Final Technical Report

for

Research Project

Assessment of Hepatitis B Surface Antigen Prevalence among Children in Maldives to evaluate the Impact of the Hepatitis B Vaccination Program

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Final Technical Report

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Assessment of the Prevalence of Hepatitis B Surface Antigen among Children of Grade One (2022-2023) in the Maldives to Evaluate the Hepatitis B Vaccination Program

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1. INTRODUCTION

Hepatitis B is the most common serious liver infection in the world, affecting an estimated 296 million people, which includes over 6 million children under the age of five (1). The global disease burden of Hep B infection is high with 1.5million new infections, 820 thousand deaths and 18 million prevalent cases in the WHO South-East Asia Region alone in 2019 (2). Compared to the horizontal disease transmission mode (through exposure to infected blood or sexual intercourse), vertical transmission (perinatal or Mother-to-child transmission (MTCT)) is responsible for more than one third of chronic HBV infections worldwide. MTCT occurs through three routes of transmission: transplacental transmission in the uterus, transmission during delivery and postpartum transmission during childcare and breastfeeding. Hence, screening pregnant women for HBV infection, providing infant postexposure prophylaxis, and maternal treatment with antiviral medications are the main strategies for reducing MTCT transmission rates and the global burden of new chronic HBV infections.

Hepatitis B is a vaccine-preventable disease. Empirical evidence depicted in figure 1 has shown that the Hepatitis B infection acquired in adulthood leads to chronic hepatitis in less than 5% of the cases, whereas infection in infancy and early childhood leads to chronic hepatitis in about 95% of the cases, which highlights the importance of strengthening and prioritizing infant and childhood vaccination for Hepatitis (3). According to the latest WHO estimates, the proportion of children under five years of age chronically infected with HBV dropped to 1% in 2019 from 5% in the pre-vaccine era (1980s to the early 2000s) (4). The CDC (1) approximates that the hepatitis B vaccine can prevent 38 million deaths over the lifetime of persons born between 2000 and 2030 in 98 low- and middle-income countries.

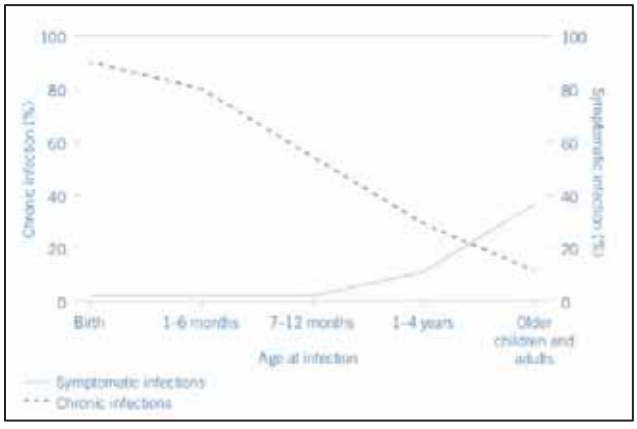


Figure 1: The Natural History of Hepatitis B

Source: Guidelines for the prevention, care, and treatment of persons with Hepatitis B infection, WHO 2015 (3)

Global initiatives on the prevention of Hepatitis B began as early in 1992 with a WHO resolution that urged its members to include the hepatitis B vaccine in their national immunization schedule. Goal 3.3 of the UN Sustainable Development Goals (SDGs) has set targets to combat viral hepatitis among other communicable diseases. Following this, WHO member states adopted the Global Health Sector Strategy (GHSS) on Viral Hepatitis 2016-2021, which set the target of eliminating viral hepatitis as a public health threat by 2030 by targeting less than 1% hepatitis B surface antigen (HBsAg) prevalence among children in 2020 and less than 0.1% HBsAg prevalence among children by 2030. In 2016, the South-East Asia Regional Immunization Technical Advisory Group recommended setting the target of reducing the prevalence of chronic hepatitis B among 5-year-old children to less than 1% by 2020 (5). The Seventy-Fifth World Health Assembly has also approved the implementation of the GHSS for the next 7 years.

In 1997, WHO introduced the concept of the elimination and eradication of diseases as a field of public health. The International Task Force for Disease Eradication (ITFDE) adapted and endorsed the elimination goals of WHO in which HBV infections are recognized as feasible targets for elimination. The reproduction value (R_0) of HBV (R_0 ¼ 4.9 –7.0) falls within the range for smallpox (R_0 ¼ 4.5), which has been eradicated globally, and polio (R_0 ¼ 6.0) and measles (R_0 ¼ 14.5), which can be prevented with vaccines and have been eliminated from several regions of the world (6). Countries are verified to having achieved the target of hepatitis B control mainly through immunization-based evidence of less than 1% prevalence of HBsAg among children measured in nationally representative serosurveys, and evidence of high hepatitis B immunization coverage. National hepatitis B serosurveys have been conducted in at least 25 countries in the WHO Western Pacific Region, with 21 countries verified by WHO to have reached the control target as of 2019. In the WHO South-East Asia Region, serosurveys have been conducted in Bangladesh, Bhutan, Nepal and Thailand and all four countries were verified for also having achieved the control goal by an independent expert panel in 2019.

1.1 SIGNIFICANCE OF THE STUDY

Global incidence of Hepatitis B is second highest and deaths related to the disease is highest in the South East Asia region to which the Maldives belong (2). Hepatitis B is a “silent epidemic” because most people do not have symptoms when they are newly infected or chronically infected. Thus, they can unknowingly spread the virus to others and continue the silent spread of hepatitis B. For people who are chronically infected but don’t have any symptoms, their liver is silently damaged which can develop into serious liver diseases such as cirrhosis or liver cancer. Since the introduction of the EPI program in the country,

interventions have always been focused on achieving high vaccination coverage. However, studies have shown that high vaccination coverage rates for individual vaccines do not necessarily imply timely vaccination or population immunity (7,8,9). Assessing vaccine coverage together with timeliness and completeness of vaccine administration is important for evaluating the effectiveness of immunization programs.

The Maldives Health Master plan 2016-2025 (21) has also highlighted the importance of building a research culture to inform health care decision making and to develop control strategies based on epidemiological evidence and maintaining the elimination status of diseases is given high priority. Information derived from this survey will provide important evidence for policy makers to understand potential delays in vaccination and which populations are most at risk for targeted interventions to improve timeliness of uptake. This report presents the findings from the first national hepatitis B Serological survey among post-vaccination cohorts in the Maldives. It has also assessed the coverage of the hepatitis B immunization program in the Maldives, to guide the immunization program strengthening in the country, and to evaluate whether Maldives has achieved the regional hepatitis B control target for the declaration of disease elimination of Hepatitis B in the Maldives.

1.2 OBJECTIVES OF THE SURVEY

The objectives of the study were twofold:

1. To measure the prevalence of HBsAg among Grade 1 school children (the majority of whom are about 6-7 years of age).
2. To collect immunization data and timeliness of vaccination and calculate effectiveness that hepatitis B vaccine has on preventing chronic infection.

1.3 COUNTRY PROFILE

Demographics: The Republic of Maldives belongs to the Southeast Asia Region of WHO. Located in the Indian ocean, it is economically categorized as an upper middle-income country. It occupies a land area of over 298 kilometers south of India consisting of 1192 small coral islands, out of which 182 islands are inhabited. Tourism is the main source of revenue for the country where it opens its borders to more than a million tourists each year. The last Census of 2021 counted a total population of 515,122 in the country, and 26% are foreigners placing 1 for every 3 locals. Population living in the capital city Male' has increased from 39% (in 2014) to 41% in 2022 (10). Children (0-14years) comprises 26% of the population while the

bulk of the population (69%) represents the working age group (15-64years) with a sex ratio of 104 men per 100 women. Life expectancy in Maldives for females is 85.68 years and for males 78.97 years in 2021.

The Maldivian health system is customized to fit its unique geography. It is designed to provide access to health services on all inhabited islands. There are a total of 190 government health facilities and 240 private health facilities, and a pharmacy is located on each inhabited island (11). The level and type of health services rendered at each government health facility depends on the population size, patient load and travel distance to the nearest Hospital from each island. While each atoll covers a population of 5,000 to 15,000 people, the regional or atoll hospitals act as the main referral centers to provide both general and specialty health care not available on an island (Figure 2). Land and sea ambulances are available at each regional level and refer cases without any cost to patients and families. In 2019, there were 2.1 medical doctors per 1000 population and 48 nurses per 10,000 population (12, 13). The crude birth rate has declined over the past 10 years from 23 live births per 1000 population in 2012 to 17 in 2020 (11). More than 99% of the births occurred at a health facility in the Maldives irrespective of type of birth or location of birth.

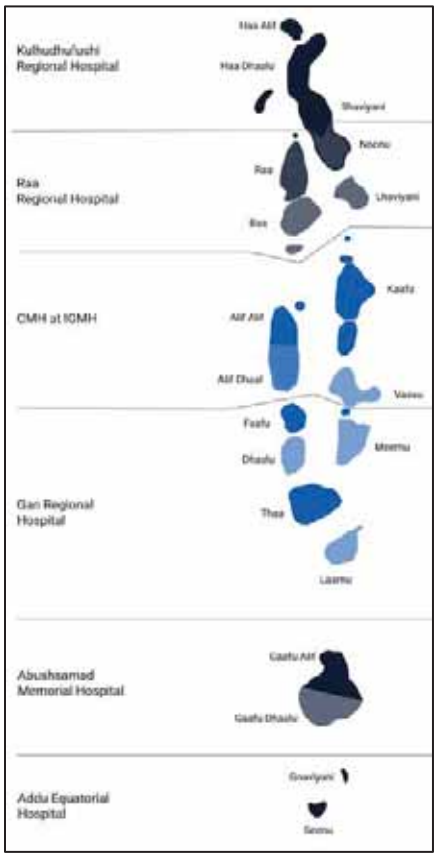


Figure 2: Regional and main referral facilities

Source: National Health Statistics 2021

Health financing Structure: Maldives is one of the few countries that offer an unlimited universal health coverage for its population since 2011. Health care services including medical examination, investigations, immunization, antenatal care, drugs etc. are provided free to all Maldivian citizens. The share of the country's GDP on health and social services is one of the highest in the Southeast Asia region. It has increased from 1.2% in 2003 to 4.6% in 2020 (20). The proportion of the government budget allocated on health spending has reached 11.2% in 2019, allowing for a twofold reduction in out-of-pocket expenditure on health from 49% in 2011 to 20% in 2017 (11). Majority of the health programs, including Maternal and Child Care and STI/HIV is almost exclusively funded by the government and without any financial implication to the people.

Maternal and antenatal services: The national ANC guidelines are implemented at all the health facilities both public and private sector. Pregnant women are advised to make at least nine ANC visits. Pregnant women are routinely screened for HIV, syphilis and hepatitis B (HBV), usually at first antenatal clinic. In addition to the routine hematology and serology tests, screening for TORCH, maternal syphilis, HIV and Hepatitis B is carried out for all pregnant women seeking antenatal care in the Maldives. The coverage of antenatal care in the Maldives is more than 97% with majority of women having their first visit in the first trimester of the pregnancy (14). The national standard for ANC service requires that pregnant women who opt out of screening complete a written signed statement. Screening for HIV, syphilis and HBV is conducted at all atolls, regional and tertiary hospitals, which provide the large majority of antenatal clinic services. Guidelines on MTCT and guidelines for management of Hepatitis positive mothers and their infants are available at all health care providers. All ANC data is collected monthly by the Health Protection Agency, which however excludes laboratory results. In case of a positive case, the national program at HPA is immediately informed and further investigations are followed. The coverage of postpartum/postnatal (PNC) visit was 94%, with 67% receiving a postnatal checkup within two days of delivery and there has been no significant discrepancies in PNC among regions and among various socio-economic status or residence. Table 1 shows the basic health indicators of the Maldives.

Table 1: Basic health indicators

VITAL STATISTICS INDICATORS	2016	2017	2018	2019	2020
Crude Birth Rate (CBR)/'000 population	19	19	18	17	17
Crude Death Rate (CDR)/'000 population	4	3	3	3	3
Infant Mortality Rate (IMR)/'000 live births	9	10	7	6	6
<5 Mortality Rate (<5MR) /'000 live births	11	11	9	8	7
Maternal Mortality Ratio (MMR)/100,000 live births	44	103	61	-	32
Still Birth Rate (SBR)/'000 live births	5	5	3	4	5
Neonatal Mortality Rate (NMR)/'000 live births	7	8	5	4	5

Source: National Health Statistics, 2021

Governance: The Health Protection Agency (HPA) is the lead agency of the Government of the Maldives implementing the disease surveillance system. The Director General of Public Health leads the HPA. It is mandated by the Public Health Act (7/2012) and functions as a department within the Ministry of Health. Each Atoll and island health facility has a public health unit. Public health units provide basic public health services, such as immunization, health awareness and advice, growth monitoring of children under 5, reproductive health services and monitoring and controlling communicable diseases. In each Atoll, the public health units of the health centers are monitored by the Hospital of the Atoll. Maldives has maintained elimination status for malaria, , lymphatic filariasis, measles, rubella, and mother to child transmission of HIV/Syphilis. In 2023, Maldives was also recognized by WHO to have interrupted transmission of Leprosy. The WHO Southeast Asia Region which includes Maldives has also been recognized to have eliminated poliomyelitis and neonatal tetanus.

Source: National Health Statistics, 2021

Prevention of mother to child transmission (PMTCT) is given highest priority and special attention by the government of Maldives and is reflected in the current Health Master Plan 2016-2025 (21). Prevention and management of STIs is a major focus of the National Reproductive Health Strategy for 2014-2018 and beyond. Management of PMTCT services in the Maldives is undertaken by a multidisciplinary team at central level. It is nationally coordinated through national focal persons at atoll and island level.. Universal screening of HIV, Syphillis, Hepatitis B and C for ANC attendees and voluntary testing have been implemented since 2006. Screening for hepatitis B is offered to all pregnant women, with near universal ANC attendance and high testing rates among ANC attendees. Infants born to mothers who test positive for Hepatitis B during pregnancy or of mothers who are already known to have chronic HBV are given hepatitis B immunoglobulin in addition to Hepatitis B birth-dose at birth. The Health Protection Agency facilitates this.

Disease Surveillance: Maldives has a well-established indicator-based surveillance system for infectious diseases. This means that data is collected on incidence of persons developing infectious diseases defined in the Notifiable Diseases List. The public health surveillance section of the Public Health Preparedness Surveillance & Epidemiology Division has the overall responsibility of managing the system. Health information generation starts at the Island Health Centre. Data on Immunization, ANC, delivery, laboratory and communicable diseases, etc. are maintained in respective registers (which are computerized in some facilities). Since 2022, Immunization module of DHIS2 has been functioning, , Data from health facilities on notifiable diseases are received daily at HPA and analysed weekly for the common epidemic-prone diseases.

Epidemiology of Hepatitis B: Viral hepatitis screening data from IGMH, the main tertiary referral center in Maldives, for the period March 2017 to August 2019 showed that “a total of 910 out of 43,773 Maldivian patients were screened out of which 2% were positive for HBV infection (HbsAg positive). Out of a total

of 466 children below the age of 5 years tested during this period, only 1 child was positive for HBV infection. Out of 9862 individuals of age group 5 to 26 years of age tested, 7 were HbsAg positive (0.07%). The rest of the positive cases (99% of the total HBsAg positive results) were in individuals older than 26 years reflecting persons born prior to introduction of the childhood Hepatitis B immunization in Maldives" (17). In the 2008 behavioral and biological survey (BBS) on HIV/AIDS, Hepatitis B was found in 6% of the MSM in Addu and 1% of MSM in Male', 4% of the seafarers, 2% among resort workers and 0.8% among IDUs in Addu (22). Antenatal screening of chronic hepatitis B is routinely conducted in all hospitals and health centers in Maldives. There is a lack of data on the prevalence of chronic hepatitis B among the general population.

