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COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

# SEROPREVALENCE OF HEPATITIS B VIRUS INFECTION AND ASSOCIATED FACTORS AMONG HEPATITIS B FULLY VACCINATED CHILDREN IN DEBRE MARKOS TOWN, NORTH-WEST ETHIOPIA

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## ABBREVIATIONS AND ACRONYMS

ASO-Adjusted Odds Ratio

CHB - Chronic Hepatitis B

COR - Crude Odds Ratio

CSA- Central Statistical Agency

DHS –Demographic Health Survey

DNA - Deoxyribonucleic Acid

ELISA- Enzyme-linked Immune-Sorbent Assay

EPI - Expanded Program for Immunization

GHSS- Global Health Sector Strategy

HBcAb -Hepatitis B core Antibody

HBcAg - Hepatitis B core Antigen

HBIG- Hepatitis B Immunoglobulin

HBsAg - Hepatitis B surface Antigen

HBV- Hepatitis B Virus

HCC- Hepatocellular carcinoma

HIV - Human Immune Virus

HRP - Horse Radish Peroxide

MAb- Monoclonal Antibody

MIU/ ml- Mili International Unit per milliliter

ORFs- Open Reading Frames

RNA - Ribonucleic Acid

RT- Reverse Transcriptase

WHO- World Health Organization

# ABSTRACT

**Background**: Hepatitis B virus is a highly infectious disease that causes serious health problems globally. Hepatitis B vaccination is recommended for all children at birth within 24 hours or during childhood to minimize the exposure as well as to immunize them to withstand the virus.

**Objective:** The main aim of this study was to assess the seroprevalence and associated factors of hepatitis B virus infection among hepatitis B vaccinated children in Debre Markos town Amahara region, North West Ethiopia.

**Methods and Materials:** A community based cross-sectional study was conducted among 165 children with 5-12 years of age from February 20/2021 to October 13, 2021. A simple random sampling technique was used to recruit study participants by proportional allocation into different Kebeles of DebreMarkos town .After 3-5 ml of venous blood was drawn from each study participants, serum sample was utilized to determine hepatitis B surface antigen (HBsAg) and anti-Hepatitis B core antibody by ELISA and associated factors were also assessed. Data were collected using a pretested structured questionnaire. The data was double entered to Epi data version 3.1 and exported to SPSS version 25 software for statistical analysis of the data. Logistic regression with 95% CI was used to show the statistical association. P value < 0.05 was considered as statistically significant association.

**Results:** The seroprevalence of HBsAg and anti-HBc anti-body among all study subjects was 4.2 % and 4.8 % respectively. Multivariable logistic regression revealed that children who were born from HBV positive mothers (AOR 3.917, 95% CI: 1.456, 5.365) (P<0.027) and those who had previous history of injectable medications (AOR 9.232, 95% CI: 1.503, 56.697) (P<0.016) were more likely to be HBsAg positive. Children with previous history of hospital admission (AOR 6.973, 95% CI: 1.495, 32.530) (P<0.013) were more likely to be anti-HBcAb positive.

**Conclusion:** There was an intermediate prevalence of HBV infection in our study area. Therefore, there should be a regular screening of children for HBV infection after vaccination.



**Key words:** Hepatitis B virus, Seroprevalence, HBsAg, Anti-HBc, DebreMarkos, North West Ethiopia

# 1. INTRODUCTION

## 1.1 Background

The hepatitis B virus (HBV) is a partially double-stranded DNA virus of the Hepadnaviridae family [1]. It's Genome is approximately 3200 bp [2]. The strands are composed from a partial positive strand and complete negative strand. The so called Dane particle and contains different proteins like inner icosahedral core antigen (HBcAg), hepatitis B surface antigens (HBsAg) and envelope antigen (HBeAg [3, 4].

HBsAg is the antigenic determinants present on the surface of HBV. It is an indicator of active HBV infection either acute or chronic. HBcAg is the antigenic specificity derived from the protein which encloses the viral nucleic acid and cannot be detected in the blood. The presence of IgM antibody to hepatitis B core antigen (IgM anti-HBc) indicates recent infection. However the presence of total anti-HBcAb shows previous or ongoing HBV infection with undefined time frame. HBeAg is the antigenic determinants closely related with the nucleocapsid of HBV. The presence of HBeAg associated with high level of viral replication and infectivity [5, 6].

The (−) strand contains four overlapping open reading frames (ORFs); (PreC/C, P, PreS/S, and X) with four regulatory promoters such as the core promoter, the PreS1 promoter, the PreS2/S promoter, and the X promoter and two enhancers (EnhI and EnhII) [7].

The longest ORF (P) encodes the viral polymerase which has reverse transcriptase (RT) activity for the generation first strand DNA genome from pregenomic RNA intermediate. PreS/S) encode the three viral envelope proteins such as large, middle and small surface antigen (HBsAg). The other ORF (PreC/C) encodes HBeAg, and HBcAg or the core proteins, which makes up the viral capsid and the smallest ORF (X), encodes the HBV X protein (HBx) (which is a small regulatory protein that is significant for HBV replication [8-11]. The virus can be transmitted through vertical and horizontal means from HBsAg carriers [12]. There are different potential risk factors for the exposure of hepatitis B infection. Infections mostly happen in infants and children due to maternal-neonatal transmission (mother-to-child transmission) or close childhood contact with family members [13].

It can also spread by reuse of contaminated sharp materials either in the community or among intramuscular drug users like contaminated syringes and needles either in the community or among intramuscular drug users or may be in the health care facility. It can also transmit through infected body fluids, blood, body piercing, and tattooing. Children with HBV infection mostly show symptoms such as yellowing skin and eyes (jaundice), low-grade fever, light colored stool, fatigue ,abdominal pain in upper right side liver ,loss of appetite, nausea, vomiting, itching , and rash appear from 25 to 180 days after exposure to the. However, majority of HBV infection in children are subclinical and about 30% to 50% develop acute hepatitis. It causes both the acute and chronic infection with hepatocellular carcinoma and cirrhosis [14].

According to the World Health Organization, one-third of the global population has serological markers of active or occult HBV infection. Moreover, approximately 780000 people die every year due to hepatitis B [15, 16]. The problem of developing CHBV infection has inversely relationship with the age. The risk of chronic infection is 90% in children younger than 1 year ; 30% for those 1–5 year [17].

World Health Assembly has taken the Global Health Sector Strategy (GHSS) on viral hepatitis in 2016 so as to minimize HBV by 2030 [18]. The target of the (WHO) GHSS is to decrease hepatitis new case from 6-10 million cases to 0.9 million cases and to reduce annual hepatitis deaths from 1.4 million to 0.5 million, by 2030. One of the five core area intervention which is documented by WHO's GHSS to eliminate hepatitis by 2030 is HBV vaccination [19].

Nowadays, greater than 200 countries have performed a universal HBV vaccination program for infants or neonates, based on the WHO recommendation [20, 21]. The purpose of such vaccination is to induce the immune response in the host and resulting in the prevention of HBV replication [22] .The aim of active immunization against HBV is to enhance the immunity in the host and results loss of HBV surface antigen (HBsAg) and control of HBV replication [23]. WHO set a plan to decrease HBV case in children under-five to below 1% by 2020, and decreasing the prevalence to 0.1% by 2030 [24].

In Ethiopia, HBV vaccination as a part of pentavalent has been introduced to the national Expanded Program for Immunization (EPI) since 2007 [25]. According to EPI schedules, HBV vaccine is given at 6, 10, and 14 weeks of age after delivery [26]. However, the vaccine is not

always protective against HBV and it may fail due to different reasons like vaccination delays, waning anti-HBs levels, vaccine escape mutations and other factors. Due to this, the children may expose to hepatitis B infection after vaccination [27]. In Ethiopia, there is ignorance about the hepatitis B virus infection determination in post vaccination in the community .Furthermore, currently there is no published data in Debre Markos town. Thus; this study aimed to fill this gap by assessing the seroprevalence of hepatitis B virus infection and associated factors among HBV vaccinated children in Debre Markos town. North West, Ethiopia.

### 1.2 Statement of the Problem

Hepatitis B is one of the most critical viral diseases affecting humans all over the world. More than a million deaths each year are resulted from hepatitis B infection. The cause of the disease the HBV-is found more than 350 million chronic carriers, which is greater than 5% of the global population [28]. Based on the recent WHO report, one- third of the world's populations have serologic evidence of present or past HBV infection [29, 30]. It is a leading cause of both chronic and acute liver disease including cirrhosis and liver cancer, which ranks as the 3$^{rd}$ cause of cancer deaths worldwide. Two billion people have been globally infected with HBV based on WHO estimation [31, 32] , and 1. 2 million die annually from chronic hepatitis, HCC [33].

It causes 60 to 80% of the world's primary liver cancers [34], and plays a significant role for morbidity worldwide. Current estimates suggest that it causes 30% of cirrhosis and approximately 50% of HCC globally [35]. Chronic infection with HBV is a paramount cause of death from cirrhosis and liver cancer, mainly in South-East Asia and sub-Saharan Africa [36, 37]. Africa has the second biggest quantity of persistent HBV carriers next to Asia and is taken into considered as a region of extraordinarily endemicity [38].

Approximately 60 million people in Africa are chronically infected with HBV, mostly acquired prenatally [39, 40] and they become persistent carriers [41]. Vertical transmission to neonates, or infections during the first years of life, results in persistent infection in more than 90% of children [42]. Babies born to a mother who are positive with HBsAg and HBeAg markers have ≥90% a probability of getting infected and becoming chronic carrier [43]. Of them , 15 to 25% have risk of dying from cirrhosis or liver cancer during adulthood [44]. Generally during childhood, liver damage due to HBV infection is mild but severe liver disease such as cirrhosis and HCC can develop for 2–7 years [45].

It is also an important cause of morbidity and mortality with a very high burden in Africa [46]. The highest rates of chronicity are seen as the result of vertical transmission (90%) and in children less than 5 years of age who are infected horizontally through household contacts (25 to 30%). The case of chronic infection is lowest after acute infection during adulthood (<10%) [47].

It is an important public health problem in Ethiopia too. The distributions vary with age, population practice, ways of exposure, and geographical area [48-50] . According to A previous national survey 10.8% of young men had HBsAg , and 73.3% had at least one HBV marker [51]. Based on the report from systematic review on HBV in Ethiopia, the prevalence of HBV was 6% and it was high among pregnant women, HIV positive patients and healthcare workers [52].

There is HBV vaccination program for children to minimize the case of HBV carrier. However, different factors like age, gender, obesity, drinking, smoking, nutritional status, poverty, socioeconomic status, lack of education, vaccination schedule and the host immune status have affect the protective efficacy (PE) of hepatitis B vaccine among children despite they took recommended dose of vaccine [53-57]. The other factor is poor Cod chain management system during transporting and storing of the vaccine. One study conducted in east Gojjam Zone showed that out of 60 health facilities, only 48.3% had correct vaccine storage temperature in the refrigerator and 38.3% of the health professionals had sufficient know how about vaccine cold chain management [58].

After vaccination 5–15 years, 15–50% of the vaccinees have undetetctable concentrations of anti-hepatitis B surface antibody and 10% still susceptible to HBV infection [59, 60]. According the studies conducted in Addis Abeba and Gondar, the vaccine response rate among HBV vaccinated children were 54.3% and 32.2% respectively. The prevalence of HBsAg among HBV vaccinated children in Jimma, Hawassa, and Gondar were, 2.1%, 4.4%, 4.2% and anti-HBc were 1.1%, 19.5%, and 6.3% respectively [61-64].

### 1.3 Justification of the study

Despite the coverage of HBV vaccination among children is high and reaches to 90% in some setting of Ethiopia including Amhara regional state, they are still susceptible to HBV infection. Though some studies have been done in some parts of the country regards to the determination of seroprevalence of HBV infection after vaccination in children, it is not done in our study area.

Because it is very crucial to determine the effectiveness of hepatitis B vaccine, to identify factors which affect the strength of hepatitis B vaccine and have contribution for HBV infection among vaccinated children. Since there is a risk of getting infection in vaccinated children when their antibody level or titter is decrease with time. As a result; they may also expose to break through infection.

Since this study will serve as a baseline for further control of HBV infection and it will also bring evidence on the seroprevalence of HBV infection and associated factors. As according to the center of disease control and prevention (CDC), persons who were born from country with HBV prevalence ≥ 2% must be screened for HBV serological markers regardless of vaccination status and our country is found under this category.

### 1.4 Significance of the study

As hepatitis B infection is one the most critical public health problem, the outcome of this study is significant to minimize the prevalence of HBV infection in the country and to decrease the magnitude of HBV infection among the general population. Furthermore, it is also crucial for policy makers for the formulation of evidence-based health policy to increase health care quality.

The outcomes of the findings will also important for other concern bodies at the national health bureaus by providing information about seroprevalence of HBV infection among children in Debre Markos town and also enhance our understanding regards to post vaccination infection in Ethiopia. It provides information about the performance of HBV vaccine in children. Therefore, it is crucial to accelerate the introduction of new vaccine, stimulate development of better HBV vaccine, and also critical to promote immunization, sustain and optimize vaccine uptake. It will motivate the administration of booster dose among vaccinated children based on the result. It will also important for researchers and practitioners.

Locally, the finding reported from this study is very interesting to minimize the morbidity and mortality rate of liver cancer following advance HBV infection in the community and to give information about prevalence and associated factors of HBV infection among children for DebreMarkos health bureau. Moreover, it enhances the establishment of community-based networks to improve HBV screening and linkage to care, treatment for those children who were positive for HBsAg.

# 2 LITERATURE REVIEW

## 2.1 Seroprevalence of HBV infection

Various studies were conducted in different part of the world on the seroprevalence of hepatitis B virus infection and associated factors among vaccinated children .For instance, a cross-sectional study was performed among 6,949 children who have been born during 2000-2008 using a stratified three level cluster technique in Vietnam, USA after the implementation of 3 dose of HBV vaccine. Blood sample was collected from each surveyed children and tested for HBsAg. Out of which the overall prevalence of HBsAg was 2.70% (95% confidence interval (CI): 2.20–3.30) [65].

A comparative study was done in Uzbekistan, Japan among 329 children with the age of 6 years old who were vaccinated at birth and were tested for HBsAg. Of which 0.8% of children were positive for HBsAg [66]. Another study was also done in Southern Iran on the seroprevalence of hepatitis B infection among 394 vaccinated school children with the age of 6-8 years. Blood samples were drawn from each study participants and serum was separated and stored -20°C until being tested .Each sample were tested for anti-HBc antibodies to confirm past or current infection and tested for HBsAg to show chronic infection. Of them 2.1% were positive for HBcAb and 0.6% were positive for HBsAg positive[67].

Moreover, there was a study conducted at Italy among 522 children who were born from hepatitis B surface antigen positive mothers from 1985 to 1994. Hepatitis B immunoglobulin (HBIG) and hepatitis B vaccine were given to them .At 5-14 years after immunization serological markers of hepatitis B infection was determined .Of which 3(0.6%) were positive for HBsAg and 17(3.3%) were positive for anti–HB core antigen [68].

A community based study also done in Brazil after 10 years of hepatitis B vaccination among 405 children with a mean age of 11.4 ±0.9. Of this 53.6% were female participants and they were tested for anti-HBc. Of them 18 (4.4%) study participants were positive for anti-HBc [69]. In Greenland, Population-based retrospective cohort study was conducted among 120 vaccinated children and 3.3%(4/120) children were positive for HBsAg (chronically infected) [70].

In thailand, epidemiological survey was performed among 2887 children aged from 6 months to 18 years using simple random sampling technique from May 2004-october 2004 so as to evaluate

the effect of universal hepatitis B vaccination programme on the seroprevalence of hepatitis B markers. Serum sample was collected from each study participants then HBsAg and anti-HBc were detected using an automated ELISA reader, of which the overall prevalence of positive markers for anti-HBc and HBsAg were 5.5%, 1.4%, respectively [71].

A study was also done in china among 1591 children who had been administered hepatitis B vaccination on the 0–1–6 month schedule and they were tested for HBV serum markers .Of this 20.49% (326 of 1591) children were positive for HBsAg and anti-HBc [72].

A case control crossectional study was conducted at rural Nigeria among 223 children whose ages were from 1-4 years and who took at least three dose of HBV vaccine at birth from February to march 2021. Blood samples were collected from them and HBsAg was detected using rapid immunoassay method according to the immunochromatographic principle. The prevalence of HBsAg was 1.3% and it was significantly higher among male participants [73].

A crossectional study was carryout at Ghana, Navrongo Health Research Center found in the Kassena Nankana District in the Upper East Region among 104 children who took full dose of hepatitis B vaccine and they were screened for HBsAg. Of which 2(1.9%) of them were HBsAg positive [74], and another cross-sectional study was performed in rural zone of niakhar, Senegal among 455 children aged from 6 months to 15 years after the introduction of three dose of HBV vaccine in the period of October to December 2018 and 7/455(1.5%),5/455(1.1%) of them were showed anti-HBcAb and HBsAg positive respectively [75].

A crossectional serological survey was carryout at two Gambian Villages among 1508 study subjects whose ages were from 1-28 years and took HBV vaccine during infancy from 1984-2003. They were tested for anti-HBc and HBsAg after blood samples were drawn them. Of whom 1251 were tested for anti-HBc serologically using ELISA and the prevalence was 10.2%. The other 1243 study subjects were tested for HBsAg using immunochromatography and the prevalence was 1.0%. Older age and being male were significantly associated with HBV infection [76].

