specimens. Because >80% of all cases of severe Hib disease occur among children <5 years old, surveillance often focuses specifically on this age group.

Table 2. Reported incidence of Hib meningitis and all invasive Hib disease among children less than five years of age in developing countries and developing areas of industrialized countries, selected studies.

Country/Area	Year	Reference	Annual incidence per 100 000 children <5 years of age	
			Hib meningitis	All invasive Hib disease
Australia/ Aboriginal	1985-1987	[10]	153	450
Chile	1985-1987	[21]	15-25	22-43
Gambia	1985-1987	[22]	60	NA
Kuwait	1981-1987	[23]	31*	NA
Senegal	1970-1979	[24]	51	NA
South Africa	1991-1992	[25]	34	47
USA/Apache	1973-1980	[26]	254	NA
USA/Navajo	1974-1980	[9]	152	NA

* Annual incidence per 100 000 children < 2 years of age.

1.4.1 Hib meningitis

Although meningitis represents only a small fraction of the burden of severe Hib disease, surveillance should focus on meningitis because its diagnosis is relatively straightforward and meningitis surveillance can yield very accurate estimates of incidence. The bacterial agents of meningitis can be identified by culture of CSF from patients with clinical symptoms of meningitis. Pathogens can also be detected by antigen detection (latex agglutination) of CSF. Because most severe meningitis among children is due to bacterial agents, the yield from cultures of CSF from patients with meningitis is greater than the yield from cultures of fluids from patients with other syndromes such as sepsis or pneumonia.

1.4.2 Hib sepsis

Identification of the bacterial agents causing sepsis is more difficult than for meningitis. Since many bacteria and non-bacterial agents (e.g. viruses, parasites, and mycobacteria) produce febrile illness, systematic collection of blood cultures from febrile children for surveillance purposes would result in an extraordinarily large burden for the laboratory and a low yield of bacterial agents relative to the cost of specimen collection and culture. As a result, blood cultures are not routinely collected from febrile children in many countries. Moreover, isolation of Hib from blood cultures may be more difficult than isolating the organism from CSF.

1.4.3 Hib pneumonia

While in many developing countries the majority of severe disease due to Hib occurs as pneumonia, identification of an etiologic agent in pneumonia cases is especially problematic. Most studies identify bacteremia in fewer than 20% of children with pneumonia [29]. Obtaining good sputum specimens from infants and children is difficult, and sputum cultures are rarely diagnostic due to growth of multiple organisms. Lung aspirates are rarely performed outside of research protocols. The sensitivity and specificity of serologic assays for diagnosing Hib pneumonia are unknown and these methods are not widely used.

1.5 Why this generic protocol focuses on Hib meningitis

This generic protocol focuses on Hib meningitis because it is the component of severe Hib disease that can be most easily quantified through surveillance. This protocol is based on population-based surveillance for bacterial meningitis, which requires:

* identification of a sufficiently large, well-defined surveillance population;

* participation of all relevant treatment centers that serve the population;

* routine use of appropriate diagnostic procedures in hospitals and laboratories.

Implementation of this generic protocol may require modification depending on the diagnostic and treatment practices in a given area and available resources.

It is important to keep in mind that Hib meningitis represents only a fraction of the total disease burden due to Hib. In many countries where mortality due to pneumonia among infants and children is high, the vast majority of severe Hib disease occurs in the form of pneumonia. A method for estimating the local burden of acute lower respiratory infections due to Hib is described in Annex 4.

2. Selecting a surveillance population

2.1 Size of surveillance population

Since bacterial meningitis is relatively uncommon among children less than five years old (perhaps 200 cases annually per 100 000 children <5 years old), the surveillance population should be large enough (e.g. 250 000 - 500 000 total persons, or about 50 000 - 100 000 children <5 years old) so that a sufficient number of cases (200 - 400 cases) would be expected during a two-year period. The surveillance population should be well defined geographically and age-specific population data should be available (e.g., total population, number of live births, and the number of children 0-4 years old) so that age-specific rates of disease can be calculated. A stable population (without many people moving in and out) is preferable since accurate surveillance and determination of rates in a highly mobile population is difficult.

If available, information on the all-cause mortality rate among children < 1 year and <5 years can be used to estimate the burden of acute lower respiratory tract infections due to Hib (see Annex 4).

2.2 Health services utilization

Residents of the surveillance population should have good access to, and a high level of utilization of, one or more health facilities for severe illness such as meningitis. The standard of care in health facilities should be such that lumbar puncture is included in the routine evaluation of children in the surveillance area who present with symptoms of meningitis. Populations where many childhood deaths occur among persons who never reach a treatment facility are less suitable for meningitis surveillance, since surveillance will underestimate morbidity and mortality due to this infection. All health facilities (including all hospitals, public and private) that diagnose and treat childhood meningitis in the surveillance population should be included in the surveillance system. The most practical population for surveillance is probably an urban population that is geographically distinct from other population centers and that is served by one or more major health centers.

