

# **Documenting the Impact of Hepatitis B Immunization: best practices for conducting a serosurvey**

**Immunization, Vaccines and Biologicals**



**World Health  
Organization**

# **Documenting the Impact of Hepatitis B Immunization: best practices for conducting a serosurvey**

**Immunization, Vaccines and Biologicals**



**World Health  
Organization**

---

**The Department of Immunization, Vaccines and Biologicals  
thanks the donors whose unspecified financial support  
has made the production of this document possible.**

This document was produced by the  
*Expand Programme on Immunization (EPI)*  
of the Department of Immunization, Vaccines and Biologicals

*Ordering code: WHO/IVB/11.08*

*Printed: November 2011*

**This publication is available on the Internet at:**

[www.who.int/vaccines-documents/](http://www.who.int/vaccines-documents/)

**Copies of this document as well as additional materials on  
immunization, vaccines and biologicals may be requested from:**

World Health Organization  
Department of Immunization, Vaccines and Biologicals  
CH-1211 Geneva 27, Switzerland

• *Fax:* + 41 22 791 4227 • *Email:* [vaccines@who.int](mailto:vaccines@who.int) •

© World Health Organization 2011

All rights reserved. Publications of the World Health Organization can be obtained from WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel: +41 22 791 3264; fax: +41 22 791 4857; email: [bookorders@who.int](mailto:bookorders@who.int)). Requests for permission to reproduce or translate WHO publications – whether for sale or for noncommercial distribution – should be addressed to WHO Press, at the above address (fax: +41 22 791 4806; email: [permissions@who.int](mailto:permissions@who.int)).

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

All reasonable precautions have been taken by the World Health Organization to verify the information contained in this publication. However, the published material is being distributed without warranty of any kind, either expressed or implied. The responsibility for the interpretation and use of the material lies with the reader. In no event shall the World Health Organization be liable for damages arising from its use.

The named authors alone are responsible for the views expressed in this publication.

**Printed by the WHO Document Production Services, Geneva, Switzerland**

---

# Contents

<i>Abbreviations</i> .....	v
<i>Glossary</i> .....	vii
<b>Introduction</b> .....	<b>1</b>
<i>Background</i> .....	1
<i>Overview of programme assessment strategies</i> .....	4
<b>Guidelines for a hepatitis B serosurvey</b> .....	<b>6</b>
<i>Survey objectives</i> .....	6
<i>Timing of the survey in relation to vaccine programme introduction</i> .....	6
<i>Age group to survey for vaccine programme evaluation</i> .....	7
<i>Age group to survey for baseline prevalence</i> .....	7
<i>Design and sampling</i> .....	8
<i>Sampling procedure</i> .....	8
<i>Sample size</i> .....	10
<i>Preparing for the field</i> .....	12
<i>Human resources</i> .....	12
<i>Field methods</i> .....	13
<i>Laboratory testing algorithm</i> .....	17
<i>Storage of blood specimens</i> .....	18
<i>Quality assurance</i> .....	19
<i>Supplies and budget</i> .....	21
<i>Data entry, cleaning, and storage</i> .....	22
<i>Data analysis</i> .....	23
<i>Interpretation of statistical analysis</i> .....	24
<i>Bias and limitations</i> .....	24
<i>Ethical considerations</i> .....	25
<i>Protocol template</i> .....	25
<b>Bibliography</b> .....	<b>26</b>
<b>Appendix 1: Laboratory considerations</b> .....	<b>28</b>
<b>Appendix 2: Prevalence of hepatitis B infection before and after the introduction of hepatitis B vaccination</b> .....	<b>31</b>
<b>Appendix 3: Example forms</b> .....	<b>32</b>
<b>Appendix 4: Sample questionnaires</b> .....	<b>35</b>
<b>Appendix 5: Practical guidance on paediatric and neonatal blood sampling</b> .....	<b>37</b>
<b>Appendix 6: Logistics</b> .....	<b>42</b>
<b>Appendix 7: Sample table for anticipated data analysis</b> .....	<b>43</b>

---

---

# Abbreviations

AEFI	Adverse events following immunization
Anti-HBc	Antibody to hepatitis B core antigen
Anti-HBs	Antibody to hepatitis B surface antigen
DE	Design effect
EDTA	Ethylenediaminetetraacetic acid
EMR	WHO Eastern Mediterranean Regional Office
EPI	Expanded programme on immunization
HAV	Hepatitis A virus
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HEV	Hepatitis E virus
HIV	Human immunodeficiency virus
OR	Odds ratio
WHO	World Health Organization
WPR	WHO Western Mediterranean Regional Office

---

---

# Glossary

Anti-HBc	Antibodies to Hepatitis B core antigen (HBcAg) – a protein found in the core of the virus.
Anti-HBs	Antibodies to the surface antigen of hepatitis B virus.
HBeAg	Hepatitis B ‘e’ antigen – indicates greater infectivity in current infection.
HBsAg	Hepatitis B surface antigen: a protein from the virus’s coat. A positive test for HBsAg indicates active HBV infection. The immune response to HBsAg provides the basis for immunity against HBV, and HBsAg is the main component of HepB.
HCC	Hepatocellular carcinoma, or primary liver cancer - a major complication of chronic HBV infection; usually fatal.
Seroprevalence	Percentage of a population positive for a specific antigen (e.g. HBsAg) or antibody (e.g. to anti-HBc).

---

---

# Introduction

## Background

### *Hepatitis B infection: natural history and burden of disease*

Hepatitis B virus (HBV) infection is a major public health problem worldwide. Approximately 30% of the world's population, or about 2 billion persons, have serologic evidence of current or past HBV infection. Of these, an estimated 360 million have chronic HBV infection and 600,000 persons die each year from HBV-related acute hepatitis, hepatocellular carcinoma (HCC, a form of liver cancer) and cirrhosis.<sup>1,2,3</sup> As a known human carcinogen, the impact of HBV infection is second only to tobacco. Persons with concomitant HIV infection are at even greater risk of HBV-related cirrhosis, end-stage liver failure and HCC.<sup>4</sup>

Infection with HBV can be asymptomatic or can cause acute hepatitis. These conditions either resolve spontaneously with subsequent immunity or lead to a chronic infection that may be lifelong. Mother-to-child transmission of HBV at birth results in chronic infection in 90% of infants of mothers with hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg), and approximately 10% of HBsAg-positive/HBeAg-negative mothers. In the absence of vaccination, more than 20% of HBV-related deaths are attributable to perinatal infection. Chronic infection develops in 80-90% of infants infected in the first year of life; this risk declines to 30-50% for children infected between 1 and 4 years of age (Figure 1). In adolescents and adults, HBV infection occurs through sexual transmission, exposure to infected blood products or contaminated needles and syringes, with 2-5% becoming chronically infected.<sup>5</sup>

Hepatitis B endemicity is defined by the seroprevalence of HBsAg in the general population. In countries with high ( $\geq 8\%$  HBsAg(+)) and intermediate (2-7%) endemicity, most of the HBV-related disease burden is due to liver cancer and cirrhosis in adulthood resulting from chronic HBV infection acquired at birth or in early childhood. Most (88%) of the global population live in areas of high or intermediate endemicity.<sup>6</sup>

---

<sup>1</sup> Goldstein ST, Zhou F, Hadler SC, Bell BP, Mast EE, Margolis HS. A mathematical model to estimate global hepatitis B disease burden and vaccination impact. *Int J Epidemiol.* 2005 Dec;34(6):1329-39.

<sup>2</sup> Shepard CW, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. *Epidemiol Rev.* 2006;28:112-25.

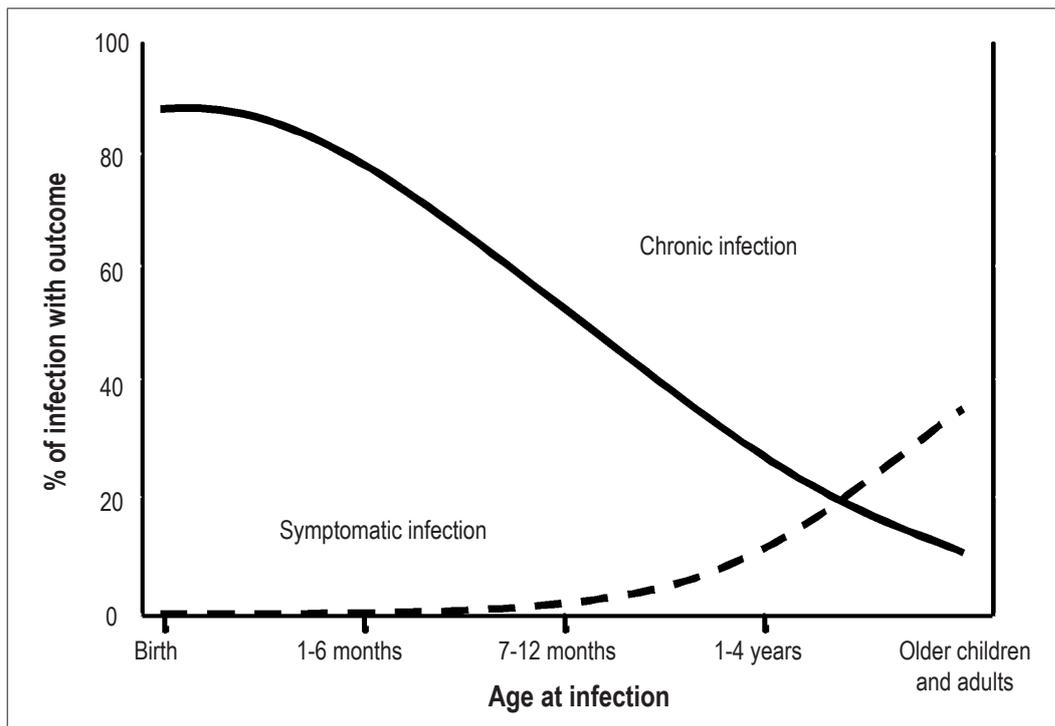
<sup>3</sup> Hepatitis B vaccines. *Wkly Epidemiol Rec.* 2009 Oct 1;84(40):405-19.

<sup>4</sup> McGovern BH. The epidemiology, natural history and prevention of hepatitis B: implications of HIV coinfection. *Antivir Ther.* 2007;12 Suppl 3:H3-13.

<sup>5</sup> <http://www.cdc.gov/hepatitis/HBV/index.htm>

<sup>6</sup> <http://www.who.int/mediacentre/factsheets/fs204/en/index.html>

Figure 1: Outcome of hepatitis B virus infection by age at infection.



These definitions based on seroprevalence of chronic infection are used because of the difficulties of measuring hepatitis B at a population level by other methods. Many infections are asymptomatic, the diseases caused (acute and chronic hepatitis, liver cancer) have multiple other causes which can only be separated with sophisticated laboratory methods and much of the disease goes undetected by health services. For all of these reasons seroprevalence surveys are also the best method of assessing the impact of control programmes.

### *Hepatitis B vaccines and immunization*

A safe and effective vaccine against hepatitis B, available since 1982, prevents HBV infection when it is given before or shortly after exposure. The main objective of hepatitis B immunization is to prevent chronic HBV infection and its consequences. The primary strategy is to prevent perinatal and early childhood HBV transmission through the timely administration of hepatitis B vaccine at birth (within 24 hours) and completion of the primary vaccination series by 6 months of life.

A course of three doses of hepatitis B vaccine induces protective levels of antibody to HBsAg (anti-HBs) in over 95% of healthy infants and children, preventing acute and chronic HBV infection. A dose at birth is particularly important to prevent perinatal infection leading to carriage. The interval between subsequent doses in the infant schedule is not critical in determining the immune response<sup>7</sup>.

<sup>7</sup> Inskip HM, Hall AJ, Chotard J, Loik F, Whittle H. Hepatitis B vaccine in the Gambian Expanded Programme on Immunization: factors influencing antibody response. *Int J Epidemiol.* 1991 Sep;20(3):764-9.

---

Hepatitis B vaccination guidelines are available (Introduction of hepatitis B vaccine into childhood immunization services and management guidelines, including information for health workers and parents. (WHO/V&B/01.31))<sup>8</sup>.

Serological markers of hepatitis B infection and immunity are summarized in Appendix 1.

### *Progress in hepatitis B control*

WHO recommends that all countries include hepatitis B vaccine in routine vaccination schedules for all children and that the first dose be given as soon as possible after birth, ideally within 24 hours. With high vaccination coverage, HBsAg seroprevalence is typically reduced to 1% or less. By the end of 2008, 177 countries (92% of WHO member states) had introduced the vaccine. However, with the global vaccination coverage for the 3rd dose of hepatitis B vaccine at 69% in 2008<sup>9</sup> and many countries not using a birth dose, millions of children remain unprotected each year, providing a reservoir for the continued transmission of HBV.

In 2007, the Western Pacific Region (WPR) became the first WHO Region to establish a hepatitis B control goal of achieving <2% prevalence of chronic hepatitis B infection by 2012. WPR has established a certification procedure to document national achievement of hepatitis B control.<sup>10</sup> Certification will be based on the HBsAg prevalence among children 5 years or older born after the start of nationwide infant hepatitis B vaccination. At least one representative serological survey is required.

In 2008, the Strategic Advisory Group of Experts on Immunization (SAGE) strongly recommended that all WHO Regions and associated countries develop goals for hepatitis B control appropriate to their epidemiologic situations. In the report of this meeting,<sup>11</sup> they establish the basis for the use of control goals and how these should be measured as follows:

“Control goals are essential for regions and countries with intermediate or high endemicity of hepatitis B virus infection or subpopulations with these levels of infection. Further, process indicators towards these goals could continue to be based on coverage of the third dose of hepatitis B vaccine and that of the birth dose (with improved birth dose definition and monitoring). However, the use of outcome measures are critical to verification of achievement of such goals. Serologic surveys of hepatitis B surface antigen (HBsAg) prevalence, representative of the target population, will serve as the primary tool to measure the impact of immunization and achievement of the control goals, supplemented by acute disease surveillance and mortality data.”

