# CHAPTER - I

## 1. INTRODUCTION

Blood donation saves lives of millions and it's true that blood transfusion plays an important role in supportive care of medical and surgical patients, however, unsafe transfusion is also putting millions of people at risk of Transfusion Transmissible Infections (TTIs) (Diro et al., 2008). While in the past, the risk of TTIs was accepted by patients and physicians as unavoidable, a low-risk blood supply is expected today (Bihl et al., 2007, Sarin et al., 1998).

Donated blood is not routinely screened for all of the possible (TTIs), although mandatory screening tests are carried out for HBV, HIV 1 & 2, HCV and Syphilis by Blood Transfusion Centers in Nepal as well as all the countries. Other screening tests are performed depending upon the disease endemic areas and if cost effective laboratory testing is available (SOP, 2006).

Viral hepatitis is a systemic disease primarily involving the liver and the term 'viral hepatitis' refers to a primary infection of the liver by any one of a heterogeneous group of 'hepatitis viruses', which consists of types A, B, C, D and E. The only common feature of hepatitis viruses is their primary hepatotropism. Additionally, well- characterized viruses that can cause sporadic hepatitis are cytomegalovirus, yellow fever virus, Epstein-Barr virus, herpes simplex virus, rubella virus and the eneteroviruses (Hayces et al., 2002, Butel, 2007). Transmission of HBV, HCV & HDV are parenteral routes may includes the chronic cases (WHO; 2002; ESAL, 1999; Williams, 2006).

Hepatitis B has also been called type B hepatitis, serum hepatitis, homologous serum jaundice. The severe pathological consequences of persistent of HBV include development of chronic hepatic insufficiency, cirrhosis and hepatocellular carcinoma (HCC). In recent

years, with the emergence of HBsAg escape mutants, the investigation of their implications for blood safety has become an important research priority (Zhang et al., 2001). HBV infection varies between 0.75 per million blood donations in Australia, 3.6 – 8.5 in the USA and Canada, 0.91 – 8.7 in Northern Europe, 7.5 – 13.9 in Southern Europe up to 200 per million donations in Hong Kong, largely reflecting the global epidemiology of HBV (Costa et al., 2005). Hepatitis B virus infection is an acute (self limiting), or chronic (long lasting) and person with acute case clear the virus within the months (Kerkar, 2005). A 6 percentage of acute hepatitis observed in Nepal and 1% of the population is asymptomatic chronic hepatitis B surface antigen carriers, 39% of patients suffer from chronic liver disease and 37% with hepatocellular carcinoma are HBsAg seropositive (Shrestha, 1990). Where as various studies reported to range from 0.3 to 4% in the general population conducted from 1990 to 2003 (Nakashima et al., 2003; Shrestha, 1990; Manandhar et al., 2000; Sawayama et al., 1999; Joshi et al., 2003; Rai et al., 1994; Bhatta et al., 2003). The inflammation of the liver is due to the infection of the hepatitis B in the liver of hominodae (Baker et al., 1996). Infected hepatocytes are characteristically enlarged and their cytoplasm has ground glass appearance (Rumbuldi et al., 2007).

Hepatitis B infection found worldwide but the prevalence varies enormously in different countries, coinfection with HCV and HIV. It is estimated that one half of the world population has experienced infection and 350 million people are chronically infected individuals. (Weinbaun, 2008) Around 40% of the chronically infected individuals or hepatitis B is responsible 1.5 million deaths per year (Pungpapong et al., 2007; CDC, 2001; CDC, 2007).

Hepatitis B is a pandemic disease having some ten times great impact than AIDS. One-fourth of 350 million people carrying virus, are dying due to cancer or cirrhosis of liver. The reported prevalence of carrier varies greatly from 0.1% in developed countries to 20% in developing countries and more 5% in South East Asia (Shrestha et al., 1993; Joshi et al., 2003). Hepatitis B infection is endemic in most region of globe, notably the part of china

and south East Asia, perinatal transmission appears to the major factor determining the overall prevalence of hepatitis B infection (Collee et al., 1996).