3. Surveillance for bacterial meningitis in children <5 years old

3.1 Case definitions

A key to accurate surveillance is the establishment of standard case definitions that are used throughout the surveillance area. Recommended definitions for a probable case and a confirmed case of bacterial meningitis are listed below.

A probable case of bacterial meningitis is defined as a child < 5 years of age presenting with clinical symptoms of meningitis (i.e., fever, headache, stiff neck, bulging fontanelle, or mental status changes) with a turbid ("cloudy") CSF, or a CSF with an elevated protein (>100 mg/dl), decreased glucose (<40 mg/dl), or leukocytosis (> 100 WBC/mm3) with >80% neutrophils (includes immature neutrophils [bands]).

NOTE: The presence of blood in a CSF specimen can complicate interpretation of cell count, protein, and glucose results. Whenever possible, non-bloody specimens should be used for chemistry tests. The cell count, protein, and glucose results from bloody CSF specimens should not be used to meet the probable bacterial meningitis case definition if the ratio of red cells to white cells is > 500:1.

For the purposes of surveillance, **a confirmed case of bacterial meningitis** is defined as a child <5 years old presenting with clinical symptoms of meningitis (i.e., fever, headache, stiff neck, bulging fontanelle, or mental status changes) and identification by culture or antigen detection of bacteria in the CSF.

3.2 Surveillance system

Figure 1 illustrates the process of collecting and recording bacterial meningitis surveillance information on each suspected case.

3.2.1   Laboratory CSF specimen logbook

Each laboratory should keep a CSF specimen logbook to record information on all children less than five years old with suspected meningitis (patients who have a lumbar puncture performed). Refer to Annex 1 for an example of a CSF logbook form. Each time a CSF specimen is sent to the laboratory it should be recorded in the CSF specimen logbook. As laboratory data (e.g., cell counts, culture results, etc.) become available, these data should be added to this CSF logbook.

Figure 1: Bacterial meningitis surveillance system

Child < 5 years with signs and symptoms of meningitis
- perform lumbar puncture
- send CSF to laboratory for culture
- enter specimen in CSF specimen logbook

CSF specimen positive by culture? * ──YES──▶ **CONFIRMED CASE**

NO ↓

Suspected bacterial by any of the following indicators?
- protein > 100 mg/dl
- glucose < 40 mg/dl
- leukocytosis with > 80% neutrophils
- turbid appearance

──YES──▶ Latex agglutination positive?

YES ↑ → CONFIRMED CASE

NO ↓ → **PROBABLE CASE**

NO ↓

Do not fill out bacterial meningitis case report form
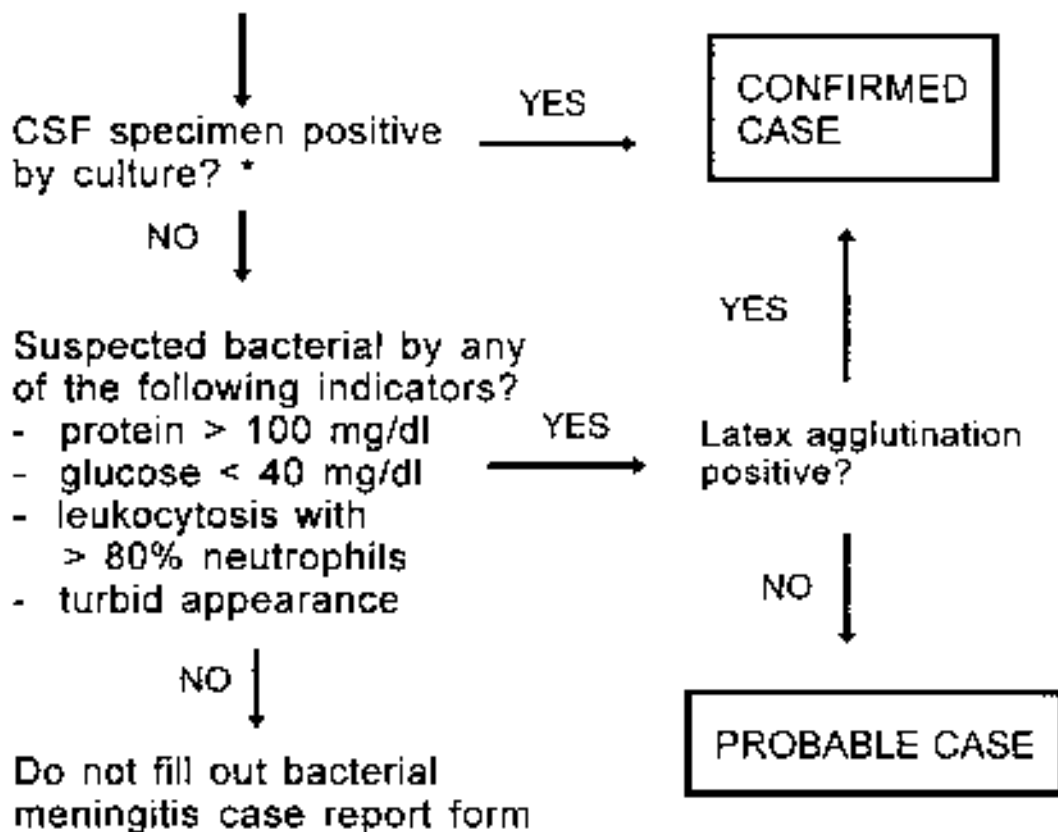
*If latex agglutination testing is routinely performed on all CSF specimens, then use the results to meet the surveillance case definitions.