A study that was conducted in Egypt showed that out of 720 children whose ages were 10 years old and who were vaccinated with hepatitis B vaccine during infancy all of them were tested for hepatitis B serologic markers including HBsAg and anti-HBc) using 3rd generation ELISA

technique. Of them 6.8% of the vaccinated children were developed hepatitis B infections. HBsAg was detected in 0.6% only of the vaccinated children [77] ,and a national community-based multistage cluster sampling design was also performed in in Beni-Suef, Egypt among 362 children who were vaccinated during the childhood from the period of July 2010 to June 2013 and 4/362(1.1%) of the were developed breakthrough infection. Two of the infected children were positive for both HBsAg and anti-HBc, other two children were positive for anti-HBc only [78].

Similar study was done in Dakahleya-Egypt among 902 children and they were selected from 2 cities and 3 villages in Dakahleya by cross-sectional study. From those children 475 boys and 423 girls whose ages were ranged from 9 months to 16 years. All of them were received three doses of HBV vaccine at the time of infancy. Serum samples were tested for hepatitis B serological markers of HBsAg and HBcAb using ELISA .The prevalence HBsAg and anti-HBc were 4/902 (0.4%), 1/902(0.11%) respectively [79].

In addition , a community based cross-sectional study was also performed in Egypt nationally among 3600 fully vaccinated children during infancy period with 9-16 years old and they were tested for HBsAg and total HBcAb using enzyme linked immunoassays. The prevalence of HBsAg and total anti-HBc in vaccinees were 0.11% and 0.36% respectively [80].

In Ethiopia a cross-sectional study was done at jimma town among 380 vaccinated children with their ages from 5-9 years old. Out of which the prevalence of HBsAg and anti-HBc were 2.1% and 1.1% respectively. The prevalence of HBsAg was higher in children who were born from illiterate mothers and urban area. The prevalence of anti-HBc was higher (4.1% vs 1.8%) among children who were born from urban than those from rural [61] ,and community-based comparative cross-sectional study design was applied among 284 vaccinated children using simple random sampling in selected kebeles in Harar, Eastern Ethiopia. Serum samples were separated after 3-5 milliliters blood collection was done in every study subjects and tested for HBsAg using ELISA. Of which the prevalence of HBsAg among study participants was 1.1%, showing chronic liver diseases [81].

Furthermore, a community based cross-sectional study was carryout using multistage sampling technique among 471 children with ages of 5-8 years old in Hawassa city, southern Ethiopia

from May to September in 2018. Blood samples were drawn from each study participants; sera were separated and used for the screening of anti-HBc and HBsAg. The overall prevalence of anti-HBc and HBsAg were 19.5 and 4.4 % respectively. All children with anti-HBc positive were also HBsAg positive. In this study, children with HBsAg seropositive mothers, family history of liver disease, and with history of injectable medication were higher risk for HBsAg markers [62]. Prospective cross-sectional study design was also conducted in Addis Abeba, Ethiopia among 383 hepatitis B vaccinated children with their mean age of 7±1 (SD) years. Of them the seroprevalence of anti-HBc and HBsAg were 5.6% and 0.4% respectively [64].

Another cross-sectional study was done in Ethiopia at Gondar city among 431 children with their ages was from 5-9 years old. Of them 49.4% (213/431) were female. Of which, 69.4% (299/431) of children had normal a body mass index (BMI), whereas 20.4% and 10.2% of children were underweight and over-weight, respectively. Out of which, 12.8% (55/431) were born to mothers who had a previous history of hepatitis B. Of them the prevalence of HBsAg and anti-HBc were 4.2% and 6.3% respectively [63].

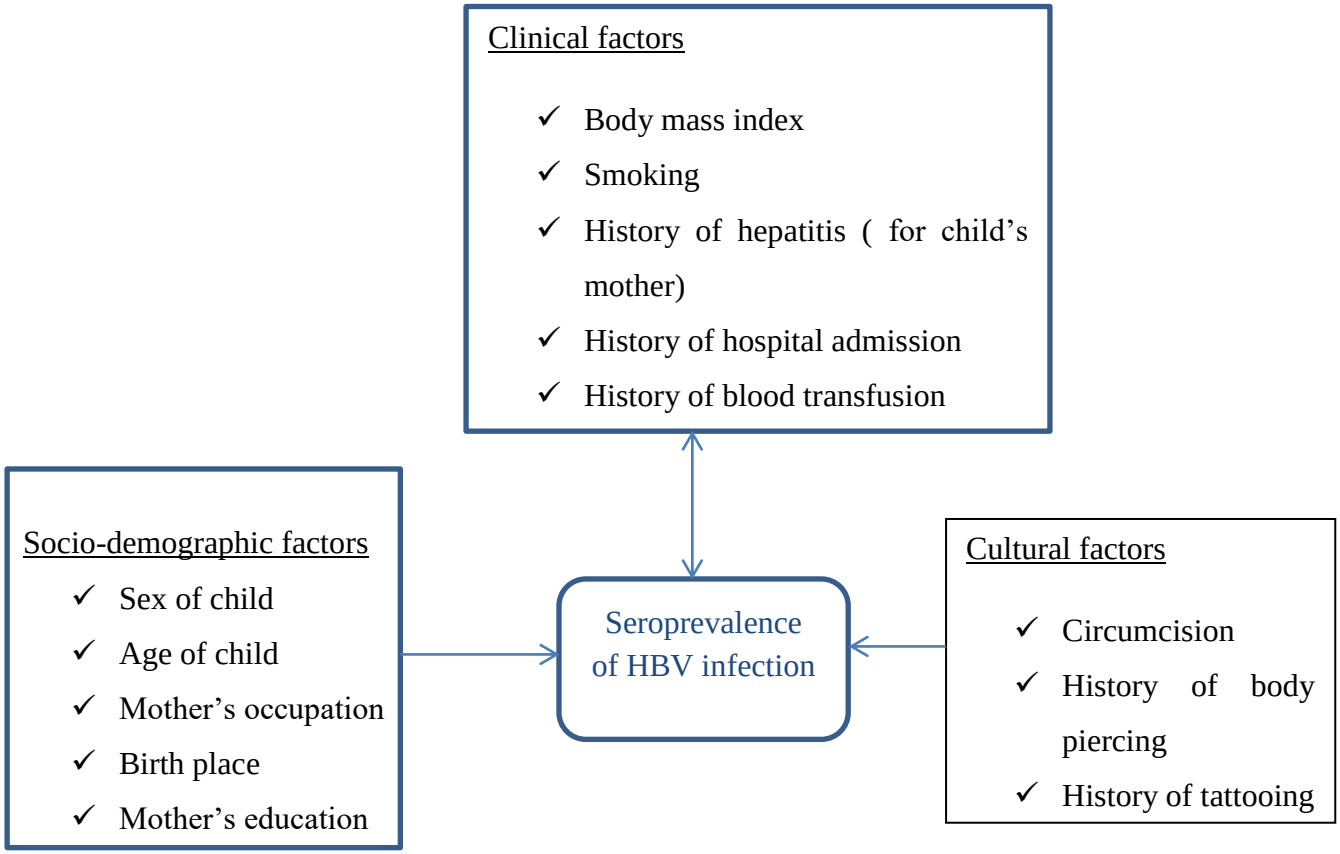



*Figure 1: Conceptual framework adopted from different literature*

# 3. OBJECTIVES

## 3.1 General objective

To assess seroprevalence of hepatitis B virus infection and associated factors among fully vaccinated children in Debre Markos town, North West Ethiopia.

## 3.2 Specific objectives

1. To determine the prevalence of HBsAg among hepatitis B vaccinated children.
2. To detect Anti-HBc antibodies (HBcAb) among hepatitis B vaccinated children.
3. To identify factors associated with seroprevalence of hepatitis B infection among children.

# 4. MATERIALS AND METHODS

## 4.1 Study design and period

A community-based cross-sectional study was conducted among hepatitis B vaccinated children in Debre Markos town, Amahara regional state, North West Ethiopia, from February 20, 2021, to October 13, 2021.

## 4.2 Study setting

Debre Markos town is the capital city of East Gojjam which is located 300Km Northwest of Addis Ababa, the capital of Ethiopia, and 265Km Southeast of Bahir Dar, the Regional capital city of Amahara national regional state. Based on the 2007 national census conducted by the Central Statistical Agency of Ethiopia (CSA) estimation, the total population size of Debre Markos town in 2012 was estimated to be 262,497, out of this 132,576 were women and 129,921 were men [82] . Based on the report from East Gojjam Zone administrative office, the number of children < 15 years was 18,345. Of which the number of girls and boys were 10,020 and 8,325 respectively. There is one comprehensive specialized hospital, three governmental health centers, seven health posts and more than 10 private clinics.

## 4.3 Source population

All hepatitis B vaccinated children whose ages were from 5-12 years and live in Debre Markos town were considered as source population.

## 4.4 Study population

Those hepatitis B vaccinated children whose ages were from 5-12 years, who fulfill eligibility criteria and were found at home during the data collection period was taken as study participants.

## 4.5 Inclusion and Exclusion criteria

### 4.5.1 Inclusion criteria

All hepatitis B vaccinated children who were volunteers to participate in the study, whose parents or legal guardians were volunteers to give assent and

those children who took full dose of hepatitis B vaccine were included in the study.

4.5.2 Exclusion criteria

Those study participant who or whose parents were severely sick during the data collection period.

Those study participants, who were on antiviral medication during the data collection period.

## 4.6 Study variables

### 4.6.1 Dependent variable

Seroprevalence of Hepatitis B virus infection

### 4.6.2 Independent variables

**Socio-demographic factors:** Sex, age, Job, birth place, and Educational status.

**Clinical factors:** Mother's history of hepatitis, History of hospital admission, History of blood transfusion, history of injectable medications, Body mass index, and smoking.

**Cultural factors:** Circumcision, History of body piercing, and History of tattooing.

## 4.7 Operational Definition

**Children:** Are group of individual whose ages are from 5 to 12 years.

**Vaccinees:** a person who took vaccine

**Legal guardian / caregiver**: is the any responsible person who gives care legally for the child who has no mother due to different reasons.

**Kebeles:** Small administrative unit in Debre Markos town.

**Fully vaccinated**: Those children who took 3 dose of HBV vaccine

**Break through infection:** an infection which occurs after vaccination

**HBsAg:** is viral surface protein in child's serum which shows active HBV infection, either acute or chronic.

**Anti-HBc:** an antibody against HBV core antigen in the serum that shows ongoing or previous HBV infection with undefined time frame.

**Seroprevalence:** is the rate of seropositivity of HBV infection in children.

**Intermediate prevalence:** When seroprevalence of HBV infection is 2-7%

## 4.8 Sample Size Determination and Sampling Techniques

### 4.8.1 Sample size determination

The required samples size for this study was calculated using 89% of prevalence in vaccine response taken from study which was conducted at Addis Abeba, Ethiopia, [83]. By using the following single population proportion formula:

$$= \frac{\left(\frac{Za}{2}\right)^2 pq}{d^2}$$

Where n= sample size

p= proportion

q= 1-p

Z= confidence interval

p =0.89 q= 0.11 with confidence interval of 95% Z= 1.96

$n= \frac{(1.96)^2*0.89*0.11}{(0.05)^2} =150$

The final sample size was adjusted as follows:

Sample size = n (sample size) + 10% non-respondent. Therefore; final sample size (n) was calculated as n = 150+15 =165. It was proportionally allocated to each selected kebeles.

### 4.8.2 Sampling Techniques

Simple random sampling technique was used to recruit the study participants until the required sample size was obtained among hepatitis B vaccinated children at the selected kebeles in Debre

Markos town. Debre Markos Town has 11 kebeles out of them 5 kebeles (45 %) were selected by lottery method so as to keep representativeness. In this way Kebeles such as 04, 05, 06, 10, and 11 were selected by this method. Then total sample size was allocated proportionally to each kebeles depending on the total number of vaccinated children from 5 to 12 years of age. Sampling frame was obtained from health extension workers' registration books.

Households in the kebeles were visited until the proportionally allocated sample size for each kebeles was fulfilled. One child was selected randomly (lottery method) from those households having two and more children. Then together with health post workers and trained nurses, we were collected data from each study participant.

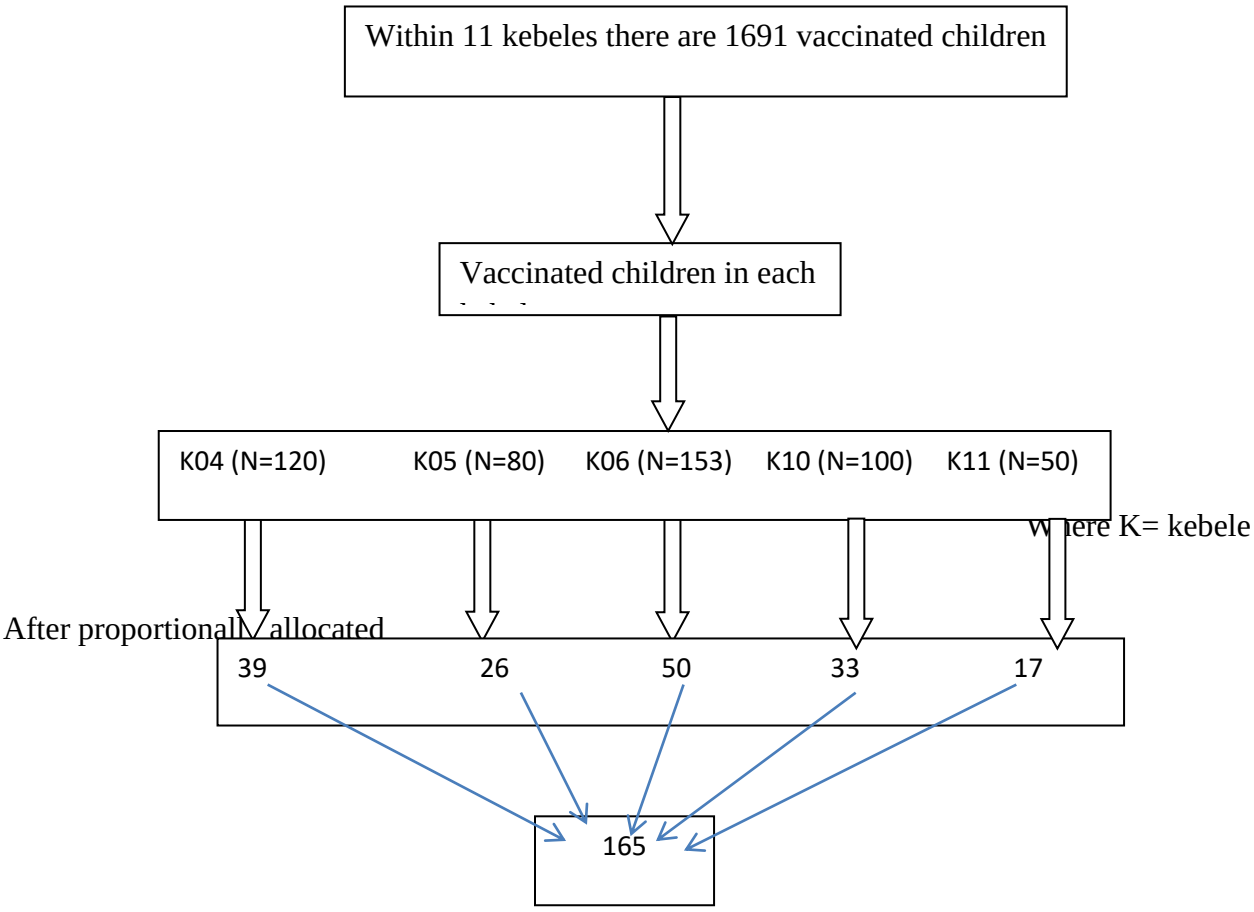



*Figure 2: Schematic presentation of sampling procedure*

### 4. 9 Data Collection Procedures

#### 4.9.1 Sociodemographic Data and Associated Factors

After the participant's family agreed to take part in the study and signed on informed written assent form, socio-demographic characteristics and associated factors data were collected by using a pretested structured questionnaire. Data about children's vaccination status were collected by health extension workers from their vaccination cards, immunization chart and history in the selected kebeles. Each child's weight and height were measured and body mass index was calculated in percentile. All those data were collected by trained data collectors who had data collection experience.

#### 4.9. 2 Laboratory Procedures

About 3-5 ml of venous blood sample was aseptically collected by making venipuncture from each study participant. The sample was labelled and packed in accordance with the existing local and international regulations for clinical specimen transportation. All collected samples from different Kebeles of Debre Markos town were transported to Debre Markos blood bank laboratory using a cold box with ice packs for storage. Serum was separated from whole blood by centrifugation, and then stored at -20oC until laboratory analysis was done. Then serum sample was transported to Armauer Hansen Research Institute (AHRI) using a cold box with ice packs for laboratory analysis.

Serum level of HBsAg and anti-HBcAb were determined using sandwich Enzyme-Linked Immunosorbent Assay (ELISA), where antigens/monoclonal antibodies were used both for capture and detection. All ELISA experiments were performed using BIO-RAD, Monolisa ELISA test kits, France with 100% sensitivity and 99.28% specificity. Each test procedure was performed according to the manufacturer's instruction based on standard operating procedures.

HBsAg was detected by Monolisa™ HBs Ag ULTRA ELISA kit .The Monolisa™ HBs Ag ULTRA microplate was pre-coated with capturing monoclonal antibody and 100 µl serum samples were added on each microplate wells. Then secondary antibody conjugate with peroxidase was added as detecting antibody and antibody-antigen-enzyme conjugated antibody complex was formed. It was detected spectrophotometrically after stopping solution was added at 450/620-700 nm optical density using a plate reader.

