The Immunological Basis for Immunization Series

Module 22: Hepatitis B

Immunization, Vaccines and Biologicals

World Health Organization

The Immunological Basis for Immunization Series

Module 22: Hepatitis B

Immunization, Vaccines and Biologicals

World Health Organization

WHO Library Cataloguing-in-Publication Data

The immunological basis for immunization series: module 22: hepatitis B.

(Immunological basis for immunization series ; module 22)

1.Hepatitis B - immunology. 2.Hepatitis B virus - immunology. 3.Hepatitis B vaccines - therapeutic use. 4.Immunization. I.World Health Organization. II.Series.

ISBN 978 92 4 150475 1 (NLM classification: WC 536)

© World Health Organization 2011
All rights reserved. Publications of the World Health Organization are available on the WHO web site (www.who.int) or can be purchased from WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland (tel.: +41 22 791 3264; fax: +41 22 791 4857; e-mail: bookorders@who.int).

Requests for permission to reproduce or translate WHO publications –whether for sale or for non-commercial distribution– should be addressed to WHO Press through the WHO web site (www.who.int/about/licensing/copyright_form/en/index.html).
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.

Printed in Switzerland

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

This module was produced for Immunization, Vaccines and Biologicals, WHO, by:

Koen Van Herck (MD, PhD) and Pierre Van Damme (MD, PhD) at the Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, a WHO Collaborating Centre for the Prevention and Control of Infectious Diseases, based at the University of Antwerp, Belgium.

Printed in December 2012

Copies of this publication as well as additional materials on immunization, vaccines and biological 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 •

© World Health Organization 2010

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). 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).

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

Abbreviations and acronyms

ALT (SGPT)	alanine aminotransferase (serum glutamic pyruvic transaminase)
BCG	bacille Calmette-Guérin (vaccine)
CDC	Centers for Disease Control and Prevention (US)
DNA (cccDNA)	deoxyribonucleic acid (covalently closed circular DNA)
DTP	diphtheria-tetanus-pertussis (vaccine)
EPI	Expanded Programme on Immunization
GACVS	Global Advisory Committee on Vaccine Safety
HAART	highly active antiretroviral therapy
HBcAg (anti-HBc)	(antibody to) hepatitis B core antigen
HBeAg (anti-HBe)	(antibody to) hepatitis B e antigen
HBIG	hepatitis B immunoglobulins (passive immunization)
HBsAg (anti-HBs)	(antibody to) hepatitis B surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
Hib	Haemophilus influenzae type b
HIV	human immunodeficiency virus
Ig	immunoglobulin (IgM)
MHC	major histocompatibility complex
OCC	out of the cold chain
OPV	oral polio vaccine
ORF	open reading frame
PATH	Program for Appropriate Technology in Health
PE	protective efficacy
RNA (mRNA)	ribonucleic acid (messenger RNA)
SAGE	Strategic Advisory Group of Experts (on immunization)
UNICEF	United Nations Children's Fund
VVMs	vaccine vial monitors
WHO	World Health Organization
YF	yellow fever (vaccine)

Preface

This module is part of the series *The Immunological Basis for Immunization*, which was initially developed in 1993 as a set of eight modules focusing on the vaccines included in the Expanded Programme on Immunization (EPI)[1] . In addition to a general immunology module, each of the seven other modules covered one of the vaccines recommended as part of the EPI programme — diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. The modules have become some of the most widely used documents in the field of immunization.

With the development of the Global Immunization Vision and Strategy (GIVS) (2005–2015) (http://www.who.int/vaccines-documents/DocsPDF05/GIVS_Final_EN.pdf) and the expansion of immunization programmes in general, as well as the large accumulation of new knowledge since 1993, the decision was taken to update and extend this series.

The main purpose of the modules — which are published as separate disease/vaccine-specific modules — is to give immunization managers and vaccination professionals a brief and easily-understood overview of the scientific basis of vaccination, and also of the immunological basis for the World Health Organization (WHO) recommendations on vaccine use that, since 1998, have been published in the *Vaccine Position Papers* (http://www.who.int/immunization/documents/positionpapers_intro/en/index.html).

WHO would like to thank all the people who were involved in the development of the initial *Immunological Basis for Immunization* series, as well as those involved in its updating, and the development of new modules.

[1] This programme was established in 1974 with the main aim of providing immunization for children in developing countries.

1. The organism, the disease and the vaccines

1.1 Hepatitis B virus

Hepatitis B virus (HBV) is a 42-nm deoxyribonucleic acid (DNA)-virus of the family Hepadnaviridae. It consists of an inner nucleocapsid of the hepatitis B core antigen (HBcAg), surrounding the small, circular, partially double-stranded DNA genome and the DNA polymerase of HBV, and an outer lipoprotein envelope containing the hepatitis B surface antigen (HBsAg) and pre-S1 and pre-S2 proteins. With a genome of about only 3200 base pairs, HBV is one of the smallest DNA viruses known *(1)*. The HBsAg protein is produced in excess amounts, resulting in the assembly of 22-nm spherical and tubular HBsAg-particles that circulate in the blood of infected persons. The hepatitis B e protein (HBeAg) is not a part of virus particles, but can be detected in the serum of patients with active HBV replication in either an acute or a chronic form of hepatitis B. HBeAg is only synthesized under conditions of high viral replication. For the last few years HBV DNA has been used as an indicator for viral replication, and is expressed as IU/ml or copies/ml.

Three major determinants of HBsAg have been described. The so-called a determinant contains a neutralizing epitope; one of the other determinants has either *d* or *y* specificity and the other has *w* or *r (2)*. Thus, four serotypes of HBV are defined: *adw, adr, ayw* and *ayr*. This serologic subtype classification of HBV has transitioned to the more precise genotype genetic classification. HBV has been classified into eight genotypes (A–H) on the basis of intergenotypic difference of >8% in the entire nucleotide sequence *(3)*. These HBV genotypes have a distinct geographic distribution, with genotype A being most common in northern Europe and the United States of America (USA), B and C in Asia, and D in Mediterranean countries and the Middle East. Chronic infection with genotype B appears to have a better prognosis than genotype C. Precore mutant infection is also most common in genotypes B, C and D, and this explains why precore mutant infection is more common in Asia and southern Europe.

Upon infection, the hepatotropic HBV attaches to hepatocytes, is uncoated in the cytoplasm, and its nucleocapsid is transported to the nucleus of the infected liver cell. The viral DNA induces the production of four messenger ribonucleic acids (mRNAs), each of which encodes the different viral proteins: one for the X protein; one for pre-S2 and HBsAg proteins; one for pre-S1, pre-S2 and S proteins, and one for HBcAg, HBeAg and polymerase *(2,4)*. Newly-assembled viruses either exit the hepatocyte or are reimported into its nucleus to restart the viral replication process.

Compared to other DNA viruses, mutations occur more frequently in HBV, due to its replication via an RNA intermediate and reverse transcriptase which apparently lacks a proofreading function *(5,6)*. HBV mutations occur in all open reading frames (ORFs), and thus in all viral genes and regulatory elements. Certain mutants may be associated with a specific course of the infection, and this could lead to reduced viral clearance by the immune system ("immune escape") or by antiviral therapy ("therapy escape"), and may affect the detectability ("diagnosis escape") or the recognition by neutralizing antibodies ("vaccine escape") *(6)*.

Certain amino-acid substitutions within the a determinant of HBsAg, particularly in the region of amino acids 139–147, may render the a determinant unrecognizable by common screening tests, as well as by vaccine-induced antibodies *(6)*. Indeed, a number of mutants have been described, some of which have been proved to be stable, infectious and pathogenic in chimpanzees *(7)*. To what extent these mutants occur naturally, or are due to pre-existing mutants having a selective advantage, remains unclear *(6)*. Despite the initial concern about the potential of HBV variants to escape prevention, detection, immune response to infection or therapy, HBV mutants have not proved to be of public-health significance, and mathematical modelling predicts that it would take decades before an effective vaccine escape mutant would become dominant *(8–11)*.

1.2 Hepatitis B disease

Hepatitis B virus is not directly cytopathic but it causes infected hepatocytes to dysfunction (12). Infection with HBV often leads to a strong T-cell response against multiple viral epitopes; consequently, these patients will succeed in overcoming their acute hepatitis B infection. However, the virus most often persists in infants infected at birth (due to neonatal immune tolerance to viral antigens), and less frequently also in older children and adults who have a very weak (or undetectable) cellular immune response, suggesting that neonatal immune tolerance or a less efficient T-cell response may result in the persistence of HBV infection and the development of a chronic hepatitis. This may explain the findings of a recent meta-analysis that showed an increased death rate in HBV co-infected human immunodeficiency virus (HIV)-positive people, both before and after starting highly active antiretroviral therapy (HAART) *(13)*. In the long run, activation of cellular oncogenes or inflammation and hepatocyte regeneration caused by chronic liver injury can lead to the development of hepatocellular carcinoma (HCC) in chronically infected persons *(14,15)*.

Indeed, HBV infection can have a variety of consequences, ranging from subclinical infection without, or with minimal symptoms, to acute (clinically apparent) hepatitis and even fulminant and potentially life-threatening acute hepatitis B *(16)*. Moreover, HBV infection can persist as chronic hepatitis (defined as HBsAg positivity and thus HBV infection, for at least six months) and may lead to chronic liver disease, liver cirrhosis and ultimately hepatocellular carcinoma (HCC) *(17)*.

Both the clinical manifestations caused by HBV infection during the acute phase, and the probability of developing chronic hepatitis are strongly dependent on the age at infection. Clinical signs and symptoms of **acute** hepatitis B occur in about 1% of perinatal HBV infections, 10% of infections in children 1–5 years of age and 30% of older children or adults *(18)*. Acute hepatitis B may lead to fulminant liver failure in 0.5%–1.0% of infected adults but rarely in infected children or infants *(19,20,21)*. Mortality due to fulminant hepatitis B can get as high as 70% in children, although liver transplantation is linked to substantially higher survival rates *(22,23,24)*.

