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# Detection of Hepatitis B Surface Antigen among Health Care Workers in Atabra locality River Nile State

**الكشف عن فيروس التهاب الكبد الوبائي ( النوع ب ) بين العاملين في مجال الرعاية الصحية في محلية عطبرة ولاية نهر النيل**

**A dissertation submitted in partial fulfillment for the requirements of M.Sc degree in Medical Laboratory Science (Microbiology)**

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# الآيـــة

قال تعالى:

﴿ يَا أَيُّهَا النَّاسُ اتَّقُواْ رَبَّكُمُ الَّذِي خَلَقَكُم مِّن نَّفْسٍ وَاحِدَةٍ وَخَلَقَ مِنْهَا زَوْجَهَا وَبَثَّ مِنْهُمَا رِجَالاً كَثِيراً وَنِسَاء وَاتَّقُواْ اللّهَ الَّذِي تَسَاءلُونَ بِهِ وَالأَرْحَامَ إِنَّ اللّهَ كَانَ عَلَيْكُمْ رَقِيباً ﴾

صدق الله العظيـم

سورة النساء الآية (1)

# DEDECATION

**TO**

**MY Mother**

**A strong and gentle soul who tought me to trust in Allah ,believe in hard work and that so much could be done with little**

**My Father**

**For earing an honest living for us and for supporting and encouraging me to believe in my self**

**My sisters**

**Whom support and love me**

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Thanks to the **ALMIGHTY ALLAH** for giving me the strength and patience to complete this work successfully.

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I would like to thank all those who assisted me, encouraged me to complete this research and provided me with the necessary information required to complete this study.

Last but not least many thanks for the **Health Care Workers** at Atbara locality for their Kindness and blood sample.

## ABSTRACT

Hepatitis B infection is one of the world's major infectious diseases. Health care workers (HCWs) have a high risk of occupational exposure to many blood- borne viruses including HIV, Hepatitis B and Hepatitis C viruses. This was cross-sectional study was conducted during period from March to June 2018., to determine the sro-prevalence of Hepatitis B virus (HBSAg) and to determine the possible association between Hepatitis B virus and selected risk factors among health care workers in Atbara Teaching Hospital and selected health centers. Blood samples were obtained from eligible participants, sera separated by centrifugation and structured questionnaire was used to collect both demographic and clinical data. Enzyme Linked Immunosorbent Assay (ELISA) technique was used to detection Hepatitis B surface antigen (HBsAg). Ninety two (n=92) HCWs participated in this study, the males were 47/92 (51.1%) and females 45/92 (48.9%), 23/92 (25%) were vaccinated and 69/92 (75%) were not vaccinated. The sero-positivity was higher in female than male, and the infection according to occupational practice was higher among laboratory technologist 3/(3.3%) followed by cleaning staff 3/(3.3%) and Nurses 2/(2.2%). Three of the vaccinated participants were infected and the disease was more frequent in those who were exposed to an accidental injury during their work (4 out of 8 infected participants ).

**ملخـــص البحث:**

عدوي الكبد الوبائي هي احد الأمراض المعدية الرئيسية في العالم. ويتعرض العاملون في مجال الرعاية الصحية لخطر كبير من التعرض المهني للإصابة من الفيروسات المنقولة عن طريق الدم فيما ذلك فيروس نقص المناعة المكتسبة، فيروس الكبد (ج) وفيروس الكبد الوبائي(ب). هذه الدراسة مقطعية أجريت في الفترة من شهر مارس إلى يونيو 2018م لتحديد الانتشار المصلي لفيروس التهاب الكبد الوبائي ولتحديد احتمالية الارتباط بين فيروس التهاب الكبد الوبائي و عوامل الخطر المحددة للعاملين بالرعاية الصحية الأولية بمستشفى عطبرة التعليمي والمراكز الصحية المختارة. جمعت عينات الدم من المتطوعين المستهدفين وفصلت بجهاز الطرد المركزي وجمعت معلومات بواسطة استبيان منتظم لجمع البيانات الديموغرافية والسريرية معاً، استخدمت تقنية اليزا للكشف عن مستضد الكبد السطحي. اثنان وتسعون من العاملين في مجال الرعاية الصحية شاركوا في هذه الدراسة . وكان عدد الذكور 47 والإناث 45 ،مجموعهم 92. 23 منهم تم تطعيمهم و69 لم يتم تطعيمهم . وكان من المستغرب أن 3 من المشاركين الذين تم تطعيمهم كانوا مصابين . كانت ايجابية المصل اعلي في الإناث من الذكور. وجدت زيادة العدوى وفقا الممارسة المهنية 3من بين اختصاصي المختبرات الطبية ،3 من موظفي التنظيف و 2 من الممرضين .كما كان المرض أكثر انتشارا في أولئك الذين تعرضوا الوخز بالإبر إثناء فترة العمل وكان عدد المصابين4.

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## LIST OF ABBREVIATIONS

| Abbreviations | Full name |
|---|---|
| ALT | Alanine Transaminase |
| CDC | Center for Disease Control |
| CCCDNA | Covalently closed circular Deoxyribo Nucleic Acid |
| C.O | Cut-Off |
| DNA | Deoxyribo-Nucleic Acid |
| ELISA | Enzyme Linked Immuno sorbent assay |
| EIA | Enzyme Immune Assay |
| HBcAg | Hepatits B core antigen |
| HBeAg | Hepatits B envelope antigen |
| HBIG | Hepatitis B immunoglobulin |
| HBsAb | Hepatitis B surface antibody |
| HBsAg | Hepatits B surface antigen |
| HBV | Hepatits B virus |
| HCC | HepatoCellular Carcinoma |
| HCV | Hepatitis C virus |
| HCW | Health Care Workers |
| HDV | Hepatitis Delta Virus |
| HRP | HorseRadish Peroxidase |
| IgG | Immunoglobulin G |
| IgM | Immunoglobulin M |
| NC | Negative Control |
| OD | Optical Density |
| PCR | Polymerase Chain Reaction |
| RNA | RiboNucleic Acid |
| SPSS | Statistical Package for the Social Scinces |
| TMB | TetraMethylBenzidine |
| WHO | World Health Organization |

# Chapter One

## Introduction

## Rationale

## Objectives

# Chapter one

# INTRODUCTION

## 1.1. Introduction

Hepatitis B viral infection is a major global health problem with predilection for the liver and is known to commonly lead to chronic infection after acute infection. The chronic infections increases risk of death from childhood hepatic failure, cirrhosis of the liver and liver cancer (Shepard *et al*., 2006; Mustaphas *et al.,* 2007). The earliest recognition of the public health importance of hepatitis B virus infection is thought to have occurred when it appeared as an adverse event associated with a vaccination campaign (WHO, 2011).More than 300 million people have chronic liver infections globally and about 600,000 people die annually from acute or chronic complications of hepatitis B infection. The highest prevalence of hepatitis B infection is in sub-Saharan Africa and East Asia, majority of the people in these regions become infected during childhood and between 5–10% of the adult population is chronically infected(WHO,2009). Approximately three million health care workers (HCW) are exposed to percutaneous blood – borne viruses each year. It is estimated that 66000 hepatitis B virus (HBV) are acquired annually (Kermode *et al*., 2005).The infections are important risk factors for hepatocellular carcinoma and other liver related morbidity (Omer *et al*., 2001). The HBV carrier rate varies widely from 0.01% to 20% in different geographical regions of the world. The HCW including clinicians, nurses, laboratory technicians, other hospital technicians, administration and cleaning staff are exposed to an increased risk of occupational infection with HBV (Tarantola*et al*., 2006). Health care workers (HCWs) are defined as all paid and unpaid persons working in health-care settings who have the potential for exposure to patients and/or to infectious materials, including body substances, contaminated medical supplies and equipment, contaminated environmental surfaces, or contaminated air. HCWs might include physicians, nurses, nursing assistants, therapists, technicians, emergency medical service personnel, dental personnel,

pharmacists, laboratory personnel, autopsy personnel, students and trainees, contractual staff not employed by the healthcare facility, and persons (e.g. clerical, dietary, housekeeping, laundry, security, maintenance, administrative, billing and volunteers) not directly involved in patient care but potentially exposed to infectious agents that can be transmitted to and from HCWs and patients (CDC, 2011). While performing their duties, healthcare workers (HCWs) are frequently exposed to dangerous infectious agents. The risk of transmission of vaccine-preventable infections, both from patients to HCWs and from personnel to patients, other HCWs, and visitors is substantial (Almuneef *et al*., 2006).Health care workers are at a high risk of exposure to blood and body fluids. Needle stick injuries, cuts and splashes are common occupational accidents exposing health care providers to different blood borne pathogens. Transmission of hepatitis B virus, human immune deficiency virus (HIV), and hepatitis C virus (HCV) has been related to injuries and frequency of exposure. According to world health organization (WHO), 2.5% of HIV cases, 40% of both and HCV cases worldwide are the result of occupational exposure among health care workers (CDC,1999) Adherence to standard precautions, awareness about post exposure prophylaxis is poor in developing countries among HCWs and documentation of exposures is suboptimal (WHO, 2002). Healthcare workers have been historically recognized as being at increased risk of HBV infection, effective vaccines are available to prevent HBV infection and universal immunization programs are now advocated, needle stick injuries are one of the most efficient modes of HBV transmission, most transmission in the healthcare setting probably occurs in the absence of a documented percutaneous injury, there is evidence from a Cochrane Library systematic review to support occupational health guidelines that all healthcare workers should be offered HBV vaccination and that the vaccine is safe (Jefferson *et al.,* 2003). Healthcare workers who have not been immunized, HBIG and HBV vaccine are recommended after a significant exposure. Although the effectiveness of HBIG and HBV vaccine has not been evaluated in the occupational health setting, the

increased efficacy of this combination compared with HBIG alone in preventing prenatal transmission is presumed to apply to the occupational health setting (Beasly *et al*., 1983).