3.2.2 Bacterial meningitis case report form

An example of a simple bacterial meningitis case report form is included in Annex 2. This form should be adapted for local use. For each case of confirmed bacterial meningitis identified (either by culture or latex agglutination test), a bacterial meningitis case report form with clinical and demographic information should be completed. For culture-confirmed cases, the isolate should be stored for further testing (serotyping and antibiotic susceptibility patterns). For probable bacterial meningitis, a bacterial meningitis case report form should also be filled out. A case report form should not be filled out for other patients with CSF specimens.

3.3 Role of the surveillance coordinator

To accurately describe the burden of Hib meningitis, surveillance must be complete in identifying children who have meningitis, in obtaining CSF specimens for testing, and in identifying Hib with high sensitivity from CSF culture. Accomplishing these objectives requires regular communication between each reporting site and a central surveillance coordinator. The surveillance coordinator should establish a contact person in each reporting site (e.g., laboratory or hospital) who will be responsible for logging CSF specimens into the CSF specimen logbook and completing bacterial meningitis case report forms.

The surveillance coordinator (or other surveillance project personnel) should periodically contact sites (every 1-4 weeks) to collect information and review laboratory records.

Case report forms should be reviewed for completeness and accuracy as they become available to the surveillance coordinator. The surveillance coordinator may have to track down missing information. Data from the case report forms should be entered into a computer database, whenever possible.

4. Laboratory procedures

Sensitive and specific laboratory diagnosis of meningitis and its etiology are essential for assessing the burden of meningitis due to Hib (and other bacteria). Every child less than five years old who presents with signs and symptoms of meningitis should have a lumbar puncture performed, with the CSF evaluated for cell count (including differential), protein, and glucose, or visually inspected for turbidity. Gram-stains on smears of CSF should also be done. Culture for bacterial pathogens should be performed on all CSF specimens.

4.1 CSF culture methods

Complete laboratory methods are provided in Annex 3. Briefly, to isolate *H. influenzae*, undiluted CSF should be streaked on chocolate agar plates supplemented with X and V factors, and incubated at 35-37°C in a 5%-10% CO_2 environment (by CO_2 incubator or candle jar) for at least four days before discarding the sample.

Use of chocolate agar plates supplemented with X and V factors for cultures is essential. Growth of *H. influenzae* is unreliable in some chocolate agar preparations that have not been supplemented with X and V factors. In some instances, hospitals that reportedly had no cases of *H. influenzae* disease at all were later shown to be using media that would not support the growth of *H. influenzae* [30]. However, after their culture media was supplemented with X and V factors, the laboratory successfully grew *H. influenzae*. To reduce variability, one central laboratory may be designated to prepare chocolate agar plates supplemented with X and V factors and to distribute them to all other clinical laboratories in the surveillance area. Alternatively, X and V factor-supplemented chocolate agar plates can be purchased directly from a distributor of laboratory supplies.

4.2 CSF latex agglutination testing

Latex agglutination tests can be useful for diagnosis of bacterial meningitis, but they are expensive (up to US$ 5 per test) and interpreting results may be difficult for an inexperienced technician [31]. In areas where latex agglutination tests are being routinely performed on all CSF specimens the results of these tests should be used to fulfil the criteria for confirmed bacterial meningitis. However, in areas where latex agglutination tests are not routinely performed on all CSF specimens it may be more cost-effective to limit the number of CSF specimens that must be tested with latex agglutination kits. Specifically, a sample of one milliliter of all CSF specimens should be frozen at the time that the specimen is obtained. For patients whose cultures are negative after 4 days, this frozen specimen should be sent to a central laboratory for latex agglu-

tination testing at a later time. Storing isolates and testing them in a single lab will minimize costs and variability in results. The cost-effectiveness could be increased further by restricting the specimens for latex agglutination testing to specimens from patients with probable bacterial meningitis (i.e., CSF with a purulent appearance, elevated protein, decreased glucose, or leukocytosis with >80% neutrophils).