The probability of becoming **chronically** infected with HBV is inversely related to age. Indeed, approximately 80%–90% of people infected perinatally, about 30% of children infected before the age of six years, and 5% or less of otherwise healthy, HBV-infected adults will develop chronic HBV infection *(25,26)*. As explained above, the T-cell response to HBV infection is believed to play an important role. This is further illustrated by the increased risk of developing chronic hepatitis in patient groups such as the HIV infected, those with renal failure and diabetics *(26,27)*.

The acute hepatitis caused by HBV is clinically similar to that induced by other causes of viral hepatitis. Apart from the incubation period (75 days on average, but ranging from 30 to 180 days), or other epidemiological characteristics which may be suggestive for HBV, confirming the diagnosis requires laboratory testing.

After a prodromal phase with malaise, nausea, mild fever, myalgia and/or fatigue which usually lasts 1–2 weeks, jaundice, dark urine and pale stools become evident. Patients may simultaneously suffer abdominal pain in the right upper quadrant. Symptoms usually resolve within 1–3 months.

HBsAg is one of the early HBV markers to be found, some 4 weeks (range: 6–60 days) after infection; its presence indicates current infection with HBV and thus, infectiousness *(28,29)*. Additionally, HBeAg as a marker for high viral replication, can be detected in the initial phase of acute HBV infection. Its presence indicates that the blood and body fluids of the infected person are highly contagious. HBV DNA is another marker of viral load and is directly correlated with infectivity. Subsequently, at the onset of symptoms or abnormalities in laboratory tests for liver function, anti-HBc antibodies become detectable (IgM-type at first). Total anti-HBc usually persists, and is a marker for past infection. Upon recovery from symptoms, HBeAg and HBsAg disappear and are replaced, during convalescence, by anti-HBe and anti-HBs, respectively. The presence of anti-HBs indicates the status of immunity to HBV infection.

By far the major part of the disease burden caused by hepatitis B infections is caused by chronic infections. In perinatally-acquired HBV infections, clinical signs and symptoms during the acute phases are rare, and most infected infants will develop a chronic hepatitis. Such chronic infection is characterized by the persistence (>6 months) of HBsAg (with or without concurrent HBeAg). Presence of HBeAg indicates that the individual concerned has high viral replication, is more highly infectious and at greater risk of developing chronic consequences. Persistence of HBsAg (or HBV DNA) are the principal markers of risk for development of chronic liver disease and hepatocellular carcinoma later in life. Spontaneous clearance of the virus — demonstrated by loss of HBsAg — is rare (less than 1% per year in untreated patients).

For infants and young children, the development of chronic hepatitis B starts with a phase of immune tolerance with active virus replication, without active liver disease, which can last 10–30 years *(30,31,32)*. Subsequently, active liver disease develops, while viral replication remains high. In adults, there is no such immune tolerance phase, and active liver disease develops rather shortly after infection. This chronic inflammation may result in the development of fibrosis of the liver, which may lead to cirrhosis. Each year, some 10% of patients spontaneously move into a low-replicative phase (i.e. they become HBeAg negative and develop anti-HBe). During this phase, virus replication as well as active liver disease, are absent, or occur at low levels.

1.3 Treatment

The main goal of therapy for chronic HBV infection is to significantly suppress replication of HBV, thus preventing liver disease progression to cirrhosis with its complications. Treatment of chronic HBV infections has some success. In patients who are HBeAg-positive, the goal of treatment is HBeAg seroconversion with sustained suppression of HBV DNA and rarely HBsAg seroconversion. In those who are HBeAg-negative, the goal of treatment is sustained suppression of HBV DNA and liver injury as measured by alanine aminotransferase (ALT) levels as well as HBsAg seroconversion (which is achieved only on rare occasions). Residual HBV DNA in the form of intranuclear covalently closed circular DNA (cccDNA) may still be present in patients who lost HBsAg and seroconverted to anti-HBs, a situation which leads to occult HBV infection.

Guidelines for the treatment of chronic HBV infections have been issued by several professional, international hepatology organizations, and are updated regularly *(33,34,35)*. Published guidelines often require diagnostic methods (e.g. liver biopsy) to determine eligibility for treatment.

Currently, seven antiviral agents are approved for the treatment of chronic HBV infection in developed countries, and have been shown to delay progression of cirrhosis, reduce the incidence of HCC and improve long-term survival *(33)*. Depending on the defined outcome, approximately one-third of patients respond to a one-year α-interferon therapy which is mainly used in HBeAg positive patients. Pegylated interferon-based therapy appears to have several advantages compared with nucleos(t)side analogues: the relatively higher rate of anti-HBe seroconversion; the limited duration of treatment compared with nucleos(t)ide analogues and the potential, albeit rare, HBsAg loss after a one-year therapy, the absence of resistance and the lower overall cost *(36,37)*. Treatment with nucleos(t)ide analogues is very effective in suppression of viral load but the end-point of treatment is undetermined and long-term treatment, which remains costly and unavailable to the majority of those affected, is required. Combination therapy of available antiviral drugs does not lead to an increased efficacy or a durable post-therapy benefit. Long-term antiviral treatment may result in elimination of viral replication in 40%–50% of cases with chronic HBV infection *(38)*. Nevertheless, HBV treatment is costly, and is therefore not always readily accessible in many resource-constrained settings which is where most people infected with HBV live. Treatment is often complicated by the toxicity of the drugs, by the selection of HBV mutants or the induction of antiviral resistance, and by high relapse rates.

1.4 Epidemiology

Even if HBV has a global spread, the geographical patterns of its prevalence vary hugely. High(est) endemicity of hepatitis B (currently defined as ≥8% of the population HBsAg-positive) is found in areas of sub-Saharan Africa, south-east Asia, the eastern Mediterranean countries, south and western Pacific islands, the interior of the Amazon Basin and in certain parts of the Caribbean; in these areas up to 20% of the population may be chronically infected. Intermediate endemicity (2%–8% of the population HBsAg-positive) are located in south-central and south-west Asia, eastern and southern Europe, the Russian Federation and most of Central and South America. In Australia, New Zealand, northern and western Europe and North America, the prevalence of chronic HBV infection is low (<2% of the population HBsAg-positive).

Humans are the only reservoir of HBV. The virus is highly contagious and is transmitted by percutaneous and permucosal exposure to blood, and in other body fluids, including saliva, semen and vaginal secretions. The highest concentrations of the virus can be found in blood and serous exudates (up to 109 virions/ml). Four different modes of HBV transmission exist: from mother to child (perinatal), from child to child (horizontal), through unsafe injections and blood transfusions (parenteral), and through sexual contact (sexual). The relative importance of each of these depends on the endemicity.

In highly endemic regions, HBV transmission mostly occurs early in life, either from mother to child at birth, or from person to person during early childhood. Infection later in life however, and especially by sexual transmission or by the use of contaminated needles (e.g. among injecting drug users), is relatively more important in situations of low(er) endemicity. Nevertheless, perinatal transmission may account for 15% of HBV-related deaths, even in low-endemic areas. Other parenteral or percutaneous routes of HBV transmission, such as needlestick injury and mucous membrane splash, or through contaminated multidose vials in the health-care setting, as well as tattooing, piercing, or sharing razors or toothbrushes, may play an important role in spreading the virus. Worldwide, unsafe injection practices are thought to account for over 21 million HBV infections each year *(39)*. While transfusion-related infections have currently become very rare in industrialized countries following the implementation of serological and molecular screening of donated blood and plasma, surgery and dental care may still be a source of HBV infection.

Global estimates indicate that at least two billion people have been infected with HBV, and that approximately 360 million people are chronic carriers (i.e. 6% of the world's population) *(40,41)*. On the basis of HBV epidemiological data, a mathematical model was developed to estimate the global hepatitis B disease burden and vaccination impact *(42)*. This model estimated that 620 000 HBV-related deaths occurred globally in the year 2000; 580 000 (94%) from chronic HBV infection and 40 000 (6%) from acute hepatitis B. Over the lifetime of the year 2000 global birth cohort, without vaccination, the model estimated there would be 64 766 000 HBV infections, 9 733 000 chronic infections and 1 405 000 HBV-related deaths. Without vaccination, 69% of all HBV-related deaths in the 2000 birth cohort were estimated to result from the combination of perinatal and early childhood infection *(42)*.

1.5 Hepatitis B vaccines

1.5,1 Vaccine formulations

In the 1970s it became clear that the antibodies to the hepatitis B surface antigen (anti-HBs) were protective against HBV infection, and thus that HBsAg could serve as an immunogen *(43,44,45)*. This led to the development of plasma-derived vaccines using purified HBsAg from the plasma of chronically HBV-infected people which was subsequently adsorbed on to alum salts as an adjuvant. This type of vaccine became commercially available in 1982.

Since 1986, new vaccines have been licensed based on recombinant DNA technology that expresses HBsAg in yeast or mammalian cells, by inserting the gene coding for HBsAg (sometimes including pre-S envelope genes) using plasmids. As a result, several recombinant DNA vaccines against hepatitis B have become available from different manufacturers *(2,46)*. The HBsAg produced self-assembles into immunogenic, spherical particles. Subsequently, the HBsAg is harvested, purified and adsorbed on to alum salts (and in certain formulations thiomersal is added). Recombinant hepatitis B vaccines have gradually replaced the plasma-derived vaccines.

More recently, so-called third-generation hepatitis B vaccines — based on the S, pre-S1, and pre-S2 antigens, or using new adjuvants — have been, and are being, developed. These vaccines specifically aim to enhance the immune response in immunocompromised patients and non-responders.

In current hepatitis B vaccine production, only the S-gene is brought to expression, with production of the major HBsAg protein. Vaccines containing pre-S1 and pre-S2 epitopes have been developed with the expectation that these vaccines would be more efficient in non-responders. So far, divergent and conflicting results have been obtained with these vaccines, while the high cost of production would limit their use *(47,48)*.