Anti-HBcAb was detected by Monolisa™ Anti-HBc PLUS ELISA kit. Total antibodies to hepatitis B virus core antigen were simultaneously detected indirectly in the serum. There was pre-coated hepatitis B core antigen with microplate wells. Then 100 µl serum samples were added on each well. Then antibodies labeled with peroxidase were added to human IgG and IgM and antigen-antibody-enzyme labeled antibodies complex was formed. After the reaction was stopped, the absorbance values were read using a spectrophotometer at 450/620-700 nm [84].

### 4.10 Data Quality Control

For the generation of quality and reliable data, all quality control was done. All the questions in a structured questionnaire were prepared in a clear and precise way and translated into the local language (Amharic).The training was given for data collectors on how to collect data from study participants. Supervision was conducted on data collector and a pre-test was applied to identify the potential problems and to assure the completeness, simplicity, and validity of the questionnaire during data collection. It was also done before to data collection to optimize the experimental set up. This was done in samples obtained from 5% (8.0) of study participants.

Before sample collection, each test tube was properly labeled the participant's ID, age, sex and date of collection for easily identification of the sample. Sample transportation was also performed with a cold box and preserved at deep freezes to maintain the quality. The laboratory environment like temperature and humidity of refrigeration was also recorded. We were also strictly followed the procedural manuals during reagent preparation, testing, and incubation times. Pipette performance was also optimized by choosing right the pipette for the work and using the correct pipette tip with the correct size and style.

All the laboratory procedures were conducted with experienced professionals and quality control and blank samples were run along with study samples. Standards were checked for each ELISA kit with known positive and negative control samples according to the kit protocol before the real laboratory analysis was done. Laboratory results were read, reported, and interpreted based on the kit protocol and correct reference range.

### 4.11 Data Processing and Analysis

After collection data were checked, corrected then entered and cleaned into a computer by using EpiData software version 3.1 and export to SPSS software vision 25 for analysis. Data were

analyzed by using SPSS version 25 software package. The results were presented by tables. Frequencies and percentages were calculated using descriptive statistics to summarize the results of categorical variables.

Logistic regression was used to show the strength and statistical association between the dependent and independent variables. Crud odds ratio (COR) was analyzed by binary logistic regression and those Variables with their p-value <0.25 were candidates for multivariate logistic regression. Then multivariate logistic regression was used in terms of adjusted odds ratio (AOR) with 95% confidence intervals to minimize the confounding effect and p-value < 0.05 was considered as statistically significant.

### 4.12 Ethical consideration

This study was approved after it was ethically reviewed by the research and ethical committee of Debre Markos University, College of Health Science. A Permission letter was written from the Debre Markos town health office to each kebele. The Participant's guardian was asked to sign on the written informed assent. All the information obtained from the study subjects were coded to keep confidentially.

### 4.13 Dissemination and utilization of result

The result of this research will be submitted and presented to the Department of Medical Laboratory Sciences, College of Health Sciences, Debre Markos University. Moreover the results of the study will be discussed with potential collaborators and any concerned bodies. The results of this study will finally be submitted for publication in a peer-reviewed journal.

# 5 RESULTS

## 5.1. Socio-demographic characteristics

A total of 165 children with the age range of 5-12 years were participated in this study. The median ages of the study participates were 8.00 ± 2.58. About 45.5% of the study participants were found within age group of 5-7 years .Among our study participants, majority of them i.e. 89 (53.9%) were males. One hundred fifty (90.9%) of participants were from urban area (table 1)

*Table 1: Socio-demographic characteristics of children and their parents at Debre Markos town, North West Ethiopia in 2021*

| Socio-demographic variables | Age category | Frequency | Percent |
|---|---|---|---|
| Age in years | 5-7 | 75 | 45.5 |
| | 8-10 | 49 | 29.7 |
| | ≥11 | 41 | 24.8 |
| Sex of child | Male | 89 | 53.9 |
| | Female | 76 | 46.1 |
| Birth place of child | Rural | 15 | 9.1 |
| | Urban | 150 | 90.9 |
| Educational status of parents | Illiterate | 50 | 30.3 |
| | Primary school | 20 | 12.1 |
| | Secondary school | 40 | 24.2 |
| | College and above | 55 | 33.3 |
| Occupation of parents | Farmer | 21 | 12.7 |
| | Government employer | 43 | 26.1 |
| | Private | 24 | 14.5 |
| | Daily laborer | 17 | 10.3 |
| | House wife | 29 | 17.6 |
| | Merchant | 31 | 18.8 |
| Total | | 165 | 100.0 |

### 5.2 Seroprevalence of HBsAg and Anti-HBc

The overall prevalence of HBsAg among all study subjects was 7/165 (4.2%) (95% CI: 1.0- 7.0) .From seven children who were positive for HBsAg, 6.7% of them were male participants and found within the age group of 5-7 years. There was zero prevalence of HBsAg among children whose ages were ≥ 11. From the total prevalence of HBsAg, 6/150(4.0%), 3/50 (6.0%) were from the urban residence and illiterate parents, respectively. The prevalence of HBsAg was 2/17 (11.8%) among children whose parents were daily laborer.

The overall prevalence of anti-HBc antibody among the study participants were 8/165 (4.8%) (95% CI: 2.0-8.0). From total prevalence of anti-HBc antibody, 4/76 (5.3%) was from female participants. About 9.8 % anti-HBc antibody prevalence was from children whose ages were found within the age group of ≥ 11 years. All anti-HBc antibody positive children were born from urban residence. The prevalence of anti-HBc antibody among children whose parents' educational status primary school was 2/20 (10.0%) and it was zero among children who were born from government employer (table 2).

*Table 2: The prevalence of HBsAg and anti-HBcAb with socio-demographic variables at Debre Markos town, North West Ethiopia in 2021*

| Variables | Category | HBsAg status | | | Anti-HBc | Status | |
|---|---|---|---|---|---|---|---|
| | | Positive | Negative | Total | Positive | Negative | Total |
| Age of child | 5-7 | 5(6.7%) | 70(93.3%) | 75 | 2(2.7%) | 73(97.3%) | 75 |
| | 8-10 | 2(4.1%) | 47(95.9%) | 49 | 2(4.1%) | 47(95.9%) | 49 |
| | ≥11 | 0(0.0%) | 41(100%) | 41 | 4(9.8%) | 37(90.2%) | 41 |
| Sex of child | Male | 6(6.7%) | 83(93.3%) | 89 | 4(4.5%) | 85(95.5%) | 89 |
| | Female | 1(1.3%) | 75(98.7%) | 76 | 4(5.3%) | 72(94.7%) | 76 |
| Birth place of child | Rural | 1(6.7%) | 14(93.3%) | 15 | 0(0.0%) | 15(100.0%) | 15 |
| | Urban | 6(4.0%) | 144(96.0%) | 150 | 8(5.3%) | 142(94.7%) | 150 |
| Educational status of parents | Illiterate | 3(6.0%) | 47(94.0%) | 50 | 2(4.0%) | 48(96.0%) | 50 |
| | Primary | 1(5.0%) | 19(95.0%) | 20 | 2(10.0%) | 18(90.0%) | 20 |
| | Secondary | 1(2.5%) | 39(97.5%) | 40 | 2(5.0%) | 38(95.0%) | 40 |
| | College and above | 2(3.6%) | 53(96.4%) | 55 | 2(3.6%) | 53(96.4%) | 55 |
| Occupation of parents | Farmer | 1(4.8%) | 20(95.2%) | 21 | 2(9.5%) | 19(90.5%) | 21 |
| | Government employer | 1(2.3%) | 42(97.7%) | 43 | 0(0.0%) | 43(100.0%) | 43 |
| | Private | 1(4.2%) | 23(95.8%) | 24 | 2(8.3%) | 22(91.7%) | 24 |
| | Daily laborer | 2(11.8%) | 15(88.2%) | 17 | 1(5.9%) | 16(94.1%) | 17 |
| | House wife | 1(3.4%) | 28(96.6%) | 29 | 1(3.4%) | 28(96.6%) | 29 |
| | Merchant | 1(3.2%) | 30(96.8%) | 31 | 2(6.5%) | 29(93.5%) | 31 |

Of the total prevalence of HBsAg, about 33.3% was from children who were born from mothers with previous history of HBV infection. The prevalence of HBsAg among obese children was 2/7(28.6%). However, it was zero among overweight children and those with previous history of

tattooing. All anti-HBc antibody positive study participants, 8/128 (6.2%) were found within normal body mass index. The prevalence of anti-HBc antibody among children with previous history of injectable medication was 1/15(6.7%). All anti-HBc positive children i.e.8/153 (5.2%) were without previous history of tattooing (table 3).

*Table 3: The prevalence of HBsAg and anti-HBcAb status by clinical and cultural variables*

| Clinical or cultural variables | Category | HBsAg status | | | Anti-HBc status | | |
|---|---|---|---|---|---|---|---|
| | | Positive | Negative | Total | Positive | Negative | Total |
| Full dose of HBV vaccine | Yes | 7(4.2%) | 158(95.8%) | 165 | 8(4.8%) | 157(95.2%) | 165 |
| | No | 0(0.0%) | 0(0.0%) | 0 | 0(0.0%) | 0(0.0%) | 0 |
| Mother's HBV status | Yes | 1(33.3%) | 2(66.7%) | 3 | 1(33.3%) | 2(66.7%) | 3 |
| | No | 6(3.7%) | 156(96.3%) | 162 | 7(4.3%) | 155(95.7%) | 162 |
| BMI status in percentile | Normal | 3(2.3%) | 125(97.7%) | 128 | 8(6.2%) | 120(93.8%) | 128 |
| | Uw | 2(11.1%) | 16(88.9%) | 18 | 0(0.0%) | 18(100.0%) | 18 |
| | Ow | 0(0.0%) | 12(100.0%) | 12 | 0(0.0%) | 12(100.0%) | 12 |
| | Obese | 2(28.6%) | 5(71.4%) | 7 | 0(0.0%) | 7(100.0%) | 7 |
| Smoking | Yes | 0(0.0%) | 0(0.0%) | 0 | 0(0.0%) | 0(0.0%) | 0 |
| | No | 7(4.2%) | 158(95.8%) | 165 | 8(4.8%) | 157(95.2%) | 165 |
| Hospital admission | Yes | 2(9.5%) | 19(90.5%) | 21 | 4(19.0%) | 17(81.0%) | 21 |
| | No | 5(3.5%) | 139(96.5%) | 144 | 4(2.8%) | 140(97.2%) | 144 |
| Blood transfusion | Yes | 1(33.3%) | 2(66.7%) | 3 | 1(33.3%) | 2(66.7%) | 3 |
| | No | 6(3.7%) | 156(96.3%) | 162 | 7(4.3%) | 155(95.7%) | 162 |
| Injectable medications | Yes | 3(20.0%) | 12(80.0%) | 15 | 1(6.7%) | 14(93.3%) | 15 |
| | No | 4(2.7%) | 146(97.3%) | 150 | 7(4.7%) | 143(95.3%) | 150 |
| Circumcision | Yes | 5(6.8%) | 69(93.2%) | 74 | 4(5.4%) | 70(94.6%) | 74 |
| | No | 2(2.2%) | 89(97.8%) | 91 | 4(4.4%) | 87(95.6%) | 91 |
| Body piercing | Yes | 3(13.0%) | 20(87.0%) | 23 | 1(4.3%) | 22(95.7%) | 23 |
| | No | 4(2.8%) | 138(97.2%) | 142 | 7(4.9%) | 135(95.1%) | 142 |
| Tattooing | Yes | 0(0.0%) | 12(100.0%) | 12 | 0(0.0%) | 12(100.0%) | 12 |
| | No | 7(4.6%) | 146(95.4%) | 153 | 8(5.2%) | 145(94.8%) | 153 |

NB: % = percentile, BMI= Body Mass Index, Normal weight BMI = 5th-85th %ile, Underweight (Uw) = <5th %ile, Overweight (Ow) = 86 th-95th %ile, Obese = ≥96th %ile

### 5.3 Factors associated with HBsAg and anti-HBcAb positivity

In bivariate logistic regression analysis, children who were born from mothers with history of hepatitis B infection (COR 13.000, 95% CI: 1.030, 16.040) (P<0.047), children with history of hospital admission (COR 2.926, 95% CI: 0.530, 16.155) (P<0.218), blood transfusion (COR 13.000, 95% CI: 1.030, 16.040) (P<0.047), injectable medications (COR 9.125, 95% CI: 1.827, 45.582) (P<0.007), and body piercing (COR 5.175, 95% CI: 1.078, 24.842) (P<0.040) were significantly associated with hepatitis B surface antigen positivity. Indeed, children who had previous history of hospital admission, blood transfusion (COR 8.235, 95% CI: 1.885, 35.979, COR 11.071, 95% CI: 0.893, 37.203) (P<0.005 and 0.061) respectively were significantly associated with anti-hepatitis B core antibody positivity.

Furthermore, multiple logistic regression analysis was also carry out after adjusting the potential confounding effects to identify significantly associated factors. In multiple logistic regression, children born from mothers with HBV infection were 3.9 times (AOR: 3.917, 95% CI: 1.456, 5.365) (P<0.027) more likely to have HBsAg seropositive as compare to those who were born from mothers without previous history of HBV infection. Children who had previous history of injectable medications were 9 times (AOR: 9.232, 95% CI: 1.503, 56.697) (P<0.016) more likely to be HBsAg positive than those without previous history of injectable medications. Moreover, children who had previous history hospital admission were 6.9 times (AOR: 6.973, 95% CI: 1.495, 32.530) (P<0.013) more likely to be anti-HBc antibody positive as compare to their counterparts (table 4 and 5).

*Table 4: Bivariate and Multivariate logistic regression of associated factors for HBsAg positivity at Debre Markos town, North West Ethiopia in 2021*

| Variables | Category | HBsAg status | | | | | |
|---|---|---|---|---|---|---|---|
| | | Positive | Negative | COR(95%CI) | P-V | AOR(95%CI) | P-V |
| Mother's | Yes | 1(33.3%) | 2(66.7%) | 13.000(1.030,16.040) | 0.047* | 3.917(1.456,5.365) | 0.027** |
| HBV status | No | 6(3.7%) | 156(96.3%) | Ref | | Ref | |
| Hospital | Yes | 2(9.5%) | 19(90.5%) | 2.926(0.530,16.155 | 0.218* | 1.539(0.185,12.828) | 0.690 |
| admission | No | 5(3.5%) | 139(96.5%) | Ref | | Ref | |
| Blood | Yes | 1(33.3%) | 2(66.7%) | 13.000(1.030,16.040 | 0.047* | 4.035(0.161,10.900) | 0.396 |
| transfusion | No | 6(3.7%) | 156(96.3%) | Ref | | Ref | 0.396 |
| Injectable | Yes | 3(20.0%) | 12(80.0%) | 9.125(1.827,45.582) | 0.007* | 9.232(1.503,56.697) | 0.016** |
| medication | No | 4(2.7%) | 146(97.3%) | Ref | | Ref | |
| Body | Yes | 3(13.0%) | 20(87.0%) | 5.175(1.078,24.842 | 0.040* | 4.264(0.640,28.405) | 0.134 |
| Piercing | No | 4(2.8%) | 138(97.2%) | Ref | | Ref | |

NB: *: Candidate variable for multivariate analysis at P < 0.25 and **: significant variable by the multivariate analysis at P < 0.05, COR: crude odds ratio, AOR: adjusted odds ratio, CI: confidence interval, P-V: p-value, Ref: reference

*Table 5: Bivariate and Multivariate logistic regression of associated factors for anti-HBcAb positivity*

| Variables | Category | Anti- HBc status | | | | | |
|---|---|---|---|---|---|---|---|
| | | Positive | Negative | CRO(95%CI) | P-V | AOR(95%CI) | P-V |
| Hospital | Yes | 4(19.0%) | 17(81.0%) | 8.235(1.885,35.979) | 0.005* | 6.973(1.495,32.530) | 0.013** |
| admission | No | 4(2.8%) | 140(97.2%) | Ref | | Ref | |
| Blood | Yes | 1(33.3%) | 2(66.7%) | 11.071(1.893,37.203) | 0.061* | 4.154(0.265,65.228 | 0.311 |
| transfusion | No | 7(4.3%) | 155(95.7%) | Ref | | Ref | |

NB: *: Candidate variable for multivariate analysis at P < 0.25 and **: significant variable by the multivariate analysis at P < 0.05, COR: crude odds ratio, AOR: adjusted odds ratio, CI: confidence interval, P-V: p-value, Ref: reference

## 6. DISCUSSION

HBV infection is one of the most important serious public health problems globally with very high burden of morbidity and mortality rate in Africa, especially in developing countries including Ethiopia [46] .To minimize this rate, above 200 countries have exercised universal HBV vaccination program for infants based on the WHO recommendations [20],and the vaccine has been introduced to national Expanded Program for Immunization (EPI) as a part of pentavalent in Ethiopia since 2007 [25].

However, different factors such as age, obesity, nutritional status, vaccination schedule, host immune status, cold chain management system etc. will affect the protective efficacy (PE) of hepatitis B vaccine among children despite they took three dose of vaccine. After many years the protective rate will decrease and 15–50% of the vaccinees have protective level of anti-HB antibodies <10 mIU/ml and 10% of vaccinees still remain at risk for HBV infection [59, 60].