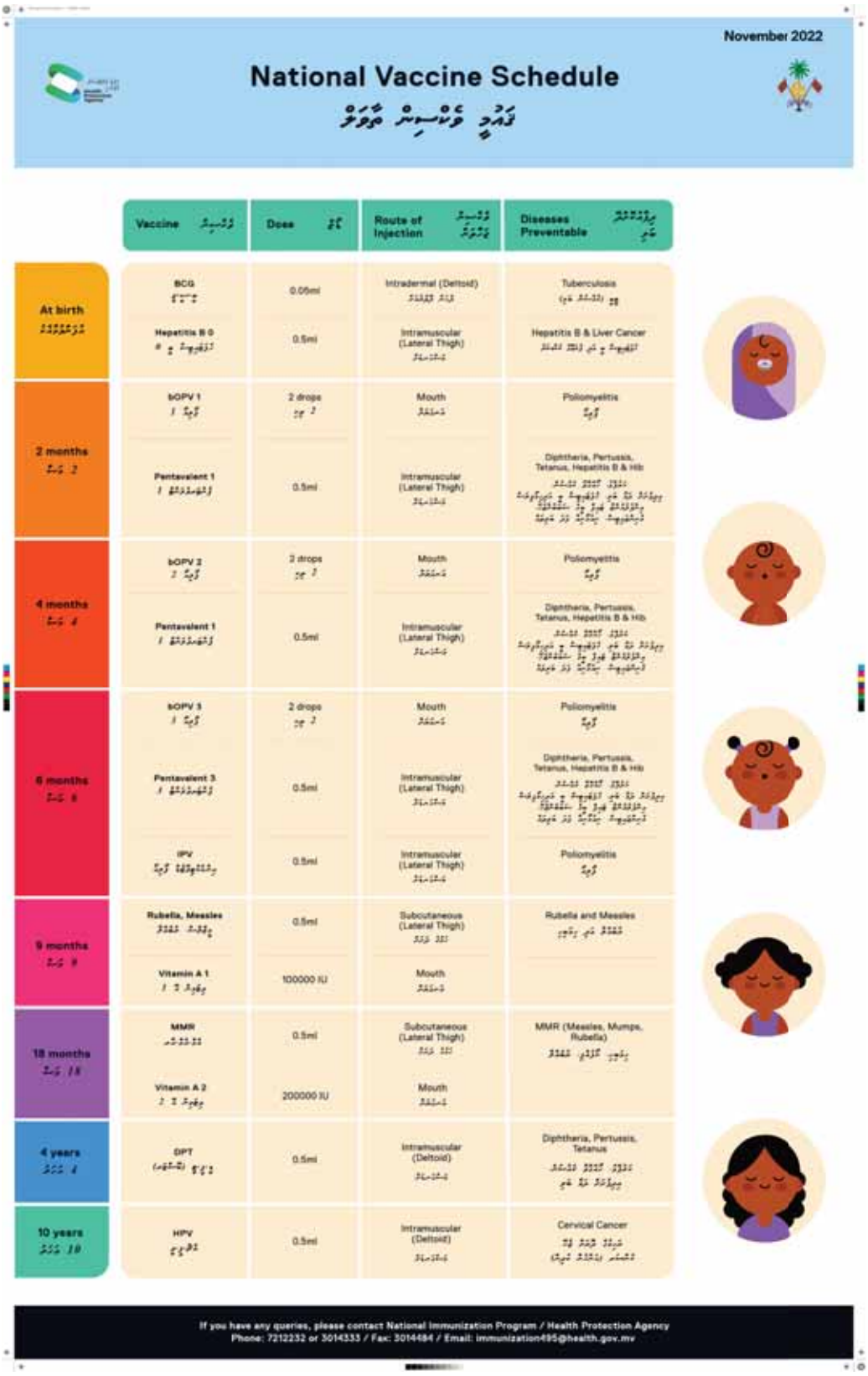
Hepatitis B serologic surveys: WHO has published technical guidelines to support countries to conduct nationally representative serologic surveys to evaluate the impact of hepatitis B immunization programs. The guidelines recommend testing children at least five years of age when they have passed through infancy and early childhood, when the risks of developing chronic hepatitis B is the highest. The children under 5 years of age can be assumed to have lower prevalence, if high HepB birth dose and HepB coverage has been maintained in cohorts born after the children included in the survey. The guidelines stipulate the width of the 95% confidence intervals to be narrower than 1% to precisely measure the national prevalence of chronic hepatitis B.

A school-based survey would be logistically efficient and provides a nationally representative sample in countries where primary school attendance rates are high. School-based hepatitis B serosurveys have been conducted in the large majority of Pacific Island countries in the WHO Western Pacific Region. In countries with small populations, all students in the selected grades were tested for HBsAg (i.e., a census). In other countries, schools were selected randomly and students in the selected schools were tested for hepatitis B. Maldives offers universal primary education since 2012 and has high primary school attendance rates (over 97% since 2014) (18). Conducting a school-based survey of Grade 1 students should result in a nationally representative sample.

1.4 ROUTINE IMMUNIZATION SCHEDULE: Since the launch of EPI in 1985, Maldives have achieved and maintained high immunization coverage of children less than 2 years of age. Figure 3 shows the history of immunization in the Maldives with Hep B vaccine introduced in 1993. The national immunization program of the Maldives includes 9 vaccines to be given to a child given to in the in the order depicted in table 2 to protect against certain diseases.

Figure 3: History of Immunization in the Maldives.

Figure 4: Routine Immunization schedule of the Maldives.



Source: Ministry of Health, 2023 (29)

In Maldives, routine immunization begins at birth, and includes vaccines against 17 diseases. Based on MDHS 2016-17 findings, 77% of children aged 12-23 months had received all the basic vaccinations in the National Immunization Schedule (14). In 2022, the status of immunization showed a high coverage of more than 95% for all the vaccines except HPV. Coverage of hepatitis B immunization was as high as 99% for the birth dose, as well as HepB3 (Pentavalent 3rd dose).

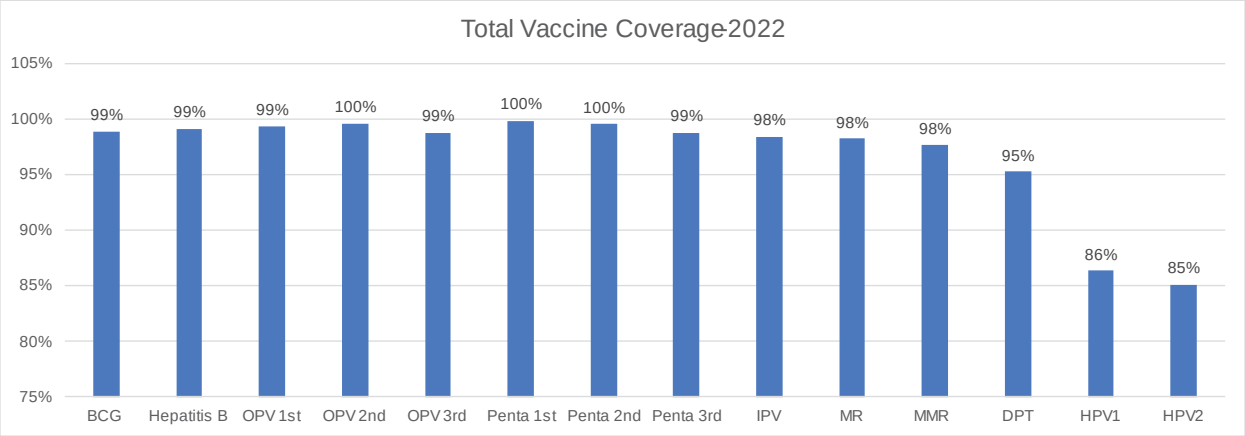


Figure 5: Immunization coverage in the Maldives 2022

Source: Health Protection Agency

Hepatitis B immunization: Hepatitis B vaccine, including the birth dose, was introduced nationwide in Maldives in 1993, and high immunization coverage has been sustained (Figure 5). The hepatitis B birth dose (HepB BD) coverage is ≥97% since 2001 and coverage of 3rd dose of Hepatitis B (HepB3) is over 96% since 2000. The HepB BD is given to all infants regardless of their mothers' hepatitis B status. The National Immunization Technical Advisory Group recommends the birth dose to be given within 24 hours, followed by 3 subsequent doses given at 2, 4 and 6 months of age. In 2013, combination DTP-HepB-Hib vaccine replaced the monovalent hepatitis B vaccine and DTP vaccine given at 2, 4, 6 months of age.

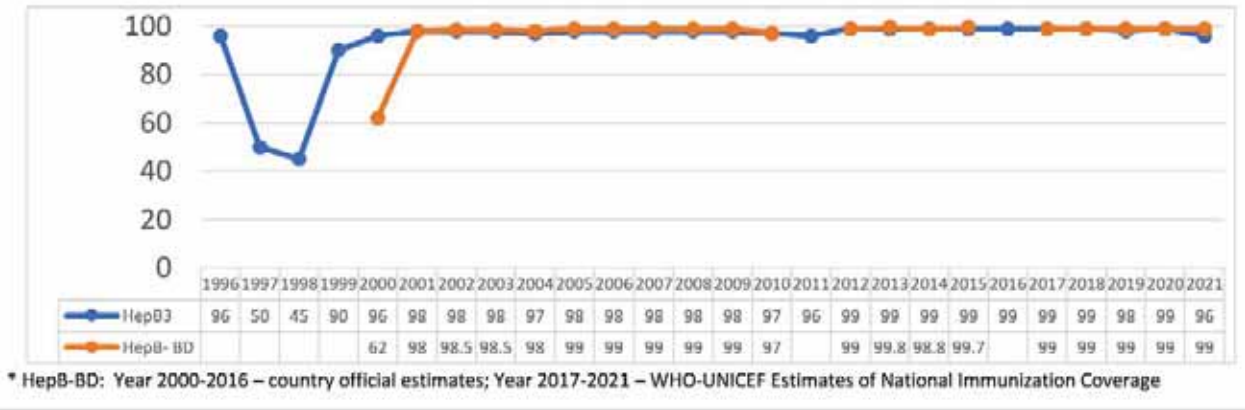


Figure 6: National immunization coverage for HepB BD and HepB3, 1996-2021 (24)

At present, rapid diagnostic test for HBsAg is available in the majority of the islands. Laboratory based immunoassay for HBsAg testing is available in tertiary hospitals in the capital and some regional hospitals. Additional hepatitis B serology such as HBe antigen testing, anti- HBe, anti- HBs and anti- HBc are available only in the tertiary facilities in the capital. Hepatitis B DNA viral load testing is available at Indira Gandhi Memorial Hospital, the main tertiary hospital in Maldives. Ultrasound scan facilities are available only in regional hospitals and some atoll hospitals in addition to hospitals in the capital (17). Fibroscan services were started in IGMH in 2021 with WHO support.

2. METHODOLOGY

2.1. SURVEY DESIGN

A national cross-sectional school-based cluster survey was conducted to estimate the prevalence of HBsAg among school children attending Grade 1 in Maldives. The basic demographic information and immunization history was collected for who consented to participate in the study. Children whose parents/legal guardians provided informed consent were tested for HBsAg. A pre-testing of the survey protocol was conducted in a school not selected into the sample prior to the national implementation of the survey. The same procedures described in the following sections were performed during the pre-testing. The purpose of the exercise was to test and validate the questionnaires and do a test run of the rapid diagnostic kits (RDTs) to be used. Since the pre- testing was done using focal points and laboratory focal points who conducted the survey, it gave them the opportunity to understand and familiarize themselves with the survey implementation. The exercise was also accompanied by intensive training on all the components of survey implementation.

Definitions of terms

- Chronic hepatitis B virus (HBV) infection: participants with a positive HBsAg test and a positive confirmatory test.

- Not currently infected with HBV: participants with a negative HBsAg test

Delimitations and limitations:

This study does not measure the antibodies to HBsAg, and therefore does not assess the immunogenicity of the hepatitis B vaccine. This study does not test or ask the mothers for their HBV infection status. Thus, it does not measure the rate of perinatal infection, or the effectiveness of the vaccine in preventing perinatal

infection. While the data generated could be ultimately cross tabulated to calculate vaccine efficacy, the survey will not have been statistically powered with that intention. As the school attendance rate is very high in the Maldives in the selected age group, the survey was focused on this population which may however present a negligent selection bias. According to the Census of 2022, the out of school rate for children of primary school age was 0.6% (10).

2.2. SAMPLE SIZE

The prevalence of HBsAg is expected to be 1%, and the desired precision of the estimate is to have the 95% confidence interval narrower than 1%. The confidence interval is calculated using the Wilson method with continuity correction and the formulas of the upper and lower limits of the confidence interval are (19):

$$\text{Upper limit} = \frac{2n\hat{p} + z^2_{1-\frac{\alpha}{2}} - \frac{1}{n}\left(2 + z^2_{1-\frac{\alpha}{2}}\right) + 1 + z_{1-\frac{\alpha}{2}}\sqrt{z^2_{1-\frac{\alpha}{2}} - \frac{1}{n} + 2 + \frac{1}{n} + 4\hat{p}(n\hat{q} - 1)}}{2\left(n + z^2_{1-\frac{\alpha}{2}}\right)}$$

$$\text{Lower limit} = \frac{2n\hat{p} + z^2_{1-\frac{\alpha}{2}} - \frac{1}{n}\left(2 + z^2_{1-\frac{\alpha}{2}}\right) - 1 - z_{1-\frac{\alpha}{2}}\sqrt{z^2_{1-\frac{\alpha}{2}} - \frac{1}{n} + 2 - \frac{1}{n} + 4\hat{p}(n\hat{q} + 1)}}{2\left(n + z^2_{1-\frac{\alpha}{2}}\right)}$$

where n was the effective sample size, and N is the total population. R software was used to calculate the minimum sample size that will give an estimate with the confidence intervals narrower than a set value (1% here). Based on the parameters considered:

− Prevalence $\hat{p}$=1%,

− 95% confidence interval as standard value, giving $Z_{1-\alpha/2}$=1.96,

− Upper limit - lower limit of the confidence interval=1%,

− N=9311 which is the number of Grade 1 students in 2022 (Ministry of Education1, 2022),the minimum required effective sample size is 1512.

− Minimum sample size=1512*design effect/ (1-response rate).

− Assuming a design effect of 1.3 and an overall response rate of 80% the sample size would be 2121.

− Applying differential response rate for the strata2, and rounding up to the next integer the minimal sample size required was 2523.

2.3. SAMPLING STRATEGY

A stratified single-stage cluster survey approach was used. The sampling frame is the list of the 219 schools that enrolled 9311 Grade 1 students in 2022-2023 in Maldives (25). The schools are stratified based on the islands on which they are located. Three strata were created: Greater Malé area, atoll centers and atoll others. Greater Malé consists of three islands: Male', Hulhumalé, and Villimalé. Atoll centers consist of islands where the atoll hospitals are located (which is the main population centre for the atoll), and 'atoll others' consist of all other inhabited islands. There are 20 schools in Greater Malé, 36 schools in atoll centers, and 163 schools in atoll others, but the number of students by school vary widely by stratum (18). Based on the average number of students in each school in Grade 1 in 2022 by strata, a sample size of 1140 is required for Greater Malé stratum, 465 in atoll centers stratum, and 918 in atoll others stratum is required (total sample size 2523). The number of the schools to be selected was allocated to each stratum proportionally to the number of students in Grade 1 within each stratum and rounded up to the next integer. Therefore, 78 schools were selected with a sampling fraction of 40% for Greater Malé (14 out of 20 schools), 35% for atoll centers (13 out of 36 schools), 35% for atoll others (57 out of 163 schools). Number of grade one students in each school was obtained from the Ministry of Education which totaled to 4286 students. As this exceeded the target sample size (2523), a random selection of schools were done while other schools were kept in reserve if there were low response rate from the randomly selected schools. Considering the expected low response rate in Greater Malé, as a contingency measure, 2 additional schools were selected in this stratum.

Recruitment of children: In order to ensure high participation rates among children in the schools, information about the purpose and methods of the survey were provided to school officials and families in advance of the survey. All students in the survey schools are selected so there were no additional selections within schools. Under the school health program based in the Ministry of Education, each school has school health focal points and health assistants who were part of the current research. Hence, school health focal points and health assistants facilitated the administrative work required from the school side. Schools have detailed records of all students.

The school focal points were trained before starting the survey by the focal point (who were trained in the central level training) in the island on the survey and they were part of the survey teams in their respective school. The school health assistant and/or a member of the senior management of the school was involved in administrative arrangements for the survey, where they organized sessions for parents, and made the screening arrangements, which were meant to maximize parents convenience and was held at the school as it would increase participation.