In order to enhance immunogenicity, new adjuvants, rather than changes in protein composition, were tested. Several adjuvant systems turned out to be more immunogenic than their non-adjuvanted counterparts: monophosphoryl lipid A, a derivative of the highly immunogenic bacterial cell wall component lipopolysaccharide, in combination with aluminium hydroxide, has been used in adults with renal insufficiency *(49)*. Other hepatitis B vaccines containing oil-in-water emulsions MF59 have also been developed *(50,51)*.

The quantity of HBsAg used for individual vaccine doses varies with the manufacturer, ranging from 3 mcg to 10 mcg per dose for paediatric formulations, and from 10 mcg to 40 mcg per adult dose, due to differences in the manufacturing process of the vaccines *(2)*. The HBsAg content, therefore, cannot be used to assess the relative efficacy of the vaccines, and no international standard exists to express vaccine potency *(52)*.

When immunizing against HBV at birth, only monovalent hepatitis B vaccine should be used; the other antigens found in combination vaccines are currently not approved for use at birth. In addition to monovalent vaccines against hepatitis B, a broad range of combination vaccines include an HBV component for vaccination during infancy, early childhood or for adults. Most of these simultaneously immunize against tetanus, diphtheria and pertussis (with either a whole-cell or an acellular component); they may also include antigens for vaccination against polio and/or Haemophilus influenzae type b, or hepatitis A virus. A list of Hepatitis B vaccines prequalified by the World Health Organization can be located at the vaccine prequalification site (http://www.who.int/immunization_standards/vaccine_quality/PQ_vaccine_list_en/en/index.html).

1.5.2 Vaccine stability

Similar to diphtheria-tetanus-pertussis (DTP) vaccines, currently licensed HBV vaccines require a shipping and storage temperature between 2°C and 8 °C. Within this range, vaccines are generally stable for 3–4 years from the manufacturing date (Table 1).

Studies have shown that hepatitis B vaccines are relatively heat stable, and have been shown to maintain their immunogenicity and reactogenicity profile after exposure to 37 °C for one month, and to 45 °C for up to one week *(53,54)*. A recent review on "out of the cold chain" (OCC) storage of HBV vaccines concluded that vaccine potency and immunogenicity were maintained after prolonged exposure to ambient temperatures.

In contrast to its heat stability, hepatitis B vaccines have been shown to be sensitive to freezing. Accidental freezing (as low as –0.5 °C) causes the HBsAg to dissociate from its alum adjuvant and thus to lose its immunogenicity and potency *(52)*. Currently, the clinical consequences of freezing on vaccine efficacy have not been evaluated.

In view of the available data on the heat stability of HBV vaccines, and because of the investments required for the facilities with refrigeration, OCC use may be (economically) more favourable *(55,56,57)*. A recent paper considered single-dose presentations of thermostable vaccines potentially cost-effective for low-resource settings in Africa and Asia *(58)*. The authors therefore recognize a clear need for additional information on the thermostability of HBV vaccines, as:

- research to improve the thermostability of new vaccines, including hepatitis B vaccine, is still ongoing;
- the translation from currently available evidence on HBV vaccine thermostability into licensing and guidance may also improve;
- several international organizations (WHO, UNICEF, PATH) advocate the use of vaccine vial monitors (VVMs) and prevention of vaccine freezing for a number of vaccines, including hepatitis B vaccines, to reduce vaccine wastage and to prevent the use of heat-damaged stock (ref http://www.who.int/vaccines-documents/DocsPDF02/www716.pdf).

Manufacturers are recommended to include stability data in the product literature accompanying each HBV vaccine. In the near future, monovalent HBV vaccines may then be stored OCC, provided that the heat exposure is appropriately monitored *(56)*.

1.5.3 Vaccine safety

In placebo-controlled studies, with the exception of local pain, reported events such as myalgia and transient fever have been less frequent than in the placebo group (<10% in children, 30% in adults). Reports of severe anaphylactic reactions are very rare; their risk has been estimated at 1.1 cases/million doses administered (95% CI: 0.1–3.9 cases/million doses administered) based on American data from the Vaccine DataLink project *(59)*.

For other severe adverse events that have been reported rarely after hepatitis B vaccination, expert review of the available data either concluded that evidence was insufficient to reject or accept a causal association with hepatitis B vaccine — for Guillain–Barré syndrome — or that a causal association could be rejected — for multiple sclerosis *(60)*. No causal association has been demonstrated between hepatitis B vaccines and chronic conditions such as chronic fatigue syndrome, rheumatoid arthritis, leukaemia, autoimmune disorders, neurologic disorders, asthma, sudden infant death syndrome or type-1 diabetes *(60–68)*. There is no evidence of toxicity in infants, children or adults exposed to thiomersal or thimerosal (USA) in vaccines *(69–76)*. The Global Advisory Committee on Vaccine Safety (GACVS)[1] will continue to monitor vaccine safety concerns (ref http://www.who.int/vaccine_safety/en/).

Hepatitis B vaccine is contraindicated for individuals with a history of allergic reactions to any of the vaccine's components. Neither pregnancy nor lactation is a contraindication for use of this vaccine.

1.5.4 Route of administration

Currently licensed hepatitis B vaccines are administered by intramuscular injection. For infants and children <2 years, the anterolateral thigh is the preferred injection site; for older children and adults it is the deltoid muscle *(77–80)*.

Administration in the buttock is not recommended because this route of administration has been associated with decreased protective antibody levels *(81)*.

Intradermal administration of hepatitis B vaccine has been studied as a potentially dose-sparing and cost-saving option. A recent review by the joint WHO-PATH project "Optimize" and two previous meta-analyses concluded that intradermal administration is broadly equivalent to intramuscular administration in terms of inducing an immune response, but that reduced-dose intradermal immunization may be less effective than intramuscular injection of a full dose *(82,83)*. Intradermal HBV vaccination has also been found successful in overcoming the lack of response to standard intramuscular vaccination observed in patients on haemodialysis *(84)*.

Considerable progress has been made in the (transient) expression of HBsAg in transgenic plants. While a number of important issues (e.g. choosing the most suitable plant carrier, improving the HBsAg expression level) still need to be addressed, currently available evidence suggests that edible HBV vaccines may form an additional route of administration in the future *(85–89)*.

1.5.5 Co-administration

The hepatitis B vaccine does not interfere with the immune response to many other vaccines, and vice versa. Specifically, the birth-dose of hepatitis B can be given safely together with oral polio vaccine (OPV) and bacillus Calmette–Guérin (BCG) vaccine; BCG does not interfere negatively with the response to hepatitis B vaccine *(52,90)*.

[1] The Global Advisory Committee on Vaccine Safety (GACVS) was established in 1999 to respond promptly, efficiently, and with scientific rigour to vaccine safety issues of potential global importance. Results are reported (and updated) through the GACVS website (http://www.who.int/vaccine_safety/en/).

Several studies have indeed confirmed that simultaneous administration of hepatitis B vaccine with BCG, DTP-polio or measles vaccines, did not induce any negative effects with regard to the safety or the respective immune responses *(91–94)*. Only with yellow fever (YF) vaccine, one study found that simultaneous administration of hepatitis B vaccine resulted in significantly lower YF antibodies; a later study however found comparable immune responses *(95,96)*.

1.5.6 Vaccination schedules

A series of three (intramuscular) vaccinations are known to induce immunity against HBV. A large variety of hepatitis B vaccination schedules have been shown to induce seroprotective anti-HBs levels[2] in over 95% of healthy infants and children. These include schedules where doses are administered at birth, 1 and 6 months of age; at 2, 4, and 6 months of age, and 6, 10, and 14 weeks of age *(78,97–102)*. In general, the minimum recommended interval between the doses is four weeks. Longer dose intervals may increase the final anti-HBs titres but not the seroconversion rates.

In any age group, an HBV vaccination schedule that is interrupted should be continued as soon as possible, while respecting the minimum interval between the remaining doses. Restarting the whole series of HBV vaccine is not necessary *(103–108)*; nevertheless, some studies report a higher proportion of non-response in vaccinees with a delayed second dose in the immunization schedule *(109,110)*.

Children who respond to hepatitis B vaccine are protected against acute and chronic forms of hepatitis B *(52)*. Additionally, most recommended infant vaccination schedules result in a high protective efficacy against chronic HBV infection, conditional on the timing of the first vaccine doses and/or an adequate dosage of hepatitis B immunoglobulins (HBIG) at birth *(110,111)*.

Hepatitis B vaccines can be co-administered with other infant vaccines. Therefore, except for the birth dose, hepatitis B vaccination can easily be added to existing infant vaccination schedules without requiring additional visits *(52)*.

1.5.7 Protection against hepatitis B at birth

Recommended vaccination schedules that include a birth dose will prevent most perinatally-acquired infections and offer early protection from horizontal transmission. Indeed, depending on the vaccination coverage for the complete series, according to model-based predictions, universal HBV infant immunization would prevent up to 75% of global deaths from HBV-related causes. Adding a birth dose to prevent perinatal transmission would increase that proportion to 84% *(42)*.

At least in the perinatal setting, HBIG alone has been shown to be about 75% effective in preventing symptomatic HBV or the development of chronic infection in neonates born to HBsAg-positive mothers. Hepatitis B vaccination also later demonstrated an at-least similar effectiveness, while combining HBIG and the vaccine increases efficacy to 85%–95% *(112,113)*.

[2] Seroprotection is defined as having an anti-HBs level >10 IU/L, when measured 1–3 months after a completely administered hepatitis B vaccination schedule (i.e. three or four doses).

2. The response to immunization

2.1 Description of the serological response

The extensive use of both plasma-derived and recombinant HBV vaccines has confirmed their excellent safety and tolerability in newborns and infants *(114)*. Anti-HBs antibodies are used as a marker for immunity. Vaccine efficacy studies demonstrated virtually complete protection against acute and chronic hepatitis B in immunocompetent people with anti-HBs levels ≥10 IU/L after vaccination. Therefore, seroprotection against HBV infection is defined as having an anti-HBs level ≥10 IU/L, when measured 1–3 months after having received a complete immunization schedule *(115–122)*.