The overall seroprevalence of HBsAg positivity among children from 5–12 years was 4.2% (95% CI: 1.0- 7.0). This prevalence of HBsAg tells us the study area is found under the category of intermediate endemicity for HBV infection [85]. This finding is consistent with similar studies conducted in Jimma (2.1%), Hawassa (4.4%), Gondar (4.2%), and Harar (1.1%), [61-63, 81]. It is also comparable with other studies conducted in other countries such as the green land [70], Vietnam, USA [65], Nigeria [73], Beni-suef-Egypt [78], Ghana-Nankana health research center [74], Gambia [76], Thailand [71], and Niakhar-Senegal [75], the prevalence was 3.3%, 2.7%, 1.3%, 2.0%,1.9%, 1.0 %,1.4%, and 1.1%, respectively.

However, the seroprevalence of HBsAg in the present study is relatively higher the results reported from Addis Abeba (0.4%) [64], Uzbekistan-Japan (0.8%) [66], Iran (0.6% [67], Italy (0.6%) [68], Egypt (0.6%) [77], and Dakahleya-Egypt (0.4%) [79]. In other words, this finding is lower than the result reported from china (20.49%) [72]. These variation may be explained by difference in host genetic factors, ethnicity, cultural practice, lifestyles, socioeconomic status, and regional variations, .Moreover, this variation also might be because of the difference in educational level of the family, family history of HBV, clinical practice, immune status, immunization coverage of HBV vaccine, inclusion of birth dose of HBV vaccine within 24 hours

, age range of study participants, sample size, sampling technique and type of laboratory method used for HBsAg detection.

According to our study, males were more susceptible for HBV infection (6.7 vs 1.3%) than females. This result is similar with the study conducted at Gambia [76]. This is may be due to the fact that females usually develop more strong innate and adaptive immune responses for viral infections. The other reason might be the effect of sex hormones on immune responses against virus. For instance, androgens have immune-suppressing effect whereas estrogens have immune-stimulating effect [86]. Based on the current findings, the prevalence of HBsAg was high among children who were born from illiterate parents and whose ages were from 5-7 years. This is in accordance with the previous result reported from Jimma [61]. This is may be due to the variation of cultural practice among study subjects and lack of knowledge, poor understanding and low level of awareness by illiterate parents about the transmission and consequences of HBV infection for their children.

The prevalence is also high in children who were born from daily laborer and rural residence. This could be because of; a lower scocio-economic status, high rate of illiteracy, lack of adequate health facility, poor public health awareness about the transmissions of HBV infection in rural community, use of traditional activities like using blade shaved by local barbers and so on.

In the current study, associated factors were also assessed and there are some independent variables associated with hepatitis B infection in post-vaccination. Among these predictors, previous history of chill's mother for HBV infection and child's injectable medications were identified as important determinants for HBV infection. Children who were born from mothers with previous history HBV infection were 3.9 times more likely to be HBsAg positive than those who were born from HBV infection negative mothers.

In addition, Children with previous history of injectable medications had 9 times higher risk of having HBsAg seropositive as compared to their counterparts. This is in accordance with the findings reported from Hawassa [62]. This is could be due to maternal-neonatal transmission (mother-to-child transmission) such as placental transmission in utero, natal transmission during delivery, and postnatal transmission during care for those children born from HBV infected mothers. There might be because of there was unsafe and reuse of syringes and needles without

sterilization during therapeutic injections for those children who had history injectable medications [87, 88]. However factors such as history of hospital admissions, blood transfusion, circumcision, and tattooing had not significant contributions for HBsAg positivity.

The overall seroprevalence of anti-HBc positivity in this study was 4.8% (95% CI: 2.0-8.0). This finding is comparable with previous results reported from Addis Abeba (5.6%) [64] and Gondar (6.3%) [63] .This finding is also comparable with the results reported from other countries such as Brazil (4.4%) [69], Thailand (5.5%) [71], Italy (3.3%) [68], Iran (2.1%) [67], and Benisuef-Egypt (2.0%) [78]. On the other hand, the current finding of anti-HBc seroprevalence in our study is relatively higher than the previous findings reported in studies conducted from Jimma (1.1%) [61], Niakhar-Senegal (1.5%) [75], and Dakahleya-Egypt (0.11%) [79]. In contrast, higher results were reported from Hawassa city (19.5%) [62] and other country; Gambian Villages (10.2%) [76]. This variations would be explained by difference in; geographical area, age range of study participants, vaccination rate of HBV vaccine , income level, socio cultural practices, inclusion of birth dose of HBV vaccine within 24 hours, type of laboratory technique used for anti-HBcAb detections etc.

According to the present study, the prevalence of anti-HBc is higher (5.3 % vs 0.0%) in urban children than rural children. This is consistent with the earlier finding which was reported from Ethiopia, Jimma (4.1% vs 1.8%) [61]. This could be due to the fact that the larger numbers of children who were involved in both studies were from the urban area. Based on the previous finding reported from Hawassa, Ethiopia, all HBsAg positive children were also positive for anti-HBc [62]. Contrary to this, the current study showed that children who were positive with HBsAg were not positive for anti-HBc. This is may be because of those individuals in our study have active HBV infection.

In this study, children who had history of hospital admission were 6.9 times more likely to be anti-HBc antibody positive as compare to their counterparts. This might be due to the horizontal transmission of HBV infection among patients, from healthcare workers and unsafe utilization of medical materials such as syringes, needle stick during care for those who had history of hospital admissions. Children who were born from HBsAg positive mothers were not significantly associated with anti-HBc positivity.

In contrast, children with mother history of HBsAg positive were more likely to be anti-HBc positive based on the study conducted in Hawassa, Ethiopia [62]. This discrepancy might be due to disparity in the early screening of mothers for HBV infection during pregnancy and administrations of Immunoprophylaxis i.e. Hepatitis B Immune Globulin (HBIG) accompanied with first dose of hepatitis B vaccine to infants who were born from HBsAg positive mothers within 24 hours after delivery [89].

# 7. CONCLUSIONS AND RECOMMENDATIONS

The seroprevalence of hepatitis B infection is intermediate among vaccinated children in the study area. Previous history of injectable medications and mother's HBV status were significantly associated with HBsAg positivity. Furthermore, history of hospital admission was significantly associated with anti-HBc antibody positivity. Thus, to decrease HBV infection, we recommended that there should be early screening of mothers for HBV infection to prevent vertical transmission. Beside to this, health care workers should use medical equipment like syringes and needles properly during drug administration through injection for their patients. They should also give health education about the horizontal transmission of HBV infection among hospital admitted clients.

In addition, there should be regular early screening of fully vaccinated children for HBV infection and determination of levels of anti-HBs antibodies should be also done, then booster doses must be given for non-responders. Even though this study has paramount public health importance, it was done with small sample size. As a result, it is difficult to infer seroprevalence of HBV infection to the entire population with this sample size. Therefore, further studies should be done with larger sample size in this study area.

## 8. LIMITATION OF THE STUDY

In the current study, only HBsAg and anti HBc antibody was detected. Other HBV infection markers like hepatitis B envelope antigen (HBeAg) and anti-HBeAg were not tested due to lack of ELISA kit. Therefore, the rate of viral replication and degree of infectivity were not determined. Positive samples were not rechecked because of shortage of ELISA kit. Detection and quantification of HBV DNA for positive samples were not done since HBV primers and kit were not available. Levels of Anti-HBs antibodies were not also determined due to lack of ELISA kit.

## 9. REFERENCES


1. Kidd-Ljunggren K, Miyakawa Y, Kidd AH: Genetic variability in hepatitis B viruses. *Journal of General Virology* 2002, 83(6):1267-1280.
2. Valaydon ZS, Locarnini SA: The virological aspects of hepatitis B. *Best Practice & Research Clinical Gastroenterology* 2017, 31(3):257-264.
3. Ganem D: Hepadnaviridae: the viruses and their replication. *Fields' virology* 2001, 2:2923-2969.
4. Jules L, Dienstag M: Hepatitis B virus infection. 2008.
5. Mast EE, Margolis HS, Fiore AE, Brink EW, Goldstein ST, Wang SA, Moyer LA, Bell BP, Alter MJ: A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) part 1: immunization of infants, children, and adolescents. *MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports* 2005, 54(Rr-16):1-31.
6. Shikata T, Karasawa T, Abe K, Uzawa T, Suzuki H, Oda T, Imai M, Mayumi M, Moritsugu Y: Hepatitis B e antigen and infectivity of hepatitis B virus. *J Infect Dis* 1977, 136(4):571-576.
7. Liao H, Liu Y, Li X, Wang J, Chen X, Zou J, Li Q, Liu L, Wang J, Huang B: Monitoring of serum HBV RNA, HBcrAg, HBsAg and anti-HBc levels in patients during long-term nucleoside/nucleotide analogue therapy. *Antiviral therapy* 2019, 24(2):105-115.
8. Chen H, Kaneko S, Girones R, Anderson R, Hornbuckle W, Tennant B, Cote P, Gerin J, Purcell R, Miller R: The woodchuck hepatitis virus X gene is important for establishment of virus infection in woodchucks. *Journal of virology* 1993, 67(3):1218-1226.
9. Keasler VV, Hodgson AJ, Madden CR, Slagle BL: Enhancement of hepatitis B virus replication by the regulatory X protein in vitro and in vivo. *Journal of virology* 2007, 81(6):2656-2662.
10. Lucifora J, Arzberger S, Durantel D, Belloni L, Strubin M, Levrero M, Zoulim F, Hantz O, Protzer U: Hepatitis B virus X protein is essential to initiate and maintain virus replication after infection. *Journal of hepatology* 2011, 55(5):996-1003.

11. Tsuge M, Hiraga N, Akiyama R, Tanaka S, Matsushita M, Mitsui F, Abe H, Kitamura S, Hatakeyama T, Kimura T: HBx protein is indispensable for development of viraemia in human hepatocyte chimeric mice. *Journal of General Virology* 2010, 91(7):1854-1864.
12. Banatvala J, Van Damme P, Oehen S: Lifelong protection against hepatitis B: the role of vaccine immunogenicity in immune memory. *Vaccine* 2000, 19(7-8):877-885.
13. Li Z, Hou X, Cao G: Is mother-to-infant transmission the most important factor for persistent HBV infection? *Emerging microbes & infections* 2015, 4(1):1-9.
14. Baumert TF, Thimme R, von Weizsäcker F: Pathogenesis of hepatitis B virus infection. *World journal of gastroenterology* 2007, 13(1):82-90.
15. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY: Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *The lancet* 2012, 380(9859):2095-2128.
16. Rapti I, Hadziyannis S: Risk for hepatocellular carcinoma in the course of chronic hepatitis B virus infection and the protective effect of therapy with nucleos (t) ide analogues. *World journal of hepatology* 2015, 7(8):1064.
17. Behrman RE, Vaughan III VC: Nelson textbook of pediatrics: WB Saunders company; 1983.
18. World Health Organization (WHO), 2016 .Global health sector strategy on viral hepatitis 2016-2021. Towards ending viral hepatitis.
19. Taylor M, Alonso-González M, Gómez B, Korenromp E, Broutet N: World health organization global health sector strategy on sexually transmitted infections: an evidence-to-action summary for Colombia. *Revista colombiana de obstetricia y ginecologia* 2017, 68(3):193-201.
20. Anderson CL, Remschmidt C, Drobnitzky F-P, Falkenhorst G, Zimmermann R, Wichmann O, Harder T: Hepatitis B immune status in adolescents vaccinated during infancy: A retrospective cohort st+udy from a pediatric practice in Germany. *Human vaccines & immunotherapeutics* 2016, 12(3):779-784.
21. Vickers NJ: Animal communication: when i'm calling you, will you answer too? *Current biology* 2017, 27(14):R713-R715.

22. Das S, Ramakrishnan K, Behera SK, Ganesapandian M, Xavier AS, Selvarajan S: Hepatitis B vaccine and immunoglobulin: key concepts. *Journal of Clinical and Translational Hepatology* 2019, 7(2):165.
23. Lok AS, Zoulim F, Dusheiko G, Ghany MG: Hepatitis B cure: from discovery to regulatory approval. *Journal of hepatology* 2017, 67(4):847-861.
24. World Health Organization (WHO), 2016. Monitoring health for the SDGs sustainable development goals.
25. Siddiqi N, Khan A, Nisar N, Siddiqi A: Assessment of EPI (expanded program of immunization) vaccine coverage in a peri-urban area. *Jpma* 2007, 57(8):391-395.
26. Shiferaw F, Letebo M, Bane A: Chronic viral hepatitis: policy, regulation, and strategies for its control and elimination in Ethiopia. *BMC Public Health* 2016, 16(1):769.
27. Huang M-L, Liao W-L, Ho M-S: HBV serological markers of vaccinated children in remote areas of Taiwan: emphasis on factors contributing to vaccine failure. *Vaccine* 2007, 25(34):6326-6333.
28. André FE, Zuckerman AJ: Protective efficacy of hepatitis B vaccines in neonates. *Journal of medical virology* 1994, 44(2):144-151.
29. Hu K-Q, Pan CQ, Goodwin D: Barriers to screening for hepatitis B virus infection in Asian Americans. *Digestive diseases and sciences* 2011, 56(11):3163.
30. Cheesbrough M: District laboratory practice in tropical countries, part 2: Cambridge university press; 2006.
31. Abdulla SA, Abdulla ZA: Effect of an educational program on nurses' knowledge and practices toward Hepatitis B virus in emergency hospitals in Erbil City. *Zanco Journal of Medical Sciences (Zanco J Med Sci)* 2014, 18(1):618_624-618_624.
32. Romanò L, Paladini S, Galli C, Raimondo G, Pollicino T, Zanetti AR: Hepatitis B vaccination: Are escape mutant viruses a matter of concern? *Human vaccines & immunotherapeutics* 2015, 11(1):53-57.
33. Negero A, Sisay Z, Medhin G: Prevalence of Hepatitis B surface antigen (HBsAg) among visitors of Shashemene General Hospital voluntary counseling and testing center. *BMC research notes* 2011, 4(1):35.
34. Parkin DM, Bray F, Ferlay J, Pisani P: Estimating the world cancer burden: Globocan 2000. *International journal of cancer* 2001, 94(2):153-156.

35. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP: The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *Journal of hepatology* 2006, 45(4):529-538.
36. Lai CL, Yuen MF: The natural history of chronic hepatitis B. *Journal of viral hepatitis* 2007, 14:6-10.
37. Liaw Y-F, Chu C-M: Hepatitis B virus infection. *The lancet* 2009, 373(9663):582-592.
38. Kim H, Shin AR, Chung HH, Kim MK, Lee JS, Shim J-J, Kim B-H: Recent trends in hepatitis B virus infection in the general Korean population. *The Korean journal of internal medicine* 2013, 28(4):413.
39. Wild CP, Hall AJ: Primary prevention of hepatocellular carcinoma in developing countries. *Mutation Research/Reviews in Mutation Research* 2000, 462(2-3):381-393.
40. Sylla BS, Wild CP: A million Africans a year dying from cancer by 2030: what can cancer research and control offer to the continent? *International journal of cancer* 2012, 130(2):245-250.
41. Beasley RP, Hwang LY, Stevens CE, Lin CC, Hsieh FJ, Wang KY, Sun TS, Szmuness W: Efficacy of hepatitis B immune globulin for prevention of perinatal transmission of the hepatitis B virus carrier state: final report of a randomized double-blind, placebo-controlled trial. *Hepatology* 1983, 3(2):135-141.
42. Ganem D, Prince AM: Hepatitis B virus infection—natural history and clinical consequences. *New England Journal of Medicine* 2004, 350(11):1118-1129.
43. Bakthavatchalu S: Hepatitis B Surface Antigen Carrier State among Asymptomatic Pregnant Women and Its Correlation with Vertical Transmission. *International Journal of Research in Pharmacy & Science* 2012, 2(3).
44. Lam N-C, Gotsch PB, Langan RC: Caring for pregnant women and newborns with hepatitis B or C. *American family physician* 2010, 82(10):1225-1229.
45. Tran TT, Martin P: Hepatitis B: epidemiology and natural history. *Clinics in liver disease* 2004, 8(2):255-266.
46. Memirie ST, Desalegn H, Naizgi M, Nigus M, Taddesse L, Tadesse Y, Tessema F, Zelalem M, Girma T: Introduction of birth dose of hepatitis B virus vaccine to the immunization program in Ethiopia: an economic evaluation. *Cost Effectiveness and Resource Allocation* 2020, 18(1):1-10.

47. Yim HJ, Lok ASF: Natural history of chronic hepatitis B virus infection: what we knew in 1981 and what we know in 2005. *Hepatology* 2006, 43(S1):S173-S181.
48. Tegegne D, Desta K, Tegbaru B, Tilahun T: Seroprevalence and transmission of Hepatitis B virus among delivering women and their new born in selected health facilities, Addis Ababa, Ethiopia: a cross sectional study. *BMC research notes* 2014, 7(1):239.
49. Howell J, Lemoine M, Thursz M: Prevention of materno-foetal transmission of hepatitis B in sub-Saharan Africa: the evidence, current practice and future challenges. *Journal of viral hepatitis* 2014, 21(6):381-396.
50. Walle F, Asrat D, Alem A, Tadesse E, Desta K: Prevalence of hepatitis B surface antigen among pregnant women attending antenatal care service at Debre-Tabor Hospital, Northwest Ethiopia. *Ethiop J Health Sci* 2008, 17(17):13-20.
51. Abebe A, Nokes D, Dejene A, Enquselassie F, Messele T, Cutts F: Seroepidemiology of hepatitis B virus in Addis Ababa, Ethiopia: transmission patterns and vaccine control. *Epidemiology & Infection* 2003, 131(1):757-770.
52. Yazie TD, Tebeje MG: An updated systematic review and meta-analysis of the prevalence of hepatitis B virus in Ethiopia. *BMC infectious diseases* 2019, 19(1):1-13.
53. Young KM, Gray CM, Bekker LG: Is obesity a risk factor for vaccine non-responsiveness? *PloS one* 2013, 8(12):e82779.
54. Yang S, Tian G, Cui Y, Ding C, Deng M, Yu C, Xu K, Ren J, Yao J, Li Y *et al*: Factors influencing immunologic response to hepatitis B vaccine in adults. *Scientific reports* 2016, 6:27251.
55. André FE, Zuckerman AJ: Review: protective efficacy of hepatitis B vaccines in neonates. *J Med Virol* 1994, 44(2):144-151.
56. Al-Eryani BM: Child malnutrition in Yemen: the role of female education. 2006.
57. Roome AJ, Walsh SJ, Cartter ML, Hadler JL: Hepatitis B vaccine responsiveness in Connecticut public safety personnel. *Jama* 1993, 270(24):2931-2934.
58. Bogale HA, Amhare AF, Bogale AA: Assessment of factors affecting vaccine cold chain management practice in public health institutions in east Gojam zone of Amhara region. *BMC public health* 2019, 19(1):1-6.