4.3 Blood cultures

In the event that vaccines against Hib become a routine part of the immunization programme, information on bacteremic Hib disease may be particularly helpful for monitoring the impact of vaccination over time. Thus, if blood cultures are routinely collected from children <5 years old with suspected sepsis at the health care facilities under surveillance, these data may supplement the meningitis data. However, latex agglutination tests of blood cultures should not be routinely carried out, due primarily to the high cost and lower yield of this procedure. In addition, for countries where blood cultures are not routinely collected from children <5 years old with suspected sepsis, we recommend that all surveillance efforts concentrate on identification and laboratory diagnosis of bacterial meningitis, not on improving surveillance for bacteremic Hib disease.

4.4 Serotyping *H. influenzae* isolates

All *H. influenzae* isolates from CSF or blood (or another normally sterile site) should be routinely sent to a central laboratory for serotyping, if serotyping is not being performed at the reporting hospital. Surveillance coordinators may wish to consider arrangements to have serogrouping of *N. meningitidis* and serotyping of *S. pneumoniae* done on sterile site isolates.

4.5 Antimicrobial susceptibility testing

Some surveillance areas may want to assess the antibiotic susceptibility patterns of their *H. influenzae* and *S. pneumoniae* isolates. Since antibiotic resistance may diminish a patient's response to treatment, information on the prevalence of antibiotic resistant organisms in a community may be useful to clinicians. However, standardizing laboratories to accurately carry out antibiotic susceptibility testing is difficult. To insure the quality of the antibiotic susceptibility data, interested surveillance areas should have isolates tested in a reference laboratory with recognized experience in assessing antibiotic susceptibility patterns.

4.6 Storage of *H. influenzae, N. meningitidis*, and *S. pneumoniae* isolates from CSF specimens

Some surveillance areas may wish to store isolates from CSF specimens for additional study in the future. Studies of the genetic relatedness of strains, the prevalence of antibiotic resistance, and the relationship between virulence factors and outcome are some examples of future studies that might be carried out with stored *H. influenzae, N. meningitidis,* or *S. pneumoniae* isolates from CSF specimens. For details on the storage and transportation of isolates refer to Annex 3.

4.7 Laboratory resources

Estimates of the expected overall incidence of bacterial meningitis among children <5 years old and the proportion of all CSF cultures that are positive for bacteria allow us to estimate the laboratory resources necessary to conduct surveillance for bacterial meningitis. Assuming that the overall incidence of bacterial meningitis among children <5 years old (in the absence of a meningococcal epidemic) is approximately 200 cases per 100 000 per year, and that 20-25% of all CSF cultures done on suspected meningitis patients yield a bacterial agent, we estimate that treatment centers and laboratories will need to prepare for approximately 800-1000 CSF specimens for each 100 000 children <5 years old in the surveillance population. Thus, for a surveillance population of 1 million, with 200 000 children <5 years old (assuming 20% of the population is <5 years old), the surveillance area laboratories can anticipate approximately 1600-2000 CSF specimens per year for bacterial meningitis surveillance. For bacteremia surveillance, the estimated number of blood cultures should be about five times the number of CSF cultures for bacterial meningitis.

4.8 Laboratory selection and laboratory quality control

Assessing the adequacy of existing laboratory methods, improving and standardizing laboratory practices, and periodic assessment of proficiency are vital for surveillance. Once all participating centers have been identified, several laboratory quality control procedures should be performed, including assessment of the laboratory's ability to grow Hib and sustain viable cultures of the organism, and the ability of laboratory personnel to use agglutination kits and interpret the results.

5. Conducting periodic data analysis and monitoring quality of surveillance system

5.1 Data analysis

Surveillance data should be analysed periodically. Analyses of surveillance data will be important to provide feedback to reporting sites on their performance, to identify potential problems with surveillance and to provide information to policy makers and physicians. Simple analyses of the data such as overall and age-specific incidence rates should be calculated periodically. Surveillance updates may be reported through existing newsletters or Ministry bulletins, wherever possible.

5.2 Quality indicators to monitor

Periodically reviewing the data can also help to identify problem areas in the surveillance process. Listed below are some indicators that a problem may exist somewhere in the surveillance process, and where to check for problems.