Reviews on the use of HBV vaccine in neonates and infants report seroprotective levels of anti-HBs antibodies at one month after the last vaccine dose for all schedules in >95% of healthy vaccinees *(123,124)*. Another review, including studies conducted mainly in newborns, reported seroprotection rates ranging from 92.6% to 100% one month after the 0, 1, 6 month schedule, and from 97% to 98% one month after an accelerated 0, 1, 2 month or 0, 1, 3 month schedule *(125)*. These data demonstrate that HBV vaccines are highly immunogenic in newborns and infants and have the advantage of providing higher seroprotection rates at early childhood compared to older populations. Indeed, antibody response rates decline gradually after the age of 40 *(126)*.

Similar to the immune response to HBV infection, T-cell dependence of the immune response to hepatitis B vaccination has been demonstrated, and it was shown that non-responding infants had a reduced capacity to adequately expand and differentiate TH cells *(127)*. However, almost all individuals who did not respond sufficiently to a primary 3-dose immunization series did respond to a 3-dose revaccination series *(128)*.

Hepatitis B vaccine administered at birth substantially lowers the risk of hepatitis B infection in infants born to HBV-infected mothers (relative risk 0.28, 95% CI 0.20–0.40), as concluded by a meta-analysis in 2006 *(129)*.

2.2 Determinants of the immune response to immunization

While HBV vaccines in general induce an adequate immune response in over 95% of fully vaccinated healthy persons, a large interpersonal variability has been demonstrated in the immune response to HBsAg in healthy subjects. As such, fast/high, intermediate, slow/poor and even non-responders can be distinguished based on the magnitude and the kinetics of the immune response to HBV vaccination *(130,131)*. The antibody response to hepatitis B vaccine has been shown to depend on the type, dosage and schedule of vaccination used, as well as the age, gender, genetic factors, co-morbidity and the status of the immune system of the vaccinee *(132)*.

2.2.1 *Vaccine-related factors*

Both plasma-derived and recombinant DNA hepatitis B vaccines are similar with respect to safety, immunogenicity and efficacy; also in the long term *(52,133,134)*.

Several studies have shown that hepatitis B vaccine can be administered simultaneously with other routinely-used childhood vaccines without interaction on their respective immune responses *(52,92,93,135)*.

A 1994 review paper has clearly demonstrated a link between hepatitis B vaccine antigen dosage and protective efficacy against chronic HBV infection in neonates born to HBeAg positive mothers. In this review, administration of lower hepatitis B antigen dosages resulted in lower protective efficacy, unless HBIG was (simultaneously) administered at birth *(111)*.

2.2.2 *Host-related factors*

Enhancement of the immune response was found if the infant was older at the time of initial vaccine injection or if the booster dose was given later *(136–139)*. This has been linked to the immaturity of the infant immune system *(140,141,142)*.

Immunization against HBV can induce protection in infants in the presence of passive immunity from maternal origin *(143,144)*.

Premature infants do not have a reduced immune response to hepatitis B vaccination *(145)*. Some infants born prematurely with low birth weight (<2000 g) may not respond well to vaccination at birth *(146)*. The long-term effectiveness of hepatitis B vaccination in these children with a birth weight <2000 g needs to be further documented, to evaluate if a primary series of three doses offers sufficient protection. However, by one month of chronological age, all premature infants, regardless of initial birth weight or gestational age, are likely to respond adequately *(147)*.

Several immunosuppressive conditions, such as advanced HIV infection, chronic liver disease, chronic renal failure and diabetes have been demonstrated to be associated with reduced immunogenicity of hepatitis B vaccine. Indeed, HIV-infected infants, infants with malignancies and infants undergoing haemodialysis have a lower probability of having an adequate immune response *(148,149,150)*. The immune response to hepatitis B vaccination in HIV-infected people is known to be associated with the prevaccination CD4 counts, and HAART therapy is known to improve the capacity of the immune system to respond to hepatitis B vaccination *(151,152,153)*. Additionally, a recent study on HIV-infected patients showed a positive association of double-dose revaccination with serologic immune response to hepatitis B vaccination *(152)*. Therefore, immunocompromised individuals may require the administration of additional injections or higher vaccine doses, and the anti-HBs antibody titres should be followed up after immunization.

For adults with renal insufficiency, a recombinant HBV vaccine containing a higher antigen dose and an additional adjuvant is available. This vaccine is more reactogenic, but was shown to induce a stronger, more rapid and longer-lasting immune response compared to a 4-dose series of double-dose standard hepatitis B vaccine *(154)*.

A complete series of hepatitis B vaccine induces protective antibody levels in >95% of infants, children and young adults. After the age of 40, protection following the primary vaccination series drops below 90%; by 60 years, protective antibody levels are achieved in only 65%–75% of vaccinees.

As with many other vaccines, higher antibody levels are obtained in females than in males *(77)*.

In addition to the factors mentioned above, non-response to hepatitis B vaccination has also been linked to genetic causes and to a number of major histocompatibility complex (MHC) class I, II, and III alleles and haplotypes *(155–158)*.

2.3 Duration of immunity

After primary immunization with HBV vaccine, anti-HBs concentrations wane quite rapidly within the first year and more slowly thereafter *(124,159)*. People with a lower peak anti-HBs level after the complete primary vaccination series have a higher probability of falling below the 10 IU/L anti-HBs level more rapidly. Among children who previously responded to a complete series of hepatitis B vaccination with antibody levels >10 IU/L, up to 50% of vaccinees have low or undetectable concentrations of anti-HBs (anti-HBs loss) between five and 15 years after vaccination *(124,160)*. However, even if anti-HBs concentrations decline to below 10 IU/L, immune memory continues to persist over a longer time period *(161,162)*. Persistence of this vaccine-induced immune memory has been demonstrated among vaccinees who responded up to 22 years earlier to a primary childhood vaccine series starting at birth. In 67% to 76% of these persons, an anamnestic response could be induced by an additional vaccine dose *(163)*. Even an absent anamnestic response following such booster vaccination may not necessarily mean that such people are again susceptible to HBV *(164)*. From a public-health perspective, these data indicate that almost all children vaccinated at early childhood retain immune memory and would develop an anti-HBs response on exposure to HBV later in life. The Hepatitis Working Group concluded at the April 2009 WHO Strategic Advisory Group of Experts (SAGE) meeting that there is high-quality evidence to support effectiveness of a primary series of hepatitis B vaccine to prevent acute and chronic HBV infection at 15 years post vaccination of infants, and low-quality evidence to support effectiveness at 22 years post vaccination of infants[3]. Given the incubation period of HBV, stimulation of memory cells should trigger antibody production rapidly enough to prevent HBV infection or at least its clinical consequences *(165)*.

[3] Source: April 2009 WHO SAGE meeting, grade table 4 of the Hepatitis Working Group. Available online at http://www.who.int/immunization/sage/4_Grade_table_Hep_B.pdf.

Protection against clinically significant breakthrough HBV infection and chronic carriage is expected to last longer than detectable antibodies after immunization. The longest follow-up of protection conferred by HBV vaccines has been conducted in populations with an initially high endemicity of HBV infection *(160)*. Studies of cohorts of immunocompetent persons vaccinated as children or infants indicate that, despite anti-HBs loss years after immunization, nearly all vaccinated persons who respond to a primary series remain protected from HBV infection, and HBV immunization remained highly efficacious in reducing the HBsAg positivity rate, despite significant proportions who had anti-HBs levels <10 IU/L 15 years or more after the primary vaccination series *(166,167)*. No clinical cases of HBV disease have been observed in follow-up studies conducted 15–20 years after vaccination among immunocompetent vaccinated persons with antibody levels >10 IU/L after the primary schedule *(168–171)*.

2.4 Vaccine efficacy

Since the first aim of hepatitis B immunization programmes is to effectively prevent chronic HBV infections, and their potential as a reservoir for further transmission of HBV, prevalence of chronic HBV infections in young children is the most reliable way to measure the impact of such vaccination programmes. The effectiveness of hepatitis B infant immunization programmes has already been demonstrated in a variety of countries and settings *(52)*. This has been reviewed by the Hepatitis Working Group and was discussed at the April 2009 WHO SAGE meeting[4], and in a 2008 Cochrane review by Lee et al *(113,129)*.

Protection from disease has been defined either in serological or epidemiological terms. In the former, seroconversion after immunization has been equated with protection from disease; in the latter, vaccine efficacy is estimated as the percentage reduction in disease incidence attributable to immunization.

The protective efficacy after primary vaccination starting at birth in neonates born to HBeAg positive mothers has been evaluated, for instance in Thailand *(172,173)*. Here, no child became a chronic carrier beyond the age of three years, showing that the vaccine provides immediate protection against HBsAg carriage.

Efforts to reduce the chronic carrier rate and to prevent late infections in infants of HBsAg-positive mothers focused on a combination of passive and active immunization *(174,175)*. Passive immunization with HBIG offers protection from perinatal transmission, but the passively-administered antibodies disappear during the first year of life. Importantly, the long-term protective efficacy did not significantly differ between neonates born to carrier mothers who were given HBV vaccine alone, or concomitantly with HBIG *(172,173,176)*. Another serological investigation of long-term immunity conducted in children born to HBV carrier mothers (albeit in a region of low endemicity) demonstrated that most children vaccinated at birth retain immunological memory to hepatitis B vaccine for 15 years, but those who did not were more likely to have received HBIG at birth, suggesting that passive antibody may interfere with the induction of immunological memory *(177)*.

[4] Source: April 2009 WHO SAGE meeting, session Hepatitis B vaccine. Background documents and grade tables available online at http://www.who.int/immunization/sage/previous_apr2009/en/index.html.