59. Leuridan E, Van Damme P: Hepatitis B and the need for a booster dose. *Clinical Infectious Diseases* 2011, 53(1):68-75.
60. Yonghao G, Jin X, Jun L, Pumei D, Ying Y, Xiuhong F, Yanyang Z, Wanshen G: An epidemiological serosurvey of hepatitis B virus shows evidence of declining prevalence due to hepatitis B vaccination in central China. *International Journal of Infectious Diseases* 2015, 40:75-80.
61. Kedir R, Taye BD, Kassa T, Teshager L, Aseffa A, et al: SERO-PREVALENCE OF HEPATITIS B VIRUS INFECTION AND SERO-PROTECTION OF HEPATITIS B VACCINE AMONG CHILDREN IN JIMMA TOWN, SOUTHWEST ETHIOPIA. *Ethiopian medical journal* 2019: 147-158.
62. Argaw B, Mihret A, Aseffa A, Tarekegne A, Hussen S, Wachamo D, Shimelis T, Howe R: Sero-prevalence of hepatitis B virus markers and associated factors among children in Hawassa City, southern Ethiopia. *BMC infectious diseases* 2020, 20(1):1-7.
63. Ayalew G: Seroprotection level of Hepatitis B vaccine among children living in Gondar town, Northwest Ethiopia. 2017: 139-146
64. Teshome S, Biazin H, Tarekegne A, Abebe T, Bekele F, Mihret A, Aseffa A, Howe R: Antibody response against hepatitis B virus after vaccination and seroprevalence of HBV in children in Addis Ababa, Ethiopia. *Ethiopian medical journal* 2019: 167-173
65. Nguyen TH, Vu MH, Nguyen VC, Nguyen LH, Toda K, Nguyen TN, Dao S, Wannemuehler KA, Hennessey KA: A reduction in chronic hepatitis B virus infection prevalence among children in Vietnam demonstrates the importance of vaccination. *Vaccine* 2014, 32(2):217-222.
66. Avazova D, Kurbanov F, Tanaka Y, Sugiyama M, Radchenko I, Ruziev D, Musabaev E, Mizokami M: Hepatitis B virus transmission pattern and vaccination efficiency in Uzbekistan. *Journal of medical virology* 2008, 80(2):217-224.
67. Hashemi S, Moghadami M, BAGHERI LK, Alborzi A, Mahboudi A: The efficacy of hepatitis B vaccination among school age children in Southern Iran. 2010.
68. Mele A, Tancredi F, Romanò L, Giuseppone A, Colucci M, Sangiuolo A, Lecce R, Adamo B, Tosti ME, Taliani G: Effectiveness of hepatitis B vaccination in babies born to hepatitis B surface antigen-positive mothers in Italy. *The Journal of infectious diseases* 2001, 184(7):905-908.

69. Fagundes GD, Tabalipa FdO, Silva Jd: Antibody levels in children after 10 years of vaccination against hepatitis B: a brazilian community-based study. *Revista da Sociedade Brasileira de Medicina Tropical* 2012, 45:260-262.
70. Børresen ML, Koch A, Biggar RJ, Ladefoged K, Melbye M, Wohlfahrt J, Krause TG: Effectiveness of the targeted hepatitis B vaccination program in Greenland. *American journal of public health* 2012, 102(2):277-284.
71. Chongsrisawat V, Yoocharoen P, Theamboonlers A, Tharmaphornpilas P, Warinsathien P, Sinlaparatsamee S, Paupunwatana S, Chaiear K, Khwanjaipanich S, Poovorawan Y: Hepatitis B seroprevalence in Thailand: 12 years after hepatitis B vaccine integration into the national expanded programme on immunization. *Tropical Medicine & International Health* 2006, 11(10):1496-1502.
72. Li X, Xu Y, Dong Y, Yang X, Ye B, Wang Y, Chen Y: Monitoring the efficacy of infant hepatitis B vaccination and revaccination in 0-to 8-year-old children: Protective anti-HBs levels and cellular immune responses. *Vaccine* 2018, 36(18):2442-2449.
73. Odusanya O, Alufohai F, Meurice F, Wellens R, Weil J, Ahonkhai V: Prevalence of hepatitis B surface antigen in vaccinated children and controls in rural Nigeria. *International Journal of Infectious Diseases* 2005, 9(3):139-143.
74. Dassah S, Sakyi SA, Frempong MT, Luuse AT, Ephraim RK, Anto EO, Oduro A: Seroconversion of hepatitis B vaccine in young children in the Kassena Nankana District of Ghana: A cross-sectional study. *PloS one* 2015, 10(12):e0145209.
75. Perieres L, Coste M, Ndiour S, Halfon P, Sokhna C, Ba E, Diallo A, Boyer S: Hepatitis B vaccination status and vaccine immune response among children in rural Senegal. *European Journal of Public Health* 2019, 29(Supplement_4):ckz185. 592.
76. Mendy M, Peterson I, Hossin S, Peto T, Jobarteh ML, Jeng-Barry A, Sidibeh M, Jatta A, Moore SE, Hall AJ: Observational study of vaccine efficacy 24 years after the start of hepatitis B vaccination in two Gambian villages: no need for a booster dose. *PloS one* 2013, 8(3):e58029.
77. Shatat H, Kotkat A, Farghaly A, Omar S, Zayton S: A study of hepatitis B vaccine efficacy 10 years after compulsory vaccination in Egypt. *The Journal of the Egyptian Public Health Association* 2005, 80(5-6):495-508.

78. Sami SM, Salama II, Hassanin AI, Hamid ATA, Abd El Haliem NF, Ibrahim NA: Hepatitis B infection, seroprotection, and anamnestic response among children vaccinated during infancy in Beni-Suef, Egypt. *Medical Research Journal* 2014, 13(2):86-92.
79. Hamid ATA, Said ZN: Persistence of protection to hepatitis B vaccine and response to booster dose among children and adolescents in Dakahleya-Egypt. *Egyptian Journal of Immunology* 2014, 21(1):13-26.
80. Salama II, Sami SM, Said ZNA, El-Sayed MH, El Etreby LA, Rabah TM, Elmosalami DM, Hamid ATA, Salama SI, Mohsen AMA: Effectiveness of hepatitis B virus vaccination program in Egypt: Multicenter national project. *World journal of hepatology* 2015, 7(22):2418.
81. Usman S, Seyoum B, Mengiste B, Tarekegne A, Bekele F, et al: Serum levels of anti-hepatitis B surface antigen and HBV associated factors among vaccinated and unvaccinated children in Harar, eastern Ethiopia: A community-based study. *Ethiopian medical journal* 2019: 122-129.
82. https://en.wikipedia.org/wiki/Debre_Marqos.
83. Tsega E, Horton J, Nordenfelt E, Hansson B, Tafesse B, et al, antibody levels in Ethiopian children five years after vaccination with two different doses of hepatitis B vaccine: Is there a need for booster vaccine? *Canadian journal of gastroenterology* 1998, 12(1):57-60.
84. Yucel F, Akcael E: Development of sandwich ELISA systems for the diagnosis of hepatitis B virus surface antigen and its antibody in human sera. *J Microbiol Exp* 2018, 6(2):77-82.
85. Alter MJ: Epidemiology of hepatitis B in Europe and worldwide. *Journal of hepatology* 2003, 39:64-69.
86. Ruggieri A, Gagliardi MC, Anticoli S: Sex-dependent outcome of hepatitis B and C viruses infections: synergy of sex hormones and immune responses? *Frontiers in immunology* 2018, 9:2302.
87. Khan AJ, Luby SP, Fikree F, Karim A, Obaid S, Dellawala S, Mirza S, Malik T, Fisher-Hoch S, McCormick JB: Unsafe injections and the transmission of hepatitis B and C in a

periurban community in Pakistan. *Bulletin of the World Health Organization* 2000, 78:956-963.

88. Ma L, Alla NR, Li X, Mynbaev OA, Shi Z: Mother-to-child transmission of HBV: review of current clinical management and prevention strategies. *Reviews in medical virology* 2014, 24(6):396-406.

89. World Health Organization (WHO), 2020 .Prevention of mother-to-child transmission of hepatitis B virus: guidelines on antiviral prophylaxis in pregnancy: web annex A: systematic review of the efficacy and safety of antiviral therapy during pregnancy.

# 10. ANNEX

A DATA COLLECTION FORM

DEBRMARKOS UNIVERSITY COLLEGE OF HEALTH SCINCE

DEPARTMENT OF MEDICAL LABORATORY SICNCES

**Annex I: General Information for the Study Participants English Version**

**Date.....**

Self-Introduction

1. My name is Adane Adugna …… and I am MSc student of DebreMarkos University, college of health Sciences department of medical laboratory Sciences. I am doing a research entitled "seroprevalence of hepatitis B infection and associated factors among hepatitis B vaccinated children in Debre Markos town ----------

**Purpose and description of the study**

The objective of this research is to assess the seroprevalence and associated factors hepatitis B infection among HBV vaccinated children in Debre Markos town .If you are agree to involve in the study, you will give us the important information and 3-5 milliliter blood sample will be taken from you for laboratory analysis.

Hepatitis B is a chronic disease which affects the liver and cause cirrhosis and hepatocellular carcinoma. Children are highly susceptible for this disease due to the vertical transmition of mother to child. WHO recommends all children for hepatitis B to be vaccinated but the vaccine is not always effective for the protection of Hepatitis B virus due to different factors .Therefor; this study will determine the seroprevalence and associated of HBV infection after vaccination.

**Will my information be kept confidential?**

All the data obtained will be kept strictly confidential and locking the data, only study personnel will have access to the files. Anonymous testing will be undertaken, that means samples will be coded and positive results will not be identified by names. There will not be any payment or

direct benefit for participating and you are not asked to pay for the laboratory examination. Your result will be reported back to the physicians if it is found significant for further diagnosis and treatment.

**Protection of your private life**

We will do everything we can to protect your privacy. Information acquired from you will be treated as strictly confidential locking the data, only study personnel will have access to the files. The information will be stored in an electronic database, and Anonymous testing will be undertaken, that means samples will be coded and positive results will not be identified by names. The information you provided will not be shared with anyone, except the study researchers .If it is found significant for further diagnosis and treatment, your result will be reported back to the concern bodies.

**What about my rights to decline participation or withdraw from the study?**

Your participation in this study is entirely voluntary, and you may stop the participation at any time or you may refuse to answer some of the questions if you feel uncomfortable .Even after you have signed this informed assent form. Your access to treatment will not be dependent on your participation in the study. If you are not comfortable please feel free to stop it at any level of the study.

**Contact person in case of questions**

If you have questions regarding this study or would like to be informed of the results after its completion, you can contact now, during, or after the study please contact me through the following address:

Investigator name: Adane Adugna

Department: Medical laboratory science, Debre Markos University

Phone no: 0937401165

**Annex II: General Information for the Study Participants Amaharic Version**

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***ረስን ማክተዋወቅ***

1. ስሜ አዳነ አዳሾ ..... እኔ ደብረሚቆስ ዩኒቨርሲቲ የህክምና ላብራቶሪ ሳይንስ ኮሌጅ የጤና ሳይንስ ኮሌጅ ኤምሲሲ ተማሪ ነኝ ፡፡ በደብረ ሚቆስ ከተማ ውስጥ የሄፐታይተስ ቢ ክትባት ከወሰዱ ሕፃናት ጫከል "የሄፐታይተስ ቢ ኢንፌክሽን እና ተያያዥ ምክንያቶች" በሜል ርዕስ ምርምር አደርጋለሁ ነው፡፡

**የጥናቱ ዓላማ እና ማለጫ**

የዚህ ምርምር ዓላማ በደብረ ሚቆስ ከተማ የሄፐታይተስ ቢ ክትባት በወሰዱ ልጆች ላይ የሄፐታይተስ ቢ ኢንፌክሽን እና ተያያዥ ምክንያቶች ማጥናት ነው፡፡ በጥናቱ ውስጥ ለማተፍ ከተስማሙአስፈላጊ ሚጃውን ይሰጡናና ከ3-5 ሚሊ ሊትር የደም ናሙና ይወስዳል ፡፡ ለእርስዎ ላቦራቶሪ ትንታኔ ፡፡

ሄፐታይተስ ቢ ሥር የሰደደ በሽታ ሲሆን ይህም በጉበት ላይ ተጽዕኖ የሚሳድር ሲሆን ለኮምትሬ እና ለሄፐታይተስ ሴል ካንሰር ያስከትላል። ልጆች ከእናት ወደ ልጅ በሚተላለፈው ሽግግር ምክንያት ልጆች ለዚህ በሽታ በጣም የተጋለጡ ናቸው፡፡ የዓለም ጤና ድርጅት ለሁሉም ልጆች ለሄፐታይተስ ቢ እንዲከተቡ ይመክራል ነገር ግን ክትባቱ ሁል ጊዜ ለሄፐታይተስ ቢ ለማከላከል ልዩ ውጤታማ አይደለም፡፡ ይህ ጥናት ከክትባቱ በኋላ የሄፐታይተስ ቢ ኢንፌክሽን ምርሙን ይለካል ፡፡

**ሚጃዬ በማጥር ይቀመጣል?**

የተገኘው ሚጃ ሁሉ በጥብቅ በማጥር ይቀመጣል እና ሚጃውን ይቆልፋል ፣ የጥናቱ ሰራተኞች ብቻ ወደ ፋይሎቹ መዳረሻ ይኖራቸዋል። ስምአልባ ምርሙ ይካሄዳል ፣ ያ ማለት ናሙዎች ኮድ ይደረግባቸዋል እንዲሁም አዎንታዊ ውጤቶች በስም አይታወቁም ማለት ነው፡፡ ለማተፍ ምንም ክፍያ ወይም ቀጥተኛ ጥቅም አይኖርም እንዲሁም ለላቦራቶሪ ምርሙ እንዲከፍሉ አይጠየቁም፡፡ ለተጨማሪ ምርሙ እና ሕክምና ከፍተኛ ሆኖ ከተገኘ ውጤትዎ ለሐኪሞቹ ሪፖርት ይደረጋል ፡፡

**የግል ሕይወትዎ ጥበቃ**

የእርስዎን ግላዊነት ለማክበቅ የምችለውዓ ሁሉ እናደርጋለን ፡፡ ከእርስዎ የተገኘ ሚጃ ወዚቡን እንደ ሚጥራዊነቱ ይቆልፋል ተብሎ ይጠብዳል ፣ የጥናቱ ሰራተኞች ብቻ ወደ ፋይሎቹ ማዳረሻ ይኖራቸዋል። ሚጃው በኤሌክትሮኒክ የሚጃ ቋት ወስጥ ይቀመጣል እንዲሁም ስምአልባ ማክራ ይከሄዳል ፣ ያም ማለት ናሙዎች ኮድ ይደረግባቸዋል እንዲሁም አዎንታዊ ውጣፎች በስም አይታወቁም ፡፡ እርስዎ ያቀረቡት ሚጃ ከጥናቱ ተሚራሚዎች በስተቀር ለማንም አይጋራም ፡፡በተጨማሪ ምሚሙ እና ህክምና ለህክምና ከፍተኛ ሆኖ ከተገኘ ውጣኔዎ ለሚጠለከታቸው አካላት ሪፖርት ይደረጋል ፡፡

**ተሳትፎን ላለማክበል ወይም ከጥናቱ ለማቋረጥ ስለ ማበቦቻስ?**

በዚህ ጥናት ወስጥ ያለዎት ተሳትፎ ሙሉ በሙሉ በፈቃደኝነት የሚረግ ሲሆን በማናቸውም ጊዜ ተሳትፎዓን ሊያቆሙ ይችላሉ ወይም የማይመች ሆኖ ከተሰማዎት ለአንዳንድ ጥያቄዎች ሜልስ ለማክጠት እማኪ ማለት ይችላሉ። የሕክምና ተደራሽነትዎ በጥናቱ ተሳትፎዎ ላይ የተመካ አይሆንም ፡፡ ካልተሞዎት እባክዎን በማናቸውም የጥናት ደረጃ ለማቆም ነፃነት ይሰማዎት ፡፡

**ጥያቄዎች ካሉ ሰው ያነ ጋግሩ**

ይህንን ጥናት አስሜልክቶ ጥያቄዎች ካሉዎት ወይም ከተጠናቀቀ በኋላ ስለ ውጣኔቹ ማወቅ ከፈለጉ ፣ አሁኑ ፣ በጥናቱ ወቅት ወይም በኋላ ማጋገር ይችላሉ እባክዎን በሚከተለው አድራሻ ያነ ጋግሩኝ ፡፡

የሚሚው ስም ፡ አዳነ አዳሩ

የትምህርት ክፍል – የሕክምና ላብራቶሪ ሳይንስ ፣ ደብረ ሚቆስ ዩኒቨርሲቲ

ስልክ ቁጥር– 0937401165

**Annex III: Assent form English version**

**Dear participants,**

I have been informed about the objective of the study entitled **"hepatitis B seroprevalence** and associated factors among hepatitis B vaccinated children in DebreMarkos town ". I am also informed that all information contained within the questionnaire is to be kept confidential.