- **If < 10% of CSF specimens yield a bacterial agent**, investigate the following: (1) the ability of all laboratories to identify *H. influenzae* and other bacterial species from inoculated cultures (i.e., positive controls); (2) the criteria for taking CSF specimens (be sure that only patients with signs and symptoms of meningitis are having lumbar punctures performed); (3) the pattern of antibiotic use among patients prior to admission, and (4) the possibility of epidemics of other nonbacterial diseases (such as dengue fever, malaria, leptospirosis) that might lead to an increase in lumbar punctures.

- **If < 25% of confirmed bacterial meningitis cases are due to *H. influenzae*,** investigate the following: (1) the ability of all laboratories to identify *H. influenzae* and other bacterial species; and (2) the possibility of an ongoing epidemic of meningococcal infection or tuberculosis.

6. The total burden of severe Hib disease

Severe Hib disease can be generally categorized into one of the three following clinical groups: (i) meningitis, (ii) other invasive disease, (e.g. septic arthritis, periorbital cellulitis, epiglottitis, etc.), and (iii) pneumonia (bacteremic and nonbacteremic).

The generic protocol outlined in this document for conducting bacterial meningitis surveillance measures only a portion of the burden of severe Hib disease in a population. Meningitis is the most severe type of Hib disease with a case fatality rate (CFR) of 3-40% [6]. Meningitis leads to severe, disabling sequelae in 10-40% of survivors [32]. Most importantly, meningitis is the type of severe disease due to Hib which can be most reliably estimated from surveillance data (Table 3).

Other Hib invasive disease syndromes are generally less severe (CFR 1-5%) and do not often lead to sequelae. Several studies have demonstrated that Hib conjugate vaccines are highly effective in preventing both meningitis and bacteremic disease syndromes (>90%) [33-35].

Table 3.   Characteristics of the three major clinical syndromes of severe disease caused by *Haemophilus influenzae* **type b.**

	Clinical syndrome due to *Haemophilus influenzae* type b		
	Meningitis	**Other invasive disease**	**Pneumonia**
Accuracy of estimates from surveillance	Very good	Fair/good	Not easily estimated by routine surveillance
Case fatality rate	3-40%	1-5%	2-10%
Frequency of long-term disability following recovery	10-40%	---	1%
Efficacy of Hib conjugate vaccines for prevention	>90%	>90%	80%*

* 80% efficacy against bacteremic pneumonia; efficacy against nonbacteremic pneumonia is unknown.

In population-based studies from industrialized countries, meningitis usually accounts for 50-60% of all invasive Hib disease (excluding bacteremic pneumonia) [6]. The remaining 40-50% of invasive Hib disease is a mixture of bacteremia without focus, septic arthritis, peritonitis, periorbital cellulitis, epiglottitis, and other focal infections.

Pneumonia due to Hib is less severe (CFR 2-10%) than meningitis and may result in some long-term sequelae among survivors (1%) [36]. However, since it is not possible to establish the incidence of Hib pneumonia through routine surveillance, estimating the burden of Hib pneumonia requires extrapolation from other data (e.g., the incidence of Hib meningitis, the overall incidence of acute lower respiratory tract infections). A method for estimating the burden of Hib pneumonia is provided in Annex 4. The efficacy of Hib conjugate vaccines against bacteremic pneumonia is high (80%) [37]; however, efficacy against non-bacteremic pneumonia has not yet been measured in vaccine trials.

While data on the rate of Hib meningitis or the proportion of bacterial meningitis due to Hib cannot be directly extrapolated to estimate the proportion of ALRI due to Hib, the isolation of Hib from patients with meningitis is evidence of severe Hib disease in the population. In The Gambia and Papua New Guinea, two developing countries where the etiologic agents of meningitis and pneumonia have been studied, the proportion of bacterial meningitis due to Hib was 40% and 49%, respectively, and the proportion of bacteremic pneumonia due to Hib was 15% and 44%, respectively [8,38,39]. Thus, although no data exist to indicate the proportion of meningitis due to Hib is directly related to the proportion of pneumonia due to Hib, these data do indicate that severe disease due to Hib occurs, and that the spectrum of severe Hib disease will likely include pneumonia.

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Annex 1.