The above-mentioned findings indicate that in high endemic countries that cannot assume a safe and affordable HBIG immunotherapy, and where mothers may not always be (or "are usually not") screened for HBV infection, routine administration of only HBV vaccine at birth is highly protective, even for very high-risk infants of HBeAg-positive mothers *(123,178,179)*. This was confirmed in a Chinese study that found timely administration of the birth dose and hospital birth to be independently associated with children being HBsAg-negative *(180)*. A systematic review of studies on the effect of HBV vaccines and HBIG in newborn infants of mothers positive for HBsAg showed that HBV vaccine, as well as the combination of vaccine plus HBIG given to newborn infants of HBsAg positive mothers, are both effective in preventing the occurrence of HBV *(113)*. Results of this meta-analysis found the combination of vaccine plus HBIG to be superior to vaccine alone, but it should be noted that, in this analysis, the primary outcome was preventing the occurrence of HBV, defined as serological presence of HBsAg, HBeAg or anti-HBc, and not protection against chronic carriage.

The long-term protection provided by infant HBV vaccination was further confirmed by a cross-sectional serological study of HBV infection in children of various ages in the Gambia, which demonstrated that vaccine efficacy against chronic carriage remained high (94%) over 14 years; efficacy against infection up to 14 years post primary vaccination was 82% *(181)*.

Some studies have documented breakthrough infections over time, although generally not earlier than three years after vaccination, as detected by the presence of HBV serological markers (anti-HBc antibodies or HBV DNA) in a limited percentage of vaccinated persons with low antibody levels *(160,161,172)*. These infections are usually transient and asymptomatic and no case of acute HBV has been recorded in a successfully immunized individual. Chronic infections have been documented only very rarely *(182)*. Breakthrough infections resulting in chronic infection were observed among infants born to HBsAg-positive mothers only, and some of these appeared to be HIV-positive *(183)*.

2.5 Booster vaccine requirements

Based on evidence supporting the effectiveness of a primary series of hepatitis B vaccine in infants for up to 22 years, and on the persistence of protection from HBV infection beyond the persistence of vaccine-induced antibodies (see section 0 on page 19), the current scientific evidence does not support the use of booster doses of HBV vaccine among immunocompetent individuals who have responded to a complete primary vaccination series *(161,183,184,185)*. The WHO, as well as advisory groups in Europe and the USA, do not recommend a booster dose in immunocompetent individuals *(183,186,187)*. However, some reports focusing on long-term protection provided by HBV vaccination in infancy, conclude that continued surveillance and long-term monitoring of HBV disease trends in these children, especially in those immunized with only three vaccinations in the first year of life, have to be conducted as they grow older in order to follow up if a booster dose might become necessary later in life *(173,181,183,188,189)*.

3. Future prospects

3.1 Discussion (research needs)

There is extensive evidence on the excellent safety and immunogenicity profile of hepatitis B vaccines. However, future research projects could address a few relevant issues and, as such, build additional evidence to fine-tune recommendations to improve the current impact of hepatitis B vaccination campaigns.

- In view of the higher probability of developing chronic HBV infection and its consequences when infected in the earliest phase of life, prevention of perinatal transmission of HBV should have the highest priority. Therefore, additional evidence on the following research questions should be pursued.
  - It has been demonstrated that timely administration of the birth dose results in the highest vaccine effectiveness. However, there are several regions in the world where birth at home is common and thus where immediate administration of the birth dose may be difficult to achieve *(190)*. This calls for additional research to assess how long after birth the first dose of HBV vaccine can be given and still result in comparable protection to when the first dose is given <24 hours at birth.
  - How can we improve the delivery of hepatitis B vaccine for newborns who are born at home (i.e. a call for programmatic research)? In this respect, increasing the knowledge base about the potential impact of cold-chain deviations may also prove helpful.
  - In settings where countries have successfully implemented HBV vaccination during infancy or childhood, with high vaccination rates, what could be the added value of adolescent and adult vaccination?
- Infant hepatitis B vaccination schedules: there are only very few studies reported that had applied a "standard EPI" (i.e. 6, 10, 14 weeks or 0, 6, 14 weeks) vaccination schedule. Nevertheless, field evidence has clearly demonstrated the efficacy of the Expanded Programme on Immunization (EPI) schedules, at least in preventing HBV infections early in life and, as such, preventing the large majority of chronic HBV infections. However, there is a discrepancy between the WHO-proposed vaccination schedules and the Centers for Disease Control and Prevention (CDC) recommendations for the interval between the first and last dose of HBV vaccine (i.e. at least a 16-week interval). The evidence-base for this issue should be enlarged.

- The available evidence on the co-administration of HBV vaccines with other infant vaccinations should be complemented with data on the co-administration with other vaccines that are (or may be) proposed to be given at birth or early infancy (e.g. meningococcal, malaria, pertussis, polio) or with other injections (e.g. vitamin A, vitamin K).
- There is substantial evidence regarding the persistence of vaccine-induced anti-HBs and immune memory. Based on the currently available data, the duration of protection is assumed to last for life. However, further research is needed to better document the exact duration of protection, especially for those vaccinated as infants, and to validate the current statement that booster doses are not required for fully vaccinated, immunocompetent individuals. Similarly, there is a need to study the long-term immunogenicity and efficacy of the standard EPI schedules, especially since the existing universal hepatitis B vaccination programmes will have substantially reduced the endemicity of HBV in these populations, and thus the probability of their being naturally boosted.
- Additional research may also help to improve the available vaccines and the strategic options to administer them, by:
  - needle-free administration of (hepatitis B) vaccines;
  - vaccines with increased immunogenicity (e.g. to minimize non-response and for people with immunocompromised conditions);
  - vaccines with increased (or at least better documented) thermostability, including better translation of the available knowledge into guidelines and recommendations.

References

1) Carman W, Thomas H, Domingo E. Viral genetic variation: hepatitis B virus as a clinical example. *Lancet*, 1993, 341(8841):349–353.

2) Mast E, Ward J. Hepatitis B vaccines. In: Plotkin S, Orenstein WA, Offit PA, eds. *Vaccines*, 5th ed. Saunders Elsevier, 2008:205–242.

3) Norder H et al. Genetic diversity of Hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology*, 2004:47(6): 289–309.

4) Lau JYN, Wright TL. Molecular virology and pathogenesis of hepatitis B. The *Lancet*, 1993, 342(8883):1311–1340.

5) Hunt CM et al. Clinical relevance of hepatitis B viral mutations. *Hepatology*, 2000, 31(5):1037–1044.

6) Francois G et al. Mutant hepatitis B viruses: a matter of academic interest only or a problem with far-reaching implications? *Vaccine*, 2001, 19(28–29): 3799–3815.

7) Ogata N et al. Infectivity and pathogenicity in chimpanzees of a surface gene mutant of hepatitis B virus that emerged in a vaccinated infant. *The Journal of Infectious Diseases*, 1997, 175(3):511–523.

8) Wilson JN, Nokes DJ, Carman WF. Current status of HBV vaccine escape variants — a mathematical model of their epidemiology. *Journal of Viral Hepatitis*, 1998, 5(Suppl. 2):S25–S30.

9) Wilson JN, Nokes DJ, Carman WF. The predicted pattern of emergence of vaccine-resistant hepatitis B: a cause for concern? *Vaccine*, 1999, 17(7–8): 973–978.

10) Wilson JN, Nokes DJ, Carman WF. Predictions of the emergence of vaccine-resistant hepatitis B in the Gambia using a mathematical model. *Epidemiology and Infection*, 2000, 124(2):295–307.

11) Hsu H et al. No increase in prevalence of Hepatitis B surface antigen mutant in a population of children and adolescents who were fully covered by universal infant immunization. *The Journal of Infectious Diseases*, 2010, 201(8): 1192–1200.

12) Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. *Annual Review of Immunology*, 1995, 13:29–60.

13) Nikolopoulos GK et al. Impact of hepatitis B virus infection on the progression of AIDS and mortality in HIV-infected individuals: a cohort study and meta-analysis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 2009, 48(12):1763–1771.

14) Beasley RP. Hepatitis B virus. The major etiology of hepatocellular carcinoma. *Cancer*, 1988, 61(10):1942–1956.

15) *Hepatitis viruses*. Lyon, International Agency for Research on Cancer, 1994.

16) Wright TL, Lau JY. Clinical aspects of hepatitis B virus infection. *Lancet*, 1993, 342(8883):1340–1344.

17) Hoofnagle JH. Chronic hepatitis B. *The New England Journal of Medicine*, 1990, 323(5):337–339.

18) McMahon BJ et al. Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. *The Journal of Infectious Diseases*, 1985, 151(4):599–603.

19) Pappas SC. Fulminant viral hepatitis. *Gastroenterology Clinics of North America*, 1995, 24(1):161–173.

20) Hoofnagle JH et al. Fulminant hepatic failure: summary of a workshop. *Hepatology*, 1995, 21(1):240–252.

21) Chang M-H. Hepatitis B virus infection. *Seminars in Fetal and Neonatal Medicine*, 2007, 12(3):160–167.

22) Chen HL et al. Pediatric fulminant hepatic failure in endemic areas of hepatitis B infection: 15 years after universal hepatitis B vaccination. *Hepatology*, 2004, 39(1):58–63.

23) Bernuau J et al. Multivariate analysis of prognostic factors in fulminant hepatitis B. *Hepatology*, 1986, ;6(4):648–651.

24) O'Grady JG et al. Early indicators of prognosis in fulminant hepatic failure. *Gastroenterology*, 1989, 97(2):439–445.

25) Edmunds WJ et al. The influence of age on the development of the hepatitis B carrier state. *Proceedings. Biological sciences / The Royal Society*, 1993, 253(1337):197–201.

26) Hyams KC. Risks of chronicity following acute hepatitis B virus infection: a review. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*, 1995, 20(4):992–1000.

27) Hadler SC et al. Outcome of hepatitis B virus infection in homosexual men and its relation to prior human immunodeficiency virus infection. *The Journal of Infectious Diseases*, 1991, 163(3):454–459.

28) Krugman S et al. Viral hepatitis, type B. Studies on natural history and prevention re-examined. *The New England Journal of Medicine*, 1979, 300(3):101–106.

29) Hoofnagle JH, Di Bisceglie AM. Serologic diagnosis of acute and chronic viral hepatitis. *Seminars in Liver Disease*, 1991, 11(2):73–83.