Moreover, I have been well informed of my right to refuse information, decline to cooperate, and drop out of the study if I want and none of my actions will have any bearing at all on my

overall health care. Therefore, with a full understanding of the situations, I agree to give the entire necessary information on behalf of my child and my child will give blood sample for laboratory analysis. I have had the chance to ask questions about the project and received explanation to my satisfaction in a language I understand.

I am also told that results for the hepatitis B infection seroprevalence and associated factors among vaccinated children in Debre Markos town will be given to concern bodies and that I may ask the information if I want. This questionnaire and sample collection is designed for the research study. The aim of this questionnaire and sample collection is to study the seroprevalence of hepatitis B virus infection and associated factors among children in Debre Markos city used for partial fulfillment of MSC in Medical Microbiology.

Whatever information you provide will be kept strictly confidential. The success of the study depends on your real responses to the questions. Please listen carefully and respond to the questions honestly. The questionnaires spend out 30 minutes.

I have read this form or the form has been read to me in the language I understand and I have understood the conditions stated above. I am willing to participate in this study.

Thank you very much for your cooperation!!

**To be completed by the participant**

Date: ……

Name of participant: ……

Signature (or thumbprint) of participant: ……

**To be completed by the witness** (in case illiterate participant)

Date: ……

Name of witness: ……

Signature of witness: ……

**To be completed by the person obtaining the informed consent**

I, understand, …… confirm that I have informed the participant (and/or the legal guardian) about all the relevant aspects of this study. I confirm that he/she has consented voluntarily to participate in the study.

Date: …… Signature: ……

**Annex IV: Assent form Amharic version**

ውድ ተሳታፊዎች

**በሚል ርዕስ ስለ ጥናቱ ዓላማ ሚጃ ተሰጥቶኛል**

በጥናቱ ዓላማ ላይ "የሄፐታይተስ ቢ ክትባት ከወሰዱ ሕፃናት ጫካከል "የሄፐታይተስ ቢ ኢንፌክሽን እና ተያያዥ ምክንያቶች" በደብረሚቆስ ከተማ በሚል ርዕስ ሚጃው ደርሶኛል ፡፡በተጨማሪም በጫንይቁ ውስጥ የተካተቱት ሚጃዎች በሙሉ በሚጥር የተያዙ መሆን እንዳለባቸው ተነግሮኛል ፡፡

በተጨማሪም ፣ሚጃን ላለመቀበል ፣ ለማቋበር አለመቀበል እና ከፈለግኩ ጥናቱ የሚቋረጥ ጫጌ በሚባ ተነግሮኛል እናም ድርጊቶቼ በአጠቃላይ በልጄ ጤና ላይ ምንም ዓይነት ተጽዕኖ አይኖራቸውም ፡፡ስለሆነም በሀኔታዎች ላይ ሙሉ ግንዛቤ በማዝ ልጄን ውስዬ አስፈላጊ ሚጃዎችን በሙሉ ለጫጠት እስማማለሁ እናም ልጄ ለላቦራቶሪ ትንተና የደም ናሙና ደሰጣል ፡፡ስለፕሮጀክቱ ጥያቄዎችን የመየቅ ዕድል አግኝቻለሁ እና በተረዳሁት ቋንቋ እንደ እርካታዬ ማብራሪያ አግኝቻለሁ ፡፡

በተጨማሪም በደብረ ሚቆስ ከተማ ውስጥ "የሄፐታይተስ ቢ ኢንፌክሽን ውጥቆች እና ተያያዥ ምክንያቶች ለሚለከታቸው አካላት እንደሚጡ ተነግሮኛል እኔም ከፈለግኩ ሚጃውን መየቅ እችላለሁ ፡፡ይህ መየቅ እና የናሙና ስብስብ ለምንም ጥናቱ የተቀየሱ ናቸው ፡፡ የዚህ መየቅ እና የናሙና አሰባሰብ ዓላማ የሄፐታይተስ ቢ ኢንፌክሽን በደብረ ሚቆስ ከተማ ውስጥ በሚኙ የህፃናት ላይ በሚካል ሞይክሮባዮሎጂ ለኤም.ኤስ.

የሚጠኑ ማንኛውም ሚጃ በጥብቅ በሚጥር ይቀመጣል ፡፡የጥናቱ ስኬት የሚወሰነው ለጥያቄዎቹ በእውተኛ ምላሾችዎ ላይ ነው ፡፡ እባክዎን በጥንቃቄ ያዳምጡ እና ለጥያቄዎች በቅንነት ይመልሱ ፡፡ መየቆቹ 30 ደቂቃዎችን ያጠፉሉ ፡፡

ይህንን ቅጽ አንብቤዋለሁ ወይም ቅጹ በሚባኝ ቋንቋ ተነበበኝ እና ከላይ የተገለጹትን ሀኔታዎች ተረድቻለሁ ፡፡ በዚህ ጥናት ውስጥ ለመሳተፍ ፈቃደኛ ነኝ ፡፡

ስለ ትብብራችሁ በጣም አመሰግናለሁ !!

በተሳታፊው እንዲሞፍቀቅ

ቀን .....

የተሳታፊ ስም Name

የተሳታፊ ፊርማ (ጣይም አሻራ)፡ .....

በምክክሩ ማጠቃለቅ (ማንበብ የማይችል ተሳታፊ ከሆነ)

ቀን .....

የምክክርነት ስም .....

የምክክርነት ፊርማ .....

በሚጀ የተደገፈ ፈቃድን በሚገኝ ሰው እንዲጠቀቅ

እኔ ለሁሉም ፣ ለተሳታፊው (እና / ጣይም ለህጋዊ ሞግዚት) ማህወቄን አረጋግጣለሁ የዚህ ጥናት አግባብነት ያላቸው ገጽታዎች ፡፡ በጥናቱ ለማተፍ በፈቃደኝነት ፈቃደኛ ሞሆኑን አረጋግጣለሁ ፡፡

ቀን ..... ፊርማ .....

**Annex V: Questionnaire English version**

Section 1: Demographic Information**:**

Patient ID

| Serial No. | Questions | Coding categories |
|---|---|---|
| Q 001 | Age of your child | _____ |
| Q 002 | Sex of your child | 1.Male<br>2. Female |
| Q 003 | Job (Children's parent/legal guardian) | 1. Farmer<br>2. Civil servant<br>3. Self-employee<br>4. Daily labor<br>5. House wife<br>6. Merchant |

| | | |
|---|---|---|
| Q 004 | Educational status (Children's parent/legal guardian) | 1. Illiterate<br>2. Primary<br>3. Secondary<br>4. College/university |
| Q 005 | Birth place of your child | 1. Rural<br>2. Urban |
| Q 006 | Does your child took 3 dose of HBV vaccine | 1. Yes<br>2. No |
| Q 007 | Do you have history of hepatitis? ( for child's mother) | 1. Yes<br>2. No |
| Q 008 | Weight | |
| Q009 | Height | |
| Q 010 | Does your child smoke? | 1. Yes<br>2. No |
| Q 011 | Does your child have history of hospital admission? | 1. Yes<br>2. No |
| Q 012 | Does your child have history blood transfusion? | 1.Yes<br>2.No |
| Q 013 | Does your child has history of injectable medication | 1. Yes<br>2. No |

| | | |
|---|---|---|
| Q 014 | Does your child circumcised by traditional way ( If the child is male) | 1. Yes<br>2. No |
| Q 015 | Does your child have of body piercing? | 1.Yes<br>2. No |
| Q 016 | Does your child has history of tattooing | 1. Yes<br>2. No |

**VI: Questionnaire Amharic version**

**በደብረሚቆስ ዩኒቨርሲቲየጤና እና ህክምና ሳይንስ ኮሌጅ**

**የሜዲካል ላቦራቶሪ ሳይንስ ትምህርት ክፍል**

**የተማሪ ጥናት የቃለ-መጠይቅ ፎርም**

ክፍል አንድ

የስነ ህዝብ ሚጃ

| | | |
|---|---|---|
| 001 | እድሜ | _____ |
| 002 | ፆታ | 1. ወንድ<br>2. ሴት |
| 003 | ስራ(ለህጋዊ አሳዳጊ ብቻም ሞግዚት) | 1. ግብርና<br>2. የጣጣስት ሰራተኛ<br>3. ይግል ስራ<br>4. የጉልበት ሰራተኛ<br>5. የቤት እመቤት |

| | | |
|---|---|---|
| | | 6. ነጋዴ |
| 004 | የትምህርት ሁኔታ | 1. ያልተማረ<br>2. የመጀመሪያ ደረጃ<br>3. ሁለተኛ ደረጃ<br>4. ኮሌጅ ዉይም ዩኑቨርሲቲ |
| 005 | የትዉልድ ቦታ | 1. ገጠር<br>2. ከተማ |
| 006 | ልጅዎ የጉበት በሽታ ክትባት 3 ጊዜ ወስዷል? | 1. አዎ<br>2. የለም |
| 007 | የጉበት በሽታ ታማሚነዎት (የልጅ እናትን የሚመለከት) ? | 3. አዎ<br>4. የለም |
| 008 | ክብደት | |
| 009 | ቁመት | |
| 010 | ልጀዎያጨሉ ? | 1. አዎ<br>2. የለም |
| 011 | ልጀዎ ከዚህ በፊት ሆስፒታሌ ተኝቶ ያወቃል ወይ? | 1. አዎ<br>2. የለም |
| 012 | ልጀዎ ከዚህ በፊት የላሊ ሰዉ ሞም ተሰጥቶት ያወቃል ወይ? | 1. አዎ<br>2. የለም |
| 013 | ልጅዎ ከዚህ በፊት በሚፈ ሜድሃኒት ወሰደ | 1. አዎ<br>2. የለም |

| | | |
|---|---|---|
| | ያውቃል ? | |
| 014 | ውንድልጀዎ ተገርዞ ያውቃል ወይ? | 1. አዎ<br>2. የለም |
| 015 | ልጀዎ ከዚህ በፊት ሰጫ ት ተበስቶ ያውቃል ወይ? | 1. አዎ<br>2. የለም |
| Q016 | ሌጀዎ ከዚህ በፊት ንቅሳት ተነ ቅሶ ያውቃሌ ወይ? | 1. አ ዎ<br>2. የ ለ ም |
| Laboratory results | HBsAg | 1. Negative<br>2. Positive |
| | Anti-HBc | 1. Negative<br>2. Positive |

**Annex VII: Laboratory testing procedure**

Hepatitis B serologic testing involves measurement of several hepatitis B virus (HBV) specific antigens and antibodies. Different serologic “markers” or combinations of markers are used to determine the seroprevalence of hepatitis B infection. Thus, serum HBsAg and HBcAb were tested by commercially available enzyme liked immune sorbent assays according to the manufacturer’s instructions.

**General test principles of ELISA**

ELISA test system was applied for HBsAg detection. For detection of HBsAg in human serum, the double antibody-sandwich ELISA was used. Based on the principle of the assay, anti-HBsAg monoclonal antibody (MAM 2G3) was bound to the different ELISA microplates as a capture

antibody. Anti-HBsAg linked horseradishe peroxidase (MAM 2G3–HRP) conjugate was used as detecting antibody.

Unlike the conventional ELISA systems, the same monoclonal antibody (MAM 2G3) against HBsAg was used as both capturing antibody and detecting antibody. MAb-HRP and MAb-Biotin conjugate was diluted 1:1000 in PBS, and human serum was added to the wells simultaneously and incubated for 1 h at 37°C. After repeating the washing step for 5 times with washing buffer, the enzymatic activity was detected by substrate buffer (3,3′,5,5′-tetramethyl benzidine (TMB) and hydrogen peroxide for HRP conjugate-antibody.

For the detection anti-HBc, there were three steps. A test specimen was put directly in the test well containing specimen diluent and incubated for a specified length of time in the first step. Antigen-antibody complexes were formed on the microwell surface if anti-HBc was present in the specimen. Complexes were not formed and the unbound plasma or serum proteins were avoided in the washing step if anti-HBc was not found.

Antibody conjugate was added to the test well. The antibody conjugate was a mixture of monoclonal antibodies specific for human IgG and IgM in the second stage. In the antibody portion of the antigen-antibody complexes the conjugate was bound specifically. When antigen-antibody complexes were not found, the unbound conjugate was removed by washing.

An enzyme was added to the test well. If bound conjugate was present, the enzyme was oxidized, resulting in a colored end-product in the third stage. Stop solution was added to stop the reaction. The intensity of color depends on the amount of bound conjugate and therefore was a function of the concentration of anti-HBc present in the specimen. The color intensity was measured with a microwell reader.

**Procedure**

**Reagents and consumables**

**Kit components**

- Coated 96 well strip plate
- Sample diluent
- Assay diluents (A and B)

- Detection reagents (A and B)
- Wash buffer
- TMB Substrate
- Stop solution
- Adhesive plate sealers
- Instruction manual

**Materials not provided with the kit**

- Micro plate reader with 450 nm wavelength filter
- Distilled water
- Incubator ($37^{o}$C)
- Pipettes (high precision)
- Multi-channel micropipettes
- Pipette tips (sterile)
- Absorbent paper
- Eppendrof tubes

## A. Monolisa™ HBs Ag ULTRA

1 Plate-96 tests 72346

5 plate -480 tests 72348

**KIT FOR THE DETECTION OF THE SURFACE ANTIGEN OF THE HEPATITIS B IN HUMAN SERUM OR PLASMA BY THE ENZYME IMMUNOASSAY TECHNIQUE**

**IVD** For *In Vitro* Diagnostic Use

**INTENDED USE**

Monolisa™ HBs Ag ULTRA assay is a one-step enzyme immunoassay method of the "sandwich" type for the detection of the surface antigen of the Hepatitis B virus (HBs Ag) in the human serum or plasma

**PRINCIPLE OF THE Monolisa™ HBs Ag ULTRA**

Monolisa™ HBs Ag ULTRA assay is a one-step enzyme immunoassay depend on the principle of the "sandwich" type using monoclonal antibodies and polyclonal anibodies preferred for their ability to bind themselves to the different subtypes of HBs Ag now recognized by the WHO and the most part of variant HBV strains. The Monolisa™ HBs Ag ULTRA solid phase is coated with monoclonal antibodies. The Monolisa™ HBs Ag ULTRA conjugates are based upon the use of monoclonal antibodies from mouse and polyclonal antibody from goat against the HBs Ag. These antibodies are bound to the peroxidase.

**CONTENTS OF THE Monolisa™ HBs Ag ULTRA**

All reagents are exclusively for in vitro diagnostic use

| Lable | NATURE OF THE REAGENTS | PRESENTATION | |
|---|---|---|---|
| | | **72346** | **72348** |
| **R1** | **MICROPLATE** : 12 strips of 8 wells each, coated with monoclonal antiHBs antibodies (mouse) | 1 Plate | 5 Plate |
| **R2** | **CONCENTRATED WASHING SOLUTION (20X** Tris NaCl buffer pH 7.4 Preservative : ProClinTM 300 (0.04%) | 1 Vial 70 ml | 1 Vial 135 ml |
| **R3** | **NEGATIVE CONTROL**<br>Tris HCl buffer containing BSA<br>Preservative : ProClinTM 300 (0.1 %) | 2 vials<br>2 x 2.5 ml | 2 vials<br>2 x 2.5 ml |
| **R4** | **POSITIVE CONTROL (HUMAN)**<br>Tris HCl buffer containing BSA with addition of mixture of purified HBs Ag from ad and ay subtypes<br>Preservative : ProClinTM 300 (0.1 %) | 1 Vial<br>2.5 ml | 1 Vial<br>2.5 ml |
| **R6** | **CONJUGATE DILUENT**<br>Tris HCl buffer pH 7.4 containing BSA, TweenR 20, Bovine immunoglobulins and mouse immunoglobulins<br>with sample addition control reagent<br>Preservatives : ProClinTM 300 (0,1%), Ciprofloxacine<br>(10 µg /ml) | 1 Vial<br>8 ml | 2 Vial<br>2x18ml |
| **R7** | **CONJUGATE**<br>Mouse Monoclonal anti-HBs antibodies and Goat polyclonal anti-HBs antibodies bound to the | 1 vial<br>sqf 8 ml | 2 vials<br>sqf 2 x 18<br>ml |

| | | | |
|---|---|---|---|
| | peroxidase. Lyophilized | | |
| **R8** | **SUBSTRATE BUFFER**<br>Citric acid and Sodium acetate solution pH 4.0 containing H2O2 (0.015%) and DMSO (4%) | 1 Vial<br>60 ml | 2 Vial<br>2x60 ml |
| **R9** | **CHROMOGEN PINK COLOURED**<br>Solution containing tetramethyl benzidine (TMB) | 1 Vial<br>5 ml | 2 Vial<br>2x5ml |
| **R10** | **STOPPING SOLUTION**<br>1N sulphuric acid solution | 1 Vial<br>28ml | 3 Vial<br>3x28ml |

**PRECAUTIONS**

The reliability of the results depends on correct implementation of the following Good Laboratory Practices:

- The name of the test or a specific identification number for the test, are written on the frame of each microtiterplate. This specific identification number is stated on each strip too.