Sample CSF specimen logbook

Specimen ID number	Date specimen received	Name of patient	Age of patient	CSF visual appear-ance	SF cell count	CSF % neutro-phils	CSF glucose (mg/dl)	CSF protein (mg/dl)	CSF gram stain	CSF culture result	CSF latex agglutin-ation test result

Annex 2:

Bacterial meningitis case report form

Reporting hospital: _____ Medical record No._____

Patient information

Family name: _____ First name:_____

Address:_____ City: _____ District:_____

Resident of surveillance area: Yes/No Sex: M/F

Age (months):__ __ Date of Birth : __ __ / __ __ / __ __ (day/mo/yr)

Clinical information

Date of specimen collection: __ __ / __ __ / __ __ (day/mo/yr)

Outcome at discharge: Survived / Died / Unknown

For survivors: Was there evidence of neurologic deficit, deafness, or other sequelae at discharge? Yes / No / Unknown

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Laboratory information

Appearance of CSF: Clear / Cloudy / Bloody / Not Done

Cell count:_____   % Neutrophils:____

Protein:____ (mg/dl)   Glucose:____ (mg/dl)

Gram stain result: _____ (ND, if Gram stain not done)

Bacteria identified from CSF or blood:

Method of detection (Check all that apply)

	CSF culture	CSF latex	Blood culture
Haemophilus influenzae	____	____	____
Neisseria meningitidis	____	____	____
Streptococcus pneumoniae	____	____	____
Other:_____	____	____	____

For *H. influenzae,* please complete the following:

Was the isolate serotype B?   Type B__   Not Type B__   Not done ____

For *N. meningitidis,* please complete the following:

What was the serogroup?   A __ B __ C __ Other __   Not done ____

Person completing form:

Name _____ : Signature: _____ Date:_____

Supervisor who reviewed form:

Name _____ : Signature: _____ Date:_____

Annex 3:

Microbiologic methods for identification of

H. influenzae from cerebrospinal fluid specimens

Note: This annex is adapted from the CDC/WHO publication, "Manual for the National Surveillance of Antimicrobial Resistance of *S. pneumoniae* and *H. influenzae:* Epidemiological and Microbiological Methods", which is available from by the Programme for the Control of Acute Respiratory Infections, World Health Organization, 1211 Geneva 27, Switzerland.

For the procedures described in this section it is assumed that at least one formally trained microbiologist is available. This individual should be familiar with sterilization techniques, including the use of a flame to sterilize inoculating loops and the lips of pouring containers, the use of an autoclave with quality control autoclave indicator tape or equivalent indicators; such as spore forming bacteria, and the use of filters to sterilize heat labile fluids. The microbiologist should also be familiar with the use of a good microscope to view Gram-stained slides, with viewing colonies on agar plates, and with use of a pH meter to determine acidity or alkalinity of fluids.

The microbiologist must be experienced in procedures required to select colonies of bacteria to obtain pure cultures of *H. influenzae.* He or she will also need to know the techniques required to maintain, store, and transport pure cultures of bacteria. Lastly, the microbiologist must be familiar with safety procedures, including disposal of all used and unused materials.

All microbiologists involved with the project should carefully read this section of the protocol. The projected number of samples to be tested can be obtained from the surveillance coordinator.

A. Collection of specimens

The collection of clinical specimens is an important step in the identification of bacterial agents that cause meningitis. Whenever possible, it is preferable to collect the clinical specimens before antimicrobial therapy is begun. Specimens should be processed in the bacteriology laboratory as soon as possible. If immediate culturing of clinical specimens is not possible, see Section F of this Annex for instructions on transport of clinical material.

The collection of cerebrospinal fluid (CSF) is an invasive technique and should be performed only by experienced personnel in a hospital environment. Collected and processed under proper conditions, CSF is an excellent source of *H. influenzae.* Lumbar puncture should be performed for diagnosis, not for surveillance alone. CSF should be inoculated directly onto chocolate agar (i.e., not diluted in a broth as with blood cultures).

B. Transport of clinical material

H. influenzae are fastidious and fragile bacteria. *H. influenzae* is more easily isolated if the CSF specimen is plated immediately.

C. Incubation conditions

The agar plates inoculated with CSF should be incubated in a 5-10% CO_2 incubator or candle-extinction jar at 35-37°C.

D. Inoculation of primary culture media

A portion of the CSF is used to inoculate the primary culture media. For *H. influenzae,* a chocolate agar plate (preferably, supplemented with X and V factors) must be used. A bacteriologic loop is used to streak or thin the bacteria into single colonies. The agar plates should be incubated in a 5-10% CO_2 incubator or candle-extinction jar.

E. Subculture and identification

Since the primary purpose of this protocol is to aid in the identification of *H. influenzae* meningitis, the methods described here are not designed for optimal identification of other bacteria that may be of clinical importance. For information on methods to identify *S. pneumoniae, N. meningitidis* and other bacteria, refer to clinical microbiology manuals such as the Manual on Clinical Microbiology (from the American Society for Microbiology) for procedures to identify these other bacteria. Gram stains of colonies may be helpful in identification of bacteria, especially if atypical or aberrant reactions are observed on the tests to identify *H. influenzae.* On Gram stain, *H. influenzae* are small gram-negative rods with random arrangement. Other manuals should be consulted for gram stain reactions of other bacteria.