30) Lok AS, Lai CL. A longitudinal follow-up of asymptomatic hepatitis B surface antigen-positive Chinese children. *Hepatology*, 1988, 8(5):1130–1133.

31) Hoofnagle JH et al. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. *Annals of Internal Medicine*, 1981, 94(6):744–748.

32) Liaw YF et al. Determinants for hepatitis B e antigen clearance in chronic type B hepatitis. *Liver*, 1984, 4(5):301–306.

33) Lok ASF, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology*, 2009, 50(3):661–662.

34) Liaw YF et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatology International*, 2008, 2(3):263–283.

35) European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of chronic hepatitis B. *Journal of Hepatology*, 2009, 50(2):227–242.

36) Lok AS et al. Antiviral drug-resistant HBV: Standardization of nomenclature and assays and recommendations for management. *Hepatology*, 2007, 46(1): 254–265.

37) Hoofnagle JH et al. Management of hepatitis B: summary of a clinical research workshop. *Hepatology*, 2007, 45(4):1056–1075.

38) Dienstag JL. Hepatitis B virus infection. *The New England Journal of Medicine*, 2008, 359(14):1486–1500.

39) FitzSimons D et al. Hepatitis B virus, hepatitis C virus and other blood-borne infections in healthcare workers: guidelines for prevention and management in industrialized countries. *Occupational and Environmental Medicine*, 2008, 65(7):446–451.

40) Kane MA. Global status of hepatitis B immunization. *Lancet*, 1996, 348(9029):696.

41) Lee WM. Hepatitis B virus infection. *The New England Journal of Medicine*, 1997, 337(24):1733–1745.

42) Goldstein ST et al. A mathematical model to estimate global hepatitis B disease burden and vaccination impact. *International Journal of Epidemiology*, 2005, 34(6):1329–1339.

43) Krugman S, Giles JP, Hammond J. Viral hepatitis, type B (MS-2 strain). Studies on active immunization. *JAMA : the journal of the American Medical Association*, 1971, 217(1):41–5.

44) Purcell RH, Gerin JL. Hepatitis B subunit vaccine: a preliminary report of safety and efficacy tests in chimpanzees. *The American Journal of the Medical Sciences*, 1975, 270(2):395–399.

45) Hilleman MR et al. Purified and inactivated human hepatitis B vaccine: progress report. *The American Journal of the Medical Sciences*, 1975, 270(2):401–404.

46) McAleer WJ et al. Human hepatitis B vaccine from recombinant yeast. *Nature*, 1984, 307(5947):178–180.

47) Rendi-Wagner P et al. Comparative immunogenicity of a PreS/S hepatitis B vaccine in non- and low-responders to conventional vaccine. *Vaccine*, 2006, 24(15):2781–2789.

48) Shapira MY et al. Rapid seroprotection against hepatitis B following the first dose of a Pre-S1/Pre-S2/S vaccine. *Journal of Hepatology*, 2001, 34(1):123–127.

49) Beran J. Safety and immunogenicity of a new hepatitis B vaccine for the protection of patients with renal insufficiency including pre-haemodialysis and haemodialysis patients. *Expert Opinion on Biological Therapy,* 2008, 8(2): 235–247.

50) Podda A, Del Giudice G. MF59-adjuvanted vaccines: increased immunogenicity with an optimal safety profile. *Expert Review of Vaccines*, 2003, 2(2):197–204.

51) McKee A et al. Immune mechanisms of protection: can adjuvants rise to the challenge? *BMC Biology*, 8(1):37.

52) Expanded Programme on Immunization (EPI). *Introduction of hepatitis B vaccination into childhood immunization services: management guidelines, including information for health workers and parents*. Geneva, World Health Organization, 2001 (WHO/V&B/01.31) [cited 12 December 2006]. Available at http://www.who.int/vaccines-documents/DocsPDF01/www613.pdf.

53) Van Damme P et al. Heat stability of a recombinant DNA hepatitis B vaccine. *Vaccine*, 1992, 10(6):366–367.

54) Melnick JL. Thermostability of poliovirus, measles, and hepatitis B vaccines. *Vaccine Research*, 1995, 4:1–11.

55) Levin CE et al. The costs of home delivery of a birth dose of hepatitis B vaccine in a prefilled syringe in Indonesia. *Bulletin of the World Health Organization*, 2005, 83(6):456–461.

56) Hipgrave DB, Maynard JE, Biggs BA. Improving birth dose coverage of hepatitis B vaccine. *Bulletin of the World Health Organization*, 2006, 84(1):65–71.

57) Implementation of newborn hepatitis B vaccination — worldwide, 2006. *MMWR Morbidity and Mortality Weekly Report*, 2008, 57(46):1249–1252.

58) Levin A et al. An economic evaluation of thermostable vaccines in Cambodia, Ghana and Bangladesh. *Vaccine*, 2007, 25(39–40):6945–6957.

59) Bohlke K et al. Risk of anaphylaxis after vaccination of children and adolescents. *Pediatrics*, 2003, 112(4):815–820.

60) Halsey NA et al. Hepatitis B vaccine and central nervous system demyelinating diseases. Viral Hepatitis Prevention Board. *The Pediatric Infectious Disease Journal*, 1999, 18(1):23–24.

61) DeStefano F et al. Childhood vaccinations, vaccination timing, and risk of type 1 diabetes mellitus. *Pediatrics*, 2001, 108(6):E112.

62) DeStefano F et al. Childhood vaccinations and risk of asthma. *The Pediatric Infectious Disease Journal*, 2002, 21(6):498–504.

63) DeStefano F, Verstraeten T, Chen RT. Hepatitis B vaccine and risk of multiple sclerosis. *Expert Review of Vaccines*, 2002, 1(4):461–466.

64) Ellenberg SS, Braun MM. Monitoring the safety of vaccines — assessing the risks. *Drug Safety : an international journal of medical toxicology and drug experience*, 2002, 25(3):145–152.

65) DeStefano F et al. Vaccinations and risk of central nervous system demyelinating diseases in adults. *Archives of Neurology*, 2003, 60(4):504–509.

66) Eriksen EM et al. Lack of association between hepatitis B birth immunization and neonatal death: a population-based study from the vaccine safety datalink project. *The Pediatric Infectious Disease Journal*, 2004, 23(7):656–662.

67) Maher JE et al. Infant vaccinations and childhood asthma among full-term infants. *Pharmacoepidemiology and Drug Safety*, 2004, 13(1):1–9.

68) Destefano F, Weintraub ES, Chen RT. Hepatitis B vaccine and risk of multiple sclerosis. *Pharmacoepidemiology and Drug Safety*, 2007, 16(6):705–707 (author reply 7–8).

69) Pichichero ME et al. Mercury concentrations and metabolism in infants receiving vaccines containing thiomersal: a descriptive study. *The Lancet*, 2002, 360(9347):1737–1741.

70) Hviid A et al. Association between thimerosal-containing vaccine and autism. *JAMA : the journal of the American Medical Association*, 2003, 290(13): 1763–1766.

71) Madsen KM et al. Thimerosal and the occurrence of autism: negative ecological evidence from Danish population-based data. *Pediatrics*, 2003, 112(3):604–606.

72) Heron J, Golding J and the ALSPAC Study Team. Thimerosal exposure in infants and developmental disorders: a prospective cohort study in the United Kingdom does not support a causal association. *Pediatrics*, 2004, 114(3):577–583.

73) Verstraeten T et al. Safety of thimerosal-containing vaccines: a two-phased study of computerized health maintenance organization databases. *Pediatrics*, 2003, 112(5):1039–1048.

74) Andrews N et al. Thimerosal exposure in infants and developmental disorders: a retrospective cohort study in the United Kingdom does not support a causal association. *Pediatrics*, 2004, 114(3):584–591.

75) Burbacher TM et al. Comparison of blood and brain mercury levels in infant monkeys exposed to methylmercury or vaccines containing thimerosal. *Environmental Health Perspectives*, 2005;113(8).

76) Pichichero ME et al. Mercury levels in newborns and infants after receipt of thimerosal-containing vaccines. *Pediatrics*, 2008, 121(2):e208–e214.

77) Fang JW et al. Female children respond to recombinant hepatitis B vaccine with a higher titre than male. *Journal of Tropical Pediatrics*, 1994, 40(2):104–107.

78) Greenberg DP et al. Comparative safety and immunogenicity of two recombinant hepatitis B vaccines given to infants at two, four and six months of age. *The Pediatric Infectious Disease Journal*, 1996, 15(7):590–596.

79) Li VS et al. Duration of hepatitis B antibody response in children immunized with hepatitis B and compulsory vaccines. *European Journal of Epidemiology,* 1995, 11(2):217–219.

80) Cook IF, Murtagh J. Comparative immunogenicity of hepatitis B vaccine administered into the ventrogluteal area and anterolateral thigh in infants. *Journal of Paediatrics and Child Health*, 2002, 38(4):393–396.

81) Centers for Disease Control (CDC). Suboptimal response to hepatitis B vaccine given by injection into the buttock. *MMWR Morbidity and Mortality Weekly Report*, 1985, 34(8):105–108, 13.

82) Sangaré L et al. Intradermal hepatitis B vaccination: a systematic review and meta-analysis. *Vaccine*, 2009, 27(12):1777–1786.

83) Chen W, Gluud C. Vaccines for preventing hepatitis B in health-care workers. *Cochrane Database of Systematic Reviews (Online)*, 2005, (4):CD000100.

84) Nicolas J-Fo, Guy B. Intradermal, epidermal and transcutaneous vaccination: from immunology to clinical practice. *Expert Review of Vaccines*, 2008, 7(8):1201–1214.

85) Thanavala Y et al. Immunogenicity in humans of an edible vaccine for hepatitis B. *Proceedings of the National Academy of Sciences of the United States of America*, 2005, 102(9):3378–3382.

86) Huang Z et al. High-yield rapid production of hepatitis B surface antigen in plant leaf by a viral expression system. *Plant Biotechnology Journal*, 2008, 6(2):202–209.