The school focal points along with Health Protection Agency team (in Greater Male' Region) or island public health staff (in islands outside GMA) conducted awareness sessions for parents/ guardians of prospective participants. The awareness sessions took place in the respective schools and each session was one hour long, followed by Q & A for parents/ guardians.

A leaflet containing information about the survey was given to all the parents/ guardians of children in the selected classes. The leaflet explained the purpose and methods of the survey (Annex 8). Parents/ guardians were provided the option to refuse to participate in the survey without penalty for any reason at any time before, during or after field activities. Informed consent was obtained from the parents/guardians of the participants by the school staff (who were already trained) prior to enrolling a child into the survey (Annex 5). The consent form was provided after the awareness session held in school. The school focal point coordinated and facilitated in obtaining the signed consent from parents/legal guardians and made arrangements to conduct the survey in their respective school. Parents not attending the awareness sessions were individually called. Using social media platforms to form "Class groups with parents" is very commonly practiced in the Maldives and each school has such Viber groups for following up with parents for many issues related to a child's education etc. The school focal points utilized these channels and coordinated the follow ups.

2.4. DATA COLLECTION

The survey was conducted by survey teams that have undergone training in data collection using the procedures outlined by this protocol. Each survey team had a laboratory technologist for sample collection and a trained public health staff for administration of the questionnaire. School health focal point from each school were also in the survey team to facilitate the administrative process. The survey teams were responsible for:

1. Obtaining informed consent from the parent/legal guardian for participating in the survey
2. Completing the questionnaire for each child by reviewing vaccination records (Which includes the immunization records.
3. Taking the blood sample for HBsAg test to perform the rapid test using whole blood from finger prick from each child whose parents/legal guardians have signed the consent form.

2.5 INSTRUMENTS AND MATERIALS

A survey tracking sheet (Form A in Annex 2) was used to list all eligible students in the selected class, document consent status, and if the interview and rapid test have been performed. A unique identifier was assigned to all eligible students and this code was used to link data collection forms. An immunization

history form was used to collect data on immunization history, gender, date of birth of all children in Grade 1 in the sampled schools (Form B in annex 3). Informed consent (Annex 5) was obtained from parents/legal guardians before the interview and the testing. This was done by each respective school health focal point after the parents/legal guardians awareness session has been completed. Parents/legal guardians were asked a few questions on key demographic characteristics of their children, the children's place of births, and history of blood transfusion during the survey, when the parent/legal guardian brings the child with them to school. The date and time were provided through school health focal point to all parents/legal guardians in the respective school. The results of the testing were recorded on the questionnaire once the reading of the rapid test is made (Form C in Annex 4). The questionnaire does not contain information on the name of the child or the parents/legal guardians. The questionnaire is only linked to identifiable information through a unique identifier that is kept confidential by the survey staff.

Data collection forms were pre-tested by persons unfamiliar with the survey prior to their use during the survey. Field testing the forms in this helped improve the quality of the data by identifying potential problems with the forms before data collection begins. The WHO pre-qualified rapid diagnostic test (RDT) Determine HbsAg2 manufactured by Abbott (formerly Alere) was used. According to the WHO prequalification report, the sensitivity for whole blood fingerstick samples is 97.2% (with 95% CI: 93.1-99.2) and specificity is 100% (with 95% CI: 98.2-100) (Annex 8). The test kit contains chase buffer, EDTA capillary tubes and sterilized single-use blood lancet. Other supplies provided to the survey teams included alcohol swabs for cleaning skin, disposable gloves, markers, safety boxes, biohazard bags, questionnaires, tracking sheets, leaflets, questionnaire, and consent forms, and back-up supplies. Each survey team received a package and a checklist of the supplies.

Site of survey administration and COVID-19 prevention methods: Data collection and blood sampling was conducted in the health facility or within a designated area of the school in the selected island. The parents/legal guardian had the option to be present at the time of sample collection if they wished to do so. Although the protocol contained measures to mitigate Covid-19 during the survey, by the time the survey was conducted, the rates of Covid-19 was very low and hence no Covid-19 measures for such events were in place for the community at the time.

2.6 BLOOD COLLECTION

2.6.1 Methods for collecting blood and testing

The surveyors (each team consisted of a registered laboratory technologist and a trained public health officer/nurse, school health focal point) were responsible for ensuring that the consent was obtained before the interview and taking the blood. 50 microliters of blood was collected by finger prick and tested via a

rapid test of HBsAg in the field using a simple test device (Determine® HBsAg from Abbott). The rapid test device was labelled with a unique identifier. Test results were visually interpreted at 30 minutes after adding the blood and buffer solution as directed by the manufacturer (Annex 8). The rapid test requires a drop of the buffer solution and a capillary tube, but otherwise no special laboratory equipment was required. The kit was considered simple and required minimal training, therefore it could be easily used by front-line health care workers. To ensure assay validity, a procedural control system was incorporated in the device. A red control bar appeared in the window. If the control bar does not appear by assay completion, the test result is invalid and the test was repeated using a new test strip. To interpret the test result, a second red bar in the test window and a red bar in the control window indicates a positive result; a red bar in the control window only indicates a negative result, and no bar in either window (or a red bar in only the test window) indicates an invalid test result. In the case of invalid results, the respondent was called for an appointment, on the same day or as soon as possible for counseling and informed consent for a second sample for repeat test. Survey staff were trained on how to perform and interpret the test results and record the result into the questionnaire. All medical waste and blood drawing supplies were appropriately disposed of in biohazard bags and sharps boxes, respectively.

2.6.2 Blood Processing, storage and transport

To protect the privacy of participants, data collected for the survey was made accessible only to survey staff and central survey team of the Health Protection Agency. Unique ID codes were used to identify study subjects in the database. All results were made available for the legal guardians after 01 month of the sample collection date provided in a neutral sealed envelope labelled, marked confidential (Annex 6). It should be collected by the legal guardian/parent from designated health facilities upon presenting the result collection slip (with a unique ID) and national identity card. The respondent were given 30 days to collect the result from the designated health facility. If the result is not collected within the timeframe of 30 days, the printed results were destroyed.

This timing was chosen so as to conduct the confirmatory testing at the Indira Gandhi Memorial Hospital or IGMH in Male' (ELISA) and if also positive, conduct the additional test for antibodies and IgM for core antigen (whole serology and viral load). The positive results were to be notified to the central survey team of the Health Protection Agency, Ministry of Health to facilitate additional testing for which Health Protection Agency would call the parent / legal guardian and coordinate with the designated trained personnel. However, no positive results were obtained in the survey.

When in routine screening a person gets positive for HBsAg using RDT, the results are reported to HPA. The person is offered confirmatory testing, and if outside Male', is given a choice to come to IGMH or to

collect sample at the island health facility and transport the sample to IGMH, depending on the patient's preference. Confirmatory testing is done using the Abbott ARCHITECT Immune Assay System. IGMH is – with WHO support - enrolled in EQA (twice a year) with the National Serology Reference Laboratory, Melbourne, Australia which maintains quality in serological testing, particularly for retroviral and other blood borne diseases and sets standards to provide accurate and cost-effective serological testing in screening, diagnostic and therapeutic programs. The multi-marker blood screening serology EQAS panel received for the blood bank is also used for general IQA.

All positive results were designed to be routed through HPA, as per the regular system for Hepatitis B positive screening results to reduce potential concerns and prevent stigmatization of children found to be HBsAg positive. All results were kept strictly confidential and were not be shared with any other community members, including schoolteachers or other health staff. Screening for HBV infection would be recommended for family members and household contacts of children confirmed positive for HBsAg and hepatitis B vaccination for those found to be susceptible. The survey team in each island referred the children found to have incomplete vaccinations to their local health facility to complete the vaccine series. The cases of incomplete vaccination were also informed to the national immunization program at HPA.

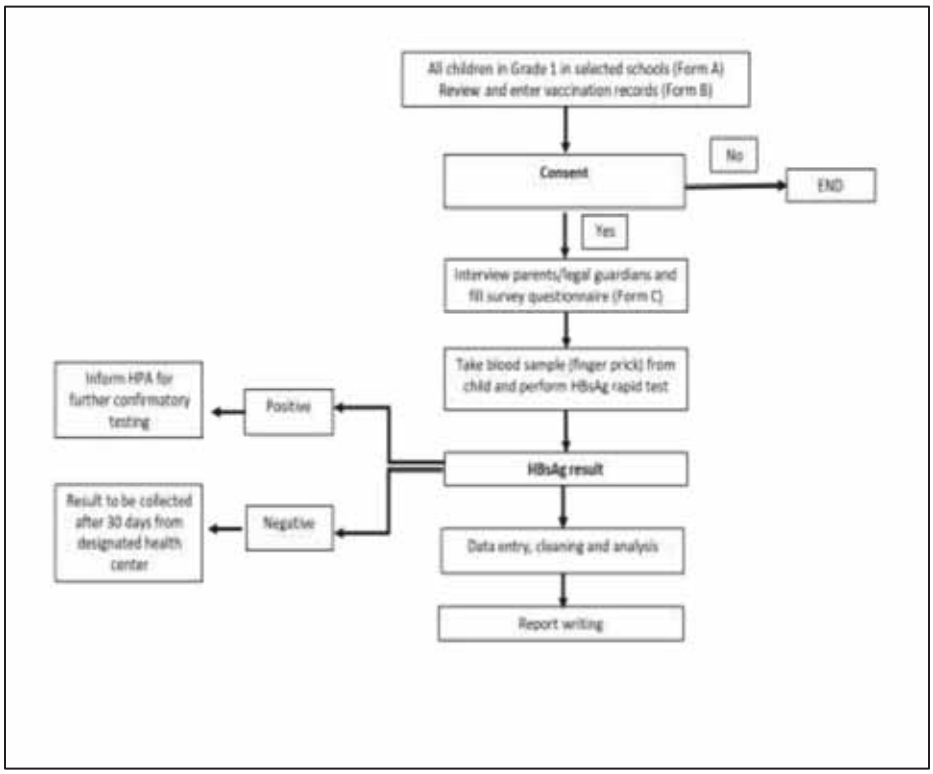


Figure 7: Flow of process

2.7 DATA ANALYSIS

Data was collected on standardized data collection forms. Survey teams reviewed the data collection forms prior to leaving the survey location to ensure that all required data elements have been collected and are recorded legibly. The name of the survey member completing the data collection forms was recorded on the forms to assist with monitoring of the quality of data collection. Data was entered into an excel spreadsheet. WHO provided technical assistance for data entry and to develop a data analysis plan. De-identified data or analytical codes were shared with WHO for cross-checking of the data analysis procedures. All data were analyzed considering the study design. Data was cleaned by a team of members from the HPA and the analytical team. Data was then imported into SPSS for statistical analysis. The data analytical framework is attached in Annex 7. Missing values in any variable have not been deleted or imputed. It has been presented as a missing value where applicable in all the analysis. Missing values did not exceed 3% of the responses.

2.7.1 Descriptive analysis

- Description of the sample of participants (by age, gender, grade, location, Country of birth, Place of birth, History of blood transfusion
- Prevalence of HBsAg
- To evaluate the timeliness of vaccination and completeness of vaccination, the following analysis were conducted:
    - the series completion rate at 12, 18, 24 months
    - Proportion of timely receipt (%)
    - Proportion of delayed receipt (%)
    - Proportion of no vaccination (%)

2.7.2 Analytical epidemiology

A number of cross-tabulations were attempted based on results of the descriptive analysis, sample size and interest of the study investigators. Key issues include analysis of the Immunization status markers by Birth dose status/timing.

Timely birth dose is defined as a hepatitis B vaccine dose given within 24 hours of birth. If the time of birth or time of the birth dose is unknown and we define day 0 as the day of birth, all Hepatitis B dose given on days 0 and 1 can be considered as timely birth dose.

Hepatitis B vaccination status was analyzed in frequency and percentages. To assess vaccine effectiveness timeliness and the series completion rate of the hepatitis B vaccine was calculated.

2.8 DATA PROTECTION AND ETHICAL CONSIDERATIONS

2.8.1 Surveyors: The surveyors attended a training provided by the central task force and participate in the pretest under the supervision of the central task force before the field work starts. For each atoll 2 staff were trained at the central level; a laboratory technician and public health focal point from the respective atoll. Trained staff after returning to their duty station oriented all relevant health workers / school staff/ community members in the island selected for sample collection in the survey implementation.

The content of training focused on:

- Basic information on hepatitis B and vaccination
- Informed consent and ethics
- Data collection forms
- Performing rapid test
- Waste disposal (according to health care waste management guideline)

2.8.2 Quality assurance: The surveyors were provided a manual with instructions on how to carry out the survey activities and reported to the central team regularly. They were obligated to make immediate reports on any adverse events, or deviations from the protocol and plans. The central task force provided supervision throughout the survey implementation phase, including conducing spot checks. Each health facility has numerous doctors, nurses, lab technicians and support staff. During the period of survey implementation, they continued to offer regular services to the catchment population. No disruption of services occurred during the planning or implementation of the survey

2.8.3 Supervision and Monitoring: Three central level supervisors were identified and assigned to supervise and monitor the survey planning and implementation in each Region (6 regions total). The 6 regional sites the central team consisted of almost 25% of the sample size. In addition, each Atoll level public health focal point were made responsible to supervise and monitor the seroprevalence survey in island selected in the catchment area. Similarly, chief of the island health center supervised the operations at each selected island. All supervisors were trained on the monitoring of the survey.

Supervision checklists were developed and used to record the observations. The supervision plan included confirmatory reading of a certain percentage (e.g. 10%) of RDTs at field sites by supervisors and respective documentation. A respective checklist was made available.

The survey was conducted in accordance with WHO and the Maldives ethical guidelines on research involving human subjects. Prior to initiation of the survey, the protocol underwent ethical review by WHO

and the Ministry of Health / Maldives which subscribes to the National Health Research Council as the country's ethics review body.

Protection of human subjects were based upon (1) minimizing risks, (2) maximizing benefits, (3) ensuring confidentiality and (4) collecting informed consent. The survey included children who were by definition a vulnerable population. However, this is justified as children are the primary target population of the immunization programs and the WHO recommendation for Hepatitis B sero-surveys is that it is conducted in children.

Risks/inconvenience: Risks and inconvenience for the participants in the survey included the time spent (about 5 minutes per child) and the discomfort associated with finger prick. Safe, single-use devices were used in compliance with standard precautions guidelines to prevent infections.

Only approximately 50 microliters amount of blood was collected from finger-prick. As a precautionary measure, participants were observed for 10 minutes after the finger prick for any adverse events.

2.8.4 Benefits of the survey:. Participants who were found to be positive for HBsAg would benefit from knowing their status. HBsAg-positive children (and their families) would be counseled about blood-borne pathogen transmission and precautions to prevent transmission among household members and close contacts. Family contacts would be screened and un-infected, unvaccinated family members of HBsAg-positive children would be recommended to receive the hepatitis B vaccine at their own cost. Maldives can assure that the Hepatitis B vaccination program as well as related HBV infection control measures have been successful.

2.8.5 Confidentiality: To protect the privacy of participants, data collected for the survey were made available only to survey staff and relevant programs of the Health Protection Agency. All data collection forms were stored in locked file cabinets. Names were not entered into the computerized database. Unique ID codes were used to identify study subjects in the database. All identifiable information were kept under lock and key by the primary investigator. Identifying links were destroyed after the completion of the survey.

c. Biologic specimens: Blood was collected only for the rapid test. They were tested as specified above. No additional testing were conducted and the used test devices were discarded according to the national health care waste management guidelines.

2.8.6 Informed consent: To provide the parents/legal guardians of potential survey participants with information to make an informed decision about whether to permit their child to participate, an information leaflet and a consent form were provided to the parents/legal guardians of each sampled child who is eligible

to participate. The leaflet and the consent form described the purpose of the survey and the methods that were used to conduct the survey. The consent forms were filled out for each child which parents/legal guardians had to sign if they wish to have their child participate in the survey. The consent forms were made available in local language and back-translated for quality assurance. Children, or their parents/legal guardians, were provided the option to elect to withdraw from the survey without penalty for any reason at any time before, during or after field activities.

3. RESULTS

3.1 DEMOGRAPHIC CHARACTERISTICS OF PARTICIPANTS

This analysis presents data from a total of 2074 participants from 50 schools stratified by Greater Male' Region, Atoll Centers and Atoll Others. Table 2 shows the response levels by the stratified samples (HPA: to fill the response rate table below).