## 1.2. Rationale

Health care workers have a high risk of occupational exposure to many blood borne viruses, hepatitis B virus is a major health problem and causes significant morbidity and mortality rate. the observation that needle-stick injuries can transmit the virus indicates that only very small amounts of blood are necessary to transmit the disease. The prevalence of disease is associated with a proper understanding of the mode of transmission of the disease. Moreover, little is known about the situation and prevalence of the disease in River Nile State especially among health workers whom may represent a source of infection. Furthermore, the proper understanding of the prevalence in study area may help in setting further control programs. Hepatitis B exposure was assessed in 311 health care workers in Uganda highly endemic country .60.1 % of health care workers have evidence of hepatitis B infection ,with 8.7 % being chronic carrier and one (0.3%) acutely infected ,needle stick injuries reported by 77% of HCW.

In Tanzania ,the prevalence of acute or chronic HBV infection in HCW was found to be 8.8%

The evidence of HBsAg among health care workers in wad madani hospital was 22% .The aim of this study was to determine the prevalence of HBsAg among health care workers in Atbara locality.

## 1.3 Objectives

### 1.3.1 General objective

To detect hepatitis B surface antigen (HBsAg) among healthcare workers in Atbara locality.

### 1.3.2 Specific objectives

1. To detect hepatitis B surface antigen (HBsAg) among health workers in Atbara locality by using ELISA technique.
2. To correlate the possible association between hepatitis B virus and selected risk factors.

# Chapter Tow

## Literature review

# CHAPTER TWO

## LITERATURE REVIEW

### 2.1 HBV properties

The hepadna viruses got their name because they cause hepatitis and they have DNA genomes. They are known as hepatitis B viruses (HBVs) and are classified in the family Hepadnaviridae. Some members infect mammals and some infect birds; examples include woodchuck HBV and heron HBV. The best known hepadnavirusis that which infects humans; it is commonly referred to as HBV, and is of major importance as an agent of disease and death. Duck HBV, on the other hand, is non-pathogenic in its natural host (Carter and Saunders, 2007). Hepatitis B virus is a member of the hepadnavirus family, it is a 42, nm enveloped virion with icosahedral nucleocapsid core containing partially double strand circular DNA genome (Levinson,2014).

### 2.1.1 Genome

Hepatitis B virus is a small DNA virus and belong to a group of hepatotropic DNA viruses (hepadnaviruses). The virus consists of nucleocapsid and an outer envelopecomposed mainly of three antigens (HBs Agthat play a central role in the diagnosis of HBV infection). The nucleocapsid contains HBc Ag, a DNA polymerase reverse transcriptase, the viral genome as well as cellular proteins (Seto*ut al*., 2011).The genome is made up of two strands of DNA, one of which is incomplete; hence the DNA is partly single stranded and partly double-stranded. A short sequence is triple-stranded as a result of a complementary sequence at the 5_ends, and this results in the DNA having a circular conformation, the genome is very small, with a length of about3.2 kb (p).At the 5_ end of each of the DNA strands there isa covalently linked molecule: a capped RNA on the short strand and a protein (P) on the long strand. (Carterand *Saunders*2007). Three types of viral particles can be visualized in the infectious serum by electron microscopy: the infectious virions and the sub viral particles. The infectious virus particles are the so-called Dane particles (Dane *et al.,* 1970), have a spherical, double shelled structure of 42-44 nm containing a

single copy of the viral DNA genome, covalently linked to the terminal protein of the virus. A hallmark of HBV infection is the presence of two additional types of particles, the spheres and the filaments, which are exclusively composed of hepatitis B surface proteins and host-derived lipids (Glebe *et al*., 2007). Since they do not contain viral nucleic acids, the sub viral particles are non-infectious. The spherical structures measure around 22 nm in diameter, while the filaments are of similar width, but of variable lengths. The viral membrane contains three viral surface proteins and is acquired by the virus during budding into the endoplasmic reticulum, whereas the viral particles are transported via the secretary pathways through the ER and Golgi. The surface proteins are named the preS1 (or large), the preS2 (or middle) and the S (or small), which correspond to the HBsAg. As with nearly all enveloped viruses, the HBV particle also contains proteins of host origin (Glebe, 2007; GlebeandUrban, 2010). The HBV genome consists of a partially double-stranded relaxed circular DNA of approximately 3200 nucleotides in length, varying slightly from genotype togenotype, that in concert with the core protein (HBcAg) forms the nucleocapsids (Nassal *et al.*,2008). Within the Dane particle the negative strand of the viral DNA is present in full-length, carrying the complete genetic information. In contrast, the positive strand spans only ~ 2/3 of the genome in length, whilst its 3' end is variable in size (Summers *et al.,* 1988*)*. The viral polymerase is covalently bound to the negative strand by a phosphotyrosine bond. At the 5' end of the positive strand a short RNA oligomer originating from the pre-genomic RNA residually remains bound covalently after the viral DNA synthesis. The negative strand also contains small redundancy of 8-9 nucleotides in length on both the 5' end and the 3' end, named the R region. These redundant structures are essential for viral replication (Seeger,1986; Nassal, 2008).

## 2.2. Replication:

Hepatocytes (liver cells) are the host cells for having the body. In the laboratory, primary cell cultures of human hepatocytes support replication, but unfortunately none of the established cell lines derived from liver tumors can be infected by HBV visions. Some cell lines, however, can be infected using HBV DNA (a procedure known as transfect ion) **(**Carter andSaunders, 2007*)*.The life cycle of the HBV is complex. Hepatitis B is one of the few known non retroviral viruses which used reverse transcription as a part of its replication process. The virus gain entry in to the cell by binding to an unknown receptor on the surface of the hepatocytes and enter it by endocytosis. Because virus multiplies via RNA made by host enzyme, the viral genomic DNA has to be transformed to the cell nucleus by host protein called chaperones. The partially double stranded viral DNA is then made fully double stranded and transform in covalently closed circular DNA (cccDNA) that serves as template, for transcription of four viral mRNAs. The largest mRNA, (which is larger than the viral genome), is used to make the new copies of the genome the capsid core protein and the viral DNA polymerase. These four viral transcripts undergo additional processing and go on to form progeny virions which are released from the cell or returned to the nucleus and recycled to produce even more copies. The long mRNA is then transported back to the cytoplasm where the virion p protein synthesized DNA via its reverse transcriptase activity (Levinson, 2014). Hepatocytes (liver cells) are the host cells for HBV in the body. In the laboratory, primary cell cultures of human hepatocytes support replication, but unfortunately none of the established cell lines derived from liver tumors can be infected by HBV virions. Some cell lines, however, can be infected using HBV DNA(a procedure known as transfection), (*Carter* and Saunders, 2007).

## 2.3 HBV Transmission

The three main modes of transmission are via blood, during sexual intercourse, and perinatally from mother to newborn (Levenson, 2014).

### 2.3.1 Risk groups for hepatitis B in developed Countries

Intravenous drug abusers, homosexual men, sexual contacts of antigen-positive persons, residents in long-stay homes for mentally handicapped
People, renal dialysis patients, recipients of multiple blood products (e.g. haemophiliacs), surgeons, dentists and morticians, and infants of infectious HBsAg positive mothers, (Bannister *et al*., 2006).

## 2.4 HBV Genotype and Its Clinical Significance

Based on an intergroup divergence of 8% or more of the complete genomes, HBV can be classified in to 7 genotypes, i.e. A-G (Okamoto *et al.*,1988; Norder*et al*.,1992). Genotype H was recently identified in central America (Arauz-RuizP*etal*.,2002), is well known that HBV genotypes have distinct geographical distributions. The geographical distributions of HBV genotypes are summarized in Table 2. The prevalent HBV strains in China are genotype B and C (Zhu *et al.*,1999). but the two genotypes distribute unevenly in China. We studied 1096 Chinese chronic HBV carriers from 9 provinces in Mainland China. Four major genotypes A, B, C and D were found and their prevalence were 1.2%,41%, 52.5% and 4.3%, respectively. In northern China, genotype C is predominant(85.1%), while in southern China, genotype B is predominant (55.0%).Genotypes A and D are also found in other areas of China. However, the genotypes E-H have not been reported in China. Recently, genotype C/D hybrid was identified in Tibet (Cui*etal.*,2002) and genotype B was found recombinated with pre C/C region of genotype C in China (Luo*et al*., 2004). Accumulated data suggest the importance of genotype, subgroup and recombination that may influence the biological characteristics of virus and clinical outcome of HBV infection. Several studies reported a correlation of HBV genotypes with HBeAg clearance, liver damage, and the response to IFN treatment. It was reported that HBeAg carrier status tends to be longer and the prevalence of HBeAg appears higher in patients with genotype C than with genotype B (Orito *et al*, 2001). HBV carriers with genotype B have lower histological activity scores and genotype C is more prevalence in patients with

cirrhosis (*Kao et al*., 2000). Furthermore, a retrospective study showed that HBV genotype B is associated with a higher rate of IFN-induced HBeAg clearance compared with genotype C (Kao *et al.*, 2000). However, whether patients with genotype B differ from those with genotype C in development of hepatocellular carcinoma remains controversial. The response of different HBV genotypes to interferon-Alfa treatment is of increasing interest because the benefit of interferon-Alfa or its pegylated form in combination with other antiviral agents is being explored in the treatment of chronic hepatitis B. In a homogeneous group of prospectively followed patients from Europe, a recent study demonstrates that genotype A responds better than other HBV genotypes to standard interferon therapy and represents an independent predictor of a therapeutic success, with a greater impact than other pre-treatment characteristics, such as HBV DNA or ALT levels (Hou*et al.*, 2000).

**2.5 Epidemiology**

There are around 350 million chronic carriers of the hepatitis B virus worldwide. The incidence of acute disease and prevalence of carriage varies considerably from country to country. In parts of south-east Asia, 10–20% of the population may be carriers, whereas most countries in Europe and North America have carriage rates below 2%.Where carriage rates are high, acute infection occurs mainly in infants and young children, mostly via intra partum and horizontal transmission within households. Skin disease and biting arthropods may facilitate the transfer of body fluids from person to person. In low-prevalence countries most infections are sporadic and arise in adults through needle stick injuries, shared syringes, bites and scratches, or by sexual contact.