---

<sup>8</sup> World Health Organization Department of Vaccines and Biologicals. Introduction of hepatitis B vaccine into childhood immunization services: Management guidelines, including information for health workers and parents. Geneva: World Health Organization; 2001. Report No.: WHO/V&B/01.31.

<sup>9</sup> Global Immunization Data October 2009. World Health Organization; 2009.

<sup>10</sup> World Health Organization Regional Office for the Western Pacific. Guidelines for certification of achievement of hepatitis B control goal in the Western Pacific Region. Manila, Philippines; 2007.

<sup>11</sup> Meeting of the immunization Strategic Advisory Group of Experts, November 2008--conclusions and recommendations. Wkly Epidemiol Rec. 2009 Jan 9;84(1-2):1-16.

---

The Eastern Mediterranean Regional (EMR) Committee, in October 2009, adopted a regional target of reduction in prevalence of chronic hepatitis B virus infection to less than 1% among children below 5 years of age by 2015.<sup>12</sup>

## Overview of programme assessment strategies

### *Purpose of the assessment*

Options for evaluating the performance or impact of a hepatitis B vaccination programme are summarized in Box 1. The purpose of the assessment will guide the selection of methods.

Performance reviews can include some or all aspects of programme implementation, vaccine coverage and vaccine effectiveness. Impact is monitored through cross-sectional measures of current (HBsAg) and past (anti-HBc) infection and surveillance for acute and chronic HBV-related disease.

The sequelae of chronic HBV infection (cancer and cirrhosis) typically manifest in adulthood, and acute infection in young children is usually asymptomatic. To evaluate hepatitis B control, the most practical and timely approach is to monitor vaccination coverage (to assess service delivery) and current infection prevalence (to assess impact). Evaluation of hepatitis B control will require both.

---

<sup>12</sup> World Health Organization Regional Committee for the Eastern Mediterranean. Resolution: The growing threats of hepatitis B and C in the Eastern Mediterranean Region: a call for action; 2009 October 2009. Report No.: EM/RC56/R.5.

---

## Box 1: Methods for assessing hepatitis B vaccination programmes

### Programme performance

- Programme review (EPI review, post-introduction evaluation)
  - policy environment (national priorities and processes, certification)
  - system-wide (stewardship, human resources, finance, service delivery)
  - programme-specific (vaccination services, surveillance and monitoring, vaccine supply and quality, logistics, training, advocacy and communications)
  - management (objectives, strategies, results, reach, resources, innovation)
  - Economic evaluation (cost-effectiveness, health services utilization)
- Vaccine coverage
  - administrative data
- coverage survey
- Vaccine effectiveness and quality
  - case-control study
  - immune response (anti-HBs)
- Surveillance for adverse events following immunization (AEFI)

### Programme impact

- Serosurveys
  - current infection (HBsAg)
  - past infection (anti-HBc)
  - infectivity (HBeAg/Ab)
- Incident disease (immunoglobulin M (IgM) anti-HBc)
- Surveillance
  - acute disease (acute hepatitis, jaundice)
- Chronic disease (cirrhosis, liver cancer)

In practice, a comprehensive EPI review or vaccine coverage survey will include assessment of hepatitis B vaccination. Where feasible, surveillance for acute disease as well as disease registries of persons with chronic infections, cirrhosis, and hepatocellular carcinoma can provide additional information, particularly for older age groups where clinical manifestation is more common. Together, these methods provide comprehensive information on hepatitis B control and vaccination programme performance.

This document is specifically about impact assessment through serosurveys. This may be a standalone method or as part of a broader evaluation of hepatitis B control. Surveys of this kind have been carried out in a number of areas of the world as illustrated in Appendix 2.

---

# Guidelines for a hepatitis B serosurvey

This document is primarily aimed at the lead investigator(s) to assist them in designing the surveys.

## Survey objectives

The first step in planning a hepatitis B serosurvey is to establish the survey objectives. This document is intended for those whose objective is either to establish baseline prevalence of hepatitis B infection among children prior to the introduction of hepatitis B vaccination, or to assess hepatitis B vaccine programme impact. So as an example the objective might be to “evaluate the prevalence of chronic HBV infection among a nationally representative sample of children born after the introduction of hepatitis B vaccine into infant immunization programmes”.

The survey objective will determine the age range of the target population. In a baseline survey, you may wish to measure prevalence infection across the whole population. You should refer to the documentation of the target established by your region to ensure that your survey will satisfy the requirements.

While you are carrying out this serosurvey, you may wish to undertake additional assessment, such as measuring the prevalence of other biological markers (e.g. HAV, HCV, HEV, HIV, tetanus, or nutritional markers), estimating vaccine effectiveness or determining the prevalence of HBsAg and HBeAg in women of childbearing age, thus assessing the importance of perinatal transmission and the need for a timely birth dose of hepatitis B vaccine in your country (see online guidance regarding these “add-on” objectives). Because this document is concerned only with the carrying out of either baseline or programme evaluation serosurveys, add-on objectives such as these will not be discussed further here.

## Timing of the survey in relation to vaccine programme introduction

Ideally you have nationally representative baseline data on disease burden from the period prior to the introduction of the vaccine programme. This is not always the case. However, it is possible to study older individuals to assess the prevalence of hepatitis B infection in a cohort born prior to vaccine introduction at the same time as carrying out your serosurvey for vaccine programme evaluation.

---

The guidelines for certification of achievement of the hepatitis B control goal in WPR offer flexibility in serosurvey timing depending on how long it is since the vaccine programme was introduced. The Technical Advisory Group for EMR has recommended that Member States who introduced vaccine more than 10 years ago assess hepatitis B vaccine impact.

In general, the longer since the vaccine programme was introduced, the more likely it is that the certification target will have been reached. This is because herd immunity increases as the proportion of the population who are immune by virtue of vaccination increases. When aiming to evaluate achievement of a control goal such as those in WPR or EMR, you should survey children at least 5 years of age once your vaccine programme is mature (i.e. you have attained at least 80% coverage of infants for at least 5 years). For example: if your programme had HepB3 coverage of Year1: 50%; Year 2: 65%; Year 3: 80%; Year 4: 82 %, Year 5 81% it might be appropriate to sample the year 3 cohort when they have reached 5 years of age.

### **Age group to survey for vaccine programme evaluation**

For vaccine programme evaluation, you should survey children who are at least 5 years of age. These children will have had an opportunity to be vaccinated and have passed through the period of highest risk of chronic infection. Note that we are assuming that you have attained high hepatitis B vaccine coverage for at least 5 years.

If baseline prevalence data were not collected prior to vaccine programme implementation, you could additionally survey older children born before the introduction of the vaccine programme. Prevalence of current infection in these older children will indicate what prevalence was before the vaccine programme was introduced. For example, if your programme was introduced 10 years ago, you could survey children 11-15 years of age to get an estimate of baseline prevalence. Do not survey children older than 15 years for the purpose of estimating baseline prevalence because other modes of transmission affect people older than 15, limiting their comparability to children aged 5-10 years in terms of exposure to hepatitis B.

When planning your survey, consult WHO region-specific requirements for evaluation of control goals to determine what vaccine coverage data might be required, and in which age groups.

You should survey both boys and girls since current infection is equally common amongst boys and girls at least up to the age of 10 years.

### **Age group to survey for baseline prevalence**

If you have yet to introduce a hepatitis B vaccine programme, or if the programme has been in place for fewer than 5 years, you may survey children between the ages of 5 and 15 years of age in order to estimate baseline prevalence of current infection before the vaccine programme will have had a major impact. These children will have passed through the period of highest risk of chronic infection. The precise age range to survey in order to determine baseline prevalence will depend on how long your vaccine programme has been in place. For estimating baseline prevalence, do not survey children born in the period when vaccine coverage was low or increasing, as results will be difficult to interpret.

---

Think carefully about what age groups to survey in light of where these children can be found (at home, in kindergarten, at school). It is easier to survey children who are all at school than to survey an age range of children that encompasses some who are in school, some who are in kindergarten and others who are at home or finished school.

## Design and sampling

The most appropriate study design is a cross sectional survey.

Make sure that the sample selected is nationally representative of the population of children. So-called “convenience samples” (e.g. children attending a clinic) are not representative of the general population. School-based sampling may be appropriate if more than 95% of children in the relevant age group attend school. If this is not the case, you will have to use some form of community-based sampling (see next section). In some situations with high school attendance it may be sufficient to sample non-school attenders in the community.

## Sampling procedure

The method of sampling is partly dependent on the resources and information available. You will almost certainly use a complex survey design (e.g. stratified multi-stage cluster sample with random selection at each stage). Consult *Sample Design and Procedures for Hepatitis B Immunization Surveys: A Companion to the WHO Cluster Survey Reference Manual*<sup>13</sup> for guidance in designing a sampling strategy for your survey.

Cluster sampling allows individuals to be selected from a sample of sub-groups (e.g. districts) and thus allows the survey team to work in focused geographic areas at lower field costs. Stratified cluster sampling allows calculation and comparison of HBsAg prevalence in different population groups, e.g. urban vs. rural areas. A discussion of the principles of survey sampling is provided in Immunization coverage cluster survey - reference manual (WHO/IVB/04.23).<sup>14</sup>

---

<sup>13</sup> <http://www.who.int/immunization/documents/en/>

<sup>14</sup> World Health Organization Department of Immunization Vaccines and Biologicals. Immunization coverage cluster survey - Reference manual. Geneva: World Health Organization; 2005. Report No.: WHO/IVB/04.23.

---

### *A note on error*

As countries move closer to certification, the prevalence of HBsAg becomes lower and less evenly distributed across the population. In this scenario certain types of what are called non-sampling errors may arise when a serosurvey is carried out. Examples of non-sampling error are non-response and coverage error. Non-response error arises when the reason for an individual not being willing or able to participate in the survey is linked to whether they are HBsAg-positive. If there is stigma associated with being HBsAg-positive, HBsAg-positive individuals may be less likely to agree to being sampled and non-response error will occur. Coverage error occurs if the likelihood of reaching population members in the first place is linked to whether they are HBsAg-positive. If HBsAg positivity is more prevalent in remote areas, which are also less likely to be sampled from because they are hard to reach, coverage error is present. Both of these types of error need to be considered, and dealt with, when designing your survey. If you believe that non-response or coverage error will be an issue for you, we strongly encourage you to seek advice from an expert in survey methodology when you are designing your survey.

### *Examples of sampling procedures*

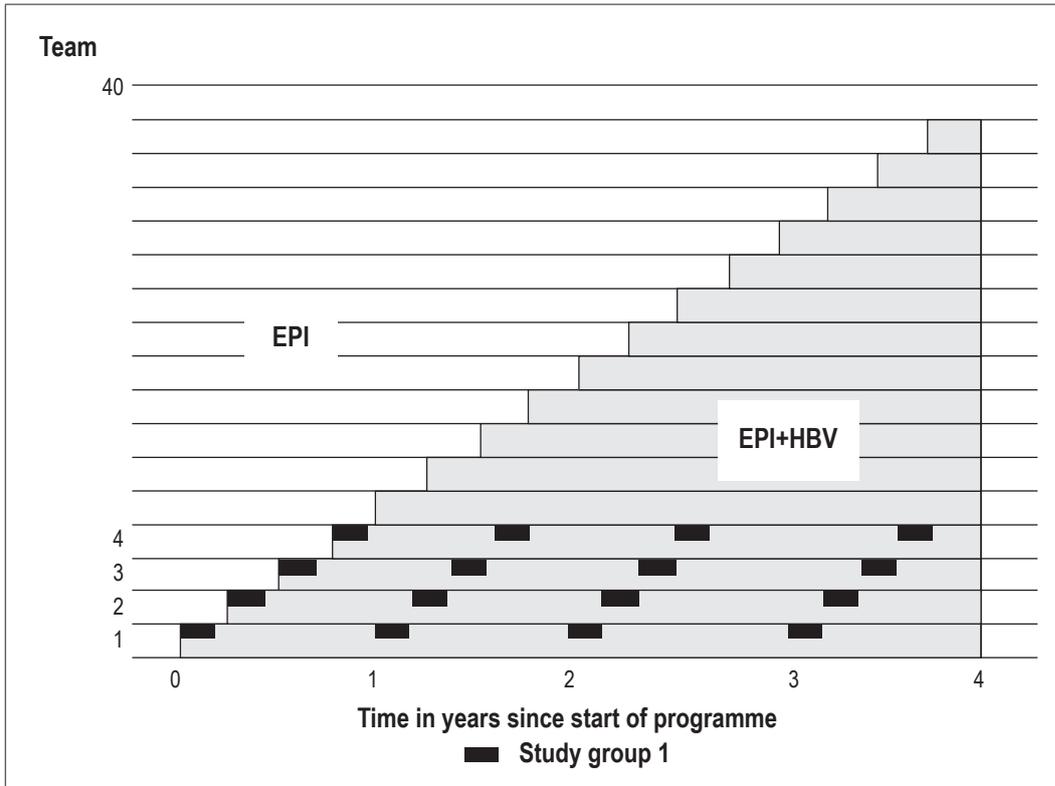
#### **Example 1: China.**

A nationally representative serological survey was carried out in China in 2006, 14 years after the introduction of the hepatitis B vaccination programme. The whole country was first divided into 3 strata based on geographical features: Eastern; Central and Western, then into urban and rural areas. Urban areas were further divided into three substrata based on the proportion of non-farming population – high, middle and low – and then further divided into three units. Rural areas were divided into three substrata based on gross domestic product (GDP) – high, middle and low – then into three units according to their total population. 160 counties were randomly selected from the urban and rural units (the ratio of urban to rural counties was 2:3). 1-4 townships were randomly selected in each county (the exact number of townships sampled in each county depended on the population of children 1-4 years in the townships). One village was randomly selected from each township and individuals were randomly selected from each village. If the village had an insufficient number of children 1-4 years of age, the nearest village was identified and the balance of children sampled from this village.