Demographic characteristics of the sample indicates that it is representative of all the 21 Atolls of the country. The gender representation of the participants were very similar with 49% females vs 51% males. Ninety six percent of the sample were Maldivians and among those delivered in the Maldives, 99% were delivered at a health facility. Ninety eight percent of the children did not have any history of blood transfusion which may have put them at risk of acquiring Hepatitis (Table 3).

Table 2: Response rate

Strata	**Number of Schools**	**Sample size required**	**Number responded**	**Non response %**
GMR	12			
Atoll Centers	12			
Atoll Others	24			
Total	**48**			

Table 3: Characteristics of the participants by Age Cohorts

	Cohort 2013		Cohort 2014		Cohort 2015		Cohort 2016		Total	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Gender										
*NA**	0	0%	0	0%	4	0%	5	1%	9	0%
Female	0	0%	1	25%	659	48%	355	52%	1015	**49%**
Male	1	100%	3	75%	717	52%	329	48%	1050	**51%**
By Birth Country										
Maldives	1	100%	4	100%	1302	96%	648	95%	1955	**96%**
Sri Lanka	0	0%	0	0%	22	2%	15	2%	37	2%
Thailand	0	0%	0	0%	6	0%	5	1%	11	1%
India	0	0%	0	0%	21	2%	7	1%	28	1%
Malaysia	0	0%	0	0%	3	0%	4	1%	7	0%
Other	0	0%	0	0%	4	0%	2	0%	6	0%
Do not know	0	0%	0	0%	1	0%	1	0%	2	0%
Place of Birth										
Island Health Center	0	0%	1	25%	19	1%	7	1%	27	1%
Atoll Hospital	1	100%	0	0%	394	29%	203	30%	598	**29%**
Male' Hospital	0	0%	3	75%	855	63%	422	63%	1280	**63%**
Home	0	0%	0	0%	11	1%	4	1%	15	1%
Other	0	0%	0	0%	76	6%	33	5%	109	5%
Do not know	0	0%	0	0%	1	0%	1	0%	2	0%
Blood transfusion History										
Yes	0	0%	0	0%	27	2%	14	2%	41	2%
No	1	100%	3	75%	1332	98%	665	98%	2001	**98%**
Do not know	0	0%	1	25%	5	0%	2	0%	8	0%
By Location (Atoll)										
Alif Alif	0	0%	0	0%	7	1%	3	0%	10	0%
Alif Dhaalu	0	0%	0	0%	75	5%	42	6%	117	6%
Baa	0	0%	0	0%	63	5%	30	4%	93	4%
Dhaalu	0	0%	0	0%	24	2%	9	1%	33	2%
Faafu	0	0%	0	0%	54	4%	23	3%	77	4%
Gaafu Alif	0	0%	0	0%	15	1%	1	0%	16	1%
Gaafu Dhaalu	0	0%	0	0%	26	2%	11	2%	37	2%
GMR	0	0%	3	75%	626	45%	304	44%	933	45%
Gnaviyani	0	0%	0	0%	12	1%	2	0%	14	1%
Haa Alif	0	0%	0	0%	26	2%	18	3%	44	2%
Haa Dhaalu	0	0%	0	0%	90	7%	43	6%	133	6%
Kaafu	0	0%	0	0%	50	4%	37	5%	87	4%
Laamu	1	100%	0	0%	44	3%	13	2%	58	3%
Lhaviyani	0	0%	0	0%	31	2%	21	3%	52	3%
Meemu	0	0%	0	0%	12	1%	9	1%	21	1%

Noonu	0	0%	0	0%	23	2%	12	2%	35	2%
Raa	0	0%	0	0%	7	1%	7	1%	14	1%
Seenu	0	0%	1	25%	72	5%	41	6%	114	5%
Shaviyani	0	0%	0	0%	65	5%	39	6%	104	5%
Thaa	0	0%	0	0%	36	3%	14	2%	50	2%
Vaavu	0	0%	0	0%	22	2%	10	1%	32	2%

*Data not available or Missing values

3.2 IMMUNIZATION HISTORY AND COVERAGE

More than 95% of the participants had in their possession their immunization record (table 4) and more than 94% of the children had completed the vaccine schedule (table 5). Immunization coverage for separate vaccines in figure 8 showed that overall, the coverage for all the vaccines in the schedule to be above 96%. It ranged from 97% for BCG and for OPV 2, 4 and 6-month doses, 96.3% for hepatitis B birth dose, 97.4% for DTP-Pentavalent 2-, 4- and 6-months dosages and 96% for measles and MMR .

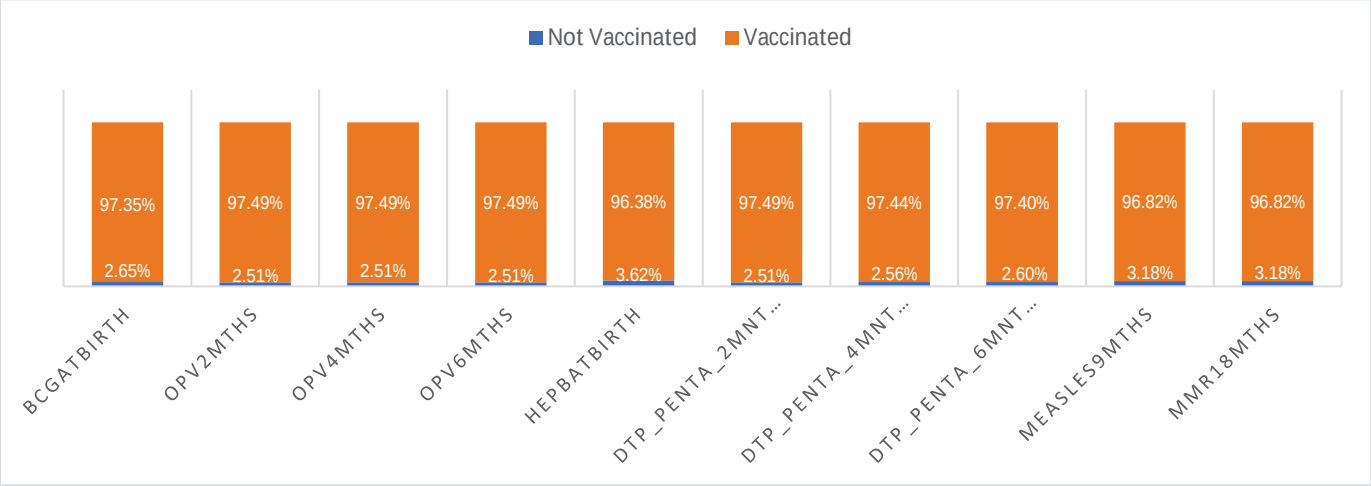
Table 4: Possession of Immunization card for review

	N	**%**
Has immunization record at school	372	**17.9**
Has immunization record at a health center	92	**4.4**
Has immunization card brought by parents	1522	**73.4**
Has immunization record from other sources	5	**.2**
Doesn't have any immunization record	42	2.0
Missing	41	2.0
Total	**2074**	**100.0**

Table 5: Series Completion Rate

	N	**%**
Yes	1958	**94.4**
No	23	1.1
Don't Know	21	1.0
Missing	72	3.5
Total	**2074**	**100.0**

Figure 8: Immunization Coverage



Assumptions: A Date = Vaccinated, 09/09/999 = Not vaccinated

3.3 HEPATITIS B IMMUNIZATION

More than 93% have received all the four doses of the hepatitis B vaccine in the sample (table 6). Only 0.4% of the participants had missed the Hepatitis B birth dose, and 4.1% missed any of Pentavalent 1,2, or 3, or Hepatitis B1, 2, or 3 doses (Table 7). Among those who missed the dosages, when asked for the reason for missing the vaccination, 4.1% did not respond and 0.3% explained that the baby was delivered in a foreign country which did not offer the vaccine (table 8)., Responses by parental recall showed that more than 92.7% (N=343) of the children received the hepatitis B vaccine at birth, 90% (n=362) received additional doses of the hepatitis B vaccine, 45% (N=842) has received the vaccine on the day of birth or the next day, but only 32% (N=822) has received all the four doses of the vaccine (table 9).

Table 6: Number of Hepatitis B doses a child received, including the birth dose and the hepatitis B vaccine in Pentavalent (comes from B6 in questionnaire)

Number of Hep B doses	**N**	**%**
0	1	.0
1	8	.4
2	2	.1
3	59	2.8
4	1938	**93.4**
Don't know	27	1.3
Missing	39	1.9
Total	**2074**	**100.0**

Table 7: Has the child missed any of the 4 doses of hepatitis B vaccines, including the pentavalent vaccine? Comes from C14 in the questionnaire

	N	%
Missed HepB BD	9	.4
Missed any of Penta1,2, or 3, or HepB1, 2, or 3	84	4.1
Unknown	31	1.5
NA	1981	94.0
Total	**2074**	**100.0**

Table 8: Reasons for missing any of the doses of Hep B vaccine

	N	%
Born in a foreign country, vaccine not offered	6	**0.3%**
No answer	85	**4.1%**
NA*	1983	95.6%
Total	**2074**	**100.0%**

* Data not available or Missing values

Table 9: Hepatitis B Immunization status by parental recall

	By parental Recall
Did the child receive the hepatitis B vaccine at birth? (N=343)	
Yes	92.71%
No	2.33%
Not Sure	4.96%
Other than the birth dose, did the child receive additional doses of hepatitis B vaccine, or the combination vaccine that prevents diphtheria, tetanus, whooping cough, Haemophilus influenzae type b, and hepatitis B (N=362)	
Yes	90%
No	3%
Not Sure	7%
How many hours or days after birth did the child receive the first dose? (n=842)	
On the day of Birth	37.53%

Next day after birth	7.60%
More than 1 day after birth	49.76%
Don't remember	5.11%
Not counting the birth dose, how many doses of hepatitis B vaccine or pentavalent vaccine did the child receive parental recall (n=822)	
0	37.71%
2	0.36%
3	25.06%
4	32.60%
8	0%
9	0%
Don't know	4.01%

Figure 9: Timeliness of Hepatitis B Birth dosage

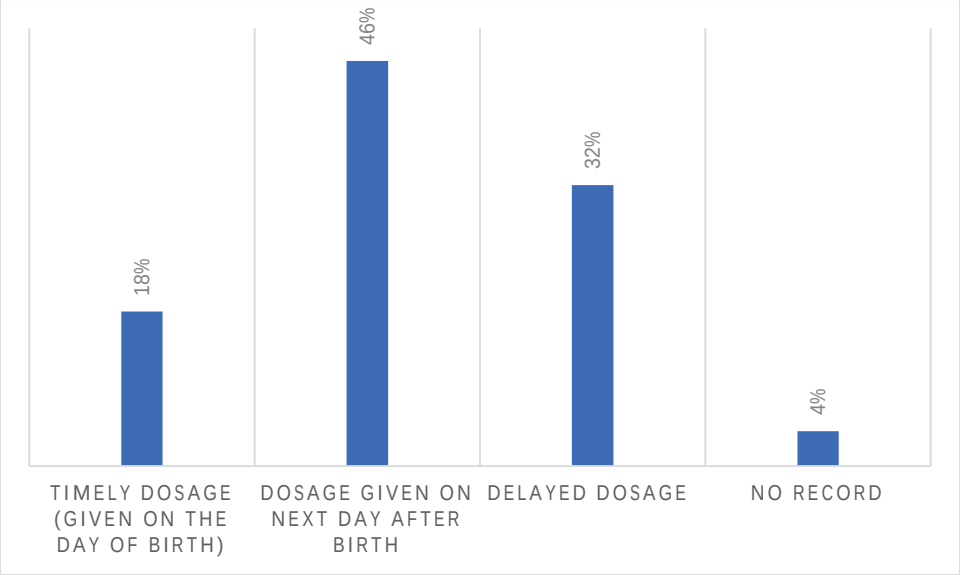


Figure 9 shows the timeliness of the hepatitis B birth dose among the participants. 18% received the birth dose on the day of the birth, while 46% received it the next day after birth. A third of the participants received the birth dose more than two days after birth.

3.4 PREVALENCE OF HELATITIS B SURFACE ANTIGEN

While 8 participants refused to take the test, the seroprevalence of Hepatitis B surface antigen was 0 among this sample of children (tables 10 and 11). Reasons for refusing the test was because the child was not corporative and the child being absent for the study period.

Table 10: Seroprevalence of Hepatitis B surface antigen

Child's HBsAg status	N	%	95% CI
Positive	0	0	-
Negative	2066	99.6	0.992 - 0.998
Invalid	0	0	-
Test not performed	8	.4	0.002 - 0.008
Total	**2074**	**100.0**	**-**

Table 11: Seroprevalence of Hepatitis B surface antigen by Age cohort

Child's HBsAg status	2013		2014		2015		2016		Total	
	N	%	N	%	N	%	N	%	N	%
Positive	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Negative	1	100.0%	4	100.0%	1373	99.5%	688	99.9%	2066	99.6%
Invalid	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Test not performed	0	0.0%	0	0.0%	7	0.5%	1	0.1%	8	0.4%
Total	**1**	**100.0%**	**4**	**100.0%**	**1380**	**100.0%**	**689**	**100.0%**	**2074**	**100.0%**

3.5 ANALYSIS OF REFUSALS

There were 8 participants who refused to take the HBsAg test and characteristics of the refusals and vaccine coverage among the refusals were analyzed. Tables 13 and 14 shows that there were no gender difference in the children, and all were Maldivians who were delivered at a health facility in the Maldives. None of the refusals had a history of blood transfusion and all of them had in their possession the immunization record and all of them had received all the four doses of hepatitis B vaccine. Vaccine completion rate among the refusals was 100% for all the vaccines in the schedule.

Table 12: Characteristics of Refusals

	N	%
By Gender		
Male	4	50%
Female	4	50%
By Age cohort		
Born in 2015	7	88%
Born in2016	1	13%
Country of Birth		
Maldives	8	100%
Place of Birth		
Male' Hospital	6	75%

Atoll Hospital	2	25%
By Location (Atoll)		
Alif Alif	1	13%
Noonu	1	13%
Faafu	1	13%
GMR	3	38%
Baa	1	13%
Dhaalu	1	13%
Blood Transfusion History		
No	8	100%
Have Immunization card		
Has immunization card brought by parents	7	88%
Has immunization record at a health center	1	13%
Number of Hep B doses including Birth dose		
4	8	100%

Table 13: Vaccine Coverage among Refusals

Received Vaccines	**N**	**%**
Hepatitis B Birth dose	8	100
BCG Birth Dose	8	100
OPV 2months	8	100
OPV 4months	8	100
OPV 6 months	8	100
Pentavalent 2 months	8	100
Pentavalent 4 months	8	100
Pentavalent 6 months	8	100
Measles 9 months	8	100
MMR 8months	8	100

4. DISCUSSION

There were two main objectives to this study; One was to measure the prevalence of HBsAg among grade one school children and the other was to evaluate the vaccination coverage for Hepatitis B. Maldives achieved universal immunization status in 1989, and to maintain these high rates, the Ministry of Education has made immunization an essential requirement for entry into government schools (28). As the Maldives offers universal education and enjoys near to 100% enrollment rates at primary level education, this study has used a nationally representative sample of 2074 school children in grade one. Study findings concludes that the seroprevalence of Hepatitis B surface antigen was 0.00% and overall immunization coverage for all the vaccines in the schedule was found to be above 96%. Immunization coverage for hepatitis B birth dose was 96.3% and 97.4% for all the three doses of DTP-Pentavalent 2-, 4- and 6-months dosages. More than 93% have received all the four doses of the hepatitis B vaccine and a negligible 0.4% of the participants had missed the Hepatitis B birth dose, while 4.1% missed any of Pentavalent 1,2, or 3, or Hepatitis B1, 2, or 3 doses. This study has identified that missed birth doses were due to children been delivered at overseas health facilities in foreign countries where the hepatitis B vaccine was not offered.

The Demographic characteristics of the study sample reveals that it is representative of all the twenty one Atolls across the country. The gender representation in the survey was also similar to that of the country's gender ratio which is 104 males to 100 females. The high rates of birth deliveries at a health facility demonstrated in the national statistics of Maldives is also depicted in the findings of this study where ninety three percent of the children were delivered at a health facility which gives them access to the necessary vaccinations.