Those most at risk include intravenous drug abusers, homosexual men, residents and staff of institutions for the mentally handicapped, surgeons, dentists, laboratory workers, morticians, renal dialysis patients and recipients of unscreened blood and blood products (Bannister *et al*., 2006). Hepatitis B is highly endemic in developing regions with large population such as South East

Asia, China, sub-Saharan Africa and the Amazon Basin, where at least 8% of the population are HBV chronic carrier. In these areas, 70–95% of the population shows past or present serological evidence of HBV infection. Most infections occur during infancy or childhood. Since most infections in children are asymptomatic, there is little evidence of acute disease related to HBV, but the rates of chronic liver disease and liver cancer in adults are high (alter,2003)Hepatitis B virus is spread from person to person primarily by blood and blood products. Blood transfusion remains a major mode of transmission in the United States; however, screening of donors has reduced the risk to 1 in 63,000 transfusions. Screening tests fail to exclude small percentage of donors who have infectious viral particles in their blood despite being negative for HBsAg. Hepatitis B virus is also found in other body fluids, including urine, bile, saliva, semen, breast milk, and vaginal secretions. It is not found in feces, however. Membrane contact with any of these body fluids can result in transmission. The virus can be spread to sexual partners, and its prevalent in homosexual men and heterosexuals with multiple partners. It can be readily spread from mother to neonate at the time of vaginal delivery—a common mode of transmission in developing countries. Intravenous drug abusers have a high incidence of hepatitis B. Reuse of needles has also led to transmission of the virus during placement of tattoos and ear-piercing. Crowded environments such as institutions for the mentally handicapped, (Frederick and Southwick, 2007).

**2.6 Pathogenesis and immunity**

After entering the blood, the virus infects hepatocytes, and viral antigens are displayed on the surface of the cells. Cytotoxic T cells mediate an immune attack against the viral antigens, and inflammation and necrosis occur. Immune attack against viral antigens on infected hepatocytes is mediated by cytotoxic T cells. The pathogenesis of hepatitis B is probably the result of this cell-mediated immune injury, because HBV itself does not cause a cytopathic effect. Antigen–antibody complexes cause some of the early symptoms (e.g., arthralgias, arthritis, andurticaria) and some of the complications in chronic hepatitis (e.g.,

glomerulonephritis, cryoglobulinemia, and vasculitis), (Levension,2014). Fully differentiated hepatocytes are the primary cell type infected by HBV. The primary cause of hepatic cell destruction appears to be the cell-mediated immune response, which results in inflammation and necrosis. The cells involved are cytotoxic T cells, which react specifically with the fragments of nucleocapsid proteins (HBcAg and HBeAg), expressed on the surface of infected hepatocytes. This response also contributes to control of the infection by eliminating virus-producing cells. Enhanced natural killer cell activity, as well as production of interferon-γ also contributes to limiting the extent of infection. Anti-HBsAg antibody, which is the neutralizing antibody, does not appear until well into the convalescence period, when it may aid in clearing any remaining circulating free virus (Harvey., Cornelissen. and Fisher, 2007).

## 2.7 Clinical Presentation/Natural History

### 2.7.1 Acute Infection

After exposure to the virus, there is a long, asymptomatic incubation period, which may be followed by acute disease(described later) lasting many weeks to months. The natural course of acute disease can be tracked using serum markers

• HBsAg appears before the onset of symptoms, peaks during overt disease, and then declines to undetectable levels in 3 to 6 months.

• Anti-HBs antibody does not rise until the acute diseases over and usually is not detectable for a few weeks to several months after the disappearance of HBsAg. Anti-HBs may persist for life, conferring immunity; this is the basis for current vaccination strategies using noninfectious. HBsAgHBeAg, HBV-DNA, and DNA polymerase appear in serum soon after HBsAg, and all signify active viral replication. Persistence of HBeAg is an important indicator of continued viral replication, infectivity, and probable progression to chronic hepatitis. The appearance of anti-HBe antibodies implies that an acute infection has peaked and is on the wane.

IgM anti-HBc becomes detectable in serum shortly before the onset of symptoms, concurrent with elevation of serum aminotransferase levels (indicative of hepatocytedestruction). Over a period of months, the IgM anti-HBc antibody is replaced by IgG anti-HBc. As in the case of anti-HAV, there is no specific assay for IgG anti-HBc, but its presence is inferred from decline of IgM anti-HBc in the face of rising levels of total anti-HB (Kumar *et al*.,2013)Initial infection with hepatitis B virus (HBV) maybe asymptomatic in up to 50 per cent of adults and90 per cent of children, When symptoms occur, they may include anorexia, vague abdominal pain, nausea, vomiting and jaundice, Fever may be absent or mild (Heymann,2008). Extra hepatic manifestations such as arthralgias, arthritis, macular rashes, thrombocytopenia or papularacrodermatitis(Gianotti- Crosti syndrome) can occur early in the course of the illness and may precede jaundice, Acute HBV infection cannot be distinguished from other forms of acute viral hepatitis on the basis of clinical signs and symptoms or nonspecific laboratory findings (American Academy of Pediatrics, 2012).

### 2.7.2 Chronic Infection

While the majority of individuals infected with HBV are able to clear the virus, some individuals fail to mount an adequate immune response, leading to chronic infection (Conly and Johnston,2007). The exact mechanisms by which chronic liver injury occurs in HBV infection are not known (Kozieland*Siddiqui*,2010). Hepatitis B virus infection becomes chronic in approximately90 per cent of infants infected at birth (American Academy of Pediatrics, 2012) If chronic infection is established, the spectrum of illness ranges from the healthy carrier state to all of the sequelae of chronic hepatitis, including mild to moderate fibrosis, compensated cirrhosis, hepatic decompensation and hepatocellular carcinoma(HCC), The single most important risk factor for HCC is cirrhosis (Pungpapong and Poterucha, 2007). Individuals who are immunosuppressed or have an underlying chronic illness are at increased risk of developing chronic infection (Heymann, 2008; American

Academy of Pediatrics, 2012). Factors that may influence the natural history of chronic infection include gender, race, alcohol use, and co-infection with hepatitis A, hepatitis C or hepatitis D viruses or humanimmunodeficiency virus (HIV) (American Academy of Pediatrics,2012). Antiviral therapy can modify the natural history of chronic HBV infection (Yimand, 2006).Super infection or co-infection is not uncommon in patients with chronic HBV infection. Acute hepatitis delta virus (HDV) may be acquired as co-infection simultaneously with HBV or as a super infection in a patient who is already a carrier of HBV (Sherman *et al*., 2007). Infection with HDV in HBV infected individuals is associated with more severe and/or progressive liver disease than is HBV mono infection. The natural course following acute hepatitis C virus(HCV) super infection has not been well studied. The long-term prognosis following acute HCV super infection is worse than that following HDV super infection (Liaw *et al*., 2004). Individuals co-infected with the parasite *Schistosoma* (Schistosomiasis) are more likely to have more severe hepatitis B manifestations and become chronic carriers of HBV (Plourde, 2008

**2.8 HBV and Hepatocellular Carcinoma** :

Epidemiologic studies have demonstrated that there is a consistent and specific causal association between HBV infection and HCC(Beasley *et al.* 1981;Chen*etal.*1996). In patients with persistent HBV infection, the risk of HCC was 100 times higher than in non-infected individuals (Beasley *et al*. 1981).The global distribution of hepatocellular carcinoma correlates with the geographic prevalence of chronic carriers of HBV, who number 400 million worldwide. The highest rates are in Southeast Asia and sub-Saharan Africa, with the HCC incidence >50/100,000 populations (Bosch *et al.*,1999).Virological factors in the pathogenesis of hepatocellular carcinoma have recently been defined. Both retrospective and prospective studies strongly supported the relation between positive HBeAg and the risk of HCC (Lin *et al*., 1991). A prospective study in Taiwan (Yang *et al*., 2000) showed that relative risk of HCC among men who were positive for both HBsAg and HBsAg were much higher than that among

men who were positive for HBsAg alone . HBV DNA was identified as the most important predictor of the development of hepatocellular carcinoma in HBsAg-positive patients with different clinical conditions, therefore, efforts at eradicating or reducing the viral load may reduce the risk for HCC. Additionally, HBV genotype might play a role in the development of HCC. The data from Taiwan showed that genotype C is associated with more severe liver disease including cirrhosis and hepatocellular carcinoma (HCC), whereas genotype B is associated with the development of HCC in young non cirrhotic patients (Ishikawa *et al*., 2001; Ikeda *et al*., 2003; Ohata*et al*., 2004).

**2.9 Occult Hepatitis B**

Occult hepatitis B is defined by the presence of HBV DNA in serum or liver in the absence of HBsAg (Hou *et al*, 2001;Hu *et al*., 2002). Serum HBV level is usually less than 104 copies/ml. Although occult HBV infection has been identified in patients with chronic liver disease two decades ago (Brechot *et al*.,1985), its precise prevalence remains to be defined. Occult HBV infection has been found in patients with HCC, past HBV infection, or chronic hepatitis C, and individuals without HBV serological markers. The frequency of the diagnosis depends on the relative sensitivity of HBV DNA assays and the prevalence of HBV infection in the population. Collectively, around 30% to35% of HBsAg-negative subjects with chronic hepatitis with or without HCC have positive serum HBV DNA (range from 5% to55%). The prevalence of HBV DNA is higher in anti-HBc positive, but anti-HBs-negative patients, ranging from 7% to 60% in populations highly exposed to HBV. HBV DNA is much less frequently identified in HBsAg-negative patients with acute, and particularly fulminant hepatitis at around 10% and 7% in serum and liver samples (Brechot*et al*., 2001). Viral DNA persistence is not, however, restricted to patients with liver disease and may be observed in subjects with normal liver parameters, including blood and/or organ donors. Overall, occult HBV infection is seen in 7%-13% of anti-HBc-positive and/or anti-HBs-positive subjects, and in 0% to 17%of blood donors. The clinical significance of occult HBV infection

remains unclear. Occult HBV infection represents a potential transmission source of HBV via blood transfusion or organ transplantation. In addition, occult HBV infection has been associated with cryptogenic chronic hepatitis and hepatocellular carcinoma. Furthermore, some studies suggested that occult hepatitis B might affect responsiveness of chronic hepatitis C to interferon therapy and disease progress (Brechot*et al*., 2001).