#### **Example 2: The Gambia**

Hepatitis B vaccine had been introduced to the EPI programme in The Gambia using a stepped wedge design (see below).

Figure 2: Gambia : HBV Vaccination



To assess the impact of the hepatitis B vaccination programme in The Gambia, a nationally representative serological survey was carried out in 2007/08, approximately 20 years after the introduction of the programme. Areas were selected deliberately to represent those where vaccine was introduced early or late, matched on geographical area. Two areas in Central region & two areas in Upper River region were selected. Villages within these areas were chosen at random. Individuals within villages were chosen at random using EPI house to house method.<sup>15</sup>

Note that these surveys were designed specifically for these settings and cannot therefore necessarily be replicated, as they are, in other settings.

### Sample size

The sample size is the minimum total number of children needed for the study. The sample size required depends on the prevalence of HBsAg to be measured, the design effect (DE) (a result of the sampling method used), the desired significance level (e.g. 0.05, for 95% confidence) and the desired width of the confidence interval around the estimated prevalence of HBsAg. You can use **this spreadsheet** to calculate the sample size you will need for your study.

<sup>15</sup> World Health Organization Department of Immunization Vaccines and Biologicals. Training for mid-level managers (MLM) Module 7: The EPI coverage survey. Geneva: World Health Organization; 2008. Report No.: WHO/IVB/08.07.

**Table 1: Sample size scenarios for a serological survey to evaluate universal hepatitis B immunization, assuming a design effect of 2 and a 95% confidence interval.**

Expected prevalence of HBsAg	Width of confidence interval		
	0.005 *	0.01	0.02
0.01	3,043	761	190
0.02	6,024	1,506	376
0.1	27,660	6,915	1,729

\* i.e. absolute precision of 0.5%

Sample size calculators are also available in EPiInfo and other software packages. See three worked examples of determining sample size below.

#### Example 1

Country X began vaccinating infants 5 years ago. They are surveying children aged 6-10 years to determine baseline prevalence of HBsAg before the start of vaccination.

You want to know the minimum number of children to sample if the expected prevalence of HBsAg in children 6-10 years old is 10% and the desired width of the confidence interval is  $\pm 3\%$ . Using **this spreadsheet**, and assuming a design effect of 2, the minimum sample size required is 768.

For a sample with 30 clusters, you need a minimum of  $768/30 = 25.6$  children per cluster – so you round up to 26 children per cluster, for a total sample size of  $30 \times 26 = 780$ . If you use 20 clusters, you need a minimum of  $768/20 = 38.4$  children per cluster, rounded up to 39 children per cluster for a total sample size of  $20 \times 39 = 780$ .

#### Example 2

Country Y is aiming for certification. You want to know the minimum number of children to sample if the expected prevalence of HBsAg in the target age group is 2% and the desired width of the confidence interval is  $\pm 0.5\%$ . Using [this spreadsheet](#), and assuming a design effect of 2, the minimum sample size required is 6024.

For a sample with 30 clusters, you need a minimum of  $6024/30 = 200.8$  children per cluster – so you round up to 201 children per cluster, for a total sample size of  $30 \times 201 = 6030$ . If you use 20 clusters, you need a minimum of  $6024/20 = 301.2$  children per cluster, rounded up to 302 children per cluster for a total sample size of  $20 \times 302 = 6040$ .

Likewise, to detect a HBsAg(+) seroprevalence of 1% with  $\pm 0.5\%$  precision, 95% confidence would require a sample size of 3043 individuals.

---

To compare estimates between regions or over time, you'll need the expected size of the difference in HBsAg prevalence between the two regions or surveys. To monitor seroprevalence in the same age group (and same sampling frame) over time, the sample size should be sufficient to detect a difference in prevalence of 1% relative to the prevalence estimated in the previous survey. Comparing estimates between regions or over time is more complex than estimating a single population proportion.

### Example 3

Country Z is carrying out a stratified cluster survey to assess baseline prevalence of HBsAg before they initiate vaccination of infants. The survey is stratified by urban and rural areas. Data from smaller studies suggest the prevalence of HBsAg will be approximately 15% in urban areas and 22% in rural areas. You want to know the minimum number of children to sample in order to be able to detect the prevalence in each area with an absolute precision of 3% so that we can detect a 6% difference in prevalence between urban and rural areas. Using **this spreadsheet**, and assuming a design effect of 2, the minimum sample size required for each area is 3159, or in other words a total sample size of 6318.

For a sample with 30 clusters per region, you need a minimum of  $3159/30=105.3$  children per cluster – so you round up to 106 children per cluster, for a total sample size for each area of  $30 \times 106 = 3180$  in each region. If you use 20 clusters, you need a minimum of  $3159/20=158$  children per cluster, for a total sample size of  $20 \times 158 = 3160$  in each region.

Note that none of the cluster sampling designs described above select individual children with a probability that is proportional to the size of the cluster: the same number of children is selected from each cluster regardless its size. You will therefore need to use selection weights in all analyses, in addition to accounting for the complex sample design. For more information on selection weights please refer to Annex A.2.1.5 in Immunization coverage cluster survey – reference manual (WHO/IVB/04.23).

For information on the assessment of vaccine coverage for the purpose of certification, consult the certification guidelines for your region.

## Preparing for the field

Before going to the field, staff need to be trained in how to complete the survey questionnaires and in blood sampling procedures. A pilot study should be conducted to identify any problems.

## Human resources

You need to decide who will do the survey. Options include a national team with external help or a national team alone. External help may include: verifying the study design; assistance with statistical issues including sample size calculation; developing or reviewing the survey protocol; training field staff; piloting the protocol; providing guidance on laboratory issues; conducting laboratory analysis.

---

### *National team with external help*

- Elements in favour may include:
  - Building national capacity
  - Engaging national stakeholders
  - External help may bridge technical gap in expertise
- Elements against may include:
  - National institution(s) may not have time to conduct a serosurvey

### *National team alone*

- Elements in favour may include:
  - Building national capacity to a greater extent than when the survey is undertaken with external help
  - Engaging national stakeholders
  - Giving full ownership of the survey to national bodies
- Elements against may include:
  - The technical nature of conducting a nationally representative hepatitis B serosurvey may be beyond national capacity
  - National institution(s) might not have time to conduct the survey

## **Field methods**

### *Questionnaire*

Trained field investigators will collect basic demographic data, vaccination history (ideally by vaccination card; if not available, by parental recall) and 5ml of blood from the subjects. A sample child questionnaire and an expanded questionnaire including questions on risk factors for infection and lack of vaccination are provided in Appendix 4.

### **Demographic data**

For all study participants name, name of father and/or mother, date of birth, sex and cluster number should be collected. A unique identification number should be given to each participant in the study and used to label their blood sample. These numbers can incorporate “check numbers” so that errors in copying them onto the different forms and the samples can be detected (see Smith and Morrow for detail on field survey methods).<sup>16</sup>

---

<sup>16</sup> Smith PG, Morrow RH. Field trials of health interventions in Developing Countries: A toolbox. MacMillan 1996

---

## Vaccination data

Hepatitis B vaccination status, and vaccination status for all other vaccines, is collected based on vaccination card documentation. If the vaccination card is available the dates of vaccinations can be copied from it. (This is collecting vaccination status “by card”). If no vaccination card is available, the caretaker will be asked whether the child received hepatitis B vaccination and, if yes, how many doses the child received. (This is collecting vaccination status “by history”). Keep separate the vaccination status information collected “by history” and “by card”. Vaccination status ascertained “by history” can be verified by comparing prevalence of anti-HBs in children with vaccination history “by card” to those “by history”.<sup>17</sup> If the two are similar it suggests vaccination “by history” is reasonably accurate. As an alternative method for verifying vaccination status “by history”, children can be tested for antibody to tetanus toxoid.<sup>18</sup>

## *Blood*

The method of obtaining a blood sample depends on how you intend to test the sample. The possibilities are a point-of-care test (i.e. a test administered and interpreted in front of the person) or standard laboratory assays. A combination of these two may be chosen. (In addition, it may be possible to obtain enough blood from a finger prick taken for a point-of-care test, onto filter paper, to allow a subset of samples to be retested for quality assurance, or to allow 1 additional assay.)

The test methods for hepatitis B surface antigen are highly sensitive and the concentration of antigen in samples may be very high – it is therefore easy to cross contaminate between samples leading to false positive results. Thus, whatever method is used, strict sterile techniques should be employed with one-use disposable equipment to eliminate contamination of samples.

Table 2 summarises the points that need to be considered in making the choice of what test to use.

---

<sup>17</sup> de la Hoz F, Perez L, Wheeler JG, de Neira M, Hall AJ. Vaccine coverage with hepatitis B and other vaccines in the Colombian Amazon: do health worker knowledge and perception influence coverage? *Trop Med Int Health*. 2005 Apr;10(4):322-9.

<sup>18</sup> Research protocol: Assessment of the impact of hepatitis B vaccination in Bangladesh, a seroprevalence study. Dhaka, Bangladesh: International Centre for Diarrhoeal Disease Research, Bangladesh; 2010.

**Table 2: Considerations for selection between laboratory assays and point-of-care tests**

Considerations	Laboratory assays	Point-of-care tests
Validity	• High sensitivity /specificity	• Moderate sensitivity, high specificity (see <b>tables S.1 and S.2 online</b> )
Blood specimens	• Venipuncture by needle	• Finger- or heel prick with lancet
Supplies	• Venipuncture supplies	• Finger or heel prick supplies • Point-of-care test kits
Sample transport	• Need the ability to separate serum prior to shipment • Transporting specimens to lab (cold chain)	• Not required
Training	• Qualified health workers	• Trained assistants
Results	• After testing by lab	• Immediate
Tests	• All HBV markers	• HBsAg only*
Feedback <sup>1</sup>	• Requires a delay	• Possible immediately~
Ethics	• Informed consent	• Informed consent
Cost	• Higher	• Lower

\* possibly 1 additional assay if collect blood on filter paper

~ Requires consideration of implications for individuals in terms of treatment, prognosis and potential stigmatization

Whatever test kit is chosen, the test should have minimum 95% specificity and 95% sensitivity. Tests selected for use should be evaluated and approved by national regulatory authorities or prequalified by WHO.

Obtaining a blood sample from participating children is the most challenging aspect of these surveys. It is critically important that sufficient quantity of blood is obtained to allow the selected tests to be carried out. There are a number of possibilities:

**Point-of-care (finger or heel prick) only** – this is the simplest method to use. A new sterile lancet is used to puncture the skin of the tip of a finger or a heel and blood squeezed on to the test strip. The test indicates whether the individual is HBsAg positive or not. As mentioned above, it is possible to squeeze sufficient blood from the finger prick onto filter paper to allow retesting of a subset of samples for quality assurance purposes or for 1 additional assay. You should discuss with the laboratory to ensure that sufficient blood is obtained for laboratory testing if you choose to do this.

Pros: opportunity to immediately feedback results; rapid; inexpensive

Cons: inability to distinguish true from false positives

Note that add-ons, such as testing for anti-HBc or testing HBsAg (+) women/mothers for HBeAg, will require venous blood.

---

**Point-of-care with venous blood sampling of HBsAg positives (for confirmation of HBsAg and anti-HBc testing)** – in this situation the test strip is used to identify those who are HBsAg positive. Then a sample of venous blood is obtained from them for confirmatory testing in the laboratory.

Pros: allows confirmatory HBsAg testing; can determine false positives (HBsAg(+)/anti-HBc(-))

Cons: more expensive than point-of-care only

### **Venous blood sampling of all participants**

Pros: low probability of false positive results for current infection since only anti-HBc(+)/HBsAg(+) are considered currently infected (anti-HBc(+)/HBsAg(-) have past, resolved infection and anti-HBc(-)/HBsAg(-) were never infected); there is the possibility to carry out a number of tests for add-on objectives; residual blood can be banked for future study

Cons: cannot provide immediate feedback to survey participants; more expensive than point-of-care; possible logistical issues with, for example, centrifuging in the field; longer to get results than with point-of-care tests

**Dried blood spot (not point-of-care) of all** – in this situation a finger prick is carried out and the blood adsorbed onto a filter paper which is then dried. This option needs careful discussion with the laboratory to ensure that sufficient blood is obtained for their analysis.

Pros: do not need to centrifuge in the field; can be used for multiple different assays

Cons: cannot immediately feedback results; takes longer to get results than with point-of-care, sufficient drying can take time; possibility of cross contamination of HBsAg between filter papers.

Venipuncture and finger-/heel-prick instructions are provided in Appendix 5.

### ***Processing and transport of blood specimens***

If venous blood is being collected, the following procedures should be followed:

#### **Labelling**

Blood specimens must be labelled with the subject identification number, name, date of sample collection, and date of birth. The use of several identifiers in this manner acts as a fail safe in the event that one is not read or transcribed clearly. Labels should be affixed to the collection tubes during sample collection. Specimens should be stored in a cold box with four frozen ice packs immediately after collection.