**Monolisa® HBs Ag ULTRA: Specific ID number = 51**

Verify the specific identification number before any use. If the identification number is missing, or different from the stated number corresponding to the assay to be tested, the strip should not be used.

- Do not use expired reagents.
- Do not mix reagents from different lots within a given test run.

REMARK: For washing solution (R2, label identification: 20X coloured green), peroxidase substrate buffer (R8, label identification: TMB buf, coloured blue), chromogen (R9, label identification: TMB 11X, coloured purple) and stopping solution (R10, label identification: 1N coloured red), it is possible to use other lots than those contained in the kit, provided the same lot is used within a given test run. These reagents can be used with some other products of our company. In addition, the wash solution (R2, label identification: 20X coloured green) can be mixed with the 2 other wash solutions included in various Bio-Rad Reagent kits (R2, label identifications: 10X coloured blue or 10X coloured orange) when properly reconstituted, provided only one mixture is used within a given test run. Contact our technical service for detailed information.

Before use, it is necessary to wait 30 minutes for the reagents to stabilise to room temperature and one hour for diluted wash buffer R2.

- ❖ Carefully reconstitute the reagents avoiding any contamination.
- ❖ Do not carry out the test in the presence of reactive vapours (acid, alkaline, aldehyde vapours) or dust that could alter the enzyme activity of the conjugates.
- ❖ Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
- ❖ Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
- ❖ The enzyme reaction is very sensitive to metal ions. Consequently, do not allow any metal element to come into contact with the various conjugate or substrate solutions.
- ❖ The development solution (substrate buffer + chromogen) must be coloured pink. The modification of this pink colour within a few minutes after reconstitution indicates that the reagent cannot be used and must be replaced. Preparation of the development solution can be made in a clean disposable single use plastic tray or glass container that has first been pre-washed with 1N HCl and rinsed thoroughly with distilled water and dried. This reagent must be stored in the dark.
- ❖ Use a new distribution tip for each sample.
- ❖ Well washing is a critical step in this procedure: respect the recommended number of washing cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
- ❖ Never use the same container to distribute conjugate and development solution.
- ❖ Check the pipettes and other equipment for accuracy and correct operation.
- ❖ Do not change the assay procedure.

**HEALTH AND SAFETY INSTRUCTION**

All the reagents included in the kit are intended for "in vitro diagnostic use".

- ➢ Wear disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
- ➢ Do not pipette by mouth.
- ➢ The positive control R4 contains purified HBs Ag from subtypes ad and ay prepared with negative human plasma for anti-HCV, anti-HIV1 and anti-HIV2 antibodies and inactivated by warming.

- Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.
- Any equipment directly in contact with specimens and reagents as well as the washing solutions should be considered as contaminated products and treated as such.
- Avoid spilling samples or solutions containing samples.
- Spills must be rinsed with bleach diluted at 10%. If the contaminating fluid is an acid, spills must be initially neutralised with sodium bicarbonate and dried with absorbent paper. The material used for cleaning must be discarded in a contaminated residue container.
- Samples and reagent of human origin, as well as, contaminated material and products must be discarded after decontamination: - either by immersion in bleach at a final concentration of 5% of sodium hypochlorite (1 volume of bleach for 10 volumes of contaminated fluid or water) for 30 minutes. - or by autoclaving at 121°C for 2 hours minimum. Autoclaving is the best method to inactivate the HIV and the HBV viruses. - DO NOT PLACE SOLUTIONS CONTAINING SODIUM HYPOCHLORITE IN THE AUTOCLAVE
- Do not forget to neutralise and/or autoclave the solutions or washing wastes or any fluid containing biological samples before discarding them into the sink.
- The Safety Data Sheet is available upon request.
- Chemicals should be handled and disposed of in accordance with Good Laboratory Practices.
- Avoid any contact of the substrate buffer, the chromogen and the stopping solution with the skin and mucosa (toxicity, irritation or burn hazard).
- Some reagents contain ProClin™ 300 (0.04%, 0.1% and/or 0.5%)

**Xi Irritant**

R43: may cause sensitization by skin contact.

S28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- The Safety Data Sheet is available upon request

**PREPARATION OF THE REAGENTS**

**NOTE**: Before use, allow reagents to reach room temperature (18-30°C).

1. **Ready for use reagents**

**Reagent 1 (R1): Microplate**

Each frame support containing 12 strips is wrapped in a sealed foil bag. Cut the bag using scissors or a scalpel 0.5 to 1 cm above the sealing. Open the bag and take out the frame. Put the unused strips back into the bag. Close the bag carefully and put it back into storage at +2-8°C.

**Reagent 3 (R3): Negative control**

**Reagent 4 (R4): Positive control**

**Reagent 10 (R10): Stopping solution**

**2. Reagents to reconstitute**

**Concentrated washing solution (20X): Reagent 2 (R2)**

Dilute 1:20 in distilled water to obtain the ready-for-use washing solution. Prepare 800 ml for one plate of 12 strips.

**Conjugate working solution (R6 + R7)**

Gently tap the vial of the lyophilized conjugate (R7) on the work-bench to remove any substance from the rubber cap.

Carefully remove the cap and pour the content of a conjugate diluent vial (R6) into the lyophilized conjugate vial (R7).

Put the cap on and let stand for 10 minutes while gently shaking and inverting from time to time to ease dissolution.

**Enzyme development solution: Reagent 8 (R8) + Reagent 9 (R9)**

Dilute 1:11 the chromogen (R9) in the Substrate Buffer (R8) (ex: 1 ml reagent R9+10 ml reagent R8). Stability is for 6 hours in the dark once prepared

**STORAGE CONDITIONS - SHELF LIFE**

The kit should be stored at +2-8°C. When stored at this temperature, each reagent contained in the kit can be used until the expiry date mentioned on the package (except for specific instructions).

**R1:** After the vacuum-sealed bag has been opened, the microwell strips stored at +2-8°C in the carefully resealed bag can be used for 1 month.

**R2:** The diluted washing solution can be stored at +2-30°C during 2 weeks. The concentrated washing solution (R2) can be stored at +2- 30°C.

**R6 + R7:** After the reconstitution, the reagents can be used for 1 month if stored at +2-8°C and 8 hours if stored at room temperature (18-30°C). R8 + R9: After the reconstitution, the reagent stored in the dark can be used for 6 hours at room temperature (18-30°C)

**COLLECTION AND HANDLING OF SPECIMENS**

Collect a blood sample according to the current practices. The test should be performed on undiluted serum or plasma (collected with EDTA, heparin, citrate, ACD-based anticoagulants). Separate the serum or plasma from the clot or red cells as soon as possible to avoid any haemolysis. Extensive haemolysis may affect test performance. Specimens with observable

Particulate matter should be clarified by centrifugation prior testing. Suspended fibrin particles or aggregates may yield falsely positive results.

The specimens can be stored at +2-8°C if screening is performed within 7 days or they may be deep-frozen at -20°C for several months.

Avoid repeated freeze/thaw cycles. Samples that have been frozen and defrozen more than 3 times cannot be used. If the specimens are to be shipped, they must be packaged in accordance with the regulations in force regarding the transport of a etiological agents.

**DO NOT USE CONTAMINATED, HYPERLIPAEMIC OR HYPEHAEMOLYSED SERA OR PLASMA.**

REMARK: Samples containing up to 90 g/l albumin, 100 mg/l bilirubin, lipemic samples containing up to the equivalent of 36 g/l triglyceride, and hemolyzed samples containing up to 1 g/l hemoglobin do not affect the results

**ASSAY PROCEDURE**

Strictly follow the proposed procedure. Use the negative (R3) and (R4) positive controls for each series of determinations to validate the test results.

Follow the following Good Laboratory Practice:

1. Carefully establish the sample distribution and identification plan.
2. Prepare the diluted washing solution.
3. Prepare the conjugate R6+R7 working solution.
4. Take out from the protective packing the support frame and the necessary number of strips (R1). Put the unused strips back in their packing and reclose it.
5. Distribute in the wells in the following order (advisable plate distribution) :

Wells A1, B1, C1 and D1: 100 µl of negative control (R3)

Well E1: 100 µl of positive control (R4)

Well F1: 100 µl of the first unknown sample if this well is not used as control well for the validation of the sample and conjugate deposition (optional)

Wells G1, H1...etc : 100 µl of unknown sample.

Depending on the used system, it is possible to modify the position of controls or the order of distribution. NB: The sample distribution can be visually controlled at this step of the manipulation: there is a difference of colouration between empty well and well with sample (Refer to section 14 for automatic verification).

6. Quickly dispense 50 µl of conjugate solution (R6 + R7) into all wells; the conjugate solution must be shaken before use. Homogenize the reaction mixture. NB: The sample distribution can also be visually controlled at this step of the manipulation, as well as the conjugate distribution: The conjugate solution (R6+R7), which is coloured red, can be visually controlled at this step of the manipulation. 7. When possible, cover the plate with new adhesive film and incubate for 1hour and 30 ± 5 minutes at 37±1°C.

8. Remove the adhesive film; empty all wells by aspiration and wash a minimum of 5 times. The residual volume must be lower than 10 µl (if necessary, dry the strips by turning them upside down on absorbent paper).

9. Quickly dispense into each well 100 µl of prepared development solution (R8+R9), freshly prepared before use. Allow the reaction to develop in the dark for 30 ± 5 minutes at room temperature (18 - 30°C). Do not use adhesive film during this incubation. N.B.: The distribution of the development solution, which is coloured pink, can be visually controlled at this step of the manipulation: There is a clear difference of colouration between empty well and well containing the pink substrate solution.

10. Add 100 µl stopping solution (R10) by using the same sequence and rate of distribution as for the substrate solution. Homogenize the reaction mixture. N.B.: The distribution of the stopping solution, which is not coloured, can be visually controlled at this step of the manipulation. After the addition of the stopping solution the pink colouration of the substrate disappears (for the negative samples) or turns from blue to yellow (for the positive samples).

11. Carefully wipe the plate bottom. Wait at least 4 minutes after stopping solution addition before reading and within 30 minutes of stopping the reaction, read the optical density at 450/620-700 nm using a plate reader.

12. Check for agreement between the spectrophotometric and visual readings and against the plate and sample distribution and identification plans.

## CALCULATION AND INTERPRETATION OF THE RESULTS

1. **Calculation of the negative control mean optical density: OD R3 Example**

| Negative control R3 | OD |
|---|---|
| 1 | 0.030 |
| 2 | 0.031 |
| 3 | 0.032 |
| 4 | 0.027 |

Total R3 OD = 0.120

Total R3 OD / 4 = 0.030 = mean OD R3

2. **Calculation of the cut-off value**

For each method, the cut-off value is equal to: OD R3 + 0.050 Example: OD R3 = 0.030 Cut-off value = 0.030 + 0.050 = 0.080

3. **Test validity conditions**

All the values of the negative control should be lower or equal to 0.080 unit of optical density. The positive control value (OD R4) should be over or equal to 1.000. If one negative control value does not respect this norm or is superior to 40% compared to the mean value of the negative controls (OD R3), disregard and recalculate the mean using the three remaining values. Only one value may be eliminated in this way.

In case of very low background for the negative control R3 (average value of negative control below 0.010 OD) do not use these rejection criteria for R3 negative control. The test must be redone if all control values are out of these norms.

4. **Calculation of ratio sample**

For each sample, calculate the ratio:

Ratio= OD sample /Cut-off value

**Interpretation of the results**

Samples with ratio values lower than 1 are considered to be negative by the Monolisa™ HBs Ag ULTRA. Results just below the cut-off value (sample ratio between 0.9 and 1) should however,

be interpreted with caution. It is advisable to retest in duplicate the corresponding samples when the systems and laboratory procedures permit.

Samples with ratio values equal to or greater than 1 are considered to be initially positive by the Monolisa™ HBs Ag ULTRA. They should be retested in duplicate before final interpretation. If after retesting of a sample, the ratio values of the 2 duplicates are less than 1, the initial result is non-repeatable and the sample is declared to be negative with the Monolisa™ HBs Ag ULTRA.

For initial reactive or doubtful (0.9 < ratio < 1) samples, if after retesting the ratio values of at least one of the 2 duplicates is equal to or greater than 1, the initial result is repeatable and the sample is declared to be positive with the Monolisa™ HBs Ag ULTRA test, subject to the limitations of the procedure, described below.

The samples which have been retested twice and found negative with Monolisa™ HBs Ag ULTRA test, but with one value near the cut-off value (ratio between 0.9 and 1) should be considered with care. It is advised to retest the patient with another method or on another sample.

In case of very low optical density for tested samples (negative OD) and when the presence of samples as well as of reagent is controlled, the results can be interpretated as negative.

To verify the specificity of the reaction, every positive result in accordance with the interpretation criterias of Monolisa™ HBs Ag ULTRA should be confirmed by a neutralisation method of the HBs Ag.

Non repeatable reactions are often caused by:

1. Inadequate microplate washing.
2. Contamination of negative samples by serum or plasma with a high HBs Ag concentration.
3. Contamination of the substrate solution by oxidising agents (bleach, metal ions, etc...),
4. Contamination of the stopping solution.

**Assay Reproducibility**

The reproducibility of Monolisa™ HBs Ag ULTRA test has been determined, by the analysis of 4 samples: 1 negative sample, 2 HBs Ag positive samples (samples 2 and 3) and 1 high HBs Ag positive sample.

**LIMITS OF THE TEST**

A negative result indicates that the tested sample does not contain detectable HBs Ag with Monolisa™ HBs Ag ULTRA test. However because very low titer of HBs Ag could not be detected, such a result does not prelude the possibility of exposure to an infection by the hepatitis B virus.

Depending on the instrument used (washer, reader, automated processor), slight performance variability may be observed.

In addition, several authors have reported in the literature cases of viral hepatitis B (acute or chronic) where in viral DNA is detectable in the absence of the surface antigen (HBs Ag negative patients). These abnormal profiles, though rare, are the consequence of possible genetic mutations, either at the S and pre-S gene level (preventing recognition of the Ag by some immunological reagents) or, usually, at the X and pol gene level, inducing weak viral replication. Testing additional markers (HBs Ag-specific antibody or, if possible, amplified viral DNA) is recommended for the final diagnosis of the infection, in those very particular cases.

To verify the specificity of the reaction, every positive result (in accordance with the interpretation criterias of Monolisa™ HBs Ag ULTRA) should be confirmed by a neutralisation method of the HBs Ag (test Monolisa™ HBs Ag confirmation for example, code number 72408)

The colorimetric method for the samples, conjugate and development solution deposition verification does not allow to verify the accuracy of the dispensed volumes. This method only shows the presence of sample, conjugate and development solution into wells. The rate of wrong answers with this method is closely linked to the accuracy of the utilized system (cumulated coefficient of variation of dispensing and reading over 10% significantly decrease the quality of the verification).

In case of very poor washing efficiency after the conjugate incubation, the automatic verification of the development solution pipetting (by reading OD of wells at 490 nm) may provide wrong results with OD above 0.100 in the absence of development solution. Never observed during evaluation on 939 tested samples.

**B. Monolisa™ Anti-HBc PLUS**

| | |
|---|---|
| 1 plate - 96 tests | 72315 |
| 5 plates - 480 tests | 72316 |

**DETECTION KIT FOR ANTIBODIES TO NUCLEOCAPSID ANTIGEN (CORE) OF THE HEPATITIS B VIRUS IN HUMAN SERUM OR PLASMA BY ENZYME IMMUNOASSA**

**PRINCIPLE OF THE TEST**

Monolisa™ Anti-HBc PLUS is an enzyme immunoassay (indirect ELISA type) for the simultaneous detection of total antibodies to hepatitis B virus core in human serum or plasma.

Monolisa™ Anti-HBc PLUS is based upon the use of a solid phase prepared with recombinant HBc antigen.

Steps of the manipulation:

1. The sera to be tested and the control sera are added to the wells. If antibodies to HBc are present, they will bind to the antigens fixed on the solid phase.
2. The peroxidase-labelled antibodies to human IgG and IgM are added after a washing step. They in turn bind to the specific antibodies captured on the solid phase.
3. After removal of the unbound enzymatic conjugate, the antigen-antibody complex is revealed by addition of substrate.
4. After the reaction has been stopped, the absorbance values are read using a spectrophotometer at 450/620-700 nm. The absorbance measured for a sample allows the presence or absence of antibodies to HBc to be determined. The colour intensity is proportional to the quantity of anti-HBc antibodies bound on the solid phase.