## 2.10 Laboratory Diagnosis

### 2.10.1 Serologic and Virologic Markers

The two most important serologic tests for the diagnosis of early hepatitis B are the tests for HBsAgand for IgM antibody to the core antigen. Both appear in the serum early in the disease (Levenson,2014).After a person is infected with HBV, the first virologic marker detectable in serum within 1–12 weeks, usually between 8 and 12 weeks, is HBsAg(Dan and Fauci,2010)Both acutely and chronically infected individuals have HBs antigenaemia. The diagnosis of acute disease is confirmed by demonstrating IgM anti-HBc in the serum. This appears2 weeks after HBsAg, and disappears a few months after uncomplicated infection. IgG anti HBc persists probably lifelong, and is a marker of previous infection. The stage of evolution of antigenaemia and antibody production is determined by EIA tests. Viral persistence can be confirmed by PCR-based detection of HBV DNA in serum. Detection of HBe is still used as a marker of enhanced infectivity and risk of chronic liver disease. (Bannister *et al.*,2006).

#### 1.10.1.1 Viral capsid surface antigen and the antibody directed against the surface antigen (anti-HBs)

The HBsAg test was the first available for detecting hepatitis B. HBsAg appears in serum within 1 to 10weeks after exposure; its disappearance within 4 to6 months indicates recovery. The persistence of HBsAg beyond 6 months indicates chronic disease. The disappearance of HBsAg may be preceded by the appearance of anti-HBs, and during this period, patients may develop a serum sickness-like illness. In a large percentage of patients, anti-HBs does not rise to detectable levels for several weeks to months after the disappearance of HBsAg.

During this window HBsAg and anti-HBs are both negative, and if these two tests alone are used for screening blood donors, a small percentage of infected donors may be missed. To prevent this occurrence, blood banks also test for IgM antibody directed against HBcAg. Anti-HBs rises slowly over 6 to 12months and usually persists for life, providing protection against re-infection.

### 2.10.1.2 Antibody directed against the core antigen (anti-HBc)

HBcAg is detected in infected hepatocytes, but is not released into serum; however, IgM antibody directed against HBcAg (anti-HBc) is usually the earliest anti-hepatitis B antibody detected in the infected patient. The IgM anti-HBc is usually interpreted as a marker for early acute disease; however, in some patients, anti-HBcIgM levels can persist for up to 2 years after acute infection, and in patients with chronic active hepatitis, IgM antibody levels can rise during periods of exacerbation. An anti-HBcIgM titer is particularly helpful for screening blood donors, because this antibody is usually present during the window between HBsAg disappearance and anti-HBs appearance. The IgG antibodies directed against the core antigen develop in the later phases of acute disease and usually persist for life.

### Secreted core antigen (HBeAg) and its antibody(anti-HBe)

Naked DNA strands and associated proteins make up HBeAg. The presence of HBeAg in serum indicates active viral replication, and it persists in patients with chronic disease, its presence correlating with infectivity. As the patient with acute hepatitis B recovers, HBeAg disappears, and anti-HBe appears. Seroconversion from HBeAg to anti-HBe usually corresponds with the disappearance of hepatitis B virus DNA from the serum.

### 2.10.3 Polymerase chain reaction (PCR) test

It is based on the use of DNA fragment called the gene probe (39). Gene probe is relatively small, single stranded DNA segment that can hunt for complementary fragment of DNA (Mumtaz*et al*,2011).To use a gene probe effectively, it is valuable to increase the DNA to be searched. The polymerase chain reaction (PCR)accomplishes this task (Pommerville, 2004).

### 2.10.3.1 Hepatitis B viral DNA (HBV-DNA)

Quantization of viral DNA in serum is most commonly used in the assessment of patients with chronic active hepatitis. In the patient with acute hepatitis, this test provides no significant advantages over that for HBeAg. Both tests indicate active viral replication. In patients with fulminant hepatitis, assays for HBV-DNA has been positive in the absence of other positive markers for HBV(Frederick and Southwick, 2007).

## 2.11 Treatment

No antiviral therapy is typically used in acute hepatitis B. For chronic hepatitis B, entecavir (Baraclude) or tenofovir (Viread) are the drugs of choice. They arenucleoside analogues that inhibit the reverse transcriptase of HBV. Interferon in theform of peginterferon alfa-2a (Pegasys) is also used. Other nucleoside analoguessuch as lamivudine (Epivir-HBV), adefovir (Hepsera), and telbivudine (Tyzeka) areused less frequently. A combination of tenofovir and emtricitabine (Emtriva) is alsoused (Levenson, 2014).

### 2.11.1 Drugs active against HBV

Lamivudine, 100 mg daily, orally (also used for HIV),**a**defovirdipivoxil, 10 mg daily, orally, tenofovir (used for HBV/HIV co-infected patients), alternative: interferon alpha, 5–10 MIU three times weekly,subcutaneously for 6 months.If an antiviral drug effective against HBV is also beingused to treat HIV co-infection, the HIV-treatment dose should be given (this is often higher than the dose for HB) (Bannister *et al*., 2006).

## 2.12 Prevention

Prevention involves the use of either the vaccineorhyperimmune globulin or both (Levenson, 2014).

### 2.12.1 Passive Immunoprophylaxis

Hepatitis B immune globulin (HBIG) contains a high titer of HBsAb. It is used to provide immediate, passive protection to individuals known to be exposed to HBsAg-positive blood (e.g., after an accidental needle-stick injury) (Levenson,2014).Immunoprophylaxis is recommended for all infants born to

HBsAg positive mothers. Current dosing recommendations are 0.13ml/kg HBIG immediately after delivery or within12 hours after birth in combination with recombinant vaccine. The combination results in a higher-than-90% level of protection against perinatal acquisition of HBV (Stevens., Taylor. And Tong. 1987). Between 3.7% to 9.9% of infants still acquire HBV infection perinatally from HBV infection mothers, despite immunoprophylaxis. Failure of passive and active immunoprophylaxis in this setting may be the result of in utero transmission of HBV infection, prenatal transmission related to a high inoculums, and/or the presence of surface gene escape mutants. To study the interruptive effect of HBIG before delivery in attempt to prevent intrauterine transmission of HBV, a large-scale, random-control study was conducted in China (Zhu*etal.*,2003). In this study, nine hundred and eighty HBsAg carrier pregnant women were randomly divided into HBIG group and control group. Each subject in the HBIG group received 200 IU or400 IU of HBIG intramuscularly at 3, 2 and 1 months before delivery, in addition to newborns receiving HBIG intramuscularly. By this way, the rate of intrauterine transmission in this group fall to 5.7%, compared to 14.3% in control group. ($P < 0.001$). However, the preventive effect of HBIG administration before delivery needs to be confirmed by more study in the future. Hepatitis B immune globulin remains a central component of prophylaxis in HBV-infected patients undergoing liver transplantation. HBIG mono therapy given at a high dosage can prevent recurrence in 65% to 80% of patients. Because the cost of long-term prophylaxis with high-dose HBIG is extremely high and combination therapy using HBIG with a nucleoside analog is more uniformly effective, the current protocol is combination HBIG with a nucleoside analog after liver transplantation. These combination protocols have reduced the rate of virologic breakthrough to 10% or less (Terrault and Vyas, 2003).

### 2.12.2 Active Immunization

Prevention of primary infection by vaccination is an important strategy to decrease the risk of chronic HBV infection and its subsequent complications. The first-generation hepatitis B vaccine, an inactive plasma-derived vaccine, became available in 1982. Consequently, the second generation of HB vaccine, a DNA recombinant HB vaccine was also available for general use in 1986. Both of the vaccines were proven to be safe and efficacious in preventing HBV infection. (WHO) recommended that hepatitis B vaccination should be included in national immunization system in all countries with a hepatitis B carrier prevalence (HBsAg). By May 2002, 154 countries hardoutine infant immunization with hepatitis B vaccine (Lavanchy, 2004).The world's first universal vaccination program for HBV infection was launched in 1984 in Taiwan (Ni *et al*. 2001). During the first 2 years of the program, coverage was provided mainly for infants whose mothers were carriers of HBsAg. Vaccination was subsequently extended, first to all newborns and then to unvaccinated preschool-age and elementary school-age children. Since1991, catch-up vaccinations have been given to children in the first grade. This program reduced the overall HBsAg prevalence rate from 9.8% in 1984 to 1.3% in 1994 among children <15 years of age. The HBV carrier population was further reduced through improved maternal screening (Chen *et al.*,1996). In 1999, vaccination rates were 80–86% for young children and higher than 90% for older children; the prevalence of HBsAg was reduced to 0.7% for children younger than 15 years of age (Ni *et al.,* 2001). To evaluate the long-term efficacy of hepatitis B (HB) vaccination in newborns, one of the longest HB vaccine follow-up studies in the world was conducted in Shanghai, China (Zhou *et al*., 2003). Children who were born in 1986 and immunized with hepatitis B vaccine at birth were followed up at least once a year. Serum HBsAg, anti-HBc and anti-HBs were tested. The positive rates of HBsAg in the vaccine group with the period of16 years were 0.46%-0.97%, the average being 0.61%, which was much lower than those of baseline before vaccination and external control.

The long-term efficacy of newborn vaccination was 85.42%. In countries such as Italy and the United States, the incidence of acute hepatitis B has declined dramatically during the past decade after vaccination program for HBV infection, particularly among persons in younger age group (Da villa, 2000).Universal HB vaccination was proven to be effective in the prevention of HCC in several large cohort studies in Southeast Asia (Chang *et al.*,1997).

# Chapter Three

## Materials and Methods

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1. Study design

This study was a descriptive cross-sectional study**.**

## 3.2. Study area:

This study was conducted in Atabra locality, is on the River Nile in Sudan, about 300 km from Khartoum.

## 3.3 Study duration

The study was carried out between March to June 2018.

## 3.4. Study population

Health care workers including (Laboratory technologist, Nurses , Pharmacist and Cleaning staff).

## 3.5. Sample size

Ninety-two(n=92) health care workers were recruited for this study .

## 3.6 Ethical consideration

Ethical approval to conduct this study in the region was obtained from the Health Services Director in Atabra locality and verbal consent was obtained from participants before collection of the blood samples.