---

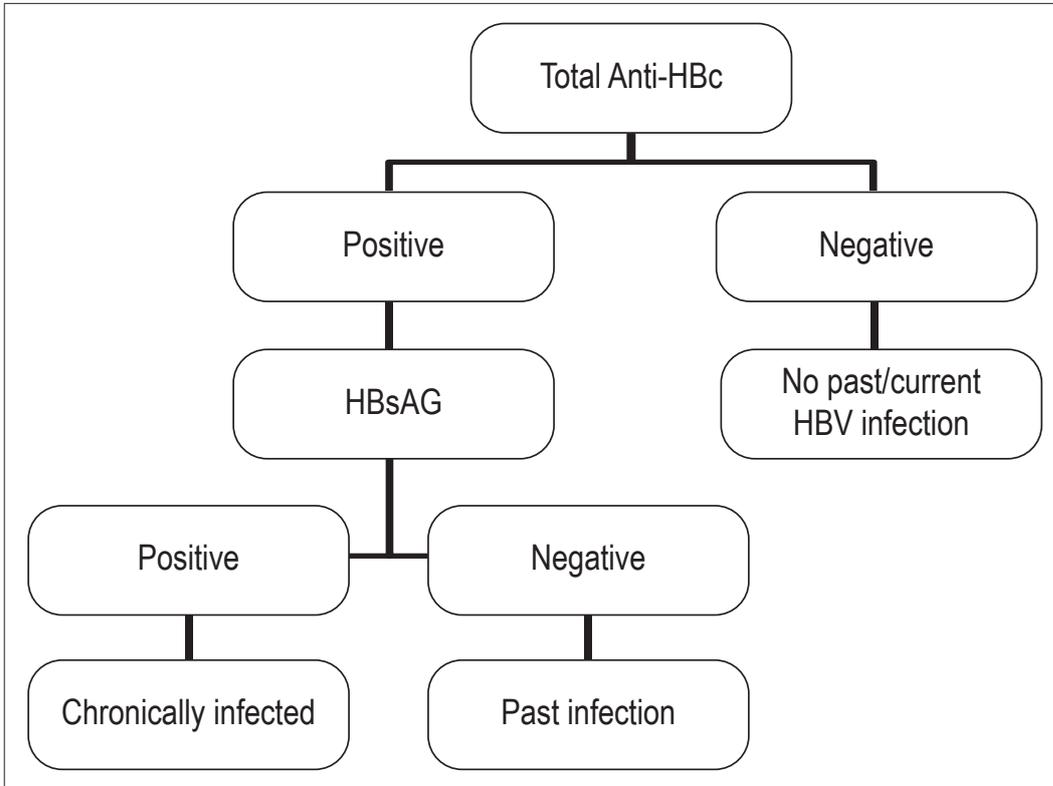
## Processing

Blood is best centrifuged for 10 minutes and serum transferred to a sterile cryovial labelled with the same information as the original label; if a centrifuge is not available, blood can be left to clot and serum poured off. We advise you to separate blood samples before driving off and agitating samples which can lyse cells. Separation should in any case be done within 24 hours of collection. Whole blood can be stored up to 24 hours at room temperature not exceeding 28°C. Serum specimens must be stored in cold-chain refrigerators and transported in specimen carriers with at least four frozen ice packs to the laboratory facility. Specimens must be kept between 2-8 degrees Centigrade during the transportation process. If whole blood is transported, it also should be done in refrigerated carriers but, to avoid haemolysis, do not put the samples directly in contact with the ice packs. Specimens should reach the testing laboratory as soon as possible but at least within one week. Point-of-care tests should either be safely discarded after use or stored if you choose to have them verified by a supervisor or kept as a quality assurance check. Should you wish to preserve samples for future studies, they should be stored at minimum -40 degrees Centigrade.

## Laboratory testing algorithm

The crucial indicator for hepatitis B vaccination programme evaluation is the prevalence of HBsAg in children. If you are planning only to, or can only afford to, test for HBsAg, use a point-of-care test. This is less expensive than testing for HBsAg in the laboratory using venous blood samples. Using a point-of-care test is also fast and allows immediate feedback of results to participants. Note however that the lower the prevalence of current infection in the country, the higher the proportion of HBsAg(+) test positives who will be false positive for current infection. In a low prevalence country it is more important to weed out these false positive HBsAg(+) results than it is in a high prevalence country. If you will test for anti-HBc or other markers of HBV infection in addition to testing for HBsAg you will need to collect venous blood. A schematic of the recommended laboratory algorithm in this case is shown in Figure 3.

**Figure 3: Testing for anti-HBc, with HBsAg testing of anti-HBc(+) samples only**



### Storage of blood specimens

**Aliquoting:** Samples may be damaged by repeated freezing and thawing. This can be avoided if samples are divided into smaller portions before freezing. The size of aliquot should be chosen so that there is enough to perform the tests required at a particular time. If only enough blood has been obtained for testing once, then aliquoting is not needed.

**Storage:** Specimens should be stored at -20 degrees Centigrade or lower (-40 if you plan to store the specimens long term). A storage record system needs to be used which allows the rapid retrieval of particular samples. If this is not done, finding particular samples can take a long time and you risk repeated freezing and thawing of specimens. In general it is best to keep samples in batches based on the dates on which they were collected or frozen. It may be helpful to keep a computerized record of the volume of serum in each sample.

**Freezer monitoring:** An uninterruptable power supply with some form of backup generator is needed for the freezers. The temperature of the freezers should be checked at least daily.

**Laboratory procedures:** A manual documenting all lab procedures from the point at which specimens are received to the completion and recording of tests should be kept including the responsibility of each staff member. A log book should be kept, where you record which test kits have been used on which dates. This log should also record any problems, such as freezer failure, which could affect the test results.

---

## Quality assurance

Before you start your survey, the survey protocol can be peer-reviewed by reviewers identified by WHO. Field and laboratory quality assurance measures are described in Box 2 below. There should be someone to coordinate the fieldwork. This person should make periodic surprise visits to the field to make sure the protocol is being followed.

### Box 2: Summary of field and laboratory quality assurance measures.

#### Quality checklist for survey

##### Field data quality

- interviewers alternate tasks of interviewing, recording and observing, checking each others' work.
- supervisors
  - observe interviews and specimen collection
  - check recorded data in the field and at the end of every day
  - check forms daily (no items are left blank)
  - ensure pre-selected clusters are used
  - ensure participants are eligible (in the correct age range)
  - check that the correct number of clusters has been surveyed
  - check each cluster has the minimum number of children
  - ensure all forms are protected (e.g. in waterproof covers)
  - complete and check summary and evaluation forms.
- coordinator
  - reviews cluster forms daily
  - ensures data is double-entered for consistency checking or
  - ensures proper completion of summary and evaluation forms.

##### Specimen processing

- every participant has a blood specimen taken
- reasons for lack or loss of specimen are recorded
- specimens are properly labelled
- specimens are properly prepared for transport
- point-of-care test results are double-checked by both interviewers and recorded
- test kits are available and all components are within the expiry date
- a quality control protocol is in place in the laboratory (e.g. all or a proportion of samples are re-tested in an alternative laboratory)
- the laboratory coordinator ensures
  - specimens are properly stored
  - results are recorded in a register
  - quality control results are reported.

---

### *Field quality assurance*

Each field team should be provided with a protocol. Ensuring quality assurance in the field has two tracts: training and supervision.

- 1) Training
  - Of field staff: in survey methodology, interview procedure (informed consent, vaccination history), blood sample collection (safety), blood sample storage and transportation, recording and reporting
  - Of field supervisors: as for field staff, with quality control procedures in addition
- 2) Supervision
  - Field supervisors should
    - Review all completed questionnaires in the field, correcting any inconsistencies / gaps by going back to the surveyed individual before leaving the cluster
    - Revisit at random a proportion (e.g. 10%) of surveyed individuals, per cluster, to verify that the information is correct

### *Laboratory quality assurance*

Laboratory quality assurance is very important. (Note laboratory quality assurance only applies if you used a laboratory. If you are using point-of-care tests, you should decide before carrying out your survey whether you will also collect bloods on filter paper, in which case laboratory quality assurance should be considered.)

There are two aspects to laboratory quality assurance: internal and external quality control. Internal quality control is continuous, concurrent control of laboratory work by the laboratory's own staff and those sending samples to the laboratory (e.g. clinicians and public health specialists). External quality control involves retrospective and periodic assessment of laboratory quality by outsiders (e.g. a reference laboratory).

To have internal laboratory quality, laboratory staff must follow correct procedures (e.g. standard operating procedures). Internal quality control encompasses everything from sample collection to final reporting. There are determinants of internal quality at each stage - pre-testing, during testing and post-testing (see a summary below).

**Figure 4: Determinants of laboratory quality**

	<b>Pre-testing</b>	<b>During testing</b>	<b>Post-testing</b>
<b>INTERNAL QUALITY CONTROL:</b> Procedures undertaken by laboratory staff and users to ensure quality of reports	<ul style="list-style-type: none"> <li>• Timing of sample within course of disease</li> <li>• Specimen type</li> <li>• Collection technique</li> <li>• Storage / transport</li> <li>• Quantity</li> <li>• Labelling</li> <li>• Laboratory choice/ capacity</li> </ul>	<ul style="list-style-type: none"> <li>• Proficiency of personnel</li> <li>• Reagents (availability, quality)</li> <li>• Equipment reliability</li> <li>• Specificity and sensitivity of test</li> <li>• Procedural reliability (standard operating procedures)</li> <li>• Use of manufacturer's controls</li> <li>• Documentation (of policies, procedures, test results)</li> <li>• Assessment (of proficiency of the laboratory)</li> </ul>	<ul style="list-style-type: none"> <li>• Recording and reporting</li> <li>• Interpretation</li> <li>• Turnaround time</li> </ul>
<b>EXTERNAL QUALITY ASSESSMENT:</b> External agency objectively checking laboratory results	<ul style="list-style-type: none"> <li>• External quality assessment scheme</li> <li>• Rechecking</li> <li>• On-site visits</li> <li>• Combination of two or three of the above</li> </ul>		
<b>Laboratory training for epidemiologists</b>			

For external quality control, a percentage of samples should be sent to a reference (or other) laboratory to confirm results. If less than a certain percentage of retested samples agree with the original test results (where the agreement threshold was decided before starting the survey), all samples should be retested at the reference lab.

You, as the principle investigator, may engage the laboratory in a dialogue and tactfully ask about quality assurance measures in place. You can also enquire about the type of assays and / or reagents being issued so that their sensitivity and specificity can be known. Note that tests selected for use should be evaluated and approved by national regulatory authorities or pre-qualified by WHO. You can take into account a track record of successful collaborations on serological surveys or outbreak investigations. However, you are not in a position to assess the reliability of the laboratory or to evaluate its quality assurance procedures, as this requires a specific expertise.

### Supplies and budget

When preparing the protocol, consider all of the things that you may need for conducting the field work. Resources required include survey and laboratory personnel, transport, specimen collection equipment, test kits and/or reagents, data collection and analysis forms and/or computers, and financial resources to support and implement the study. Plan ahead for what you will need to print a study report, disseminate the results, and advocate for the necessary action. The budget will depend on the number of survey teams deployed and the number of days required to complete the survey of the clusters. Box 3 is a checklist of things to consider when determining supplies and budget:

---

### Box 3: Checklist to inform budgeting

#### Planning for survey resources

- Determine personnel needed, length of the survey and skills required.
- A survey team has two members who check each other's work.
- One or preferably both interviewers have the skills to obtain the required specimen (i.e. venipuncture or finger-prick by lancet and point-of-care test).
- Plan for one supervisor for every two teams.
- One team completes at least one cluster per day.
- Complete the survey within 1 to 3 weeks. Balance the number of interviewers with your ability to train and supervise them for high quality work and the resources available (e.g. vehicles). For example, for 30 clusters you can deploy 4 or 5 teams.
- Deploy vaccination personnel to survey areas where they do not work routinely.
- Plan for laboratory technicians' time, training, space, supplies and supervision.
- Arrange specimen storage facilities.

#### Budget line items to plan for

- Preparation and production of data collection forms, instructions and guidelines.
- Administrative costs (personnel, supplies).
- Training expenses (room rental, equipment, materials and supplies).
- Allowances for all survey personnel as appropriate.
- Transport (vehicles, fuel).
- Laboratory and specimen collection supplies.
- Communications and results dissemination.
- Consultants, statistician as needed.

A logistics checklist is provided in Appendix 6.

### Data entry, cleaning, and storage

#### *Questionnaire data*

Keep completed questionnaires secure at all times while in the field (e.g. in a waterproof box) and when field work is completed (e.g. in a locked cabinet). If questionnaire data have been collected electronically (e.g. with a personal digital assistant), these should be backed up every few days. Double enter questionnaire data into an electronic database. Check the two sets of data for discrepancies and correct these by referring to original questionnaires. Keep all electronic data in password protected databases. Backup the electronic data every few days. Once you are ready to start the analysis, load the corrected dataset into a statistical package for analysis, first cleaning the data by conducting range checks and logical checks.

---

### *Laboratory data*

Double-enter laboratory test results into a password protected electronic database and check discrepancies between the two sets of data entry. Backup the database every few days. For analysis, laboratory data needs to be merged with questionnaire data using the unique identifying number for each study participant.

### **Data analysis**

#### *Descriptive epidemiology*

The key indicator of hepatitis B vaccine programme impact is the prevalence of HBsAg in your study sample. Start by describing your study sample in terms of age, sex, and other variables you have collected which may be related to current infection (like rural vs urban dwelling), using either tables or figures like bar charts or histograms.