87) Gomez E, Zoth SC, Berinstein A. Plant-based vaccines for potential human application: a review. *Human Vaccines*, 2009, 5(11):738–744.

88) Guan Z-j et al. Overview of expression of hepatitis B surface antigen in transgenic plants. *Vaccine*, [in press, corrected proof].

89) Rybicki EP. Plant-made vaccines for humans and animals. *Plant Biotechnology Journal*, 8(5):620–637.

90) Ota MO et al. Influence of Mycobacterium bovis bacillus Calmette-Guérin on antibody and cytokine responses to human neonatal vaccination. *Journal of Immunology*, 2002, 168(2):919–925.

91) Coursaget P et al. Simultaneous injection of hepatitis B vaccine with BCG and killed poliovirus vaccine. *Vaccine*, 1992, 10(5):319–321.

92) Chiron JP et al. Simultaneous administration of hepatitis B and diphtheria/tetanus/polio vaccines. *Lancet*, 1984, 1(8377):623–624.

93) Coursaget P et al. Simultaneous administration of diphtheria-tetanus-pertussis-polio and hepatitis B vaccines in a simplified immunization program: immune response to diphtheria toxoid, tetanus toxoid, pertussis, and hepatitis B surface antigen. *Infection and Immunity*, 1986, 51(3):784–787.

94) Coursaget P et al. Simultaneous injection of hepatitis B and measles vaccines. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 1991, 85(6):788.

95) Yvonnet B et al. Simultaneous administration of hepatitis B and yellow fever vaccines. *Journal of Medical Virology*, 1986, 19(4):307–311.

96) Coursaget P et al. Simultaneous injection of plasma-derived or recombinant hepatitis B vaccines with yellow fever and killed polio vaccines. *Vaccine*, 1995, 13(1):109–111.

97) Hadler SC, Margolis HS. Hepatitis B immunization: vaccine types, efficacy, and indications for immunization. In: Remington JS, Swartz MN, eds. *Current topics in infectious diseases*. Boston, Blackwell Scientific Publications, 1992:282–308.

98) Coursaget P, Kane M. Overview of clinical studies in developing countries. In: Ellis RW, ed. *Hepatitis B vaccines in clinical practice*. New York, Marcel Dekker, 1993:209–228.

99) Da Villa G et al. Anti-HBs responses in children vaccinated with different schedules of either plasma-derived or HBV DNA recombinant vaccine. *Research in Virology*, 1997, 148(2):109–114.

100) Aspinall S, Kocks DJ. Immunogenicity of a low-cost hepatitis B vaccine in the South African Expanded Programme on Immunization. *South African Medical Journal*, 1998, 88(1):36–39.

101) Goldfarb J et al. Comparative study of the immunogenicity and safety of two dosing schedules of Engerix-B hepatitis B vaccine in neonates. *The Pediatric Infectious Disease Journal*, 1994, 13(1):18–22.

102) Yusuf HR et al. Association between administration of hepatitis B vaccine at birth and completion of the hepatitis B and 4:3:1:3 vaccine series. *JAMA : the journal of the American Medical Association*, 2000, 284(8):978–983.

103) Keyserling HL et al. Antibody responses of healthy infants to a recombinant hepatitis B vaccine administered at two, four, and twelve or fifteen months of age. *Journal of Pediatrics*, 1994, 125(1):67–69.

104) Mangione R et al. Delayed third hepatitis B vaccine dose and immune response. *Lancet*, 1995, 345(8957):1111–1112.

105) Halsey NA et al. Hepatitis B vaccine administered to children and adolescents at yearly intervals. *Pediatrics*, 1999, 103(6):1243–1247.

106) Duval B, Deceuninck G, Middleman AB. Seroprotection rates after late doses of Hepatitis B vaccine. *Pediatrics*, 2002, 109(2):350–351.

107) Middleman AB et al. The effect of late doses on the achievement of seroprotection and antibody titer levels with hepatitis b immunization among adolescents. *Pediatrics*, 2001, 107(5):1065–1069.

108) Jackson Y et al. High immunogenicity of delayed third dose of hepatitis B vaccine in travellers. *Vaccine*, 2007, 25(17):3482–3484.

109) Sabidó M et al. Timing of hepatitis B vaccination: its effect on vaccine response in health care workers. *Vaccine*, 2007, 25(43):7568–7572.

110) Tharmaphornpilas P et al. Increased risk of developing chronic HBV infection in infants born to chronically HBV infected mothers as a result of delayed second dose of hepatitis B vaccination. *Vaccine*, 2009, 27(44):6110–6115.

111) Andre FE, Zuckerman AJ. Review: protective efficacy of hepatitis B vaccines in neonates. *Journal of Medical Virology*, 1994, 44(2):144–151.

112) (CDC) CfDCaP. Updated US Public Health Service guidelines for the management of occupational exposures to HBV, HCV, and HIV and recommendations for postexposure prophylaxis. *Morbidity and Mortality; weekly report*, 2001, 50(RR–11):1–42.

113) Lee C et al. Effect of hepatitis B immunization in newborn infants of mothers positive for hepatitis B surface antigen: systematic review and meta-analysis. *BMJ (Clinical research ed.)*, 2006, 332(7537):328–336.

114) Niu MT. Review of 12 million doses shows hepatitis B vaccine safe. *Vaccine Weekly*, 1996, 4:13–25.

115) Szmuness W et al. Hepatitis B vaccine: demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *The New England Journal of Medicine*, 1980, 303(15):833–841.

116) Szmuness W et al. A controlled clinical trial of the efficacy of the hepatitis B vaccine (Heptavax B): a final report. *Hepatology*, 1981, 1(5):377–385.

117) Francis DP et al. The prevention of hepatitis B with vaccine. Report of the Centers for Disease Control multi-center efficacy trial among homosexual men. *Annals of Internal Medicine*, 1982, 97(3):362–366.

118) Hadler SC et al. Long-term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. *The New England Journal of Medicine*, 1986, 315(4):209–214.

119) Centers for Disease Control (CDC). Update on hepatitis B prevention. *MMWR Morbidity and Mortality Weekly Report*, 1987, 36(23):353–360, 66.

120) Ambrosch F et al. Immunization against hepatitis B. *Lancet*, 1988, 1 (8590):875–876.

121) *Informal consultation on quadrivalent diphtheria-tetanus-pertussis-hepatitis B vaccine. Final report*. Geneva, World Health Organization, 1992.

122) Jack AD et al. What level of hepatitis B antibody is protective? *The Journal of Infectious Diseases*, 1999, 179(2):489–492.

123) Venters C, Graham W, Cassidy W. Recombivax-HB: perspectives past, present and future. *Expert Review of Vaccines*, 2004, 3(2):119–129.

124) Safary A, André F. Over a decade of experience with the yeast recombinant hepatitis B vaccine. *Vaccine*, 1999, 18:57–67.

125) Keating GM, Noble S. Recombinant hepatitis B vaccine (Engerix-B): a review of its immunogenicity and protective efficacy against hepatitis B. *Drugs*, 2003, 63(10):1021–1051.

126) Averhoff F et al. Immunogenicity of hepatitis B vaccines. Implications for persons at occupational risk of hepatitis B virus infection. *American Journal of Preventive Medicine*, 1998, 15(1):1–8.

127) Avanzini MA et al. Antigen-specific T cell response in infants after recombinant hepatitis B virus vaccination at birth: evaluation of T helper lymphocyte diversity. *Clinical Immunology (Orlando, Fla.)*, 2003, 107(2):122–128.

128) Tan KL et al. Immunogenicity of recombinant yeast-derived hepatitis B vaccine in nonresponders to perinatal immunization. *JAMA : the journal of the American Medical Association*, 1994, 271(11):859–861.

129) Lee C et al. Hepatitis B immunization for newborn infants of hepatitis B surface antigen-positive mothers. *Cochrane Database of Systematic Reviews (Online)*, 2006 (2):CD004790.

130) Dienstag JL et al. Hepatitis B vaccine in health care personnel: safety, immunogenicity, and indicators of efficacy. *Annals of Internal Medicine*, 1984, 101(1):34–40.

131) Alper CA. The human immune response to hepatitis B surface antigen. *Experimental and Clinical Immunogenetics*, 1995, 12(3):171–181.

132) Hollinger FB. Factors influencing the immune response to hepatitis B vaccine, booster dose guidelines, and vaccine protocol recommendations. *The American Journal of Medicine*, 1989, 87(3A):36S–40S.

133) Dentinger CM et al. Persistence of antibody to hepatitis B and protection from disease among Alaska natives immunized at birth. *The Pediatric Infectious Disease Journal*, 2005, 24(9):786–792.

134) Chan CY, Lee SD, Lo KJ. Legend of hepatitis B vaccination: the Taiwan experience. *Journal of Gastroenterology and Hepatology*, 2004, 19(2):121–126.

135) Giammanco G et al. Immune response to simultaneous administration of a recombinant DNA hepatitis B vaccine and multiple compulsory vaccines in infancy. *Vaccine*, 1991, 9(10):747–750.

136) del Canho R et al. Immunogenicity of 20 micrograms of recombinant DNA hepatitis B vaccine in healthy neonates: a comparison of three different vaccination schemes. *Journal of Medical Virology*, 1993, 41(1):30–34.

137) Mazel JA et al. Passive-active immunization of neonates of HBsAg positive carrier mothers: preliminary observations. *BMJ (Clinical research ed.)*, 1984, 288(6416):513–515.

138) Schalm SW et al. Prevention of hepatitis B infection in newborns through mass screening and delayed vaccination of all infants of mothers with hepatitis B surface antigen. *Pediatrics*, 1989, 83(6):1041–1048.

139) Stevens CE et al. Prospects for control of hepatitis B virus infection: implications of childhood vaccination and long-term protection. *Pediatrics*, 1992, 90(1 Pt. 2):170–173.