## 3.7. Data collection

A structured questionnaire was used to collect demographic and clinical data.

## 3.8 Collection of blood specimens

Under sterile condition Five ml of venous blood sample was withdrawn from each participant, then waited until sample clotted the serum was separated by centrifugation at 5000 rpm for five minutes, serum was separated into plain vacutainers then stored at -20C˚ until used.

## 3.9 Laboratory investigation

HBV surface antigen (HBsAg) was screened by HBsAg (high sensitivity) - ELISA Kit.

### 3.9.1 ELISA technique:

**Method: ELISA (Enzyme linked immune sorbent assay)**

Fortress HBsAg is an in vitro diagnostic kit for the detection of hepatitis B surface antigen (HBsAg) in human serum or plasma Intended use: -

For screening of blood donors

For monitoring individuals with a higher than normal risk of contracting hepatitis e.g. patients, Technicians or nursing personnel in renal dialysis units or clinical laboratories as an aid the diagnosis of liver disease

#### 3.9.1.1. Principle

The test is an enzyme linked immune sorbent assay based on sandwich principle. Polystyrene microtiter wells have been coated with a monoclonal HB antibodies to HBsAg patterns serum or plasma sample is added to the micro wells. During incubation the specific –immune complex formed in the case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum proteins ,Second antibody conjugate to the enzyme HRP and directed against different epitopes of HBsAg is added to the conjugated antibodies will be bound to any ant-HBs-HBsAg complexes previously formed during the first incubation, and The un bound HRP conjugate is then removed by washing after washing to unbound HRP conjugate, chromogen solutions containing TMB and urea peroxidas are added to the wells .in the presence of antibody-antigen , antibody HRP sandwich immune- complex ,the colorless chromogen are hydrolyzed by the bound HRP conjugate to blue colored product .The blue colure turns yellow after stopping the reaction by using the stop solution. The color intensity can be measured and it is proportional to the amount of the antigen captured in the wells and it is amount respectively. wells containing samples negative for HBsAg remain colorless.

### 3.9.1.2. Assay procedure:

The reagent and samples were allowed to reach room temperature Numbered of wells including two negative control e.g. (B1, C1) and one blank (e.g. A1) and one blank (e.g. A1, neither samples nor HPR conjugate should be added into the blank wells).

Then added 20ul of sample diluents to each well except the blank and mixed by toping the plate gently. and added 100ul of positive control and negative control and specimen to their respective wells by using separate disposable tip for each specimen negative control and positive control to avoid contamination. Then added 50 ul HRP conjugate to each well except the blank and mixed tapping the plate gently. And covered the plate with plate cover and incubated for 30 minutes for 37˚Cat the end of the incubation removed and discard the plate cover Washed each well 5 times with diluted wash buffer. Each time allowed the micro wells to soaked for 45 second, After the five washing and plotting paper or clean towel, and tap it to remove any remainders. After washing dispense 50ul of chromogen A and 50 ul of chromogen B solutions was added into each well including the blank and mixed by tapping the plate gently. incubated the plate at 37˚C for 15 minutes Stopped the reaction by using a multichannel pipette, added 50 ul stop solution into the each well and mixed gently the absorbance measured at 450 nm. and calculated the cut-off value and evaluated the result and read the absorbance within 5 minutes after the stopping the reaction.

**Interpretation of results:**

Each micro plate should be considered separately when calculated and interpreting result of the assay ,regardless of the number of the plate concurrently processed the results are calculated by relating each samples optical density (OD) value to the cut-off (C.O.) of the plate .if the cut-off reading spaced on single filter plate reader, results should be calculated by subtracting the blank well OD value from the print report value of samples and

controls , In case the reading spaced on dual filter plate reader, don't subtract the blank well OD from the print report values of samples and controls.

Cutoff value (C.O.) =*NC*2.1

***NC**=the mean absorbance value of two negative controls

**Negative result:** sample giving an absorbance less than the cut off value are considered negative, which indicate no HBV surface antigen has been detected with this HBsAg ELISA kits.

**Positive result:** sample giving an absorbance greater than the cut off value are considered initially reactive, which indicate HBV surface antigen has been detected with this HBsAg ELISA kit.

### 3.10. Statistical analysis

The data analysis was done through Statistical Package for the Social Scinces (SPSS) version 22 and Chi-square test was used to assess the association between various variables.

# Chapter Four

## Results

# CHAPTER FOUR

# RESULTS

## 4.1 Results

A total ninety- two health care workers (HCWs) who were considered at occupational risk of contracting HBV infection were enrolled in this study. Fourty seven (51.1%) were male and 45/92 (48.9%) were female, the sero-positivity among males was 2 (2.2%) and among females was 6 (6.5%) from the total infected participants 8 (8.7%).

**Table 4.1 The distribution of HBsAg positive according to gender**

| Sex | HBV result | | Total |
|---|---|---|---|
| | Positive | Negative | |
| Male | 2 | 45 | 47 |
| | 2.2% | 48.9% | 51.1% |
| Female | 6 | 39 | 45 |
| | 6.5% | 42.4% | 48.9% |
| Total | 8 | 84 | 92 |
| | 8.7% | 91.3% | 100.0% |

p-value = 0.122 ( p-value > 0.05) Result indicated insignificant

**Table 4.2 HBV result and vaccination**

| HBV results | vaccine | | Total |
|---|---|---|---|
| | vaccinated | Non vaccinated | |
| Positive | 3/3.3% | 5/5.4% | 8/8.7% |
| Negative | 20/21.7% | 64/69.6% | 84/91.3% |
| Total | 23/25% | 69/755 | 92/100% |

p-value = 0.393 insignificant.

Twenty three (25%) of the participants were vaccinated and 69/92 (75%) were not vaccinated ( by using questionnaire ).

**Table 4.3 Frequency of HBV results among marital status**

| HBV result | Marital status | | Total |
|---|---|---|---|
| | Married | Single | |
| Positive | 3/3.3% | 5/5.4% | 8/8.7% |
| Negative | 44/47.8% | 40/43.5% | 84/91.3% |
| Total | 47/51.1% | 45/48.9% | 92/100% |

p-value = 0.421 insignificant .

While 47 (51.1%) were married and 45(48.9%) were single .

**Table 4.4: Frequency of Hepatitis B virus result among health care workers**

| Health care workers | HBV positive result | HBV negative result |
|---|---|---|
| Lab technologist | 3 /37.5% | 37/44% |
| Nurse | 2 /25% | 31/37% |
| Pharmacist | 0 /0% | 3/3.5% |
| Cleaning staff | 3 /37.5% | 13/15.5% |

p-value = 0.289( p-value > 0.050 result indicated that in significant association between occupation practice.

**Table 4.5Distribution of HBV infection according to accidental injury**

| HBV Results | Injury | | Total |
|---|---|---|---|
| | Yes | No | |
| Positive | 4/4.3% | 4/4.3% | 8/8.7% |
| Negative | 20/21.7% | 64/69.6% | 84/91.3% |
| Total | 24/26.1% | 68/73.9% | 92/100% |

p-value 0.107 ( p-value >0.05) insignificant.

Twenty four 24 out of 92 were exposed to an accidental injury

**Table 4.6 Association of HBsAg results and blood transfusion**

| **HBV results** | **Blood transfusion** | | **Total** |
|---|---|---|---|
| | **Yes** | **No** | |
| Positive | 0/.0% | 8/8.7% | 8/8.7 % |
| Negative | 2/2.2% | 82/89.1% | 84/91 .3% |
| Total | 2/2.2% | 90/97.8% | 92/10 0% |

p- value = 0.569 ( p-value >0.05) insignificant**.**

**Table 4.7: Distribution of HBV infection according to age groups**

| AGE GROUP | HBV results | | Total |
|---|---|---|---|
| | Positive | Negative | |
| 20-40 years | 8 | 70 | 78 |
| | 8.7% | 76.1% | 84.8% |
| 41-60 years | 0 | 14 | 14 |
| | .0% | 15.2% | 15.2% |
| Total | 8 | 84 | 92 |
| | 8.7% | 91.3% | 100.0% |

p-value = 0.210 ( p-value > 0.05) insignificant.

# Chapter Five

## Discussion

## Conclusion

## Recommendations

# CHAPTER FIVE

## 5.1 Discussion

In this study the sero-prevalence of HBsAg was assessed for 92 (HCWs) atAtabra locality hospital and selected health centers. Only 8 (8.7%) were positive for HBsAg and this result is similar to those reported from Tanzania where the prevalence of hepatitis B virus among HCWs in tertiary hospital was (7%) (Mueller *et al.* 2015) and the results are similar to those reported from Yemen and Palestine which were (9.9%) and (9.60) respectively (Alhurabi*et al*., 2004; Jadallah *et al*., 2005). While, they disagree with other report from White Nile State, Sudan which was (27%) (Abuelgasim*et al*., 2013). The result is higher than other studies done in Korea which was (2.4 %) (Shin *et al.*, 2006), Morocco which was (1%) (Djeriri *et al.*, 2008), Khartoum which was (4.4%) (Abdalwhaband Nafi, 2014) and Lagos State in Nigeria which was (1.5%) (Abiola *et al*. 2016). The variation between some of the results particularly that carried at River Nile State could be attributed to the difference in the population and the sample size.

The incidence of infection in laboratory technologist (3 participants), nurses (2 participants), and cleaning staff (3 participants) could be justified by the frequent contact of those HCWs with sources of infection (e.g., accidental needle stick injuries). Such incidents might occur while giving an injection or after injection , including recapping contaminated needle, and handling infected sharps before and after disposal, contaminated blood during sampling, unsafe sharps waste management, and reuse of injection equipment to administer injection to more than one person. Although majority of the participants in this study were not vaccinated against Hepatitis B virus infection 25% only. Some of the vaccinated participants were infected by HBV and this could be due to poor response to vaccine, the participants were immunosuppressed, or they were vaccinated with non-effective vaccine. In this study only eight were positive for HBsAg which represent (8.7%) and this could be due understanding of HCWs to the safety protocols that prevent against blood borne infections.