Next, describe the prevalence of current infection by categories of each relevant variable (e.g. by age, sex, rural/urban dwelling). If you used a sampling algorithm that allowed you to determine the proportion of HBsAg test positives who are false positive (because they are HBsAg(+) and anti-HBc(-)) consider only those samples which are anti-HBc(+)/HBsAg(+) as currently infected in your analysis. Give a 95% confidence interval for each prevalence estimate. Prevalence estimates and confidence intervals will need to be calculated using statistical software, and specific commands, to take account of the complex survey design used. Standard confidence intervals should not be calculated. EpiInfo's CSAMPLE module is an example of a group of statistical commands for analyzing clustered data. To use CSAMPLE, you will need a variable in the data identifying the clusters, and another identifying the probability that a given member of the sample would be chosen, in addition to the usual outcome and stratification variables. The use of this module is well described in the EpiInfo manual. Both the program and the manual are available on-line and are free.

A sample table is provided in Appendix 7 to guide planning of your analyses.

If the survey was conducted after the hepatitis B vaccine programme had been in place for several years, you may also wish to compare the prevalence HBsAg in the current survey with the prevalence of HBsAg before the vaccine programme was introduced (i.e. at baseline). To do this, calculate the difference in prevalence between the two surveys, and the confidence interval around the difference, using statistical software that takes into account the complex survey design you used. If the confidence interval around the difference excludes 0 this is evidence of a true difference in prevalence of HBsAg between the two surveys.

Variation in prevalence between parts of the country – districts or regions – may be useful in indicating how well the vaccination programme has performed in the past. The differences may not be large enough to reach statistical significance with the sample size but can still indicate where attention is needed. For example the presence of high levels of chronic infection in vaccinated children may suggest that the vaccine could have been frozen prior to administration.

---

## Interpretation of statistical analysis

The primary objective of the analysis is to estimate the prevalence of current infection (HBsAg(+)). You should provide some interpretation for your estimate of prevalence. For instance “our best estimate of the prevalence of current infection in the sampled age group is the x.xx; there is a 95% probability that the true prevalence lies within y.yy and z.zz”. Note that when you are comparing prevalence of current infection between two groups, e.g. boys and girls, or rural and urban dwellers, if 95% confidence intervals for estimates from the two groups overlap then there is weak evidence prevalence differs between the two groups.

You can determine progress towards certification, if appropriate, by comparing your estimate of prevalence to the goal for the region. (Note that for certification, regional guidelines must be followed where they exist. For example, for WPR, the point estimate for HBsAg prevalence must be below 2% with precision +/- 0.5%.)

If you are comparing prevalence of current infection between two surveys, take care to indicate whether results from the two surveys are directly comparable and, if not, why not. It is valid to compare two surveys which were nationally representative and which used similar laboratory methods.

Interpret all your findings in light of the known limitations of the study (in design, implementation or context), and use the ancillary findings obtained from the study (e.g. geographic differences in prevalence of current infection, where stratification allows results to be reported in this way, or descriptive findings from the questionnaire) to generate hypotheses about infection risk or programme implementation. For example, if one region has much higher prevalence of current infection than others, examine the quality of hepatitis B vaccine birth dose implementation in the area.

## Bias and limitations

Limitations of your survey may include:

1) Attaining a representative sample

If vaccine coverage is not geographically homogeneous, your sample prevalence of current infection may be lower (or higher) than the true prevalence of current infection because sampled clusters were more (or less) likely to be vaccinated than unsampled clusters.

2) The choice of laboratory tests / point-of-care tests in terms of sensitivity and specificity.

3) Recall of parents for vaccination, health events.

1) Locating vaccination cards/records.

---

## Ethical considerations

You should aim to maximize benefit for, minimize risks to, and seek informed consent from, survey participants. This is irrespective of whether you will require ethical approval for your survey (we expect that in most cases you will require ethical approval).

See the *WHO ethical committee checklist (email attachment)*. You should check what local and national ethical requirements you will need to adhere to. Note that some funders also require that you seek separate ethical clearance from them. Steps to take to gain ethical approval may differ depending on the setting.

You will need to seek informed consent from sampled individuals, or their parent/guardian (and assent from children), before enrolling them in the survey, regardless of whether ethical approval was required. Sample information, consent and assent forms are available in Appendix 3. With regards to consent, there may be local differences in procedure. For example, in some settings participants may agree to participate in the survey, but then be reluctant to physically sign the consent form. In this instance it may be appropriate for the investigator to sign for the participant.

### *Confidentiality*

Ensure that individuals cannot be identified when presenting results from your analysis.

### *Biological specimens*

You will need to decide how blood specimens will be treated after the survey. Options include storing samples for future use or discarding them.

### *Non maleficence*

An important consideration is whether to provide test results to the survey participants. This is potentially a benefit to surveyed individuals. Factors which may lead you to decide not to feedback results include logistics (if venous blood is being drawn for testing later in the laboratory, it might be difficult to reach individuals at a later date to give them their results), ethics (Is there universal access to healthcare in this setting? Is treatment likely to be available to the person who is told they are HBsAg(+)?). Even if antiviral treatment is unavailable, it may be possible to provide advice on cofactors to avoid (such as alcohol and aflatoxin-contaminated maize and groundnuts). If you decide to feed results back to participants, think carefully about how to communicate an HBsAg(+) result in light of the considerations mentioned.

Another potential benefit to participants and their families is to be offered vaccination. The surveyed child may be offered vaccination if evidence of complete vaccination cannot be provided. Families of those found to be HBsAg(+) may also be offered vaccination. Note that you will only be able to offer, at most, the first dose of vaccine. You should try to refer participants to local health services for their 2nd and 3rd doses.

## Protocol template

See templates *online*.

---

# Bibliography

Al-Faleh FZ, Al-Jeffri M, Ramia S, Al-Rashed R, Arif M, Rezeig M, et al. Seroepidemiology of hepatitis B virus infection in Saudi children 8 years after a mass hepatitis B vaccination programme. *J Infect.* 1999 May;38(3):167-70.

Avazova D, Kurbanov F, Tanaka Y, Sugiyama M, Radchenko I, Ruziev D, et al. Hepatitis B virus transmission pattern and vaccination efficiency in Uzbekistan. *Journal of Medical Virology.* 2008;80(2):217-24.

Chen HL, Chang MH, Ni YH, Hsu HY, Lee PI, Lee CY, et al. Seroepidemiology of hepatitis B virus infection in children: Ten years of mass vaccination in Taiwan. *JAMA.* 1996 Sep 18;276(11):906-8.

Chongsrisawat V, Yoocharoen P, Theamboonlers A, Tharmaphornpilas P, Warinsathien P, Sinlaparatsamee S, et al. Hepatitis B seroprevalence in Thailand: 12 years after hepatitis B vaccine integration into the national expanded programme on immunization. *Trop Med Int Health.* 2006 Oct;11(10):1496-502.

Chunsuttiwat S, Biggs BA, Maynard J, Thamapalo S, Laoboripat S, Bovornsinsin S, et al. Integration of hepatitis B vaccination into the expanded programme on immunization in Chonburi and Chiangmai provinces, Thailand. *Vaccine.* 1997 Apr-May;15(6-7):769-74.

Davaalkham D, Ojima T, Nymadawa P, Tsend N, Lkhagvasuren T, Wiersma S, et al. Seroepidemiology of hepatitis B virus infection among children in Mongolia: results of a nationwide survey. *Pediatr Int.* 2007 Jun;49(3):368-74.

Da Villa G, Piccinino F, Scolastico C, Fusco M, Piccinino R, Sepe A. Long-term epidemiological survey of hepatitis B virus infection in a hyperendemic area (Afragola, southern Italy): results of a pilot vaccination project. *Res Virol.* 1998 Sep-Oct;149(5):263-70.

Harpaz R, McMahon BJ, Margolis HS, Shapiro CN, Havron D, Carpenter G, et al. Elimination of new chronic hepatitis B virus infections: results of the Alaska immunization program. *J Infect Dis.* 2000 Feb;181(2):413-8.

Hsu HM, Lu CF, Lee SC, Lin SR, Chen DS. Seroepidemiologic survey for hepatitis B virus infection in Taiwan: the effect of hepatitis B mass immunization. *J Infect Dis.* 1999 Feb;179(2):367-70.

Madani TA. Trend in incidence of hepatitis B virus infection during a decade of universal childhood hepatitis B vaccination in Saudi Arabia. *Trans R Soc Trop Med Hyg.* 2007 Mar;101(3):278-83.

Perz JF, Elm JL, Jr., Fiore AE, Huggler JI, Kuhnert WL, Effler PV. Near elimination of hepatitis B virus infections among Hawaii elementary school children after universal infant hepatitis B vaccination. *Pediatrics.* 2006 Oct;118(4):1403-8.

---

Poovorawan Y, Theamboonlers A, Vimolket T, Sinlaparatsamee S, Chaiear K, Siraprapasiri T, et al. Impact of hepatitis B immunisation as part of the EPI. *Vaccine*. 2000 Nov 22;19(7-8):943-9.

Shih HH, Chang MH, Hsu HY, Lee PI, Ni YH, Chen DS. Long term immune response of universal hepatitis B vaccination in infancy: a community-based study in Taiwan. *Pediatr Infect Dis J*. 1999 May;18(5):427-32.

Viviani S, Jack A, Hall AJ, Maine N, Mendy M, Montesano R, et al. Hepatitis B vaccination in infancy in The Gambia: protection against carriage at 9 years of age. *Vaccine*. 1999 Aug 6;17(23-24):2946-50.

# Appendix 1: Laboratory considerations

## Serological markers of hepatitis B infection and immunity

Figure A.1 illustrates the evolution of serologic markers of hepatitis B infection in the acute phase of infection. As HBsAg is cleared, anti-HBc titers rise, affording life-long protection against new hepatitis B infection. In the event of protection through vaccination, only anti-HBs will be present (although subclinical infection after vaccination can lead to anti-HBc conversions).

Figure A.1: Serologic markers of acute hepatitis B virus infection

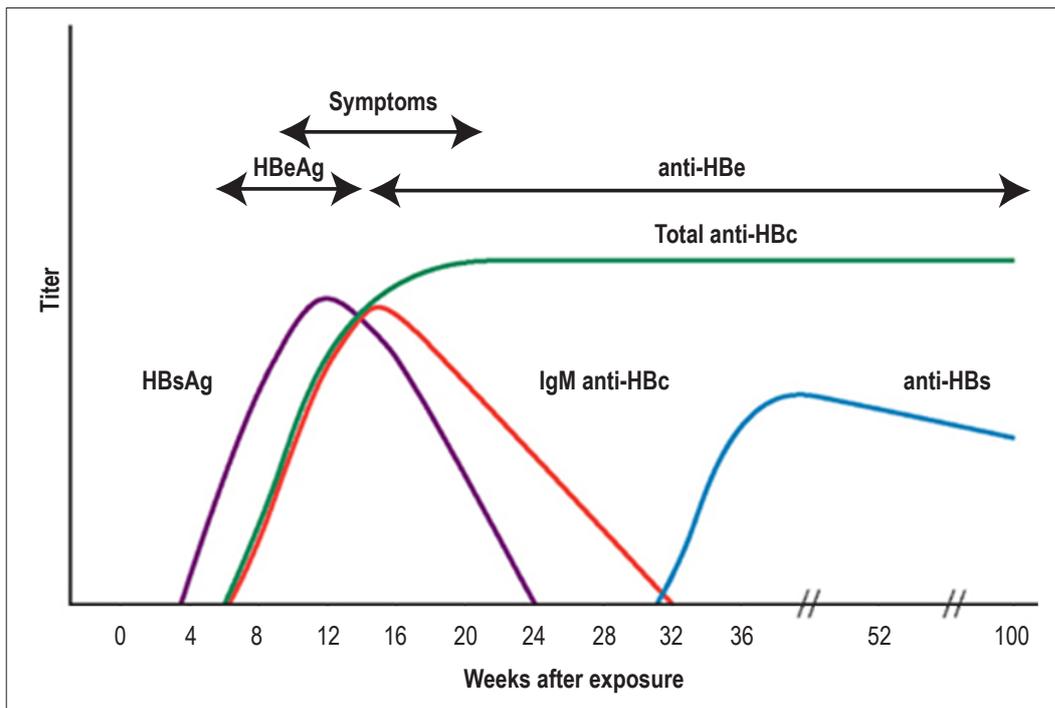


Figure A.2 illustrates the evolution of hepatitis B serologic markers with the development of chronic active infection, with replicating virus continuing to produce HBsAg and sometimes HBeAg.