For information on isolation of *Streptococcus pneumonia*, refer to the WHO/CDC document, "Manual for the National Surveillance of Antimicrobial Resistance of *S. pneumoniae* and *H. influenzae*: Epidemiological and Microbiological Methods", available through the Programme for the Control of Acute Respiratory Infections, World Health Organization, 1211 Geneva 27, Switzerland.

For information on the isolation of *Neisseria meningitidis*, refer to the WHO document, "Control of Epidemic Meningococcal Disease: WHO Practical Guidelines". This document is available through: Edition Fondation Marcel Mérieux, 17, rue Bourgelat - B.P. 2021, 69227 Lyon, France

E.1 Recognition of colonies

On chocolate agar, *H. influenzae* appear as large, flat, colorless to gray opaque colonies. No hemolysis or discoloration of the medium is apparent. Encapsulated strains appear more mucoidal than nonencapsulated strains, which appear as compact grayish colonies.

E.2 Identification of Haemophilus influenzae

H. influenzae is a fastidious organism requiring media containing haemin (X factor) and nicotinamide adenine dinucleotide (NAD, V factor). Growth occurs on choco-

late agar because NAD (V factor) is released during the heating process in the preparation of chocolate agar. Haemin is available from nonhemolyzed as well as hemolyzed cells. *H. influenzae* is identified based on growth requirements for X and V factors. A single colony from the primary isolation plate is selected and subcultured to a chocolate agar plate. After overnight incubation, a heavy suspension of cells is prepared in a suitable broth -- trypticase soy, heart infusion, or peptone water. Caution should be used in preparation of the broth so that no media are transferred to the broth; even the smallest sample of agar will affect the following tests and may lead to misidentification of the bacteria.

The suspension is used to inoculate a heart infusion or trypticase soy agar plate. A large wire loop (2 or 3 loops should be used if the loop is small) of the suspension is streaked over one-half of the plate, and paper strips or disks containing X, V, and XV factors are placed on the inoculated plate after the inoculum has dried. *H. influenzae* will grow only around the disk containing both X and V factors.

A freezer vial for permanent storage of the strain should also be prepared from the chocolate agar plate (see Section F of this Annex for instructions on preservation). If the *Haemophilus* strain is to be transported, a chocolate agar slant should also be prepared from the chocolate agar plate at this time.

F. Preservation and transport of *H. influenzae*

To confirm the identification and antimicrobial susceptibility of bacteria isolated during these studies, it may be necessary to preserve and transport the strains to national, and/or WHO reference laboratories. *H. influenzae* are fragile bacteria, and care must be employed in order to preserve and transport the bacteria.

Viability during short-term storage (one week or less) is best if *H. influenzae* are inoculated onto chocolate agar slants (with screw-capped tubes), incubated overnight at 35°C, and then maintained at 4°C. *H. influenzae* do not survive well in broth and survive only 3-5 days on primary agar plates. Thus, preservation for transport to reference laboratories must be an essential part of this programme.

Long-term storage is best accomplished by either lyophilization or freezing. Detailed instructions on both these methods are available in the "Manual for the National Surveillance of Antimicrobial Resistance of *S. pneumonia* and *H. influenzae*". Cultures stored in the frozen state must be recultured, inoculated onto chocolate agar slants in screw-capped tubes, incubated overnight, and then packaged for transport.

Cultures with proper packaging can be transported without refrigeration. The cultures should be packaged according to the regulations specified in The Laboratory Biosafety Manual, WHO, Geneva, 1993. Briefly, the vials or tubes containing the cultures must been closed in a metal container, which is then enclosed in a shipping container. An address label and an etiologic agent warning label are attached to the shipping container. The culture is packaged inside the metal container in sufficient absorbent material to absorb the entire contents of the culture if the culture tube or vial becomes broken. No more than 50 ml of culture should be shipped in one package. Lyophilized culture vials can be safely transported with proper packaging.

Annex 4:

A method to estimate local burden of acute lower respiratory tract infections due to Hib

In most countries acute lower respiratory tract infections (ALRI) are a leading cause of childhood death[1]. WHO estimates that, in developing countries/areas, over 30% of all deaths among children <5 years old are from ALRI (3.7 million deaths annually)[2]. The proportion of deaths due to ALRI in a country tends to be higher than the global average in countries that have high child mortality rates and to be lower in those with lower child mortality rates[3].