## COMPOSITION OF THE KIT

| Lable | | Description of reagent composition | Preparation 72315 | Preparation 72316 |
|---|---|---|---|---|
| **R1** | **Microplate** | 12 strips of 8 wells coated with purified recombinant antigen (expressed in E. Coli) | 1Microplate | 5 Microplates |
| **R2** | **Concentrated washing solution (20X)** | Tris NaCl buffer, pH 7.4 Preservative: ProClin™ 300 (0.04%) | 1 Vial 70ml | 1 Vial 235ml |
| **R3** | **Negative control Serum** | Human serum negative for anti-HBc antibodies Preservative: Sodium Azide (0.1%) | 1 Vial 1.5ml | 1 Vial 3ml |
| **R4** | **Positive control Serum** | Human serum containing anti-HBc antibodies. Photochemicaly inactivated. Preservative: Sodium Azide (0.1%) | 1 Vial 1.5ml | 1 Vial 3ml |
| **R6** | **Sample diluent** | Sample diluent PBS buffer with a coloured control for sample deposition (purple) Preservative: ProClin™ (0.1%) and Ciprofloxacin 10 µg/ml. | 1 Vial 30ml | 2 Vials 2x60ml |

| | | | | |
|---|---|---|---|---|
| **R7** | **Conjugate** | Peroxidase-labelled goat antibody directed against human IgG and IgM (green) Preservative: ProClin™ (0.1%) and Ciprofloxacin 10 µg/ml | 1 Vial 30ml | 2 Vials 2x60ml |
| **R8** | **Peroxidase Substrate buffer** | Citric acid and Sodium acetate solution pH 4.0 containing H2O2 (0.015%) and dimethyl sulfoxide (DMSO) 4% | 1 Vial 60ml | 2 Vials 2x60ml |
| **R9** | **Chromogen: tetramethylbenzidine (TMB) solution (11X)** | Solution containing 3.3' , 5.5' tetramethylbenzidine (TMB) | 1 Vial 5ml | 2 Vials 2x5ml |
| **R10** | **Stopping solution** | Sulphuricacid solution (H2SO4 1N) | 1 Vial 28ml | 3 Vials 3x28ml |

**HEALTH AND SAFETY INSTRUCTIONS**

All the reagents included in the kit are intended for *"in vitro"* diagnostic use.

The name of the test, as well as a specific identification number for the test, are written on the frame of each microtiterplate. This specific identification number is stated on each strip too.

**Monolisa™ Anti-HBc PLUS : Specific ID number = 14.**

1. Verify the specific identification number before any use. If the identification number is missing, or different from the stated number above, the strip should not be used.
2. Wear disposable gloves when handling reagents and samples and thoroughly wash your hands after handling them.
3. Do not pipette by mouth.
4. Human origin material used in the preparation of the negative control (R3) has been tested and found non-reactive for hepatitis B surface antigen (HBs Ag), anti-HBc

antibodies and antibodies to hepatitis C virus (HCV) and human immunodefiency virus (HIV 1 and HIV 2).

5. Human origin material used in the preparation of the positive control (R4) has been tested and found reactive for hepatitis B surface antigen (HBs Ag) and anti-HBc antibodies and non -reactive for antibodies to hepatitis C virus (HCV) and human immunodefiency virus (HIV 1 and HIV 2). It has been photochemicaly inactivated.
6. No method can absolutely guarantee the absence of the HIV, HBV, HCV viruses or other pathogens. Consider these reagents as well as patient samples, as potentially infectious and handle them with the customary precautions
7. Consider any material directly in contact with samples and reagents of human origin, as well as washing solutions, as infectious materials.
8. Avoid spilling samples or solutions containing samples.
9. Spills must be rinsed with bleach diluted at 10%. If the contaminating fluid is an acid, spills must be initially neutralized with sodium bicarbonate, then cleaned with bleach and dried with absorbent paper. The material used for cleaning must be discarded in a contaminated waste container.
10. Samples, reagents of human origin, as well as, contaminated material and products should be discarded after decontamination either by immersion in bleach at the final concentration of 5% of sodium hypochlorite (1 volume of bleach for 10 volumes of contaminated fluid or water) for 30 minutes, or by autoclaving at 121°C for 2 hours minimum.

**CAUTION:** do not introduce solutions containing sodium hypochlorite into the autoclave.

11. Avoid any contact of the substrate buffer, the chromogen or the stopping solution with the skin or mucosa.
12. Do not forget to neutralize and/or autoclave the solutions or washing wastes or any fluid containing biological samples before discarding them into the sink.

**PRECAUTIONS**

The quality of results is dependent upon following good laboratory practice:

a. Do not use expired reagents.
b. Do not mix reagents from different lots within a given test run.

REMARK: For washing solution (R2, label identification: 20X coloured green), peroxidase substratebuffer (R8, label identification: TMB buf, coloured blue), chromogen (R9, label identification: TMB 11X, coloured purple) and stopping solution (R10, label identification: 1N coloured red), it is possible to use other lots than those contained in the kit, provided the same lot is used within a given test run. All reagents must be brought to room temperature before proceeding (30 minutes).

c. Carefully reconstitute the reagents avoiding any contamination.
d. Do not carry out the test in the presence of reactive vapours (acid, alkaline, aldehyde vapours) or dust that could alter the enzymatic activity of the conjugate.
e. Use glassware thoroughly washed and rinsed with deionized water or preferably, disposable material.
f. Do not allow the microplate to dry between the end of the washing operation and the reagent distribution.
g. The enzymatic reaction is very sensitive to any metals or metal ions. Consequently, no metal lement must be allowed to come into contact with the various solutions that contain conjugate or substrate solution.
h. The development solution (substrate buffer + chromogen) must be coloured pink. The modification of this pink colour within a few minutes after reconstitution indicates that the reagent cannot be used and must be replaced.

Preparation of the development solution can be made in a clean disposable single use plastic tray or glass container that has first been pre-washed with 1N HCl and rinsed thoroughly with distilled water and dried. This reagent must be stored in the dark.

i. Use a new pipette tip for each serum.
j. Well washing is a critical step in this procedure: follow the recommended number of wash cycles and make sure that all wells are completely filled and then completely emptied. Incorrect washing may lead to inaccurate results.
k. Never use the same container to distribute the conjugate and the development solution.
l. Check the pipettes and other equipment for accuracy and correct operation.
m. Do not change the assay procedure.

**SAMPLES**

Collect a blood sample according to the usual practice.

The test shall be performed on serum or plasma (collected in EDTA, heparin, citrate, ACD, CPD and CPDA-based anticoagulants). Extract it as soon as possible to avoid haemolysis. A severe haemolysis may alter the performance of the test. Samples containing aggregates must be clarified by centrifugation prior to testing. Suspended fibrin aggregates or particles may produce false positive results. The samples should be stored at +2-8°C if the screening is carried out within 7 days or deep-frozen at -20°C.

Avoid repeated freezing/thawing. If the samples are to be shipped, pack them in accordance with the regulations regarding the transport of etiologic agents, transport them preferably frozen. **DO NOT USE CONTAMINATED, HYPERLIPEAMIC OR HYPERHAEMOLYSED SERA.**

REMARK: Samples containing up to 30 g/l albumin, 200 mg/l bilirubin, lipemic samples containing up to the equivalent of 36 g/l triolein, and hemolyzed samples containing up to 5 g/l hemoglobin do not affect the results.

**RECONSTITUTION OF THE REAGENTS - VALIDITY – STORAGE**

Before using the reagents of the Monolisa™ Anti-HBc PLUS kit, allow them to stabilize at room temperature (18-30°C) for 30 minutes.

**A. Ready- for-use reagents**

**1. HBc Ag microplate (R1)**

Each frame support containing 12 strips is wrapped in a sealed foil bag. Cut the bag using scissorsor a scalpel 0.5 to 1 cm above the sealing. Open the bag and take out the frame. Put the unused strips back into the bag. Close the bag carefully and put it back into storage at +2-8°C.

**2. Negative control serum (R3)**

**3. Positive control serum (R4)**

**4. Sample diluent (R6)**

Invert gently to homogenize before use.

**5. Conjugate (R7)**

Invert gently to homogenize before use.

**B. Reagents to be reconstituted**

1. **Washing solution (20X concentrate) : R2**

Dilute 1:20 in distilled water to obtain the ready-for-use washing solution. Prepare 800 ml for one plate of 12 strips.

2. **Working diluted substrate solution (R8 + R9)**

Dilute reagent (R9) 1:11 using reagent R8 (example: 1 ml of R9 reagent in 10 ml of R8 reagent). 10 ml are necessary and sufficient for 1 to 12 strips.

## C. Validity

The kit should be stored at +2-8°C. When stored at this temperature, each reagent contained in the kit can be used until the expiry date mentioned on the package (except for specific instructions).

R1: After the vacuum-sealed bag has been opened, the microwell strips stored at +2-8°C in the carefully resealed bag can be used for 1 month.

R2: The diluted washing solution can be stored at +2-30°C during 2 weeks. The concentrated

washing solution (R2) can be stored at +2-30°C.

R8 + R9: After the reconstitution, the reagent stored in the dark can be used for 6 hours at room

temperature (18-30°C).

## D. METHOD

1. Strictly follow the protocol.
2. Use negative and positive control sera for each test, in order to validate the test quality.
3. Apply Good Laboratory Practice.

Two methods are available with Monolisa™ Anti-HBc PLUS :

| | Method-1 | Method-2 |
|---|---|---|
| Sample incubation | 37±1°C | 40±1°C |
| | 30 ± 5 min<br>water-bath dry incubator | |

| | | |
|---|---|---|
| Conjugate incubation | 37±1°C | 40±1°C |
| | 60 ± 5 min<br>water-bath<br>dry incubator | |
| Enzymatic revelation | 30 ± 5 min<br>room<br>temperature 18 - 30°C<br>(in the dark) | |

1. Carefully define the sample distribution and identification plan.
2. Prepare the wash solution to working strength.
3. Remove the microplate frame and ready to use strips (R1) from their protective bag.
4. Add quickly, directly and in succession :

   200 µl of diluent (R6) into each well

   20 µl of negative control serum (R3) in A1, B1

   20 µl of positive control serum (R4) in C1, D1, E1

   20 µl of the first sample in F1 if this well is not used as a reagent control for the sample addition monitoring

   20 µl of the second sample in G1, etc...

Depending on the utilized system, it is possible to modify the position of the controls. Homogenize the reaction mixture by a minimum of 3 aspirations with the 20 µl pipette or by shaking the microplate after the pipetting step.

It is also possible to dispense 220 µl of a sample previously diluted to 1:11.

If the sample distribution is over 10 min, it is recommended to distribute the negative and positive controls after the samples to be tested.

NB: After the samples distribution, the purple diluent turns blue.

5. Cover the wells with adhesive film by pressing over the whole surface to ensure tightness
6. Incubate the microplate in a thermostat-controlled water-bath or in a dry microplate incubator for :

Method 1: 30 min ± 5 min at 37°C ± 1°C

Method 2: 30 min ± 5 min at 40°C ± 1°C

7. Remove the adhesive film. Aspirate the contents of all wells into a liquid waste container and add a minimum of 0.370 ml of washing solution to each well. Aspirate again. Repeat the washing step three times (4 washes). The residual volume must be lower than 10 µl (if necessary, blot the microplate by turning it upside down on absorbent paper). If an automatic washing device is used, follow the same operating cycle.
8. Distribute quickly 200 µl of the conjugate solution into all wells. The conjugate must be shaken gently before use.

NB: The conjugate is coloured green. It is possible to verify the presence of conjugate in the wells by spectrophotometric reading at 450 nm.

9. Cover with new adhesive film and incubate for :

Method 1: 60 min ± 5 min at 37°C ± 1°C

Method 2: 60 min ± 5 min at 40°C ± 1°C

10. Remove the adhesive film, empty all wells by aspiration and wash 4 times as previously described. The residual volume must be lower than 10 µl (if necessary, blot the microplate by turning it upside down on absorbent paper).
11. Prepare the substrate solution (reagent R8 + R9).
12. Quickly dispense into each well 100µl of prepared development solution (R8+R9), freshly prepared before use. Allow the reaction to develop in the dark for 30 ± 5 minutes at room temperature (18- 30°C). Do not use adhesive film during this incubation.

N.B.: The distribution of the development solution, which is coloured pink, can be visually controlled at this step of the manipulation : There is a clear difference of colouration between empty well and well containing the pink substrate solution.

13. Add 100 µl stopping solution (R10) by using the same sequence and rate of distribution as for the substrate solution. Homogenize the reaction mixture.

    **Note:** the distribution of the stopping solution, which is not coloured, can be visually controlled at this step of the manipulation. After the addition of the stopping solution the pink colouration of the substrate disappears (for the negative samples) or turns from blue to yellow (for the positive samples).

14. Carefully wipe the plate bottom. At least 4 minutes after stopping solution addition and within 30 minutes of stopping the reaction, read the optical density at 450/620-700 nm using a plate reader.
15. Before recording the results, check the correlation between the reading and the microplate and sample distribution and identification plan.

**CALCULATION AND INTERPRETATION OF THE RESULTS**

The presence or absence of anti-HBc antibodies is determined by comparing for each sample the recorded absorbance with that of the calculated cut-off value.

A. Calculate the mean of the absorbance values for the positive control serum (OD R4)

Example: Positive control R4

| Well with positive control serum | Optical density |
|---|---|
| C1 | 1.796 |
| D1 | 1.802 |
| E1 | 1.852 |
| **Total** | **5.450** |

Mean of OD R4 = Total optical density/3 =5.450/3 = 1.817

B. Calculate the cut-off value (Vs)

Vs =Mean of OD R4/5= 1.817/5 = 0.363

**Validation criteria**

1. For the negative control: each individual measured absorbance value must be <0.100.
2. For the positive control: each absorbance value must be ≥1.000 and ≤ 2.900.

NB. If one of the positive control value is out of these norms or differs by more than 30% from the mean value, perform the calculation again with the two remaining positive control values. The test should be repeated if more than one positive control value is outside the limits set above.

**Interpretation of the results**

Samples with an optical density < the cut-off value are considered to be negative with the Monolisa™ Anti-HBc PLUS test.

Samples with an optical density ≥ the cut-off value are considered to be initiallly positive with the Monolisa™ Anti-HBc PLUS test and must be retested in duplicate before the final interpretation.

However, results just below the cut-off value Vs -10% < OD should be interpreted with care (it is advised to retest the corresponding samples in duplicate when the utilized systems and laboratory procedures allow it).

For initial reactive or doubtful (0.9<ratio<1) samples, after retesting, the sample is considered to be positive with the Monolisa™ Anti-HBc PLUS test if at least one of the both measurements is positive, i.e. higher than, or equal to, the cut-off value. The sample is considered to be negative with the Monolisa™ Anti-HBc PLUS test if both values are less than the cut-off value.

**SPECTROPHOTOMETRIC VERIFICATION OF SAMPLE AND REAGENT PIPETING (OPTIONAL)**

**VERIFICATION OF THE SAMPLE PIPETING**

After the sample distribution, the purple diluent turns blue.

**Method 1: without the use of a control reagent**

The presence of sample into the well can be verified by automatic reading at 620 nm: compare the measured optical densities for each well, after the distribution of sample diluent (R6) and after the sample pipeting :

1. The OD values of the wells containing sample diluent (R6) only must be over 0.500.
2. Each well containing sample must have an OD variation greater than 0.200 (an OD variation strictly lower than 0.200 indicates a poor dispensing of the sample).

**Method 2: using control reagent**

The presence of sample into the wells can be verify by automatic reading at 620 nm with the use of a control well containing the sample diluent (R6) only: read the plate and compare the OD obtained for each sample with the OD obtained for the control well:

1. The OD value of the control well containing sample diluent (R6) only must be over 0.500.
2. The OD variation between well with sample and the control well must be greater than 0.200 (an OD variation strictly lower than 0.200 indicates a poor dispensing of the sample).

**VERIFICATION OF CONJUGATE PRESENCE**

The conjugate (R7) is coloured green.

The presence of conjugate (R7) into the wells can be controled by automatic reading at 450 nm: the OD value of each well must be greater than 0.300 (a value lower this norm indicates a poor dispensing of the conjugate).

**DEVELOPMENT SOLUTION PIPETTING VERIFICATION**

It is possible to verify the presence of pink development solution into the well by automatic reading at 490 nm: a well with development solution must have an optical density greater than 0.100 (a lower OD indicates a poor dispensing of the development solution).

**NOTE:** the acuracy of Monolisa™ Anti-HBc PLUS test has been determined by the analysis of 4 samples: 1negative sample (sample 1), 2 low anti-HBc positive samples (samples 2 and 3) and 1 high anti-HBc positive sample (sample 4). The intra assay reproducibility has been evaluated by testing these 4 samples 30 times in the same run, the inter assay reproducibility has been evaluated by testing these 4 samples 3 times on 2 microplates performed on 2 independant runs each day during 5 days.

**LIMITS OF THE TEST**

A negative result indicates that the tested sample does not contain detectable antibodies anti-HBc with Monolisa™ Anti-HBc PLUS. However, such a result does not preclude the possibility of exposure to an Hepatitis B virus infection. The colorimetric method for the samples, conjugate and development solution deposition verification does not allow to verify the accuracy of the dispensed volumes. This method only shows the presence of sample, conjugate and development solution into wells. The rate of wrong answers with this method is closely linked to the accuracy of the utilized system (cumulated coefficient of variation of dispensing and reading over 10% significantly decrease the quality of the verification).

In case of very poor washing efficiency after the conjugate incubation, the automatic verification of the development solution pipetting (by reading OD of wells at 490 nm) may provide wrong results with OD above 0.100 in the absence of development solution. However this phenomenon has not been observed during evaluation on 939 tested samples.

## Annex: VIII. Thesis report declaration sheet

I, the under signed, declared that this is my original work, will never be presented in this or any other University, and that all the resources and materials used for the thesis work, will be fully acknowledged.

Name of student: Adane Adugna Signature: _____Date: _____

This thesis will be submitted for examination with my approval as the student thesis work advisor.

Name of advisor: Ahmed Esmael (Msc, PhD candidate) Signature _____ Date: _____

Gebereselassie Demeke (Msc, Assistant Professor) Signature_____ Date_____

This thesis will be submitted for examination with my approval as the program coordinator.

Name of coordinator: _____Signature: _____Date: _____

This thesis examination result will be corrected and based on the given evaluation criteria

Name of external examiner: _____Signature: _____Date: _____

Name of internal examiner _____Signature: _____Date: _____