Figure A.2: Serologic markers of chronic HBV infection

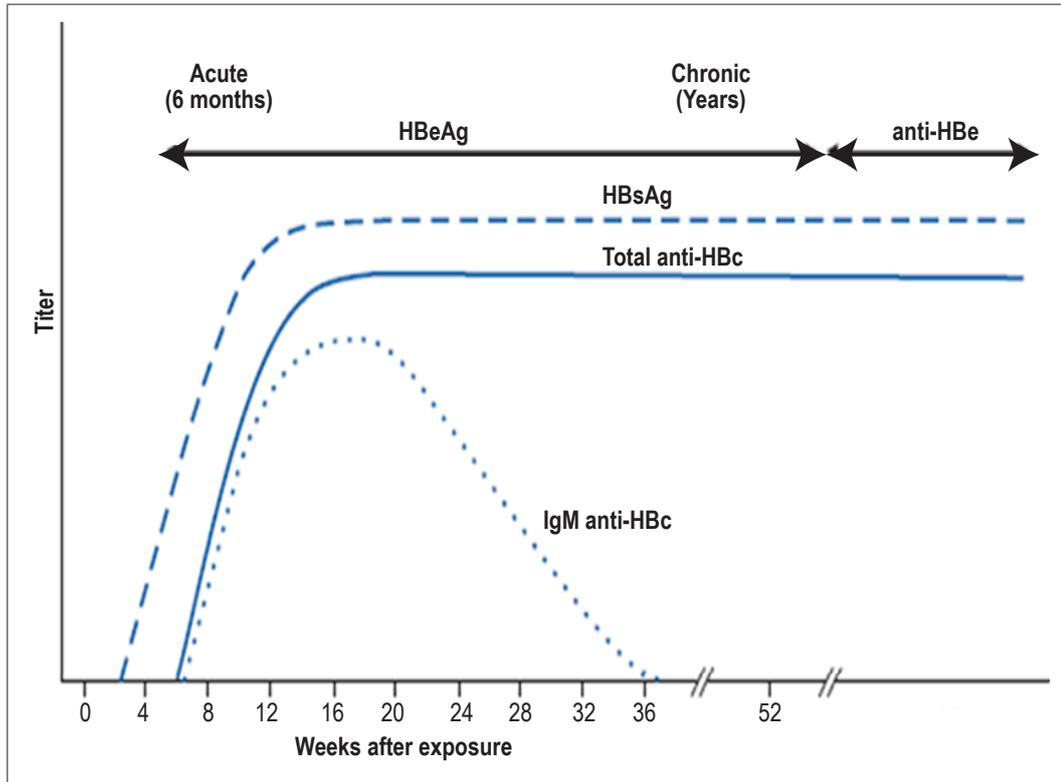


Table A.1 illustrates the interpretation of hepatitis B serologic marker test results in the general population. A hepatitis B serosurvey will often only measure HBsAg and sometimes also anti-HBc.

**Table A.1: Interpretation of serologic markers of hepatitis B virus infection**

Serological test results				Interpretation
HBsAg	anti-HBc (total)	anti-HBc (IgM)	anti-HBs (total)	
-	-	-	-	Susceptible; never infected
+	-	-	-	Early acute infection or Transient after vaccination (21 days)
+	+	+	-	Acute infection
-	+	+	+ / -	Acute resolving infection
+	+	-	-	Current infection
+	+	-	+	Current infection
+	-	-	+	Current infection   ( <i>undetectable or false negative anti-HBc</i> )
-	+	-	+	Past infection, recovered and immune**
-	-	-	+	Immune due to hepatitis B vaccination†
-	+	-	-	Previously infected: <i>Recovering from acute HBV infection</i> <i>Previously exposed, undetectable anti-HBs</i> <i>Current infection with undetectable HBsAg</i> or Susceptible: <i>Susceptible with false positive anti-HBc</i> <i>Passive transfer to an infant from HBsAg(+) mother</i>

(-) seronegative; (+) seropositive. \*\* this pattern may also be seen in successfully immunized individuals who subsequently are asymptotically infected † if titer > 10 mIU/ml, from hepatitis B vaccine or immune globulin. Table adapted from Centers for Disease Control and Prevention. National Center for HIV/AIDS, Viral Hepatitis, STD and TB Prevention. Available from Shepard et al. *Epidemiol Rev* 2006;28:112-25.

# Appendix 2:

## Prevalence of hepatitis B infection before and after the introduction of hepatitis B vaccination

**Table A.2: Examples of the prevalence of current hepatitis B infection  
before and after hepatitis B vaccination introduced**

Study site (reference)	Age group studied (years)	Time after hepatitis B vaccine introduction (years)	HepB3 coverage achieved (%)	Current infection before HepB introduction (% HBsAg(+))	Current infection after HepB introduction (% HBsAg(+))
Alaska (Harpaz)	2-10	10	98	16	0.0
The Gambia (Viviani)	9	9	NA	10	0.6
Italy (Da Villa)	6-14	15	NA.	6.8	0.7
Mongolia (Davaalkham)	7-12	13	Nationally, 47.4 % 1992, 88.5 % 1997, 98 % 2004	10-15	5.2
Saudi Arabia (Al-Faleh)	1-12	8	85	6.7	0.3
Saudi Arabia (Madani)	< 15	9	NA	6.7	0.05
Taiwan (Chen)	< 12	8-10*	85	9.8	1.3
Taiwan (Shih)	7	8-10*	89.7	9.7	0.7
Taiwan (Hsu)	6	7-9	92.4	10.5	1.7
Thailand (Chunsuttiwat)	0.7 mths – 5 yrs	5	90.4	5.4	0.8
Thailand (Poovorawan )	0.5 – 18	7 – 10†	82.3	3.4	0.7
Thailand (Chongsrissawat)	< 18	12-15‡	97.3	4.3	0.7
USA, Hawaii (Perz )	6-9	9	83 by 30 mths old, 95 by 5 yrs old	1.6	0.04
Uzbekistan (Avazova)	1-10	6	NA	5.4	0.8

\* Targeted vaccination of newborns born to HBsAg-positive mothers began 10 years prior to the study; universal vaccination of newborns began 8 years before the study. † One study region began vaccinating 9 years prior to the study, the other regions began vaccinating 7 years prior to the study. ‡ One study region began vaccinating 15 years prior to the study, the other regions began vaccinating 12 years prior to the study. NA - Not available.

---

# Appendix 3:

## Example forms

**MINISTRY OF HEALTH, MONGOLIA**  
***IMPACT ASSESSMENT OF HEPATITIS B IMMUNIZATION***  
***PROGRAMME IN MONGOLIA***  
**CONSENT OF PARENTS**

Dear citizen,

The Ministry of Health and Health Sciences University intends to conduct study in order to assess the impact of hepatitis B immunization programme in Mongolia. Hepatitis B virus infection is wide spread in the country, and is the main cause of hepatocellular carcinoma and liver cirrhosis. This investigation targets all children who are in a second grade of primary school during the school year 2004/2005. The choice of children that is going to participate has been made randomly according to a rigorous scientific method in the goal to get a representative sample of all second grade children in the school.

This investigation is going to gather information concerning your child's immunization from the actual health center, and to check your child's physical improvement, as well as intends to make blood testing for general health status evaluation, liver function, and in order to confirm the efficiency of Hepatitis B immunization in your child. Doctors and well trained nurses will obtain 5 to 10 milliliters of blood using disposable syringes.

Following venipuncture, subjects may experience mild discomfort at the site, and possible bruising and bleeding. Significant adverse events are rare but may include more prolonged bleeding. Doctors and nurses will follow this procedure and it will be managed and referred appropriately.

Specimens will not be used for unplanned studies in the future and after above mentioned testing, over left specimens will be discarded immediately.

Hepatitis B vaccine protects our dear children from dangerous diseases, and answers provided by this investigation will be useful to strengthen this immunization programme in the country. Therefore, it would be very precious if you allow your child take part in the study.

Refusing to take part in the study will not exert adverse effect to your child's health services and treatment.

All information obtained during the study will be maintained strictly confidential, and will be used exclusively for goals of this survey. A research will completed within 10 months, and after the study, records of children's name will be destroyed appropriately. We will provide you with the summary of study findings through the schools.

---

We will encourage your understanding our research and your child's participation with a gift. If an incomplete or inefficient immunization will be found in your child, we will inform to the local health centre and the necessary vaccinations will be provided to your child free of charge.

If you agree for your child's involvement to this study, please sign (or make a witnessed "mark") this document and return to us.

*I have read above information and understand the objective and procedure of the study. By signing here I state that I voluntarily allow my child to take part in this study.*

Signature(s) of subject's parent(s)/guardian \_\_\_\_\_

Subject name \_\_\_\_\_

Name of the person who received this form \_\_\_\_\_

Today's date: \_\_\_\_\_ year \_\_\_\_ month \_\_\_\_ day

For further information contact Dr. xxxxxx, [title], [department and contact details]

---

## INFORMED CONSENT

Dear parents,

The Ministry of Health and Social Protection conducts an advanced study of the immunity level among children vaccinated against hepatitis B, to assess whether the vaccination doses received so far are sufficient to protect the health of your child.

Hepatitis B is a severe disease, caused by the hepatitis B virus and is accompanied by the following symptoms: loss of appetite, tiredness, pain in muscles, nausea, vomiting, yellowing of skin and eyes (jaundice). In some cases patients recover, but often the disease evolves quietly (becomes chronic), attacks the liver and can lead to liver damage (cirrhosis), liver cancer, and in some cases death.

Worldwide over 350 million people are currently estimated to be chronically infected with the hepatitis B virus. About 250,000 persons die each year from acute and chronic sequelae of hepatitis B infection. In the Republic of Moldova almost every 10th person is a carrier of the above mentioned virus.

The evolution of the hepatitis B virus infection indirectly depends on age: the younger the age, the higher is the probability of infection becoming chronic.

Hepatitis B virus is spread out through the contact with blood or other body fluids of an infected person, often after common use of toothbrushes, razors, manicure tools, needles, and other sharp objects.

Fortunately, scientists have discovered some time ago the vaccine against hepatitis B, which is able to prevent this severe illness. This is the first vaccine against cancer, as it prevents the disease evolution to liver cancer.

This vaccine has been used in our country for the last ten years.

Vaccination schedule currently comprises three doses of vaccine. In order to assess to what extent children are protected from this severe illness, and to check whether they need additional doses, we shall verify the immunity level of children vaccinated 7-10 years ago.

That is why we shall obtain a blood sample taken from the arm of your child. The procedure is free of risk of transmitting infections since only sterile disposable needles and syringes are used for that purpose.

You will be given information on the results from the blood tests and recommendations on the need for further vaccination. The results from the blood tests will be kept confidential. Investigations are performed free of charge.

In case you agree to participate, we would like to ask to write your name and provide your signature below.

*Herewith I confirm my consent to collect a blood sample from my daughter/son:*

---

**First and second name of the child**

---

**First and second name of the parent**

---

# Appendix 4:

## Sample questionnaires

### SEROSURVEY OF CHILDREN IN MOLDOVA

Date	___/___/___	ID Number	_____
1.	Name (FIRST, LAST) _____		
2.	Sex MALE / FEMALE		
3.	Birthdate (DAY/MONTH/YEAR) ___/___/___		
4.	Location of home (RAYON) _____		
5.	Is mother of child a known carrier of hepatitis B virus? YES / NO		
5a)	When was this determined? (MONTH/YEAR) ___/___		
6.	Has child received vaccine against hepatitis? YES / NO / UNKNOWN		
6a)	How many doses? 1 2 3 4 >4 UNKNOWN		
6b)	When was first dose given? (DATE / MONTH / YEAR / SERIE OF VACCINE )	_____	_____
6c)	When was second dose given? (DATE / MONTH / YEAR / SERIE OF VACCINE )	_____	_____
6d)	When was third dose given? (DATE / MONTH / YEAR / SERIE OF VACCINE )	_____	_____
Date	___/___/___		

Subject number \_\_\_\_\_

## Questionnaire

The questionnaire asks you about a number of aspects of your child's life such as living situation, medical history, and family medical history. This will help us to understand the main risk factors for hepatitis viral infections in the country.

You are free to not answer any question. However, it would be very precious if you answer these questions, because the findings will be very useful for developing further preventive strategies and measures against transmission of this harmful disease.

### Personal history

- 1) A total number of people in the household:
- 2) Ethnicity
- 3) Housing: (please circle) 1. Ger 2. Apartment 3. House 4. Other

### Medical history

- 4) Had your child vaccinated against viral hepatitis B? 1. Yes 2. No 3. Unknown
- 5) Please answer the following questions thinking about your child's situation. (please circle)

Questions	Yes	No
Ever had blood or blood component transfusion	Yes	No
Ever had any surgeries	Yes	No
Ever had teeth treated	Yes	No
Ever been hospitalized	Yes	No
Ever got injection treatment in a hospital	Yes	No
Ever got injection treatment at home	Yes	No
Ever been injected by non disposable glass syringe or needle	Yes	No

- 6) Does your child share (even sometimes) toothbrush with family members: 1. Yes 2. No
- 7) Has your child ever had viral hepatitis? 1. Yes 2. No
- 8) Has any of your relatives ever had the following diseases? (Write "+" if yes, "-" if no, and "0" if unknown)

Subject's relative	Liver cirrhosis	Liver cancer	Viral Hepatitis	Stroke
Father				
Mother				
Brothers/ sisters				
Father's parents				
Mother's parents				
Other household member				

**Thank you for your collaboration!**

---

# Appendix 5:

## Practical guidance on paediatric and neonatal blood sampling

*(Adapted from WHO guidelines on drawing blood: best practices in phlebotomy 2010 WHO/EHT/10.01)*

### Venipuncture

- 1) Use a winged steel needle, preferably 22 or 23 gauge, with an extension tube (a butterfly):
  - i) avoid gauges of 25 or more because these may be associated with an increased risk of haemolysis;
  - j) use a butterfly with either a syringe or an evacuated tube with an adaptor; a butterfly can provide easier access and movement, but movement of the attached syringe may make it difficult to draw blood.
- 2) Use a syringe with a barrel volume of 1–5 ml, depending on collection needs; the vacuum produced by drawing using a larger syringe will often collapse the vein.
- 3) When using an evacuated tube, choose one that collects a small volume (1 ml or 5 ml) and has a low vacuum; this helps to avoid collapse of the vein and may decrease haemolysis.
- 4) Where possible, use safety equipment with needle covers or features that minimize blood exposure. Auto-disable (AD) syringes are designed for injection, and are not appropriate for phlebotomy.
- 5) Ask whether the parent would like to help by holding the child. If the parent wishes to help, provide full instructions on how and where to hold the child; if the parent prefers not to help, ask for assistance from another phlebotomist.
- 6) Immobilize the child as described below.
  - a) Designate one phlebotomist as the technician, and another phlebotomist or a parent to immobilize the child.
  - b) Ask the two adults to stand on opposite sides of an examination table.
  - c) Ask the immobilizer to:
    - i) stretch an arm across the table and place the child on its back, with its head on top of the outstretched arm;
    - ii) pull the child close, as if the person were cradling the child;
    - iii) grasp the child's elbow in the outstretched hand;
    - iv) use their other arm to reach across the child and grasp its wrist in a palm-up position (reaching across the child anchors the child's shoulder, and thus prevents twisting or rocking movements; also, a firm grasp on the wrist effectively provides the phlebotomist with a "tourniquet").