Findings from this study are in alignment with many of the national and global health statistics for the Maldives as well as with findings from national health surveys. Two decades ago, immunization coverage of Hepatitis B in 2001 was reported at 98% and since then it has slightly fluctuated from 97% in 2010 (27) to 99% in 2020 (11). The Maldives Demographic Health survey of 2009 and 2016-2017 were the first surveys that reported immunization coverage for Pentavalent vaccine (DTP+HEP B+ HIB). In 2009, the coverage in the first 12 months of a child for Hepatitis B was 98.7%, 97.5% and 91.9% compared to 2016-17 which reported 91%, 88% and 85% for the first, second and third dose respectively. Findings from this study on the unvaccinated proportions (2.6 to 3.6% across all vaccines) differ from that of the latest Demographic Health survey which reported eight percent of children age 12-23 months had not received any vaccinations in 2016-17. The declining trend in non-vaccination gives evidence of the public confidence in the Maldivian government's vaccination program and hence sustain the zero-prevalence status of hepatitis B in the country. An analysis of 2 years data at the main tertiary hospital of the Maldives

has shown that out of a total of 466 children below the age of 5 years tested, only 1 child was positive for HBV infection (17). Vaccine series completion rate was found to be very high. Over 94% of participants had completed the entire recommended hepatitis B vaccination schedule and Individual vaccine coverage for all antigens in the schedule exceeded 96%. And even the few parents who refused to take the Hep B surface antigen test proved to have completed all the vaccines in the schedule. This suggests the successful implementation of the national vaccination program, potentially contributing to a decline in hepatitis B burden.

Comparison of immunization coverage rates of four doses of hepatitis B vaccine and the prevalence rate of hepatitis B in the Maldives versus the global and regional statistics show that Maldives outperform the global and regional rates. While the coverage for four doses of Hepatitis B vaccine is estimated at 84% globally and 75% in the Southeast Asia Region (26), this study has demonstrated that the coverage for four doses of Hepatitis B vaccine in the Maldives at 97%. In addition, the prevalence rate of Hepatitis B in the SEAR region was above 8% in three countries, 2 – 7% in four countries and 2% in the other four countries (26). Findings of this study has highlighted that the Maldives has surpassed the global and regional averages for the prevalence of hepatitis B surface antigen (HBsAg) positivity rate at 0.00%. The findings provide compelling evidence for the success of the national immunization program in preventing Hepatitis B infection.

Strengths, Potential Biases and Mitigation Measures

It was not a census of grade one students which may leave out some at risk participants.

In addition, the study period saw a change in the start date of the academic year by the government of Maldives from January each year to June each year. However, when the second half of the study commenced after the start of the new academic year, the same cohort of children, who were by then in Grade 2, were screened.

The survey was conducted using a paper-based data collection tools which may have contributed to few errors which has been addressed through close supervision at island level and central level. The combined efforts of the data entry team and cleaning team together with HPA helped in identifying any errors present in the data as well as to manage missing values in the dataset as well.

With regard to response rate, there was high non-response rate in the Greater Male' region which was expected in the design stage, due to recent experiences with health surveys in the area. Hence, two additional schools were added to the list of schools in the GMR region. Even with this addition, the response rate in GMR remained 50.6% while in atolls it was 89%, giving an overall response rate of 66.3%.

With such a low response rate in one strata, there is a concern that non-responders were different to responders in terms of vaccination status. To address this, vaccination rates for this cohort was analyzed using data from school health screening (30).

School Health Screening is a program conducted by the Ministry of Education since 2013 From 2018, the integrated School Health Screening Program was started where screenings are conducted every year for students in Lower Kindergarten (LKG), Grade 1, Grade 4 and Grade 7. All students in these grades are invited for health screening.

Various assessments are conducted in the screening as appropriate for age, including assessment of hearing, vision, dental issues, etc. For some ages, blood tests and screening for mental health conditions are also performed. As part of the school health screening, vaccination status is also recorded, and children with missed doses are referred for catch-up vaccination. Vaccination status is recorded by physically checking the vaccine card. Parents are asked to bring the vaccine card when they accompany the child for the health screening appointment.

From among the 14 schools selected for the survey in Greater Male' Region, data of health screening for 11 schools were obtained from Ministry of Education. The data (Annex 10) shows that health screening has a high response rate in most schools, with over 80% coverage in 9 of the 11 schools reported. In all the schools, the vaccine coverage among those who attended the school health screening is calculated. Those who did not bring the vaccine card at the time of the screening are recorded as having missing vaccination information, and was not counted as vaccinated, even though the parent may inform that all vaccines have been completed.

For the schools where the health screening data is provided, it can be seen that the vaccination coverage for Hepatitis B birth dose and Pentavalent 3 dose is above 95%. In the single school where the coverage is below 98%, it is due to more students with missing data as they did not bring vaccine card at the time of health screening. The birth dose coverage is slightly lower than that of Penta3 coverage in 2 of the schools. This is seen when some children are born abroad, in countries where the Hepatitis B birth dose is not given. They may have been cases where the family resides in Maldives but went abroad for delivery and came back after, or those who have been residing abroad at the time of birth. For these cases, catch-up vaccination cannot be done, as by the time they arrive in the country after the birth, the time window for administering the birth dose would have passed. These children would go on to complete 3 additional doses at 2,4 and 6 months. In the data from the 11 schools, 2 students did not have Hepatitis B birth dose, while having completed all 3 pentavalent doses.

The health screening data is for the same cohort that participated in the sero-survey. As the data from the health screening shows a higher participation rate than the sero-survey, and a high vaccination coverage of Hepatitis B birth dose and Penta3, even though the response rate in some schools in GMA was low, we show that this is not due to a difference in the immunization status, and that there is no difference in the immunization status between those who participated in the survey and those who did not.

This survey with its robust sampling design, and the comprehensive collection of immunization data throughout the country makes it the first study of its kind which has enabled an estimation of the prevalence of Hepatitis B in the Maldives for any group. While the study result does not allow for a direct calculation of vaccine effectiveness, the observed findings strongly support the effectiveness of the HBV vaccine in preventing chronic infection. The high immunization coverage and lack of HBsAg-positive cases suggest that the vaccine is effectively providing protection.

5. CONCLUSIONS AND POLICY DIRECTIVES

Findings from this study has established evidence of the success of the Maldivian government's continued investment in the universal immunization program for its people since 1960. Specifically, the findings demonstrate the strength of the Hepatitis B vaccination program introduced three decades ago in 1993 in preventing hepatitis B among the Maldivian population.

The most notable findings of this study were that no participants tested positive for hepatitis B surface antigen (HBsAg - 0%), indicating a remarkable zero prevalence among all age cohorts of grade one students, an outstandingly high immunization coverage which is comparatively way above the global or regional estimates, a very high series completion rate, a high rate of immunization card retention, minimal missed doses, and unlike many other countries where there is decline in the coverage for subsequent doses of a vaccine, the coverage for all the four doses of hepatitis B was found to be stable at ninety seven percent in the Maldives which is commendable. With less than 0.1% HBsAg prevalence among children, findings of this study gives evidence of the Maldives' achievement of the GHSS target of eliminating viral hepatitis as a public health threat by 2030 and the target set by the South-East Asia Regional Immunization Technical Advisory Group as well.

Areas for improvement includes the need for targeted interventions to improve adherence, combat vaccine hesitancy, address missed doses, the need to educate new mothers about birth doses especially if they may deliver out of the Maldives and timeliness of all vaccinations can be improved by raising awareness among frontline health care personnel and parents alike. Effectiveness of the EPI program needs to be measured

through annual surveys among different sub populations in the country and success stories such as that of the extraordinary universal immunization program of Maldives needs to be documented and advocated.

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7. ANNEXES

ANNEX 1- Information leaflet

Leaflet to be given to officials and parents of children in selected schools

The National Expanded Program for Immunization, the Ministry of Health is doing a survey about hepatitis B among children to evaluate the impact of national hepatitis B vaccination program on the disease. Hepatitis B is caused by a virus that attacks the liver. Hepatitis B vaccine can prevent this infection. Hepatitis B vaccine has been recommended for all children in the Maldives starting in 1993 with the first dose given at birth.

We are doing this survey to see if children are protected against hepatitis B. This survey will help us evaluate the National Immunization Program and to help the Ministry of Health to improve it further. Similar surveys have been conducted in over 15 countries in the world.

We are asking the parents of Grade 1 students to allow us to test your child's blood to see if your child has been infected with hepatitis B.

Any information about your child's vaccination status and the results of testing will be kept strictly confidential. If the test shows that your child has been infected with the hepatitis B virus we will inform you at the end of the survey. We will only share the results of your child's blood test with you and the relevant programs of the Ministry of Health to help you with follow-up testing and care if your child is positive for hepatitis B.

It will take about 5 minutes to obtain the small amount of blood from your child's finger. Please note that the results of blood testing will be kept strictly confidential.

The benefit of knowing if your child has hepatitis B infection mainly has to do with your child's health in the future. Many people with chronic hepatitis B remain well and may not have any symptoms of being sick. However, some may develop serious liver problems later in life and being aware of symptoms and monitoring health can benefit you child in the future.

In addition, since hepatitis B can be passed to household members, these members can also be screened, and if they are not already protected – they can be vaccinated to protect them from infection.

Your decision for your child to participate is entirely voluntary. There is no penalty for declining to participate, and you can decline without having to provide a reason.

If you have any questions about the survey, please contact XX at XXXX.

Annex 2 – Form A. Tracking sheet

Form A. Hepatitis B survey tracking sheet

Atoll: Island:
School: School health focal person:
Grade: 1 Class: Number of students in class:
Please list all students in the selected class.

Response status:
1. Consented
2. Refused
3. Child absent
4. Parent absent
5. Parent not reached
9. Others

Student's Name	Gender	Date of birth (DD/MM/YYYY)	School code	Class code	Child code	Vaccination record found?	Response status	Interviewed?	Rapid test performed?	Notes
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	
	F M	__/__/____				Yes No	1 2 3 4 5 9	Yes No	Yes No	

Total number of students:_______ ___ with vaccination records ___ consented ___ interviewed ___ tested

Date of survey: Surveyor: Supervisor:

Page __ of __

Annex 3 – Form B. Immunization history

Form B. Immunization history

B1	**School code: __ __ __ Class code: __ __ Child code: __ __ __**		
B2	Gender of the child	Female Male Don't know	1 2 9
B3	Child's date of birth Time of birth (if available)	__ __/__ __/__ __ __ __ D D / M M / Y Y Y Y Source: Immunization record (preferred) Other	 1 2
B4	Does the child have immunization card/record for review? Select the one from which you took the immunization data for B5 and take a picture with name covered by codes.	Has immunization record at school Has immunization record at a health center Has immunization card brought by parents Has immunization record from other sources (please specify_______________________________) Doesn't have any immunization record	1 2 3 4 5
B5	Vaccination history *(Format: DD/MM/YYYY)*		

Vaccines	At birth	2 months	4 months	6 months	9 months	18 months
BCG	DD/MM/YYYY					
Hepatitis B	DD/MM/YYYY					
OPV	DD/MM/YYYY	DD/MM/YYYY	DD/MM/YYYY	DD/MM/YYYY		
Pentavalent		DD/MM/YYYY	DD/MM/YYYY	DD/MM/YYYY		
Measles					DD/MM/YYYY	
MMR						DD/MM/YYYY

(a) Copy dates for each vaccination from the card.
(b)Write '01/01/0101' if card shows that vaccination was given but no date recorded.
(c)For each vaccination not recorded write '09/09/9999'.
(d)If there are other immunization doses given, please specify here:

B6	How many hepatitis B doses did the child received, including the birth dose and the hepatitis B vaccine in Pentavalent?	______ doses (range 0-4, 99 if don't know)	
B7	Is the child fully vaccinated with all vaccines in the national schedule?	Yes No Don't know	1 2 **(Advise parent of catch-up immunization)** 9

Surveyor's name: Date:

Annex 4 – Form C. Questionnaire

Form C. Questionnaire

No.	Questions and filters	Coding categories	Skip
Section 1. Basic information			
C1	**School code: __ __ __ Class code: __ __ Child code: __ __ __**		
C2	Response status	Parent/guardian signed consent form 1 Refused 2 Child absent 3 Parents/guardian absent 4 Parents/guardian could not be reached 5 Others (please specify) 9	**→END** **→REVISIT IF FEASIBLE** **→REVISIT IF FEASIBLE** **→END** **→END**
C3	What is the date of birth of the child ?	__ __/__ __/ __ __ __ __ D D / M M / Y Y Y Y If not known, write 09/09/9999	
C4	What time was the child born?	__ __ : __ __ (24 h format) H H : M M If not known, write 99:99	
C5	Was the child born in Maldives or overseas?	Maldives 1 Overseas Sri Lanka 2 Thailand 3 India 4 Malaysia 5 Other foreign countries (please specify) 6 ..... Don't know 9	
C6	Where was the child born?	Island health center 1 Atoll hospital 2 Male' hospital 3 Home 4 Other (please specify) 5 ..... Don't know 9	

C7	Has the child ever received blood transfusion?	Yes 1 No 2 Don't know 9	
Section 2. Hepatitis B immunization			
C8	Check Form B. Immunization history. Does the child have a copy of the immunization record at school or in a health facility?	Yes 1 No 2 Don't know 9	**→Skip to C14**
C9	Can I see your child's health record? (If the parent presents the record, copy vaccination dates to Form B if not yet done. Take a picture of the immunization record with names covered by the child code.)	Presents the record 1 Doesn't have the record 2 No answer/refused 9	**→Skip to C14** **→Skip to C14**
C10	(If no record can be found, ask parent to recall) Did the child receive the hepatitis B vaccine at birth, that is an injection on the outside of the thigh to prevent hepatitis B disease?	Yes 1 No 2 No answer/not sure 9	**→Skip to C12** **→Skip to C12**
C11	(By parental recall) How many hours or days after birth did the child receive the first dose?	____ hours OR _____ day(s) Write days if hours unknown or over 24 hours; 0 day if given on the day of birth; 1 day if given on the next day after birth; if does not remember write 99 days.	
C12	(By parental recall) Other than the birth dose, did the child receive additional doses of hepatitis B vaccine, or the combination vaccine that prevents diphtheria, tetanus, whooping cough, Haemophilus influenzae type b, and hepatitis B? The latter is also called the DTP-Hib-HepB vaccine, or the pentavalent vaccine. It is an injection in the thigh, often given during the same visits when a drop in the mouth to prevent polio is given.	Yes 1 No 2 No answer/not sure 9	**→Skip to C14** **→Skip to C14**
C13	(By parental recall) Not counting the birth dose, how many doses of hepatitis B vaccine or pentavalent vaccine did the child receive?	_____ doses (Don't include birth dose; 99 if don't know)	
C14	Check B6, B7 of Form B or C10, C12, C13 if by recall. Has the child missed any of the 4	Missed HepB BD 1 Missed any of Penta1,2, or 3, or HepB1, 2, or 3 2	

	doses of hepatitis B vaccines, including the pentavalent vaccine? The child should receive **at least 3 doses** of hepatitis B vaccine. **Advise parent of catch-up** vaccination if <3 doses of HepB, or missing OPV, Penta (or DTP), measles, or MMR	Received all 4 doses of HepB in schedule Unknown	3 9	**→Skip to C16** **→Skip to C16**
C15	If the child missed any of the 4 doses of hepatitis B vaccine, what are the reasons? (Do not prompt. Mark all that apply)	Born at home, vaccine not offered Born in a foreign country, vaccine not offered Too far to get vaccinated Transportation cost was too high Forgot it was time for next vaccine Don't think vaccination is important Stock out at health facility Don't trust vaccination Adverse event after last vaccination Not in area when vaccine was due Did not know where to go for vaccine Too busy to take child to health facility Other (please specify) ..... No answer	a b c d e f g h i j k l m n	
Section 3. Hepatitis B surface antigen test				
C16	Child's HBsAg status	Positive Negative Invalid Test not performed	1 2 3 4	**→END** **→END** **→C17** **→C18**
C17	**Only repeat the test one more time when the first test is invalid, and the child is amenable.** Child's HBsAg status (the second test)	Positive Negative Invalid Test not performed	1 2 3 4	**→END** **→END** **→END**
C18	Reasons for not performing the test	Child uncooperative Child absent for extended period of time Parent consented to interview but refused to take blood Others (please specify)	1 2 3 9	

Surveyor's name: Date:

Annex 5 – Informed consent form

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މި ސްޓަޑީގައި ބައިވެރިވުމުން ކުރިމަތިވާނެ ބޮޑު ހިރާސައެއް ނެތެވެ. ނަމަވެސް ސުވާލުތަކަށް ޖަވާބު ދިނުމަށް ވަގުތު ހޭދަވާނެއެވެ، އަދި ބައެއް ސުވާލުތަކަކީ އިނގިރޭސިބަހުން ކުރާ ސުވާލުތަކެއް ކަމުން ބައެއް ފަރާތްތަކަށް ސުވާލުތައް ދެނެގަތުމަށް އުނދަގޫވެދާނެއެވެ. މިފަދަ ކަންތައް ދިމާވެއްޖެނަމަ ބައިވެރިވާ ފަރާތުން ހާމަކުރާ މަޢުލޫމާތު ސިއްރުކޮށް ހިފެހެއްޓޭނެ ކަމުގައި ބައިވެރިންނަށް ޔަގީންކޮށްދެމެވެ. މި އިންޓަވިއު ނިންމުމަށް ދަންނަވާ ވަގުތަކީ ގާތްގަނޑަކަށް ފަނަރަ މިނެޓެވެ. މި ސުވާލުތަކަށް ޖަވާބު ދިނުމަށް ބޭނުންނުވާނަމަ، ޖަވާބު ނުދިނުމުގެ ފުރުސަތު ވެސް ހުރިކަން ހަނދުމަފުޅުކޮށްދެމެވެ.