140) Marchant A, Newport M. Prevention of infectious diseases by neonatal and early infantile immunization: prospects for the new millennium. *Current Opinion in Infectious Diseases*, 2000, 13(3):241–246.

141) Siegrist CA. Neonatal and early life vaccinology. *Vaccine*, 2001, 19(25–26): 3331–3346.

142) Marchant A et al. Predominant influence of environmental determinants on the persistence and avidity maturation of antibody responses to vaccines in infants. *The Journal of Infectious Diseases*, 2006, 193(11):1598–1605.

143) Maupas P et al. Efficacy of hepatitis B vaccine in prevention of early HBsAg carrier state in children. Controlled trial in an endemic area (Senegal). *Lancet*, 1981, 1(8215):289–292.

144) Barin F et al. Immune response in neonates to hepatitis B vaccine. *Lancet*, 1982, 1(8266):251–253.

145) del Canho R et al. Hepatitis B vaccination and preterm infants. *The Pediatric Infectious Disease Journal*, 1993, 12(5):407–408.

146) Losonsky GA et al. Hepatitis B vaccination of premature infants: a reassessment of current recommendations for delayed immunization. *Pediatrics*, 1999, 103(2):E14.

147) Saari TN. Immunization of preterm and low birth weight infants. American Academy of Pediatrics Committee on Infectious Diseases. *Pediatrics*, 2003, 112(1 Pt. 1):193–198.

148) Zuin G et al. Impaired response to hepatitis B vaccine in HIV infected children. *Vaccine*, 1992, 10(12):857–860.

149) Entacher U et al. Hepatitis B vaccination and immune response in children with malignant diseases. *European Journal of Pediatrics*, 1985, 144(2):160–163.

150) Callis LM et al. Hepatitis B virus infection and vaccination in children undergoing haemodialysis. *Acta Paediatrica Scandinavica*, 1985, 74(2):213–218.

151) Armstrong KE et al. Role of CD4 count in immunity development after Hepatitis A and B vaccination among HIV-infected patients: Kentucky, 2002–2007. *Journal of the International Association of Physicians in AIDS Care (JIAPAC)*, 2010, 9(3):179–186.

152) Psevdos G et al. Efficacy of double-dose Hepatitis B rescue vaccination in HIV-infected patients. *AIDS Patient Care and STDs*, 2010, 24(7):403–407.

153) Sutcliffe CG, Moss WJ. Do children infected with HIV receiving HAART need to be revaccinated? *The Lancet Infectious Diseases*, 2010, 10(9):630–642.

154) Kong NC et al. A new adjuvant improves the immune response to hepatitis B vaccine in haemodialysis patients. *Kidney International*, 2008, 73(7):856–862.

155) Alper CA et al. Genetic prediction of nonresponse to hepatitis B vaccine. *The New England Journal of Medicine*, 1989, 321(11):708–712.

156) Martinetti M et al. Humoral response to recombinant hepatitis B virus vaccine at birth: role of HLA and beyond. *Clinical Immunology (Orlando, Fla.)*, 2000, 97(3):234–240.

157) Milich DR, Leroux-Roels GG. Immunogenetics of the response to HBsAg vaccination. *Autoimmunity Reviews*, 2003, 2(5):248–257.

158) Amirzargar AA et al. HLA-DRB1, DQA1 and DQB1 alleles and haplotypes frequencies in Iranian healthy adult responders and non-responders to recombinant hepatitis B vaccine. *Iranian Journal of Immunology : IJI*, 2008, 5(2):92–99.

159) Jilg W, Schmidt M, Deinhardt F. Decline of anti-HBs after hepatitis B vaccination and timing of revaccination [letter]. *Lancet*, 1990, 335(8682):173–174.

160) Mast E et al. Hepatitis B vaccine. In: Plotkin S, Orenstein WA, eds. *Vaccines*, 4th ed. Philadelphia, WB Saunders Company, 2004:299–337.

161) Banatvala JE, Van Damme P. Hepatitis B vaccine — do we need boosters? *Journal of Viral Hepatitis*, 2003, 10(1):1–6.

162) Yuen MF et al. 18-year follow-up study of a prospective randomized trial of hepatitis B vaccinations without booster doses in children. Clinical Gastroenterology and Hepatology : *the official clinical practice journal of the American Gastroenterological Association*, 2004, 2(10):941–945.

163) Petersen KM et al. Duration of hepatitis B immunity in low-risk children receiving hepatitis B vaccinations from birth. *The Pediatric Infectious Disease Journal*, 2004, 23(7):650–655.

164) Hammitt LL et al. Hepatitis B immunity in children vaccinated with recombinant hepatitis B vaccine beginning at birth: a follow-up study at 15 years. *Vaccine*, 2007, 25(39–40):6958–6964.

165) West DJ, Calandra GB. Vaccine induced immunologic memory for hepatitis B surface antigen: implications for policy on booster vaccination. *Vaccine*, 1996, 14(11):1019–1027.

166) van der Sande MA et al. Long-term protection against carriage of hepatitis B virus after infant vaccination. *The Journal of Infectious Diseases*, 2006, 193(11): 1528–1535.

167) Lu CY et al. Humoral and cellular immune responses to a hepatitis B vaccine booster 15–18 years after neonatal immunization. *The Journal of Infectious Diseases*, 2008, 197(10):1419–1426.

168) Viviani S et al. Hepatitis B vaccination in infancy in the Gambia: protection against carriage at 9 years of age. *Vaccine*, 1999, 17(23–24):2946–2950.

169) Floreani A et al. Long-term persistence of anti-HBs after vaccination against HBV: an 18-year experience in health-care workers. *Vaccine*, 2004, 22(5–6):607–610.

170) Poovorawan Y et al., eds. Long-term efficacy of hepatitis B vaccination of newborns born of hepatitis B surface antigen-positive mothers in Thailand. *Proceedings of the twelfth International Symposium on Viral Hepatitis and Liver Disease*. Paris, 2006.

171) Bialek SR et al. Persistence of protection against hepatitis B virus infection among adolescents vaccinated with recombinant hepatitis B vaccine beginning at birth: a 15-year follow-up study. *The Pediatric Infectious Disease Journal*, 2008, 27(10):881–885.

172) Poovorawan Y et al. Long-term hepatitis B vaccine in infants born to hepatitis B e antigen positive mothers. *Archives of Disease in Childhood. Fetal and Neonatal Edition*, 1997, 77(1):F47–F51.

173) Poovorawan Y et al., eds. Long-term follow-up (11 to 13 years) of high-risk neonates, born to hepatitis B e antigen-positive mothers and vaccinated against hepatitis B. *Proceedings of the tenth International Symposium on Viral Hepatitis and Liver Disease*. Atlanta, 2002.

174) Tada H et al. Combined passive and active immunization for preventing perinatal transmission of hepatitis B virus carrier state. *Pediatrics*, 1982, 70(4):613–619.

175) Beasley RP et al. Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. *Lancet*, 1983, 2(8359):1099–1102.

176) Lolekha S et al. Protective efficacy of hepatitis B vaccine without HBIG in infants of HBeAg-positive carrier mothers in Thailand. *Vaccine*, 2002, 20(31–32):3739–3743.

177) Boxall EH et al. Long-term persistence of immunity to hepatitis B after vaccination during infancy in a country where endemicity is low. *The Journal of Infectious Diseases*, 2004, 190(7):1264–1269.

178) Marion SA, Tomm Pastore M, Pi DW, Mathias RG. Long-term follow-up of hepatitis B vaccine in infants of carrier mothers. *American Journal of Epidemiology*, 1994, 140(8):734–746.

179) Mast EE, Alter MJ, Margolis HS. Strategies to prevent and control hepatitis B and C virus infections: a global perspective. *Vaccine*, 1999, 17(13–14): 1730–1733.

180) Cui F et al. Factors associated with effectiveness of the first dose of hepatitis B vaccine in China: 1992–2005. *Vaccine*, 28(37):5973–5978.

181) Whittle H et al. Observational study of vaccine efficacy 14 years after trial of hepatitis B vaccination in Gambian children. *BMJ (Clinical research ed.)*, 2002, 325(7364):569.

182) Wu JS et al. Hepatitis B vaccination in high-risk infants: 10-year follow-up. *The Journal of Infectious Diseases*, 1999, 179(6):1319–1325.

183) Banatvala J et al. Are booster immunizations needed for lifelong hepatitis B immunity? *Lancet*, 2000, 355(9203):561–565.

184) Banatvala J, Van Damme P, Oehen S. Lifelong protection against hepatitis B: the role of vaccine immunogenicity in immune memory. *Vaccine*, 2000, 19(7–8):877–885.

185) Fitzsimons D et al. Long-term efficacy of hepatitis B vaccine, booster policy, and impact of hepatitis B virus mutants. *Vaccine*, 2005, 23(32):4158–4166.

186) Immunization Practices Advisory Committee (ACIP). Hepatitis B virus: a comprehensive strategy for eliminating transmission in the United States through universal childhood vaccination. *MMWR. Recommendations and Reports : Morbidity and Mortality Weekly Report. Recommendations and reports / Centers for Disease Control*, 1991, 40(RR–13):1–25.

187) *Hepatitis B vaccine*. World Health Organization [cited October 2009]. Available at www.who.int/vaccines/en/hepatitisb.html.

188) Lu CY et al. Waning immunity to plasma-derived hepatitis B vaccine and the need for boosters 15 years after neonatal vaccination. *Hepatology*, 2004, 40(6):1415–1420.

189) Combined hepatitis B vaccines. *Proceedings of the Viral Hepatitis Prevention Board meeting*. St Julians, Malta, Viral Hepatitis Prevention Board, 2001.

190) Kramvis A, Clements CJ. Implementing a birth dose of hepatitis B vaccine for home deliveries in Africa — too soon? *Vaccine*, 28(39):6408–6410.

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, Women's and Children's Health (FWC)

World Health Organization
20, Avenue Appia
CH-1211 Geneva 27
Switzerland
E-mail: vaccines@who.int
Web site: http://www.who.int/immunization/en/

ISBN 978 92 4 150475 1