- 
- 7) If necessary, take the following steps to improve the ease of venipuncture.
    - a) Ask the parent to rhythmically tighten and release the child's wrist, to ensure that there is an adequate flow of blood.
    - b) Keep the child warm by removing as few of the child's clothes as possible and, in the case of an infant, by:
      - i) swaddling in a blanket; and
      - ii) having the parent or caregiver hold the infant, leaving only the extremity of the site of venipuncture exposed.
  - 8) Warm the area of puncture with warm cloths to help dilate the blood vessels.
  - 9) Use a transilluminator or pocket pen light to display the dorsal hand veins and the veins of the antecubital fossa.
  - 10) Collect all the equipment needed for the procedure and place it within safe and easy reach on a tray or trolley, ensuring that all the items are clearly visible. The equipment required includes:
    - a) a supply of laboratory sample tubes, which should be stored dry and upright in a rack; blood can be collected in sterile glass or plastic tubes with rubber caps (the choice of tube will depend on what is agreed with the laboratory);
      - i) vacuum-extraction blood tubes; or
      - ii) glass tubes with screw caps;
    - b) a sterile glass or bleeding pack (collapsible) if large quantities of blood are to be collected;
    - c) well-fitting, non-sterile gloves;
    - d) an assortment of blood-sampling devices (safety-engineered devices or needles and syringes, see below), of different sizes;
    - e) a tourniquet;
    - f) alcohol hand rub;
    - g) 70% alcohol swabs for skin disinfection;
    - h) gauze or cotton-wool ball to be applied over puncture site;
    - i) laboratory specimen labels;
    - j) writing equipment;
    - k) laboratory forms;
    - l) leak-proof transportation bags and containers;
    - m) a puncture-resistant sharps container.
  - 11) Ensure that the rack containing the sample tubes is close to you, the health worker, but away from the patient, to avoid it being accidentally tipped over.
  - 12) Identify the child.
    - a) If a parent or legal guardian is present, ask that person for the child's first and last names.
    - b) Check that the name, date of birth and hospital or file number are written on the laboratory form, and match them to the identity of the patient.

- 
- 13) Perform hand hygiene; that is
    - a) wash hands with soap and water, and dry with single-use towels; or
    - b) if hands are not visibly contaminated, clean with alcohol rub – use 3 ml of alcohol rub on the palm of the hand, and rub it into fingertips, back of hands and all over the hands until dry.
  - 14) After performing hand hygiene, put on well-fitting, non-sterile gloves.
  - 15) Clean the site with a 70% alcohol swab for 30 seconds and allow to dry completely (30 seconds)

Note: alcohol is preferable to povidone iodine, because blood contaminated with povidone iodine may falsely increase levels of potassium, phosphorus or uric acid in laboratory test results

    - a) Apply firm but gentle pressure. Start from the centre of the venipuncture site and work downward and outwards to cover an area of 2 cm or more.
    - b) Allow the area to dry. Failure to allow enough contact time increases the risk of contamination.

DO NOT touch the cleaned site; in particular, DO NOT place a finger over the vein to guide the shaft of the exposed needle. If the site is touched, repeat the disinfection.
  - 16) Once the infant or child is immobilized, puncture the skin 3–5 mm distal to (i.e. away from) the vein ; this allows good access without pushing the vein away.
  - 17) If the needle enters alongside the vein rather than into it, withdraw the needle slightly without removing it completely, and angle it into the vessel.
  - 18) Draw blood slowly and steadily.
  - 19) When the blood collection procedure is complete, apply firm pressure to the site to stop the bleeding
  - 20) Clean up blood spills.
  - 21) Collect all equipment used in the procedure, being careful to remove all items from the patient’s bed or cot; to avoid accidents, DO NOT leave anything behind.
  - 22) Record relevant information about the blood collection on the requisition and specimen label; such information may include:
    - a) date of collection;
    - b) subject name;
    - c) subject identity number;
    - d) subject date of birth
    - e) phlebotomist’s initials.
  - 23) Offer comfort and reassurance to the child. For example, give verbal praise, a handshake, a fun sticker or a simple pat on the back. A small amount of sucrose (0.012–0.12 g) is safe and effective as an analgesic for newborns undergoing venipuncture or capillary heel-pricks
  - 24) Adhere strictly to a limit on the number of times a paediatric patient may be stuck. If no satisfactory sample has been collected after two attempts, seek a second opinion to decide whether to make a further attempt, or cancel the sampling.

---

## Finger and heel-prick

- 1) Select the proper lancet length for the area of puncture. Lengths vary by manufacturer (from 0.85 mm for neonates up to 2.2 mm). In a finger-prick, the depth should not go beyond 2.4 mm, so a 2.2 mm lancet is the longest length typically used. In heel-pricks, the depth should not go beyond 2.4 mm.

The recommended depth for a finger-prick is:

- a) for a child over 6 months and below 8 years – 1.5 mm;
- b) for a child over 8 years – 2.4 mm.

Too much compression should be avoided, because this may cause a deeper puncture than is needed to get good flow.

- 2) First immobilize the child by asking the parent to:
  - a) sit on the phlebotomy chair with the child on the parent's lap;
  - b) immobilize the child's lower extremities by positioning their legs around the child's in a cross-leg pattern;
  - c) extend an arm across the child's chest, and secure the child's free arm by firmly tucking it under their own;
  - d) grasp the child's elbow (i.e. the skin puncture arm), and hold it securely;
  - e) use his or her other arm to firmly grasp the child's wrist, holding it palm down.
- 3) Apply alcohol (not povidone iodine) to the entry site and allow to air dry
- 4) Puncture the skin with one quick, continuous and deliberate stroke, to achieve a good flow of blood and to prevent the need to repeat the puncture.
- 5) If necessary, take the following steps to improve the ease of obtaining blood by finger-prick in paediatric and neonatal patients:
  - a) ask the parent to rhythmically tighten and release the child's wrist, to ensure that there is sufficient flow of blood;
  - b) keep the child warm by removing as few clothes as possible, swaddling an infant in a blanket, and having a mother or caregiver hold an infant, leaving only the extremity of the site of capillary sampling exposed.
- 6) Wipe away the first drop of blood because it may be contaminated with tissue fluid or debris (sloughing skin).
- 7) Avoid squeezing the finger or heel too tightly because this dilutes the specimen with tissue fluid (plasma) and increases the probability of haemolysis
- 8) When the blood collection procedure is complete, apply firm pressure to the site to stop the bleeding
- 9) Clean up blood spills.
- 10) Collect all equipment used in the procedure, being careful to remove all items from the patient's bed or cot; to avoid accidents, **DO NOT** leave anything behind.

- 
- 11) Record relevant information about the blood collection on the requisition and specimen label; such information may include:
    - a) date of collection;
    - b) subject name;
    - c) subject identity number;
    - d) subject date of birth
    - e) phlebotomist's initials.
  - 12) Offer comfort and reassurance to the child. For example, give verbal praise, a handshake, a fun sticker or a simple pat on the back. A small amount of sucrose (0.012–0.12 g) is safe and effective as an analgesic for newborns undergoing venipuncture or capillary heel-pricks
  - 13) Adhere strictly to a limit on the number of times a paediatric patient may be stuck. If no satisfactory sample has been collected after two attempts, seek a second opinion to decide whether to make a further attempt, or cancel the sampling.

---

# Appendix 6:

## Logistics

### Box A.1

#### Logistics checklist for survey fieldwork

- Transport:** reserve, borrow, arrange or rent the necessary transport for supervisors and interviewers. Allow for fuel, maintenance and servicing.
- Accommodation:** arrange safe and convenient quarters, where the team can also comfortably review the days' activities.
- Meals:** arrange meals and water for team, or allowances if food easily available.
- Safety and security:** ensure security and protection from the elements for teams and survey materials.
- Background information:** provide letters of introduction, information on the local schools and health services, dates of key local/national events helpful for probing for birth dates and vaccination dates.
- Maps, lists and directions:** ensure teams know how to find and complete the survey of each cluster.
- Survey materials:** provide data collection forms, clipboards, pens, specimen collection supplies and instructions, water-proof covers for all materials, and copies of the national vaccination schedule.
- Communication:** provide means of communication or establish set meeting places and times to find the supervisor or coordinator.
- Remuneration:** ensure timely and appropriate payment to maintain team commitment.

# Appendix 7:

## Sample table for anticipated data analysis

**Table A.3: Example table describing prevalence of current hepatitis B infection among survey participants by demographic variables and putative risk factors, <setting> , 20XX**

Risk factor		Chronically infected		OR (95% CI)
		#	% (95% CI)	
Sex	Male			
	Female			Referent
Age	10			Referent
	9			
	8			
	7			
	6			
	5			
	<5			
	Vaccination	Fully vaccinated		
	Not fully vaccinated			
Type of area	Rural			Referent
	Urban			

Table S.1. Field sensitivity and specificity of a range of point-of-care HBsAg tests from Davies et al.

Year	Author	Setting	Test kit	Medium	Total samples tested	True HBV+	Sensitivity	Specificity
2000	Lien et al <sup>2</sup>	Vietnam	Determine	Serum	328	117	100	100
2008	Lin et al <sup>3</sup>	China	Determine	Plasma	671	186	98.9 (97.4 – 100)	100
		Guinea	Determine	Plasma	579	180	96.6 (94.0 – 99.2)	100
2008	Randrianirina et al <sup>4</sup>	Madagascar	Determine	Serum	200	91	97.8 (94.8 – 100)	100
2008	Nyirenda et al <sup>5</sup>	Malawi (tested locally)	Determine (Abbott)		194	34	56 (39 – 73)	69 (62-76)
2009	Davies et al <sup>6</sup>	Malawi, samples tested in the UK	Determine (Inverness)	Serum	75	25	100	100
1998	Abraham et al <sup>7</sup>	India	Viruscheck	Serum			77	99
2008	Randrianirina et al <sup>20</sup>	Madagascar	Viruscheck	Serum	200	91	95.6 (91.4 – 99.8)	98.2 (95.7 – 100)

- <sup>1</sup> Davies J, van Oosterhout JJ, Nyirenda M, Bowden J, Moore E, Hart IJ, et al. Reliability of rapid testing for hepatitis B in a region of high HIV endemicity. *Trans R Soc Trop Med Hyg.* Feb;104(2):162-4.
- <sup>2</sup> Lien TX, Tien NT, Chanpong GF, Cuc CT, Yen VT, Soderquist R, et al. Evaluation of rapid diagnostic tests for the detection of human immunodeficiency virus types 1 and 2, hepatitis B surface antigen, and syphilis in Ho Chi Minh City, Vietnam. *Am J Trop Med Hyg.* 2000 Feb;62(2):301-9.
- <sup>3</sup> Lin YH, Wang Y, Loua A, Day GJ, Qiu Y, Nadala EC, Jr., et al. Evaluation of a new hepatitis B virus surface antigen rapid test with improved sensitivity. *J Clin Microbiol.* 2008 Oct;46(10):3319-24.
- <sup>4</sup> Randrianirina E, Carod JF, Ratsima E, Chretien JB, Richard V, Talarmin A. Evaluation of the performance of four rapid tests for detection of hepatitis B surface antigen in Antananarivo, Madagascar. *J Virol Methods.* 2008 Aug;151(2):294-7.
- <sup>5</sup> Nyirenda M, Beadsworth MB, Stephany P, Hart CA, Hart IJ, Munthali C, et al. Prevalence of infection with hepatitis B and C virus and coinfection with HIV in medical inpatients in Malawi. *J Infect.* 2008 Jul;57(1):72-7.
- <sup>6</sup> Davies J, van Oosterhout JJ, Nyirenda M, Bowden J, Moore E, Hart IJ, et al. Reliability of rapid testing for hepatitis B in a region of high HIV endemicity. *Trans R Soc Trop Med Hyg.* Feb;104(2):162-4.

Table S.1. Field sensitivity and specificity of a range of point-of-care HBsAg tests from Davies et al. *cont'd*...

Year	Author	Setting	Test kit	Medium	Total samples tested	True HBV+	Sensitivity	Specificity
1996	Mvere et al <sup>8</sup>	Zimbabwe	SimpliRED	Serum	206	15	93.7 (81.4 – 100)	100
		Zimbabwe	Dipstick-HBsAg(PATH)	Serum	206	15	93.7 (81.4 – 100)	100
2000	Lien et al <sup>18</sup>	Vietnam	Serodia	Serum	328	117	95.7 (92.0 – 99.4)	100
		Vietnam	Dainascreen	Serum	328	117	100	100
2002	Clement et al <sup>9</sup>	Belgium	Hepatitis B sAg/eAg test (Binax Inc.)	Serum	942	403	99.8 (99.3 – 100)	99.3 (98.6 – 100)
2006	Akanmu et al <sup>10</sup>	Nigeria	AMRAD	Whole blood	101	7	100	98.9 (96.8 – 100)
2008	Lin et al <sup>19</sup>	China	DRW	Plasma	671	186	99.4 (98.2 – 100)	99.1 (98.2 – 99.9)
		Guinea	DRW	Plasma	579	180	94.4 (91.0 – 97.8)	100
2008	Randrianirina et al <sup>20</sup>	Madagascar	Cypress	Serum	200	91	95.6 (91.4 – 99.8)	96.3 (92.8 – 99.8)
		Madagascar	Hexagon	Serum	200	91	96.7 (93.0 – 100)	96.3 (92.8 – 99.8)

7 Abraham P, Sujatha R, Raghuraman S, Subramaniam T, Sridharan G. Evaluation of two immunochromatographic assays in relation to 'RAPID' Screening of HBsAg. Indian J Med Micro. 1998;16(1):23-5.