ސިއްރު ހިފެހެއްޓުން

ދިރާސާއާ ގުޅިގެން ހޯދާ މަޢުލޫމާތު ސިއްރުކޮށް ހިފެހެއްޓުމަށް މަސައްކަތް ކުރެވޭނެއެވެ. ސުވާލުތަކަށް ދެއްވާ ޖަވާބުތަކުގައި ނަމާއި އެޑްރެސް ފަދަ ތަފްޞީލެއް ނުހިމެނޭނެއެވެ. ޖަމާކުރެވޭ މަޢުލޫމާތު ބޭނުންކުރާނީ ހަމައެކަނި ދިރާސާގެ މަޤްޞަދަށް ރިޕޯޓް ތައްޔާރުކުރުމަށް ކަމަށާއި މި ޖަމާކުރާ މަޢުލޫމާތު ހެލްތު ޕްރޮޓެކްޝަން އޭޖެންސީއަށް، މިނިސްޓްރީ އޮފް ހެލްތު އިން ބޭރުގައި އެހެން ފަރާތަކާ ހިއްސާ ނުކުރެވޭނެ އެވެ.

އަގު/ފައިސާ

މި ސްޓަޑީ ގައި ބައިވެރިވުމަށް އެއްވެސް އަގެއް ނުނަގާނެ އެވެ.

ބައިވެރިވުމާއި އުޒުރުވެރިވުން

މި ދިރާސާގައި ބައިވެރިވުމަކީ އިޚްތިޔާރީ ކަމެކެވެ. މި ދިރާސާގައި ބައިވެރިވުމަށް އެއްބަސްވުމަށްފަހުވެސް އެއްވެސް ހިސާބަކުން ދިރާސާއިން ވަކިވުމުގެ ފުރުސަތު ހުރެއެވެ. ބައިވެރިވުމަށް ބައިވެރިވުމަށް ހާމަކުރެވޭ ދީލައިލާކުރަން، އެއްވެސް ސަބަބެއް ބަޔާންކުރުމަކާނުލައި މި ދިރާސާ ދޫކޮށްލުމުގެ އިޚްތިޔާރު ބައިވެރިވާ ފަރާތަށް ލިބިގެން ވެއެވެ. ސްޓަޑީ ދޫކޮށްލުމުގެ ސަބަބުން ބައިވެރިވާ ފަރާތަށް އެއްވެސް ގެއްލުމެއް ނުވާނެ އެވެ.

ސުވާލު

މި ސްޓަޑީ އާއި ގުޅޭގޮތުން އެއްވެސް ސުވާލެއް އޮތްނަމަ، ހެލްތު ޕްރޮޓެކްޝަން އޭޖެންސީގެ ނަންބަރު 3014333 އަށް ގުޅުއްވުން އެދެމެވެ.

ހެޕަޓައިޓިސް-ބީ ސީރޯ ސަރވޭ 2023 ގައި ބައިވެރިވުމުގެ އެއްބަސްވުން

ސާވޭގައި ބައިވެރިވުމުގެ އެއްބަސްވުމަށް ތިރީގައި ސޮއިކުރުމުގެ ކުރިން ސަރވޭއާ ބެހޭ ސޮއިކުރުމުގެ ކަރުދާސް އެދެން.

ސަރވޭގައި ބައިވެރިވުމުގެ އެއްބަސްވުން

މި ސާވޭގެ މަޤްސަދަކީ، ދިވެހިރާއްޖޭގެ ކުދިންގެ ތެރޭގައި ހެޕަޓައިޓިސް ބީ ބަލީގެ ހާލަތު ދެނެގަތުމެވެ. ދިރާސާއާއި ބެހޭ މަޢުލޫމާތު ދަރިފުޅުގެ ބެލެނިވެރިޔާއަށް ދީ، ބަލީގެ ހާލަތާއި ބެހޭ ގޮތުން ކުރެވޭ ސުވާލުތަކަށް ޖަވާބުދިނުމަށް ދަރިފުޅުގެ ލޭ ނަމޫނާއެއް ނެގުމަށް އެދެމެވެ. މި ސަރވޭގެ މައުލޫމާތު ސިއްރުކޮށް ރައްކާކުރެވޭނެއެވެ. ސަރވޭގައި ބައިވެރިވުމަކީ ދަރިފުޅުގެ އިޚްތިޔާރެވެ. ބަލީގެ ހާލަތާއި ބެހޭ ގޮތުން ދޭ ނަތީޖާއަކީ ސިއްރު މައުލޫމާތެކެވެ. އަދި މީގެ ސަބަބުން ދަރިފުޅަށް އެއްވެސް ގެއްލުމެއް ނުވާނެއެވެ. މި ސާވޭގައި ބައިވެރިވާ ކުދިންގެ ނަން ސިއްރުކޮށް ރައްކާކުރެވޭނެ ކަމަށް ޔަޤީންކޮށްދެމެވެ.

މީގެ އިތުރުން:

- ދަރިފުޅުގެ އިންޓަވިއުކުރުން ތެރޭ ސާވޭގައި އެއް ނަގާނަން ކަމަށް އެއްބަސްވަމެވެ.
- މި ސާވޭގެ މަޢުލޫމާތު މުސްތަޤްބަލުގައި ދިރާސާކުރުމުގައި ބޭނުންކުރުމަށް 1 ގެ ކުރިންގެ ދުވަހުގައި ހެޕަޓައިޓިސް ބީ ފަރުވާދިނުމަށް ބޭނުންކުރުމަށް އެއްބަސްވަމެވެ.
- މި ސާވޭގައި ދަރިފުޅު ބައިވެރިކުރުމަށް އިޚްތިޔާރުކުރުމާއި އެއްބަސްވާކަން އެއްބަސްވަމެވެ.

ކުއްޖާގެ ނަން:.......................................

އުފަން ތާރީޚު:.......................................

ބެލެނިވެރިޔާގެ ނަން:.......................................

ކުއްޖާ އާއި ހުރި ގުޅުން:.......................................

ފޯނު ނަންބަރު:.......................................

ބެލެނިވެރިޔާގެ ސޮއި: ތާރީޚު:

ސްޓާފު ކޯޑު: ކްލަސްޓަރ ކޯޑު: ކުއްޖާގެ ކޯޑު:

بسم الله الرحمن الرحيم

Information Sheet- Hepatitis B Sero-Survey in Grade 1 Children in Maldives

National Immunization Program, Health Protection Agency, Ministry of Health, Maldives

Purpose of the survey

Hepatitis B virus infection is one of the most common cause of chronic liver disease in Maldives. The hepatitis B vaccine is very effective in preventing infection with this virus. It should be given to babies as close to birth as possible, to prevent mothers with the virus from passing it on to them. Hepatitis B vaccine is given to all infants in Maldives since 1993. The Ministry of Health is conducting a national survey to know the rates of hepatitis B among children. This research will provide important information on how common hepatitis B infection is for children in Grade 1 after the introduction of the vaccination program. Nationally, over 2000 children are being asked to participate in the research. Your child is being asked to participate because he/she attends one of the 63 schools that were selected by chance.

Procedure

We will review the vaccination records of your child. Then we would like to ask you a few questions about your child and take a few drops of blood from your child's finger by making a small prick at the tip of the finger. We will test this blood for hepatitis B. We will let you know whether your child has hepatitis B by the end of the survey.

The results of the testing will only be shared with you and the Health Protection Agency, Ministry of Health. Your participation will greatly assist all children living in Maldives. We will tell you if your child needs any more doses of hepatitis B vaccine based on their immunization records. If your child needs further vaccination, your child can receive the hepatitis B vaccine wherever you normally have your child vaccinated. We will also tell you if your child has hepatitis B. There will be no charge to you or your child for being in the survey and having the blood test. If the test result is positive, the relevant programs of Health Protection Agency will be notified and will facilitate follow-up testing and care for your child. Your child's participation in the survey is completely voluntary and optional. Being in the survey or choosing not to be in the survey will not affect the medical care or services your child or your family receives from the Ministry of Health. If you wish to know if your child has hepatitis B, but do not want your child to take part in the survey, you could bring your child to the health center/atoll hospitals and discuss with the doctors about the testing.

Notes:

- A finger will be pricked to get a few drops of blood. The procedure will only take a few minutes to complete.
- The test poses no danger to your child's health. There may be some discomfort or slight pain where the blood was taken. Please contact your doctor if you have any concerns after the test.

- Blood samples will only be tested for a marker of hepatitis B virus. It will not be used for any other purpose. The samples will be destroyed immediately after the test results are written down.
- If your child's test results show he/she is in infected with the hepatitis B virus, we will inform you and provide counseling on what to do next. You will be responsible for following the advice as you are able to.

Risks and discomfort

There is no major risk involved to participate in this study. Other than momentary, mild pain when pricking a finger and collecting a few drops of blood, temporary change in colour of surrounding skin and rare chance of infection, it will not cause any other harm to the child. We will take all precautions to prevent these problems including use of sterile, disposable lancets. In the event your child develops any problems despite our preventive efforts, we would provide adequate treatment or make arrangements for appropriate treatment of the condition at another hospital and will bear all costs related to such treatment. We will contact you in this situation and you may call the study doctor.

Confidentiality

The information we collect about your child will be kept confidential and will only be used for the purpose of this survey and for follow-up testing and care for your child if the test result is positive. The result of your child's blood test will only be shared with you and the Health Protection Agency, Ministry of Health at the end of the survey.

Costs/Payment

There is no cost, nor payment, for participation in this survey.

Right to refuse or withdraw

Your decision for your child to participate is entirely voluntary. If you decide to join the survey, you are also free to change your mind at any time for any reason. There is no penalty for declining to participate and you can decline without having to provide a reason.

Questions

If you have any questions about your child's rights as a participant in this survey or if you want to withdraw your child from this survey at any time, you may call 3014333of the Health Protection Agency.

Agreement to Participate

If you agree to participate in this study, please indicate that by putting your signature at the specified space below.

Consent Form- Hepatitis B Sero-Survey 2023

I have read this form or had this form read to me about the purpose of the survey and its possible risks and benefits. I have been able to ask questions about the survey and have had my questions answered. I understand that I can refuse to let my child to participate in this survey, even after signing this form, and it will not affect the medical care my child and family receive from the Ministry of Health.

I understand

- That my child will have a blood test collected by a finger prick.
- That the purpose of the survey is to determine the rates of hepatitis B infection in Grade 1 students in Maldives.
- that the participation of my child is voluntary

Print name of child: _____

Date of birth: _____

Print name of parent or guardian: _____

Relationship to the child: _____

Phone: _____

_____ Date:__/_____/_____

Signature of parent or guardian

School code: Class code: Child code:

Annex 6 – Letters disclosing test results

Hepatitis B Screening test result:

a. Your child tested **NEGATIVE** for hepatitis B infection. That means your child does not have hepatitis B.
b. Thank you for being part of this survey. This survey will help us evaluate the National Immunization Program and support the Ministry of Health to improve it further.
c. If you are interested in learning more about hepatitis B, you are welcome to visit the hepatitis website of the World Health Organization at http://www.who.int/hepatitis/en/

Hepatitis B Sero-Survey 2023

Hepatitis B Confirmatory Result:

a. Your child tested **positive** for hepatitis B infection.
b. It is recommended that your child has a follow-up test in 6 months to see if this is a chronic infection. This can be arranged by the Health Protection Agency of Ministry of Health.
c. Many people with chronic hepatitis B remain well and may not have any symptoms of being sick. However, some may develop serious liver problems (1 in 5 persons) later in life (30 to 50 years old).
d. Even without symptoms the virus can be passed to others. Understanding how the virus is passed can help keep others from being infected. It is most commonly spread via blood and other body fluids.
e. Children should not be excluded from any activities.
f. It is recommended that household members should also be tested for hepatitis B infection and immunity against hepatitis B, and if they are not infected and not immune against hepatitis, they should complete the hepatitis B vaccination series.

Annex 7 - Prequalification report of Determine HBsAg2

WHO Prequalification of In Vitro Diagnostics
PUBLIC REPORT

Product: VIKIA® HBs Ag
WHO reference number: PQDx 0284-016-00

VIKIA® HBs Ag with product code **31124**, manufactured by **bioMérieux SA**, **CE-marked regulatory version**, was accepted for the WHO list of prequalified in vitro diagnostics and was listed on 26 July 2018.

Summary of WHO prequalification assessment for VIKIA® HBs Ag

	Date	Outcome
PQ listing	26 July 2018	listed
Dossier review	N/A	MR
Site inspection(s) of quality management system	July 2017 May 2018	MR
Laboratory evaluation of performance and operational characteristics	4th quarter of 2017 to 1st quarter of 2018	MR

MR: Meets requirements
N/A: Not applicable

Intended use:
Rapid test for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum, plasma, or whole blood. The test is intended for laboratory use by healthcare professionals only.

Assay description:
VIKIA® HBsAg is a qualitative test based on the association of monoclonal and polyclonal antibodies specific to HBsAg. This test uses the principle of lateral immunochromatography for the detection of circulating HBs antigen. It is used for the detection of the main sub-types ad and ay in serum, plasma, and the whole blood.

The test consists of a plastic device containing:

1. A chromatography membrane to which are fixed:
   - in the test region (T), a goat polyclonal anti-HBs antibody
   - in the control region (C), a monoclonal anti-biotin antibody
2. A test strip impregnated with a conjugate consisting of:
   - a mixture of two monoclonal anti HBs-antibodies coupled to red-dyed polystyrene microspheres,
   - a BSA-biotinylated complex coupled to blue-dyed polystyrene microspheres.

The sample is added to the sample well "S" and migrates by capillarity along the membrane. If the sample contains HBs antigen, the HBs antigen forms antigen-antibody complex with the antibodies specific to this virus present on the red-dyed polystyrene microspheres. The antigen-antibody complexes migrate along the membrane and bind to the anti-HBs antibodies forming complexes revealed by a red line in the test region (T) of the membrane. To serve as a procedural control, a blue line will always appear in control region (C) if the test has been performed correctly.
The BSA-biotinylated complex coupled to blue dyed polystyrene microspheres migrates along the membrane at the same time as the sample and binds to the anti-biotin antibody forming a complex revealed by a blue line in control region (C). Absence of this colored line invalidates the test.