The Centers for Disease Control and Prevention (Atlanta, GA, USA) and the WHO Programme for Control of Acute Respiratory Infections (Geneva, Switzerland) recently reviewed the epidemiology and etiology of pneumonia in developing countries. Of the estimated 3.7 million ARLI deaths among children < 5 years old in the developing world, the CDC/WHO review estimates that 342 000 (9%) are due to Hib[2]. This estimate of Hib ALRI deaths is approximately ten times more than the estimated number of Hib meningitis deaths among children < 5 years old in developing countries (J Schillinger, CDC, personal communication 1995).

When interpreting this global estimate of the burden of Hib pneumonia it is important to keep in mind that the proportion of bacterial pneumonia due to Hib may vary considerably across geographic regions. Consequently, one may wish to consider whether or not the global estimates of the burden of Hib pneumonia are appropriate for the specific surveillance area. Ideally, this could be accomplished by using data from careful studies of the etiologic agents of pneumonia among children <5 years old in the surveillance area. However, because studies of the etiologic diagnosis of pneumonia are complicated, it is unrealistic to expect that this information will be available in every site. In areas where this information is available, it should be incorporated into estimation of the burden of ALRI due to Hib.

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[1]Greenwood B. Epidemiology of acute lower respiratory tract infections, especially those due to *Haemophilus influenzae* type b, in The Gambia, West Africa. *J Infec Dis.* 1992;165(suppl 1):S26-8.

[2]Schwartz B, Gove S, Lipman H, Lob-Levyt G. The etiology of acute lower respiratory tract infections among young children in developing countries. *Pediatr Infect Dis J.* (in press)

[3]Garenne M, Ronsmans C, Campbell H. The magnitude of mortality from acute respiratory infections in children under 5 years in developing countries. *World Health Stat Q.* 1992;45:180-91.

The burden of Hib pneumonia in a surveillance area can be calculated using the WHO estimates of the global burden of Hib pneumonia, mortality data from the Ministry of Health, and Hib meningitis surveillance data, as shown in the attached Worksheet. The first step in making these estimates is to determine the national mortality burden of ALRI in children. In many countries these data may be available from mortality surveys or death registries. Where such data are not available, ALRI mortality can be estimated from the overall, all-cause mortality rate for children <5 years old. The proportion of mortality due to ALRI increases as the overall child mortality rate increases[3], as shown by the adjustment factor in Worksheet Step 1.

The overall burden of Hib pneumonia mortality can be estimated by multiplying the estimate for the number of ALRI deaths by the proportion likely caused by Hib (9%) (Worksheet Step 2). To calculate the number of Hib pneumonia cases from the number of deaths, one would divide the estimated number of Hib ALRI deaths by 6%, the Hib pneumonia case fatality rate estimated by CDC/WHO[2] (Worksheet Step 3).

Worksheet for estimating annual mortality and morbidity rates of acute lower respiratory tract infection (ALRI) due to Hib

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Step 1. Estimate ALRI deaths per 1000 children <5 years old

Source of data: If available, use statistics from the national ALRI control programme. If information on mortality due to ALRI specifically is not available, develop an estimate of the ALRI mortality rate by multiplying the overall, all-cause mortality rate per 1000 children <5 years by the appropriate adjustment factor (adopted from Garenne et al. 1992):

All-cause deaths per 1000 children <5 years:	Adjustment factor:
>75	0.25
25-75	0.20
10-24	0.15
<10	0.10

Estimated number of ALRI deaths per 1000 children <5 years = _____ $_x$ _____   *= (A)*
                                                                        All-cause mortality   Adjustment factor

---

Step 2. Estimate Hib-ALRI deaths per 1000 children <5 years old

Source of data: A WHO review of the etiology of ALRI deaths in developing countries indicates that, on average, 9% of ALRI deaths are due to Hib (Schwartz et al. in press). To calculate the estimated number of Hib-ALRI deaths, multiply the estimated ALRI mortality rate per 1000 children <5 years old (A) by 0.09.

Estimated number of Hib-ALRI deaths per 1000 children <5 years = (A) x 0.09 = _____ *(B)*

Step 3. Estimate Hib-ALRI cases per 1000 children <5 years old

Source of data: A WHO review of the etiology of ALRI deaths in developing countries indicates that, on average, the Hib-ALRI case fatality rate is 6% (Schwartz et al. in press). To calculate the Hib ALRI incidence rate, divide the estimated Hib-ALRI mortality rate (B) by 0.06.

Estimated number of Hib-ALRI cases per 1000 children <5 years = (B) / 0.06 = _____ *(C)*

Step 4. Estimate annual rate of Hib-ALRI cases per 100 000 children < 5 years old

To make the annual rate of Hib-ALRI cases per 1000 children comparable to the rates per 100 000 children < 5 years old used for meningitis, multiply the rate (C) by 100.

Estimated number of Hib-ALRI cases per 100 000 children < 5 years = (C) x 100 = _____ *(D)*