8 Mvere D, Constantine NT, Katsawde E, Tobaiwa O, Dambire S, Corcoran P. Rapid and simple hepatitis assays: encouraging results from a blood donor population in Zimbabwe. Bull World Health Organ. 1996;74(1):19-24.

9 Clement F, Dewint P, Leroux-Roels G. Evaluation of a new rapid test for the combined detection of hepatitis B virus surface antigen and hepatitis B virus e antigen. J Clin Microbiol. 2002 Dec;40(12):4603-6.

10 Akanmu AS, Esan OA, Adewuyi JO, Davies AO, Okany CC, Olatunji RO, et al. Evaluation of a rapid test kit for detection of HBsAg/eAg in whole blood: a possible method for pre-donation testing. Afr J Med Med Sci. 2006 Mar;35(1):5-8.

**Table S.2. Sensitivity and specificity of some commercially available rapid HBsAg tests, assessed by the International Consortium for Blood Safety (<http://www.icbs-web.org/>) (access date 6 December 2010)**

Product name	Manufacturer	Total samples tested	True HBV+	Specificity	Sensitivity	Catalogue no.	Contact information
Determine™ HBsAg	Abbott Laboratories Japan Co., Ltd.	346	146	100	97.95	7D25-13	Mfd. for Abbott Laboratories by Abbott Japan Co., Ltd. Minato-Ku, Tokyo, Japan
HBsAg Line Test Device	Acon Biotech Co., Ltd., China; marketed by Transasia Bio-Medicals Ltd. Mumbai, India	346	146	100	94.52	CD-5-2362	Acon Biotech CO., Ltd, China; Imported and Marketed by TRANSASIA BIO-MEDICALS LTD., Mumbai, India
Acon HBsAg One Step Diagnostic Test Strip	Acon Laboratories, Inc.	346	146	100	97.95	IHBsg-301	Laboratories, Inc. San Diego, CA 92121, USA Website:www.aconlabs.com
HBsAg Dipstick One Step HBsAg Test	Cypress Diagnostics	346	146	100	98.63	142-310	Cypress Diagnostics; Langdorpsesteenweg 160 3201 Langdorp Belgium, Phone: +32 16 446389, Fax: +32 16 447762
HBsAg (WB)	David & Tom Biotechnology Co., Ltd. (Ind. Biotech(Qingdao) Co., Ltd.)	346	146	100	97.95		David & Tom Biotechnology Co.; Ltd. Western side songshan rd. shimao guangchang SHANTOU Guangdong 515041 China; Tel: 86 - 754 - 8736059; Fax: 86 - 754 - 8736059
Hepatitis B Antigen (HBsAg) Cassette	EQUIPAR Diagnostici Societa a Responsabilita Limitata	346	146	100	97.26	30HBGDE-A5	Equipar Diagnostici Societa a Responsabilita Limitata Equipar Via Gaudenzio Ferrari,21/N 21047 Saronno (Varese) Italy Tel.+39029605422- +39029605824 Fax+39029607106 www.equipar.it info@equipar.it
Quick HBsAg Test	Firmer Co. Ltd., manufactured by Guangzhou Wondfo Biotech Co. Ltd.	346	146	100	97.26	F3-C4	Firmer Co., Ltd. Thailand Tel.:(662)9341581-4 Fax:(662)934-1580

**Table S.2. Sensitivity and specificity of some commercially available rapid HBsAg tests, assessed by the International Consortium for Blood Safety (<http://www.icbs-web.org/>) (access date 6 December 2010) cont'd...**

Product name	Manufacturer	Total samples tested	True HBV+	Specificity	Sensitivity	Catalogue no.	Contact information
AssureR HBsAg Rapid Test	MP Biomedicals Asia Pacific Pte. Ltd.	346	146	100	97.95	43471-020	MP Biomedicals Asia Pacific Pte. Ltd. 85 Science Park Drive #04-01, The Cavendish Singapore Science Park, Singapore 118259 Tel. No.: + 65 6775 0008 Fax. No.: + 65 6775 4536 Email: enquiry_ap@mpbio.com; www.mpbio.com
i-LAB HBsAg Test	i+MED Laboratories Co., Ltd.	346	146	100	97.26	HB-131L, FL0050	i+MED Laboratories Co., Ltd., Thailand, 1201/100 Sriwara Rd., Wangthonglang, Bangkok 10310 Thailand, Phone: 0-2530-7800, Fax: 0-2934-8222, Email: marketing@imed.co.th
Hepacard One Step Rapid Visual Test For the Qualitative Detection of HBsAg in Human Serum/Plasma	J.Mitra & Co. Ltd.	346	146	100	97.95	HB010100	J.Mitra & Co. Ltd., A-180, Okhla Industrial Area, Phase-1, New Delhi-110020, India, Biomed Industries Plot No. 20-A, Sector-1, Industrial Estate, Panwano-173220, Himachal Pradesh
Quick Chaser HBsAg	Mizuho Medy Co., Ltd.	346	146	100	97.95	61040	Mizuho Medy Co., Ltd.; 5-4 Fujinoki-Machi, Tosu City, Saga 841 Japan, Phone: +81-942850301, Fax: +81-942831048
One Step HBsAg Dipstick Test	Newmarket Laboratories Ltd.	346	146	100	97.95	60091	Newmarket Laboratories Ltd: Lanwades Business Park, Kentford, Newmarket, CB8 7PN; Tel: +44 (0) 1638 552 882; Fax: +44 (0) 1638 552 375; Email: info@newlabs.co.uk; Web Site Address: www.newlabs.co.uk
Bioline HBsAg Strip - One Step Bioline Hepatitis B Surface Antigen Test Strip	Pacific Biotech Co., Ltd.	346	146	100	97.95		Pacific Biotech Co.,Ltd. Soi Ladprao 110 (sonthiwattana 3), Ladprao Rd., Wangthonglang, Bangkok 10310 , Thailand; Tel.:66-2530-2754-60 Fax.:66-2931-8340 www.pacific-biotech.com

**Table S.2. Sensitivity and specificity of some commercially available rapid HBsAg tests, assessed by the International Consortium for Blood Safety (<http://www.icbs-web.org/>) (access date 6 December 2010) cont'd...**

Product name	Manufacturer	Total samples tested	True HBV+	Specificity	Sensitivity	Catalogue no.	Contact information
Hep-alert-B One step HBsAg Card Test	Ranbaxy Laboratories Ltd.	346	146	100	97.26	1638/97	RANBAXY Laboratories Ltd. Diagnostica Division, A-3, Okhla Industrial Area, Phase-1, New Dehli-110 020
SD BioLine HBsAg (One Step HBsAg Test)	Standard Diagnostics, Inc.	346	146	100	97.95	01FK10	Standard Diagnostics, Inc. 156-68 Hagal-ri, Giheung eup, Yongin si Kyonggi-do, Korea 449-906; Tel.: 82-31-899-9700 Fax.: 82-31-899-9740 <a href="http://www.standardia.com">http://www.standardia.com</a>
HEP-CHECK-1 (Mini Clip HBsAg)	Veda Lab	346	146	100	97.26	2034	Parc du Londeau, B.P. 181, 61006 Alencon Cedex, France, Phone: (33)233275625, Fax: (33)233277060, <a href="http://www.vedalab.com">www.vedalab.com</a> , Email: <a href="mailto:vedalab@wanadoo.fr">vedalab@wanadoo.fr</a>
HEP-CHECK-1-STRIP (HBsAg)	Veda Lab	346	146	100	97.26	2071	Parc du Londeau, B.P. 181, 61006 Alencon Cedex, FrancePhone: (33)233275625, Fax: (33)233277060, <a href="http://www.vedalab.com">www.vedalab.com</a> , Email: <a href="mailto:vedalab@wanadoo.fr">vedalab@wanadoo.fr</a>
One Step HBsAg Cassette Test	World of Health Biotech Company	346	146	100	97.26		World of Health Biotech Company; 122# No.2 Building Xinjiekou Xicheng Beijing China 100035; Tel: (86,10 ) 66179934 Fax: (86,10 ) 62220041; Email: <a href="mailto:info@healthbiotech.net">info@healthbiotech.net</a>
HepaScan HBsAg Cassette Type / HepaScan HBsAg Strip Type	YD Diagnostics	346	146	100	97.95	IM702-50S, IM702-100	YD Diagnostics; Hasuco Korea: Suite No. 726, Woosung Character Ville B/D, 595-73, Shindaebang-Dong, Dongjak-Gu, Seoul, 156-849, Phone: +82-2-831-0082, +82-2-831-0477 (Customer Service), Fax: +82-2-834-5775, <a href="http://www.hasuco.com">www.hasuco.com</a> , E-mail: <a href="mailto:info@hasuco.com">info@hasuco.com</a>

## Sample size calculator

Double click in the spreadsheet below to edit to your own specifications.

	Analyzing a single survey			Comparing two regions or two surveys	
	Scenario A	Scenario B	Scenario C		Scenario D
Cluster Sample Size Calculator					
$n = DE \frac{z^{*2} p(1-p)}{d^{*2}}$	2	2	2		$n = DE \frac{z^{*2} (p1(1-p1) + p2(1-p2))}{d^{*2}}$
Design Effect (DE)				2	
Prevalence of factor under study (p)	0.01	0.01	0.01	0.15	
				0.22	
Width of confidence interval (d)	0.005	0.01	0.02	0.03	
Minimum sample size	3043	761	190	2553	
Cluster/Class Size Calculator					
20 Clusters	152	38	10	20 clusters per region	128
30 Clusters	101	25	6	30 clusters per region	85
Lab Supply Calculator					
anti-HBc prevalence (estimated)	20	5	5		
anti-HBc kits required	3043	761	190	anti-HBc kits required	2553
HBsAg kits required	669	42	10	HBsAg kits required	0
cost anti-HBc kits					
cost HBsAg kits					

- <sup>1</sup> Meeting of the immunization Strategic Advisory Group of Experts, November 2008--conclusions and recommendations. Wkly Epidemiol Rec. 2009 Jan 9;84(1-2):1-16.
- <sup>2</sup> World Health Organization Department of Immunization Vaccines and Biologicals. Training for mid-level managers (MLM) Module 7: The EPI coverage survey. Geneva: World Health Organization; 2008. Report No.: WHO/IVB/08.07.
- <sup>3</sup> Davies J, van Oosterhout JJ, Nyirenda M, Bowden J, Moore E, Hart IJ, et al. Reliability of rapid testing for hepatitis B in a region of high HIV endemicity. Trans R Soc Trop Med Hyg. Feb;104(2):162-4.

---

The World Health Organization has provided technical support to its Member States in the field of vaccine-preventable diseases since 1975. The office carrying out this function at WHO headquarters is the Department of Immunization, Vaccines and Biologicals (IVB).

IVB's mission is the achievement of a world in which all people at risk are protected against vaccine-preventable diseases.

The Department covers a range of activities including research and development, standard-setting, vaccine regulation and quality, vaccine supply and immunization financing, and immunization system strengthening.

These activities are carried out by three technical units: the Initiative for Vaccine Research; the Quality, Safety and Standards team; and the Expanded Programme on Immunization.

The Initiative for Vaccine Research guides, facilitates and provides a vision for worldwide vaccine and immunization technology research and development efforts. It focuses on current and emerging diseases of global public health importance, including pandemic influenza. Its main activities cover: i) research and development of key candidate vaccines; ii) implementation research to promote evidence-based decision-making on the early introduction of new vaccines; and iii) promotion of the development, evaluation and future availability of HIV, tuberculosis and malaria vaccines.

The Quality, Safety and Standards team focuses on supporting the use of vaccines, other biological products and immunization-related equipment that meet current inter-national norms and standards of quality and safety. Activities cover: i) setting norms and standards and establishing reference preparation materials; ii) ensuring the use of quality vaccines and immunization equipment through prequalification activities and strengthening national regulatory authorities; and iii) monitoring, assessing and responding to immunization safety issues of global concern.

The Expanded Programme on Immunization focuses on maximizing access to high quality immunization services, accelerating disease control and linking to other health interventions that can be delivered during immunization contacts. Activities cover: i) immunization systems strengthening, including expansion of immunization services beyond the infant age group; ii) accelerated control of measles and maternal and neonatal tetanus; iii) introduction of new and underutilized vaccines; iv) vaccine supply and immunization financing; and v) disease surveillance and immunization coverage monitoring for tracking global progress.

The Director's Office directs the work of these units through oversight of immunization programme policy, planning, coordination and management. It also mobilizes resources and carries out communication, advocacy and media-related work.

## **Department of Immunization, Vaccines and Biologicals**

### **Family and Community Health**

World Health Organization  
20, Avenue Appia  
CH-1211 Geneva 27  
Switzerland

E-mail: [vaccines@who.int](mailto:vaccines@who.int)

Web site: <http://www.who.int/immunization/en/>