Test kit contents for 25 tests/kit (product code 31124):

Component	Number	Product code
Sealed pouches. Each pouch contains: • a ready-to-use test device: - goat polyclonal anti-HBs antibody - mouse monoclonal anti-biotin antibody - polystyrene microspheres sensitized with mouse monoclonal anti-HBs antibody - and a BSA-biotinylated complex • a pipette • a desiccant.	25	R1
Dropper bottle for whole blood (3mL)	1	R2
Ready-to-use Phosphate Buffer pH 7.4 + 5 g/l casein + preservatives (< 0.1 % sodium azide)		
User quick guide printed on the box.	1	n/a
Package insert provided in the kit or downloadable from www.biomerieux.com/techlib.	1	n/a

Items required but not provided:

- Lancets
- Timer
- Disposable gloves
- Alcohol swabs
- Waste disposal device

Whole blood

- Equipment for blood collection by venipuncture or by fingerstick;
- 75 µL EDTA capillary tubes, bulbs or other devices with or without EDTA, to collect and dispense 75 µL of whole blood.

Storage:
The test kit should be stored at 4 to 30 °C.
Do not freeze.

Shelf-life upon manufacture:
25 months.

If stored according to the recommended conditions, all components are stable until the expiry date indicated on the box. Do not use after the expiry date.
The test device should remain in the sealed pouch until use.
R2 buffer may change color with time. This change does not affect test performance prior to the expiry date indicated on the box.

Warnings/limitations:
See manufacturer's instructions for use.

Prioritization for prequalification

Based on the established criteria, VIKIA® HBs Ag was given priority for WHO prequalification.

Product dossier assessment

In accordance with the WHO procedure for abridged prequalification assessment, bioMérieux SA was not required to submit a product dossier for VIKIA® HBs Ag as per the "*Instructions for compilation of a product dossier*" (PQDx_018 v1). Notwithstanding, certain aspects of the product dossier previously submitted for stringent regulatory review were reviewed by an assessor during the site inspection.

Commitment for prequalification:

1. Revised instructions for use to be supplied with next lot manufactured.

Manufacturing site inspection

In accordance with the WHO procedure for abridged prequalification assessment, a shortened inspection with was conducted at the site(s) of manufacture (bioMerieux, 376 Chemin de l'Orme, Marcy l'Etoile, 69280 France) of VIKIA® HBs Ag in July 2017, and to assess the implementation of some reportable changes, this inspection was followed with a short inspection conducted in May 2018 as per the "*Information for manufacturers on prequalification inspection procedures for the sites of manufacture of diagnostics*" (PQDx_014 v1).

The inspection found that the manufacturer had an acceptable quality management system and good manufacturing practices in place that ensured the consistent manufacture of a product of good quality.

The manufacturer's responses to the nonconformities found at the time of the 2017 inspection were accepted on 19 February 2018. The May 2018 inspection is in the post-inspection phase.

Based on the site inspection and corrective action plan review, the quality management system for VIKIA® HBs Ag meets WHO prequalification requirements.

Laboratory evaluation

VIKIA HBsAg (bioMérieux) was evaluated by WHO in the 4th quarter of 2017 to 1st quarter of 2018 using plasma specimens. From this evaluation, we drew the following conclusions:

VIKIA HBsAg is a rapid immunochromatographic assay for the detection of HBsAg in human serum, plasma or whole blood. A volume of 75 µL of specimen is needed to perform the assay. This type of assay requires no sophisticated equipment and can therefore be

performed in laboratories with limited facilities. Reading of the results can be done visually.

Performance characteristics in comparison with an agreed reference standard		
	Initial (95% CI)	**Final (95% CI)**
Sensitivity %	99.5% (97.3% - 100%)	99.5% (97.3% - 100%)
Specificity %	97.8% (95.4% - 99.1%)	99.04% (97.2% - 99.8%)
Invalid rate %	0	
Inter-reader variability %	0	

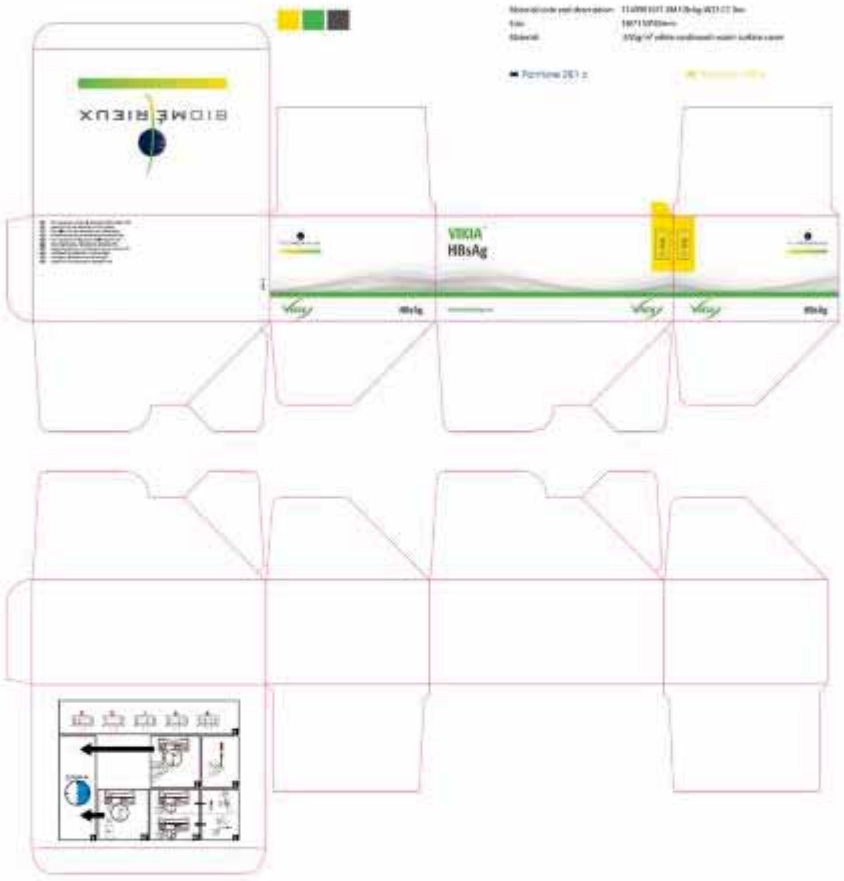
Additional performance characteristics	
Sensitivity during seroconversion on six seroconversion panels in comparison with a benchmark assay; Monolisa Ag HBs Plus [Bio-Rad Laboratories]).	Seroconversion sensitivity index of +1.5, therefore detection is 1.5 days later than the benchmark assay
Analytical sensitivity on WHO reference preparations 03/262 and the 12/226	Detected 2IU/ml
Analytical sensitivity on a mixed titer panel in comparison with an agreed reference standard	15 of 15 specimens were correctly classified.
Lot to lot variation on a dilution panel in comparison with an agreed reference standard	Acceptable

Labelling

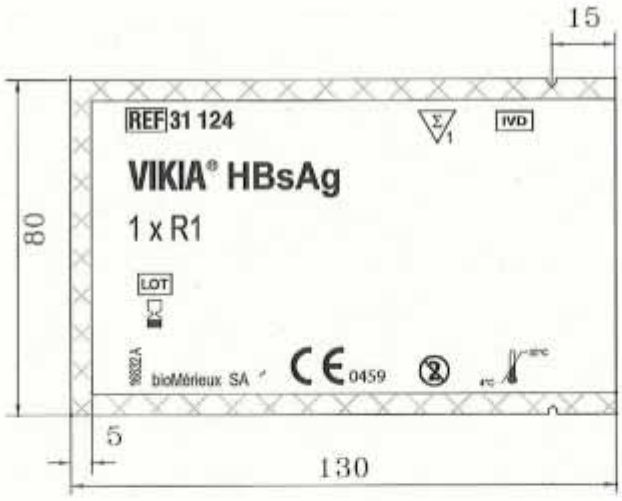
1. **Labels**
2. **Instructions for use**

1. Labels

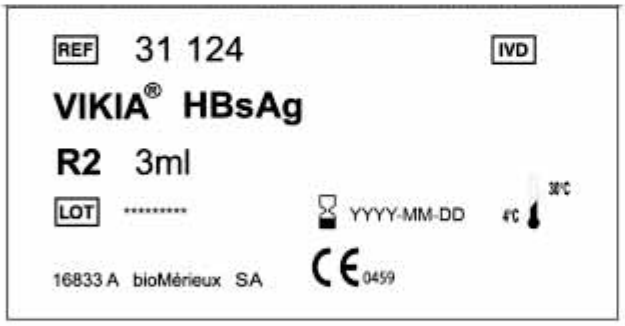
Kit box



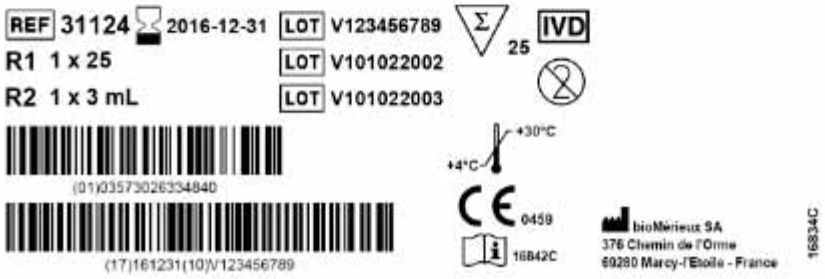
R1 :



R2



Kit label



2. Instructions for use

VIKIA® HBsAg

IVD

INTENDED USE

Rapid test for the qualitative detection of hepatitis B surface antigen (HBsAg) in human serum, plasma, or whole blood.
The test is intended for laboratory use by healthcare professionals only.

PRINCIPLE

VIKIA® HBsAg is a qualitative test based on the association of monoclonal and polyclonal antibodies specific to HBsAg. This test uses the principle of lateral immunochromatography for the detection of circulating HBs antigen. It is used for the detection of the main sub-types ad and ay in serum, plasma, and whole blood.
The test consists of a plastic device containing:

1. A chromatography membrane to which are fixed:
- in the test region (T), a goat polyclonal anti-HBs antibody
- in the control region (C), a monoclonal anti-biotin antibody

2. A test strip impregnated with a conjugate consisting of:
   - a mixture of two monoclonal anti-HBs antibodies coupled to red-dyed polystyrene microspheres,
   - a BSA-biotinylated complex coupled to blue-dyed polystyrene microspheres.

The sample is added to the sample well "S" and migrates by capillarity along the membrane.
If the sample contains HBs antigen, the HBs antigen forms an antigen-antibody complex with the antibodies specific to this virus present on the red-dyed polystyrene microspheres.
The antigen-antibody complexes migrate along the membrane and bind to the anti-HBs antibodies forming complexes revealed by a red line in the test region (T) of the membrane.
To serve as a procedural control, a blue line will always appear in control region (C) if the test has been performed correctly.
The BSA-biotinylated complex coupled to blue dyed polystyrene microspheres migrates along the membrane at the same time as the sample and binds to the anti-biotin antibody forming a complex revealed by a blue line in control region (C). Absence of this colored line invalidates the test.

CONTENT OF THE KIT (25 TESTS):

25 Sealed pouches	R1	Each pouch contains: - a ready-to-use test device (goat polyclonal anti-HBs antibody + mouse monoclonal anti-biotin antibody + polystyrene microspheres sensitized with mouse monoclonal anti-HBs antibody and a BSA-biotinylated complex). - a pipette. - a desiccant.
1dropper bottle for whole blood 3 mL	R2	Ready-to-use. Phosphate Buffer pH 7.4 + 5 g/l casein + preservatives (< 0.1% sodium azide).
1 User quick guide printed on the box		
1 Package insert provided in the kit or downloadable from www.biomerieux.com/techlib		

MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- Disposable gloves
- Alcohol swabs
- Waste disposal device

Whole blood
- Equipment for blood collection by venipuncture or by fingerstick.
- 75 µL EDTA capillary tubes, bulbs or other devices with or without EDTA, to collect and dispense 75 µL of whole blood.

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use only.**
- **For professional use only.**
- The kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions.
- Do not ingest. Do not inhale.
- All specimens should be considered infectious and handled following the recommended precautions (CLSI® M29-A, Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline – Current revision). For additional information on handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories – HHS Publication – Latest edition", or the current regulations in the country of use.

- As the specimens are potentially infectious, wear gloves when handling them.
- Do not touch the test device chromatography membrane with your fingers.
- Do not touch the test device during the test.
- The test device should be stored in the sealed pouch containing the desiccant until use.
- Do not use reagents after the expiry date indicated on the box.
- Do not use the test device if the pouch is damaged.
- The test device and the pipette are for single use only; these components should not be reused.
- Do not mix reagents from different lots.
- Kit reagents contain sodium azide which can react with lead or copper plumbing to form explosive metal azides. If any liquid containing sodium azide is disposed of in the plumbing system, drains should be flushed with water to avoid build-up.

STORAGE AND STABILITY

- Store the kit at +4 to +30°C.
- **DO NOT FREEZE.**
- If stored according to the recommended conditions, all components are stable until the expiry date indicated on the box. Do not use after the expiry date.
- The test device should remain in the sealed pouch until use.
- R2 buffer may change color with time. This change does not affect test performance prior to the expiry date indicated on the box.

SPECIMEN COLLECTION AND PREPARATION

Specimen type and collection

It is the responsibility of each user to validate the sampling tube and/or capillary used.

If the capillary tube is used with a non disposable bulb, the user is responsible for checking that the bulb has not been contaminated by previously collected samples.

1. Collection of serum, plasma

Use sera or plasma collected in lithium heparin or EDTA. Store the serum or plasma separated from the pellet. Following good laboratory practice, clarify samples by centrifugation before use.
The results obtained were not found to be influenced for icteric samples (bilirubin concentrations up to 500 µmol/l), for hemolyzed samples (hemoglobin (monomer) concentrations up to 300 µmol/l), for lipemic samples (up to 30 mg/mL equivalent in triglycerides) and for samples spiked with biotin (up to 2 mg/l).
Do not inactivate samples.

2. Whole blood by venipuncture

Use whole blood collected in lithium heparin or EDTA.
The other anticoagulants have not been validated.

3. Whole blood by fingerstick

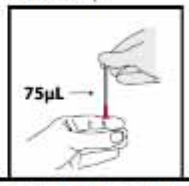
Use a capillary tube or another device (please refer to the section **MATERIAL REQUIRED BUT NOT PROVIDED**) to collect blood from the fingertip. The sample should be tested extemporaneously.

Specimen stability

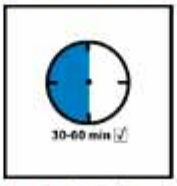
- Samples (serum and plasma) can be stored for 5 days at +2 to +8°C and 4 hours at +15 to +37°C. If longer storage is required, freeze at -25 ± 6°C. A study performed on samples frozen for 2 months showed that the quality of results is not affected. Avoid successive freezing and thawing (one cycle has been validated).
- The whole blood collected by venipuncture can be stored for 5 days at +2 to +8°C and 4 hours at +15 to +37°C. Do not freeze whole blood samples.
- **Whole blood collected by fingerstick should be tested immediately.**

INSTRUCTIONS FOR USE

- Allow the required reagents to come to room temperature before use.
- Remove the test device from the sealed pouch and use it immediately.
- Perform the test on a flat clean vibration-free surface.

Serum or plasma	Whole blood by venipuncture	Whole blood by fingerstick
1. 	1. 	1. To facilitate blood drop formation, massage the fingertip from the base to the tip. Collect 75 µL of sample using the capillary tube or another device (please refer to the section **MATERIAL REQUIRED BUT NOT PROVIDED**). 
2. Using the pipette, transfer 3 drops of sample (75 µL) to the sample well (S) on the test device without trapping bubbles. 	2. a) Using the pipette, transfer 3 drops of whole blood (75 µL) to the sample well (S) on the test device without trapping bubbles. The sample well turns red. 	2. a) Deposit the sample in the sample well (S) on the test device without trapping bubbles. The sample well turns red. 
	2. b) Dispense one drop of buffer (40 µL) without trapping bubbles in the sample well (S). 	

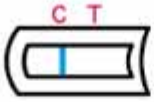
- **Start the timer. Read the result at 30 minutes.**
- Do not interpret the result after 60 minutes.
- During the migration period, the test device must not be handled or moved.



Note: For most positive samples, the line in the test region (T) can appear before 30 minutes. However, test reading and interpretation should only be performed after 30 minutes following sample deposit.

RESULTS AND INTERPRETATION

POSITIVE: Two distinct lines appear: a blue line in the control region (C), and a red line in the test region (T).
A pink to red line (T), even if it is thin, indicates a positive result.



NEGATIVE: A blue line appears in the control region (C).
No line appears in the test line region (T).