## 5.2 Conclusions

In conclusion, this study has shown that only 8.7% of the HCWs at Atabra locality were positive for HBsAg. Laboratory technologist were the most affected and only one from those who had blood transfusion was positive. HCWs with frequent injuries had higher prevalence of HBV infection than others

## 5.3. Recommendations

1- HCWs should be screened regularly for Hepatitis B virus and other blood-borne infections.

2- Further studies should be conducted with larger sample size to confirm these results.

3- HCWs should be vaccinated against Hepatitis B virus ( HBV ) and ensure they are assessed for immunity ( post-vaccination management ).

# Chapter Six

## References

## Appendices

# Chapter Six

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**جامعة شندي**

**كلية الدراسات العليا**

**استبيان**

## APPENDICES (1)

**الكشف عن فيروس الكبد الوبائي (النوع ب) بين العاملين في مجال الرعاية الصحية الأولية في محلية عطبرة . ولاية نهر النيل**

## Questionnaire on Detection of Hepatitis B Virus among health care workers in Atbara locality ,River Nile State –Sudan.

### Data collection Sheet

### General data

1. ID. number……
2. Gender: Male ( ) female ( )

3.Age……..

4. Locality ……..

Urban ( ) rural ( )

5. Marital status

Married( ) single( )

6.Type of occupation……...

7. duration in hospital…….

8. Vaccine: Yes ( ) No ( )

9.Have you taken a sharp instrument? Yes ( ) No ( )

If the answer is yes ,what is the procedure used in the hospital to treat the injured person?.....

10.Blood transfusion Yes ( ) No ( )

11.Surgical operation Yes ( ) No ( )

12.Renal dialysis

Yes ( ) No( ).

# إقرار بالموافقة

الاسم : .....

العمر : ..... العنوان : .....

أوافق أنا بمحض إرادتي في المشاركة في البحث العلمي المتعلق بدراسة الكشف عن فيروس الكبد الوبائي (النوع ب) بين العاملين في مجال الرعاية الصحية الأولية في محلية عطبرة. ولاية نهر النيل

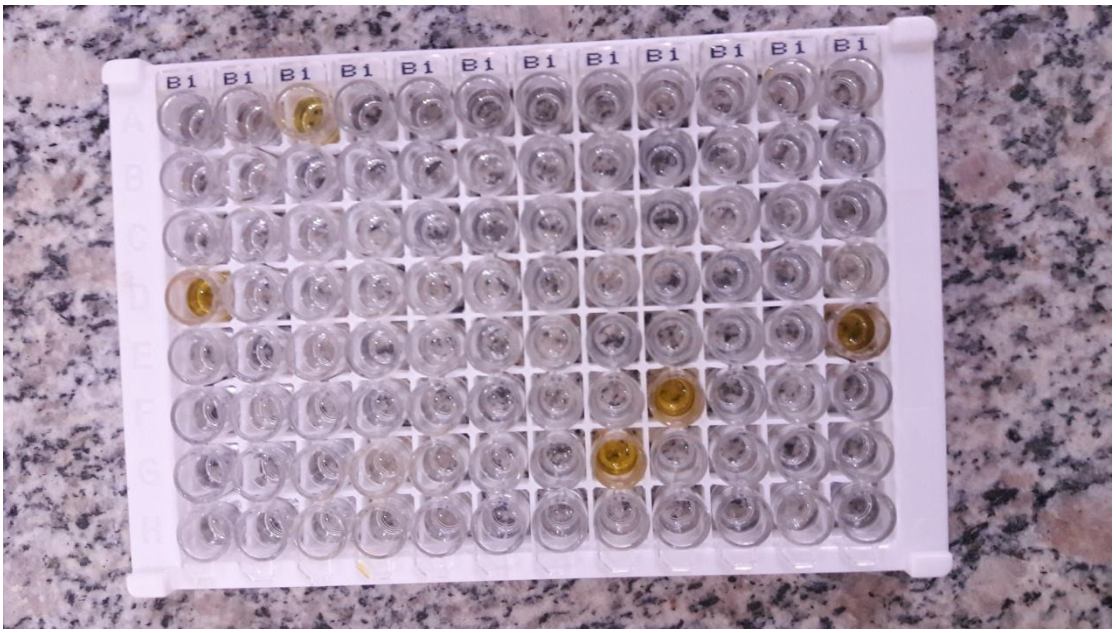
**إيناس محمد احمد الطيب محمد**

بعد أن شرح لي انه لا يترتب عليه أي أذى جسدي أو نفسي واعلم أن المشاركة في هذا البحث لن تؤثر بأي حال من الأحوال كما يحق لي بدون إبداء أسباب الانسحاب من هذا البحث في أي مرحله من المراحل

**البحث بإشراف :**

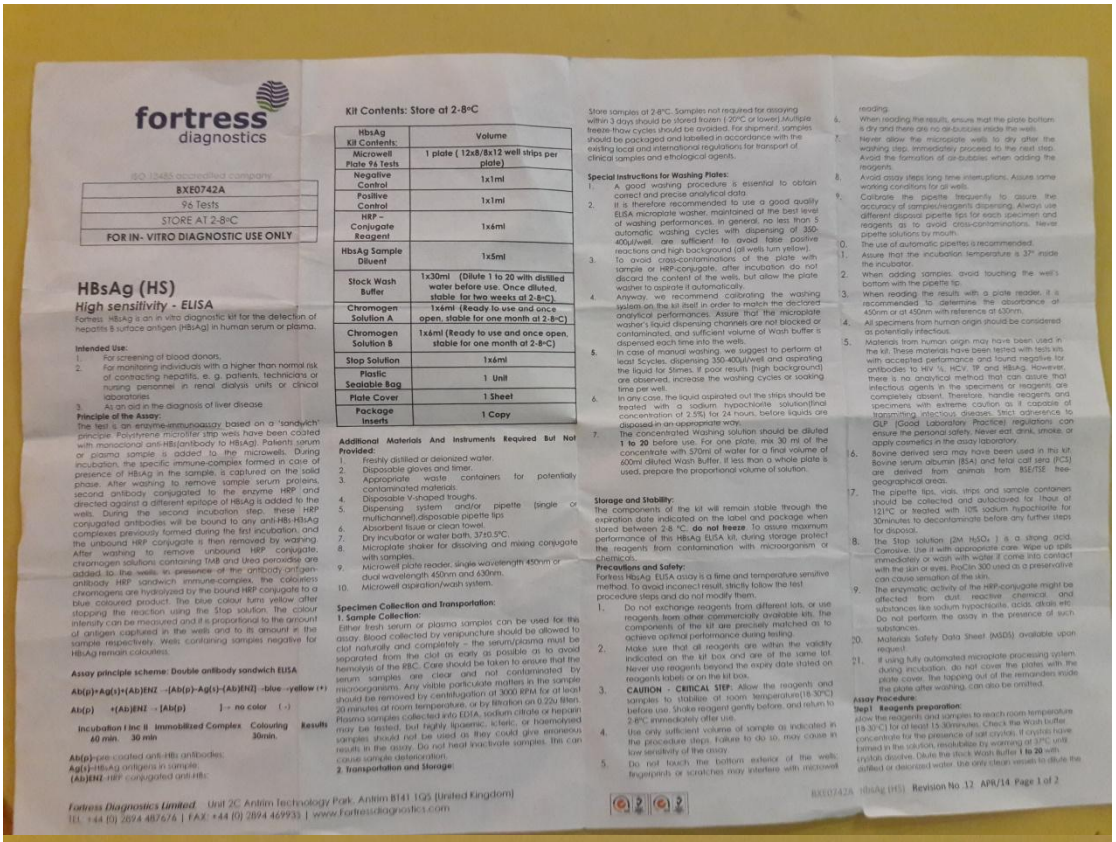
**د. ليلي محمد احمد عبد القادر**

## Appendix ( 2 )




# ELISA Results

# Appendix ( 3 )



fortress diagnostics

ISO 13485 accredited company

BXE0742A

96 Tests

STORE AT 2-8°C

FOR IN- VITRO DIAGNOSTIC USE ONLY

## HBsAg (HS)

*High sensitivity - ELISA*

Fortress HBsAg is an in vitro diagnostic kit for the detection of hepatitis B surface antigen (HBsAg) in human serum or plasma.

**Intended Use:**

1. For screening of blood donors.
2. For monitoring individuals with a higher than normal risk of contracting hepatitis, e. g. patients, technicians or nursing personnel in renal dialysis units or clinical laboratories
3. As an aid in the diagnosis of liver disease.

**Principle of the Assay:**

The test is an enzyme-immunoassay based on a "sandwich" principle. Polystyrene microtiter strip wells have been coated with monoclonal anti-HBs(antibody to HBsAg). Patients serum or plasma sample is added to the microwells. During incubation, the specific immune-complex formed in case of presence of HBsAg in the sample, is captured on the solid phase. After washing to remove sample serum proteins, second antibody conjugated to the enzyme HRP and directed against a different epitope of HBsAg is added to the wells. During the second incubation step, these HRP conjugated antibodies will be bound to any anti-HBs-HBsAg complexes previously formed during the first incubation, and the unbound HRP conjugate is then removed by washing. After washing to remove unbound HRP conjugate, chromogen solutions containing TMB and Urea peroxide are added to the wells. In presence of the antibody antigen-antibody HRP sandwich immune-complex, the colorless chromogens are hydrolyzed by the bound HRP conjugate to a blue coloured product. The blue colour turns yellow after stopping the reaction using the Stop solution. The colour intensity can be measured and it is proportional to the amount of antigen captured in the wells and to its amount in the sample respectively. Wells containing samples negative for HBsAg remain colourless.

**Assay principle scheme: Double antibody sandwich ELISA**

Ab(p)+Ag(s)+(Ab)ENZ →(Ab(p)-Ag(s)-(Ab)ENZ) -blue →yellow (+)

Ab(p) +(Ab)ENZ → [Ab(p) ]– no color (-)

| Incubation I inc II | Immobilized Complex | Colouring | Results |
|---|---|---|---|
| 60 min. 30 min | | 30min. | |

Ab(p)-pre coated anti-HBs antibodies.
Ag(s)-HBsAg antigens in sample.
(Ab)ENZ-HRP conjugated anti HBs

**Kit Contents: Store at 2-8°C**

| HbsAg Kit Contents: | Volume |
|---|---|
| Microwell Plate 96 Tests | 1 plate ( 12x8/8x12 well strips per plate) |
| Negative Control | 1x1ml |
| Positive Control | 1x1ml |
| HRP – Conjugate Reagent | 1x6ml |
| HbsAg Sample Diluent | 1x5ml |
| Stock Wash Buffer | 1x30ml (Dilute 1 to 20 with distilled water before use. Once diluted, stable for two weeks at 2-8°C). |
| Chromogen Solution A | 1x6ml (Ready to use and once open, stable for one month at 2-8°C) |
| Chromogen Solution B | 1x6ml (Ready to use and once open, stable for one month at 2-8°C) |
| Stop Solution | 1x6ml |
| Plastic Sealable Bag | 1 Unit |
| Plate Cover | 1 Sheet |
| Package Inserts | 1 Copy |

**Additional Materials And Instruments Required But Not Provided:**

1. Freshly distilled or deionized water.
2. Disposable gloves and timer.
3. Appropriate waste containers for potentially contaminated materials.
4. Disposable V-shaped troughs.
5. Dispensing system and/or pipette (single or multichannel),disposable pipette tips
6. Absorbent tissue or clean towel.
7. Dry incubator or water bath, 37±0.5°C.
8. Microplate shaker for dissolving and mixing conjugate with samples.
9. Microwell plate reader, single wavelength 450nm or dual wavelength 450nm and 630nm.
10. Microwell aspiration/wash system.

**Specimen Collection and Transportation:**

**1. Sample Collection:**

Either fresh serum or plasma samples can be used for this assay. Blood collected by venipuncture should be allowed to clot naturally and completely – the serum/plasma must be separated from the clot as early as possible as to avoid hemolysis of the RBC. Care should be taken to ensure that the serum samples are clear and not contaminated by microorganisms. Any visible particulate matters in the sample should be removed by centrifugation at 3000 RPM for at least 20 minutes at room temperature, or by filtration on a 0.22µ filter. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipoemic, icteric, or haemolysed samples should not be used as they could give erroneous results in the assay. Do not heat inactivate samples. This can cause sample deterioration.

**2. Transportation and Storage:**

Store samples at 2-8°C. Samples not required for assaying within 3 days should be stored frozen (-20°C or lower).Multiple freeze-thaw cycles should be avoided. For shipment, samples should be packaged and labelled in accordance with the existing local and international regulations for transport of clinical samples and ethological agents.

**Special Instructions for Washing Plates:**

1. A good washing procedure is essential to obtain correct and precise analytical data.
2. It is therefore recommended to use a good quality ELISA microplate washer, maintained at the best level of washing performances. In general, no less than 5 automatic washing cycles with dispensing of 350-400µl/well, are sufficient to avoid false positive reactions and high background (all wells turn yellow).
3. To avoid cross-contaminations of the plate with sample or HRP-conjugate, after incubation do not discard the content of the wells, but allow the plate washer to aspirate it automatically.
4. Anyway, we recommend calibrating the washing system on the kit itself in order to match the declared analytical performances. Assure that the microplate washer's liquid dispensing channels are not blocked or contaminated, and sufficient volume of wash buffer is dispensed each time into the wells.
5. In case of manual washing, we suggest to perform at least 5x cycles, dispensing 350-400µl/well and aspirating the liquid for 5times. If poor results (high backgrounds) are observed, increase the washing cycles or soaking time per well.
6. In any case, the liquid aspirated out the strips should be treated with a sodium hypochlorite solution(final concentration of 2.5%) for 24 hours, before liquids are disposed in an appropriate way.
7. The concentrated Washing solution should be diluted **1 to 20** before use. For one plate, mix 30 ml of the concentrate with 570ml of water for a final volume of 600ml diluted Wash Buffer. If less than a whole plate is used, prepare the proportional volume of solution.

**Storage and Stability:**

The components of the kit will remain stable through the expiration date indicated on the label and package when stored between 2-8 °C. **do not freeze**. To assure maximum performance of this HBsAg ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

**Precautions and Safety:**

Fortress HbsAg ELISA assay is a time and temperature sensitive method. To avoid incorrect result, strictly follow the test procedure steps and do not modify them.

1. Do not exchange reagents from different lots, or use reagents from other commercially available kits. The components of the kit are precisely matched as to achieve optimal performance during testing.
2. Make sure that all reagents are within the validity indicated on the kit box and are of the same lot. Never use reagents beyond the expiry date stated on reagents labels or on the kit box.
3. **CAUTION - CRITICAL STEP:** Allow the reagents and samples to stabilize at room temperature(18-30°C) before use. Shake reagent gently before, and return to 2-8°C immediately after use.
4. Use only sufficient volume of sample as indicated in the procedure steps. Failure to do so, may cause in low sensitivity of the assay.
5. Do not touch the bottom exterior of the wells; fingerprints or scratches may interfere with microwell reading.
6. When reading the results, ensure that the plate bottom is dry and there are no air-bubbles inside the wells.
7. Never allow the microplate wells to dry after the washing step, immediately proceed to the next step. Avoid the formation of air-bubbles when adding the reagents.
8. Avoid assay steps long time interruptions. Assure same working conditions for all wells.
9. Calibrate the pipette frequently to assure the accuracy of samples/reagents dispensing. Always use different disposal pipette tips for each specimen and reagents as to avoid cross-contaminations. Never pipette solutions by mouth.
10. The use of automatic pipettes is recommended.
11. Assure that the incubation temperature is 37° inside the incubator.
12. When adding samples, avoid touching the well's bottom with the pipette tip.
13. When reading the results with a plate reader, it is recommended to determine the absorbance at 450nm or at 450nm with reference at 630nm.
14. All specimens from human origin should be considered as potentially infectious.
15. Materials from human origin may have been used in the kit. These materials have been tested with tests kits with accepted performance and found negative for antibodies to HIV ½, HCV, TP and HBsAg. However, there is no analytical method that can assure that infectious agents in the specimens or reagents are completely absent. Therefore, handle reagents and specimens with extreme caution as if capable of transmitting infectious diseases. Strict adherence to GLP (Good Laboratory Practice) regulations can ensure the personal safety. Never eat, drink, smoke, or apply cosmetics in the assay laboratory.
16. Bovine derived sera may have been used in this kit. Bovine serum albumin (BSA) and fetal calf sera (FCS) are derived from animals from BSE/TSE free geographical areas.
17. The pipette tips, vials, strips and sample containers should be collected and autoclaved for 1hour at 121°C or treated with 10% sodium hypochlorite for 30minutes to decontaminate before any further steps for disposal.
18. The Stop solution (2M $H_2SO_4$ ) is a strong acid. Corrosive. Use it with appropriate care. Wipe up spills immediately or wash with water if come into contact with the skin or eyes. ProClin 300 used as a preservative can cause sensation of the skin.
19. The enzymatic activity of the HRP-conjugate might be affected from dust, reactive chemical, and substances like sodium hypochlorite, acids, alkalis etc. Do not perform the assay in the presence of such substances.
20. Materials Safety Data Sheet (MSDS) available upon request.
21. If using fully automated microplate processing system during incubation, do not cover the plates with the plate cover. The tapping out of the remainders inside the plate after washing, can also be omitted.

**Assay Procedure:**

**Step1 Reagents preparation:**

allow the reagents and samples to reach room temperature (18-30°C) for at least 15-30minutes. Check the Wash buffer concentrate for the presence of salt crystals. If crystals have formed in the solution, resolubilize by warming at 37°C until crystals dissolve. Dilute the stock Wash Buffer **1 to 20** with distilled or deionized water. Use only clean vessels to dilute the buffer.



to the

**Step2 Numbering Wells:**

Set the strips needed in strip-holder and sufficient number of wells including three negative controls (all), C1, D1), two Positive Controls (c1, F1), and one Blank (H1, H1) and one Blank (e,g,A1, neither samples nor HRP Conjugate should be added into the blank well, if the results will be determined by using dual wavelength plate reader, the requirement for use of Blank well could be omitted. Use only number of strips required for the test.

**Step3 Adding Sample Diluent:**

Add 20ul of Sample Diluent to each well except the Blank, and mix by tapping the plate gently.

**Step4 Adding Sample:**

Add 100ul of Positive control, negative control and specimen into their respective wells, Note: Use a separate disposable tip for each specimen, Negative control and Positive control to avoid cross contamination.

**Step5 Incubation:**

Cover the plate with the plate cover and incubate for **60 minutes at 37°C**. It is recommended to use thermostat-controlled water bath to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

**Step6 Adding HRP Conjugate:**

Add 50ul HRP Conjugate to each well except the Blank and mix by tapping the plate gently.

**Step7 Incubation II:**

Cover the strips with the plate cover and incubate for 30 minutes for 37°C as in Step 5.

**Step8 Washing:**

At the end of the incubation, remove and discard the plate cover. Wash each well **5 times** with diluted wash buffer. Each time, allow the microwells to soak for 30-60seconds. After the final washing cycle, turn the plate down onto blotting paper or clean towel, and tap it to remove any remainders.

**Step9 Colouring:**

Dispense **50µl** of Chromogen A and **50µl** Chromogen B solution into each well including the **Blank**, and mix by tapping the plate gently. Incubate the plate at **37°C for 15minutes avoiding light**. The enzymatic reaction between the Chromogen solutions and the HRP-Conjugate produces blue colour in Positive control and HBsAg positive sample wells.

**Step10 Stopping Reaction:**

Using a multichannel pipette or manually, add **50µl** Stop Solution into each well and mix gently. Intensed yellow colour develops in Positive control and HBsAg positive sample wells.

**Step11 Measuring the Absorbance:**

Calibrate the plate reader with the blank well and read the absorbance at **450nm**. If a dual filter instrument is used, set the reference wavelength at **630nm**. Calculate the Cut-off value and evaluate the results (**Note:** read the absorbance within **5** minutes after stopping the reaction).