INVALID: The control line fails to appear or no line appears in the control region (C) and the test region (T).
Insufficient specimen volume or incorrect procedural techniques are the most likely reasons.
Review the procedure and repeat the test with a new test device.
If the problem persists, discontinue using the kit and contact your local distributor.

NOTE: The intensity of the red line in the test region (T) may vary depending on the concentration of HBs antigen in the sample. However, the concentration of antigen in the sample cannot be determined by this qualitative test.

If testing whole blood, check for a red color in the sample well which indicates that the sample has definitely been dispensed.

Interpretation of test results should be made taking into consideration the patient's history, and the results of any other tests performed.

For all the samples tested, no significant difference in performance was observed for readings performed at 30 minutes and 60 minutes.

QUALITY CONTROL

Internal procedural controls are included in the test. A blue colored line appearing in the control region (C) confirms proper sample migration. If the control line does not appear, the test result is invalid.

Note

It is the responsibility of the user to perform Quality Control in accordance with any applicable local regulations.

LIMITATIONS OF THE METHOD

- A negative VIKIA® HBsAg result does not rule out the possibility of hepatitis B infection.
- The serum HBs antigen concentration may in fact be below the analytical sensitivity of the reagent.
- The presence of a modified HBs antigen (variant) cannot be excluded; the antigen may, in this case, have been incorrectly recognized or not recognized by the antibodies in the reagent.
- In case of a negative results, it is recommended to confirm using another technique, particularly in the presence of clinical symptoms and/or risk factors.
- The results of this assay must be interpreted taking into consideration the patient's history, and the results of any other tests performed (Elisa and neutralization, HBV DNA, other serological markers, etc.).
- If both HBs antigen and anti-HBs antibodies are present, the quantity of antigen may be reduced, or negative in rare cases.
- This test has been validated for serum, plasma and whole blood. It should not be used for other biological fluids such as saliva, cerebrospinal fluid (CSF), or urine.
- This test should not be used with specimens collected post-mortem.
- Do not use sample pools.
- Interference may be encountered with certain sera containing antibodies directed against reagent components. For this reason, assay results should be interpreted taking into consideration the patient's history, and the results of any other tests performed.

PERFORMANCE

Studies performed using VIKIA® HBsAg gave the following results:

1. ANALYTICAL SENSITIVITY

The analytical sensitivity was determined using the WHO Standard (Second International Standard for HBsAg, subtype adw2, genotype A), it is less than or equal to 2 IU/mL.

2. STUDIES PERFORMED IN WEST AFRICA, SOUTH AMERICA AND ASIA

To take into account the different HBV genotypes, VIKIA® HBsAg performance was studied as part of an international multicenter evaluation performed in West Africa (Burkina-Faso), South America (Brazil) and Asia (India and China).
Plasma or serum sample status (EDTA) was established using the ELISA method.
VIKIA® HBsAg performance: relative specificity and sensitivity on fresh plasma, serum and venous whole blood, was established in comparison with the ELISA method.
An equivalence study was performed for fresh plasma, venous whole blood, and capillary blood, on a limited number of paired samples.
Furthermore, VIKIA® HBsAg was evaluated using plasma or serum in comparison with another immune-chromatographic test (ICT).

2.1 Clinical specificity and sensitivity

1800 paired plasma and venous whole blood samples and 150 serum samples, (1528 negative and 422 positive) from different populations: donors, pregnant women, asymptomatic patients at testing centers, positive HBs antigen patients known to have chronic or acute hepatitis, were tested using the VIKIA® HBsAg test.

	West Africa		South America (Brazil)		Asia		Number of samples tested		
Sample status	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Total
Asymptomatic patients and donors:									
- Plasma or serum	475	138	399	0	507	0	1381	138	1519
- Venous whole blood	475	138	399	0	407	0	1281	138	1419
Pregnant women:									
- Plasma or serum	48	2	49	1	50	0	147	3	150
- Venous whole blood	48	2	49	1	50	0	147	3	150
Positive HBs Ag patients:									
- Plasma or serum	0	20	0	107	0	154	0	281	281
- Venous whole blood	0	20	0	107	0	104	0	231	231
Total:									
- Plasma or serum	523	160	448	108	557	154	1528	422	1950
- Venous whole blood	523	160	448	108	457	104	1428	372	1800
Total number of samples per country:									
- Plasma or serum	683		556		711		1950		
- Venous whole blood	683		556		561		1800		

A) PLASMA OR SERUM

Reading at 30 minutes

		Elisa		Total
		Positive	Negative	
VIKIA® HBsAg	Positive	418	3	421
	Negative	4	1525	1529
Total		422	1528	1950

Relative specificity of the VIKIA® HBsAg test compared to the ELISA method:

99.80% [99.41% - 99.93%] on 1528 negative plasma or serum samples, with a 95%CI.

Relative sensitivity of the VIKIA® HBsAg test compared to the ELISA method:

99.05% [97.55% - 99.64%] on 422 positive plasma or serum samples, with a 95%CI. The same relative sensitivity was observed at 15 minutes.

B) VENOUS WHOLE BLOOD

Reading at 30 minutes

		Elisa		Total
		Positive	Negative	
VIKIA® HBsAg	Positive	368	3	371
	Negative	4	1425	1429
Total		372	1428	1800

Relative specificity of the VIKIA® HBsAg test compared to the ELISA method:

99.79% [99.37% - 99.93%] on 1428 venous whole blood samples, with a 95% CI.

Relative sensitivity of the VIKIA® HBsAg test compared to the ELISA method:

98.92% [97.22% - 99.59%] on 372 positive venous whole blood samples, with a 95% CI. The same relative sensitivity was observed at 15 minutes.

2.2 Comparison with another immunochromatography test (ICT)

This comparison is performed using plasma or serum samples only.

A) SAMPLES WITH A NEGATIVE STATUS

		VIKIA® HBsAg (reading at 30 minutes)		Total
		Positive	Negative	
Other ICT	Positive	0	0	0
	Negative	3	1522	1525
Total		3	1522	1525

3 samples with a negative status were found to be positive with the VIKIA® test.

Agreement: 99.8%

B) SAMPLES WITH A POSITIVE STATUS

		VIKIA® HBsAg (reading at 30 minutes)		Total
		Positive	Negative	
Other ICT	Positive	412	1	413
	Negative	6	3	9
Total		418	4	422

6 samples with a positive status that were detected by the VIKIA® test, were not detected by the other ICT.

Agreement: 98.34%

The same percentage of agreement was observed for reading at 15 minutes.

2.3 Equivalence study of the different types of specimens

This study was performed using VIKIA® HBsAg on 126 samples: 95 samples with a negative status and 31 paired samples with a positive status: fresh plasma, venous whole blood and capillary whole blood.

		VIKIA® HBsAg		
Sample status	**Number of samples tested**	**Plasma**	**Venous whole blood**	**Whole blood by fingerstick**
Negative	95	94	94	94
Positive	31	32*	32*	32*
Total	126			

*One sample with a negative status was found to be positive with the VIKIA® HBsAg test whatever the type of specimen used.

No discrepancies were observed between the different types of specimens.

3. STUDIES PERFORMED IN EUROPE

Studies performed in Europe using the VIKIA® HBsAg test gave the following results:

Population	**Number of samples tested negative for HBs**	**Sample type**	**Negative by VIKIA® HBsAg Reading at 30 minutes**
Blood donors	1000 (including 60 fresh* samples) non selected, and collected from 2 blood transfusion centers	Serum	999** **Specificity of the VIKIA® HBsAg test for this population : 99.90% 95% CI [99.44 - 100]**
Hospitalized patients	200 (including 174 fresh* samples)	Serum	199** **Specificity of the VIKIA® HBsAg test for this population : 99.50% 95% CI [97.25 - 99.99]**
Pregnant women	208 including 14 samples from multipar women and 25 fresh samples*	Serum	208** **Specificity of the VIKIA® HBsAg test for this population : 100% 95% CI [98.24 - 100]**

* Collected less than 24 hours previously.
** Within each selection, only one sample gave a false positive result at 30 minutes.

3.1. Diagnostic sensitivity:

a) Diagnostic sensitivity of ELISA methods

419 serum samples with a positive HBs antigen status, including 37 fresh samples collected less than 24 hours previously, were tested.

Population	**Number of samples tested positive for HBs**	**Sample type**	**Positive with VIKIA® HBsAg Reading at 30 minutes**
Europe	419 (including 37 fresh* samples)	Serum	412* Sensitivity: 98.33% 95% CI [96.59 - 99.33]

* Among the 7 samples that gave a false negative result for VIKIA® HBsAg, 6 have very low titers of HBs-Ag and are therefore considered to be undetectable with the VIKIA® HBsAg test owing to its analytical sensitivity.

b) Comparison with another ICT

438 serum samples with a positive HBs antigen status, including 6 native HBsAg mutant samples, were tested in comparison with another CE-marked ICT.
The following results were obtained for this population:

		ICT		
		Positive	Negative	**TOTAL**
VIKIA® HBsAg Reading at 30 min	Positive	406	22	**428**
	Negative	0	10*	**10**
	TOTAL	**406**	**32**	**438**

* 10 samples, detected as false negative, were tested using an EIA method: the results obtained using the EIA suggest that the samples had very low titers of HBsAg; these 10 samples are therefore considered to be undetectable with the VIKIA® HBsAg test owing to its analytical sensitivity.

The diagnostic sensitivities of each of the 2 devices for this population are the following:
Sensitivity of the VIKIA® HBsAg test for this population : 97.72% 95% CI [95.84 - 98.90]

Sensitivity of the ICT for this population: 92.69 % 95% CI [89.84 - 94.95]

The diagnostic sensitivity of the VIKIA® HBsAg test for this population is significantly better than that of the ICT.

3.2. Sensitivity on seroconversion panels

34 commercial seroconversion panels were tested. The results obtained with VIKIA® HBsAg test at 30 minutes were compared to the results of the commercial EIA mentioned in the package inserts for the tested panels.
The results obtained for the 34 seroconversion panels with VIKIA® HBsAg at 30 minutes were considered to be satisfactory: all of the differences in precocity observed between the VIKIA® HBsAg test and the commercial EIA, concerned seroconversion samples with low titers of HBs-Ag that could not be detected using the VIKIA® HBsAg test, owing to its analytical sensitivity.

3.3 Cross-reactivity

307 HBsAg-negative samples from patients whose disease states are likely to interfere with the VIKIA® HBsAg test were tested.
The following results were obtained with VIKIA® HBsAg at 30 minutes:

	Number of tests with a positive VIKIA® result / Total number of samples tested
Description of the interferent	
anti-Toxo.gondii antibody	0/10
anti-Trepo.pallidum antibody	0/10
anti-RUB antibody	0/10
anti-HSV antibody	0/10
anti-HCV antibody	0/6
anti-HAV antibody	1/10
Rheumatoid factor	0/10
anti-EBV antibody	0/10
anti-CMV antibody	0/10
anti-nuclear antibody	0/10
anti-HIV antibody	3/211

3.4. Serum/plasma equivalence study

The study was carried out using 30 HBs Ag-negative sample sets, consisting of one serum specimen (plain tube) and one lithium-heparin specimen.
All of the 30 sets were tested using VIKIA HBsAg before and after spiking with HBs Ag (2 spiked concentrations were tested to simulate weakly HBs Ag-positive and moderately HBs-Ag-positive samples).
The results obtained during this study with VIKIA HBsAg are strictly equivalent, regardless of the type of sample tube used (serum/plasma), and no discrepancy was observed.

Hook effect:
A range of concentrations for each of the 2 ad and ay Genelabs antigens was tested using the VIKIA® HBsAg kit. No hook effect was observed up to 750 µg/mL.

Precision:
Three samples with different antigen levels (one negative, one positive at the detection limit level and one positive above the detection limit) were tested 72 times on the same day: using 2 lots performed in triplicate by 2 operators and at 2 separate times of the day (morning, afternoon). The tests were read visually by 3 different people.
The repeatability (intra-lot) and the total precision of the antigen levels for the 2 lots was evaluated.
The agreement between the results obtained and the expected results for the three different levels is 100%:

Sample	Lot	Number of concordant results	Percentage of concordant results
Negative	1	36	100% 95% CI [90.26 -100.0]
	2	36	100% 95% CI [90.26 -100.0]
Low positive	1	36	100% 95% CI [90.26 -100.0]
	2	36	100% 95% CI [90.26 -100.0]
Positive	1	36	100% 95% CI [90.26 -100.0]
	2	36	100% 95% CI [90.26 -100.0]

WASTE DISPOSAL

Dispose of used or unused reagents as well as any other contaminated disposable materials following procedures for infectious or potentially infectious products.
It is the responsibility of each laboratory to handle waste and effluents produced according to their nature and degree of hazardousness and to treat and dispose of them (or have them treated and disposed of) in accordance with any applicable regulations.

LITERATURE REFERENCES

1. LAVANCHY D., Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention. Journal of clinical virology 34 suppl. 1 (2005) S1-S3.
2. FATTOVICH G., BORTOLOTTI F., DONATO F. Natural history of chronic hepatitis B : Special emphasis on disease progression and prognostic factors. Journal of hepatology 48. (2008) p 335-352.
3. LLOVET J.M., BRUIX J. Novel advancements in the management of hepatocellular carcinoma in 2008. Journal of hepatology 48 (2008) suppl S20-S37.
4. BLUMBERG B.S., ALTER H.J., VISNICH S. JAMA, A "New" Antigen in Leukemia Sera, 1965, 191, 541-546.
5. PRINCE A.M., An antigen detected in blood during the incubation period of serum hepatitis, Proc Natl Acad Sci USA, 1968, 60, 814-821.

INDEX OF SYMBOLS

Symbol	Meaning
REF	Catalog number
IVD	*In Vitro* Diagnostic Medical Device
	Manufacturer
	Temperature limit
	Use by date
LOT	Batch code
	Do not use if package damaged
	Consult Instructions for Use
	Contains sufficient for <n> tests
	Do not re-use
	Date of manufacture

LIMITED WARRANTY

bioMérieux warrants the performance of the product for its stated intended use provided that all procedures for usage, storage and handling, shelf life (when applicable), and precautions are strictly followed as detailed in the instructions for use (IFU).
Except as expressly set forth above, bioMerieux hereby disclaims all warranties, including any implied warranties of merchantability and fitness for a particular purpose or use, and disclaims all liability, whether direct, indirect or consequential, for any use of the reagent, software, instrument and disposables (the "System") other than as set forth in the IFU.

REVISION HISTORY

Change type categories

N/A:	Not applicable (First publication)
Correction:	Correction of documentation anomalies
Technical change:	Addition, revision and/or removal of information related to the product
Administrative:	Implementation of non-technical changes noticeable to the user
Note:	*Minor typographical, grammar, and formatting changes are not included in the revision history*

Release date	Part Number	Change Type	Change Summary
2015/07	16842 - B	Technical	Content of the kit (25 tests) Materials required but not provided Warnings and Precautions Limitations of the method Results and Interpretation
		Administrative	Intended use Principle Storage and Stability Specimen collection and preparation Instructions for use Performance Revision history Index of symbols
2016/06	16842 - C	Administrative	Instructions for use Index of symbols Limited Warranty

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Annex 8- Vaccine Coverage Data, selected schools in the Greater Male' Region- School Health Screening of Grade 1: 2022-2023.

School	Total in Grade 1	Total HS Screening	Health Screening Coverage	HepB Birth dose given (no.)	HepB birth dose coverage	Penta 3 given no.	Penta3 coverage	No data number	No data
TS	117	113	97%	113	100%	113	100%	0	0%
Aminiyya	237	177	75%	167	94%	168	95%	8	5%
Billabong	67	60	90%	60	100%	60	100%	1	2%
Dharumavantha	53	50	94%	49	98%	49	98%	0	0%
Hiriya	152	147	97%	147	100%	147	100%	0	0%
Iskandhar	198	176	89%	176	100%	176	100%	0	0%
Izzudheen	102	84	82%	83	99%	83	99%	0	0%
Jamaludheen	207	171	83%	170	99%	171	100%	0	0%
Kamil Didi (original students)	193	134	69%	134	100%	134	100%	0	0%
Majeedhiya	136	125	92%	123	98%	124	99%	1	1%
Muhiyuddhin	44	23	52%	23	100%	23	100%	0	0%