**Interpretation of Results:**

Each microplate should be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. The results are calculated by plotting each sample's optical density (OD) value to the Cut-off value (C.O.) of the plate. If the Cut-off reading is based on single filter plate reader, the results should be calculated by subtracting the Blank well OD value from the print report values of samples and control. In case the reading is based on dual filter plate reader, do not subtract the Blank well OD from the print report values of samples and control.

**Cut-off value (C.O.) = *Nc × 2.1**

*Nc: = the mean absorbance value for three negative controls

**Important: if the mean OD value of the negative control is lower than 0.05, take it as 0.05 .If higher than 0.05 see the Quality control range.**

**Example:**

1.C.Calculation of Nc:

| Well No | B1 | C1 | D1 |
|---|---|---|---|
| Negative controls OD value | 0.02 | 0.012 | 0.016 |

Nc= 0.016 (the Nc Value is lower than 0.05 so take it as0.05

Calculation of Cut-off value: Cut off(C.O)=0.05x2.1=0.105

If one of the Negative control values does not meet the Quality control range specifications, it should be discarded and the mean value is calculated again using the remaining two values. If more than one negative control OD value does not meet the Quality control range specifications, the test is invalid and must be repeated.

**2. Quality control range:**

The test results are valid if the Quality Control criteria are verified. It is recommended that each laboratory must establish appropriate quality control system with quality control material similar to or identical with the patient sample being analyzed.

1. The OD value of the Blank well, which contains only Chromogens and Stop solution, is less than 0.080 of 450 nm.
2. The OD value of the Positive control must be equal to or greater than 0.800 at 450/630nm, or at 450nm after blanking.
3. The OD value of the Negative control must be less than 0.100 at 450/630nm or at 450nm after blanking.

**3. Interpretations of the results:**

(S = the individual absorbance (OD) of each specimen)

**Negative Results (S/C.O. <1):** samples giving an absorbance less than the Cut-off value are considered negative, which indicates that no hepatitis B surface antigen has been detected with this HBsAg ELISA kit, therefore the patient is probably not infected with hepatitis B virus.

**Positive Results (S/C.O.≥1):** samples giving an absorbance greater than or equal to the Cut-off value are considered initially reactive, which indicates that HBV surfaces antigen has probably been detected with this HBsAg ELISA kit. Any initially reactive samples should be retested in duplicates. Repeatedly reactive samples could be considered positive for HBsAg, therefore the patient is probably infected by HBV and the blood unit should not be transfused.

**Borderline:** samples with absorbance to Cut-off ratio between 0.9 and 1.00 are considered borderline samples and retesting is recommended. Repeatedly positive samples can be considered positive for HBsAg.

**Fortress HBsAG 3rd Gen Performance:**

**Clinical Specificity:** The clinical specificity of this assay was determined by a panel of samples obtained from 4476 healthy blood donors and 6344 hospitalized patients.

| | Sample | - | + | Specificity |
|---|---|---|---|---|
| Donors | 4476 | 4471 | 5 | 99.89 % |
| Patients | 6344 | 6340 | 4 | 99.94 % |

**Clinical Sensitivity: 1)** A panel of 40 serum samples including 26 positive confirmed samples.

| PANEL | BACKGROUND | FORTRESS HBSAG 3RD GEN | |
|---|---|---|---|
| | | + | - |
| CDC | + | 26 | 0 |
| | - | 0 | 14 |
| DETECTION RATE | | 100 % | |

2. A panel of 108 samples sequenced by PCR method.

| BACKGROUND | | NUMBER | FORTRESS HBSAG 3RD GEN |
|---|---|---|---|
| adr(+) | Wildtype | 35 | 33 |
| | 4 mutations | 5 | 4 |
| adw(+) | Wild type | 37 | 34 |
| | 16 mutations | 25 | 24 |
| ayw(+) | Wild type | 2 | 2 |
| | 2 mutations | 2 | 2 |
| ayr(+) | 2 mutations | 2 | 2 |
| TOTAL | | 108 | 101 |

3. Two seroconversion panels from BBI

| CODE | DAYS | FORTRESS HBSAG 3RD GEN |
|---|---|---|
| PHM909 | 0 | 0.03 |
| | 4 | 0.07 |
| | 7 | 0.16 |
| | 9 | 1.64 |
| | 14 | 5.09 |
| | 16 | 17.17 |
| | 21 | 27.10 |
| PHM920 | 0 | 0.04 |
| | 5 | 0.03 |
| | 26 | 2.46 |
| | 35 | 27.62 |
| | 37 | 29.00 |
| | 42 | 28.40 |

**Analytical Specificity:**

1. No cross reactivity observed with samples from patients infected with HAV, HCV, HIV, CMV, and TP.
2. No interference from rheumatoid factors up to 20000 IU/ml observed.
3. No high dose hook effect up to HBsAg concentrations of 20000ng/ml observed during clinical testing.
4. Frozen specimens have been tested too to check for interference due to collection and storage.

**Analytical Sensitivity** (lower detection limit).The sensitivity of the assay has been calculated by a panel of series of dilutions of WHO reference standard. The assay shows that lower detection limit reaches 0.1 IU/ml.

| CONCENTRATION LEVEL | FORTRESS HBSAG 3RD GEN |
|---|---|
| 0.5 IU/ml | + |
| 0.2 IU/ml | + |
| 0.1 IU/ml | + |
| 0.05 IU/ml | +/- |
| 0.025 IU/ml | - |

**Limitations:**

1. Non-reproducible positive result may occur due to the general biological and biochemical characteristics of ELISA assays. The test is design to achieves very high performance characteristics of sensitivity and specificity. However, in very rare cases some HBV mutants or subtypes can remain undetectable. Antigens may be undetectable during the early stages of the disease and in some immunosuppressed individuals.
2. If, after retesting of the initially reactive samples, the assay results are negative, these samples should be considered as non-repeatable (false positive) and interpreted as negative. As with many very sensitive ELISA assays, false positive results can occur due to several reasons, most of which are related but not limited to inadequate washing steps.
3. Any positive results must be interpreted in conjunction with patient clinical information and other laboratory testing results.
4. Common sources for mistakes are beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, wrong volumes, sample nature and quality.
5. The prevalence of the marker will affect the assay's predictive values.
6. This kit is intended ONLY for testing of individual serum or plasma samples. Do not use it for testing of cadaver samples, saliva, urine or other body fluids, or pooled (mixed) blood.
7. This is a qualitative assay and the results cannot be use to measure antigens concentrations.

**Indications of Instability or Deterioration of the Reagents:**

1. Values of the Positive or Negative controls, which are out of the indicated Quality control range, are indicator of possible deterioration of the reagents and/or operator or equipment errors. In such case, the results should be considered as invalid and the samples must be retested. In case of constant erroneous results classified as due to deterioration or instability of the reagents, immediately substitute the reagents with new ones.
2. If after mixing of the Chromogen A and B solutions into the wells, the, the colour of the mixture turns blue within few minutes, do not continue carrying out the testing and replace the reagents with fresh ones.

**Reference:**

1. Stevens, C. E., P. E. Taylor, and M. J. Tong. 1988. Viral hepatitis and liver disease. Alan R. Liss, New York, N.Y. 142.
Stevens, C. E., P. E. Taylor, M. J. Tong, P. T. Toy, G. N. Vyas, P. V. Nair,
2. J.Y. Weisman, and S. Krugman. 1987. Yeast-recombinant hepatitis B vaccine. Efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. JAMA 257:2612-2616. 143. Stevens, C. E., P. T. Toy, P. E. Taylor, T. Lee, and M. F. Yip. 1992. Prospects for control of hepatitis B virus infection: implications of childhood vaccination and long term protection. Pediatric Research 31:70-75.
3. Hurie, M. B., E. E. Welzel, and J. P. Davis. 1992. Horizontal transmission of hepatitis B virus infection to U.S. born children of Hmong refugees. Pediatrics 89:268-273.



1. Calculation of Cut-off value

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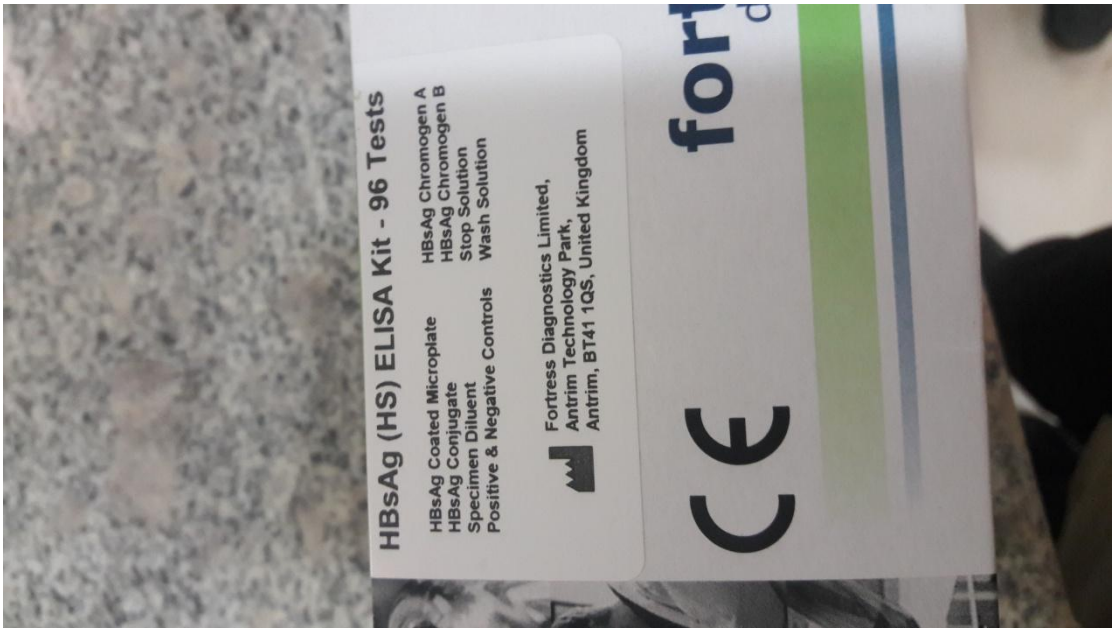
ELISA sheet

**Appendix ( 4 )**




ELISA Kit in study