HBV can pose a serious problem in transfusion medicine if timely analysis is not made on the prevalence of various possible infections. In our country, attempts have been made to study prevalence of HBV infection. The study is hence carried out to understand the current scenario of most prevalent infection and the likely co-infections that is seen. Overall HBV seroprevalence can reveal the problem of concerned infections in healthy looking part of general population. Co-infection between hepatitis B, hepatitis C and HIV are very rare. HCV infections are common worldwide. It is estimated that 3% of world population have HCV and about 4 millions carries in Europe alone (Viral hepatitis prevention board, 1995). On another study at Tanzania 167 HIV infected children, 88(52.7%) males and 79(47.3%) females were enrolled. The overall prevalence of hepatitis co-infection was 15%, with the seroprevalence of HBV and HCV being 1.2% and 13.8%, respectively. Hepatitis virus co-infection was not associated with any of the investigated risk factors and there was no association between HBV and HCV. Elevated ALT was associated with hepatitis viral co-infection but not with ART usage or immune status (Safila et al., 2007).

When a plasma-derived hepatitis vaccine became available in 1981, a vaccine demonstration project was instituted in the hyper-endemic western Alaska region (Barrett et al., 1977; Heyward et al., 1979). The seroprevalence of HBsAg among the blood donors on a cross-sectional study was 0.53%, higher seroprevalence was observed on the Nepalese male blood donors (0.58%) than the female blood donors (0.18%) and seroprevalence higher in age group of 41-50 years (0.88%). The rate of hepatitis C co-infection with HBV-infected blood donors was 1.67% when the research was conducted on Nepal Red Cross Society, Central Blood Transfusion Service, Exhibition Road, Kathmandu (Karki et al., 2008).

Prevalence of any infection does vary with time and place of study. So, it is necessary to monitor the prevalence in given area in course of time to understand the current scenario. The findings of present study not only update on the epidemic situation of the infections, but also give a clear picture of trends of that infection over period of time. It is not reasonable to study a large population to know the figures at extra cost of investigation. However, blood banks besides meeting the requirements of blood supply are important centers for investigations of some infections that are of concern to general public. The world can be divided into three areas where the prevalence of chronic HBV infection is high (>8%), intermediate (2-8%), and low (<2%). There is no seasonal trend for HBV infection and no high predilection for any age groups such as parenteral drug abusers, institutionalized persons, health care personnel, multiply transfused patients, organ transplant patients, hemodialysis patients and staff, highly promiscuous persons, and new born infants born to mothers with hepatitis B (Butel, 2007).

A person who is infected with both the hepatitis B and the HIV viruses is said to have a HBV /HIV Co-infection. Approximately 10% of the HIV-infected population worldwide is infected with hepatitis B. This figure may approach 20% in Southeast Asia and 5% in North America and Western Europe. Since both the hepatitis B virus and the HIV virus share similar transmission routes, it is not surprising that there is a high frequency of co-infection. Sexual activity and/or injection drug use are the most common routes of transmission of the hepatitis B virus among those also infected with HIV (WHO, 2002).

Blood donors represent the part of general population who are considered to be healthy looking and are ignorant of their infections. The infected individuals from such population are the potential source of infections in community. Study of HBV among blood donors at the centre can be a cost-effective approach to monitor the prevalence, distribution, and trends of these infections among such healthy looking individuals. Similarly, the study on seroprevalence and co-infection rates of HBV among the blood donors would provide data important in formulating the strategies for improving the management of safe blood supply

in the country (Nakashima et al., 2003; Shrestha, 1990; Manandhar et al., 2000; Sawayama et al., 1999; Joshi et al., 2003; Rai et al., 1994; Bhatta et al., 2003).

Therefore, the study was designed to explore seroprevalence of HBV among the blood donors of Jhapa District of Nepal. The study outcomes may be helpful in managing Transfusion Transmissible Disease in Nepal.

# CHAPTER - II

## 2. OBJECTIVES

### 2.1. General objectives

To determine seroprevalence of HBV infection among blood donors of Jhapa district.

### 2.2. Specific objective

- To detect the HBsAg in serum samples of the blood donors using commercial rapid diagnostic kits.
- To study the seroprevalence on male and female blood donors and their co-relations.

# CHAPTER - III

## 3. LITERATURE REVIEW

### 3.1 Hepatitis B Virus

HBV causes the hepatitis B. Hepatitis B is the most common form of parenterally transmitted viral hepatitis, and an important cause of acute and chronic infection of the liver. Hepatitis B Virus (HBV) is the only Hepadna virus causing infection in humans. It cannot yet be grown but can be transmitted to certain primates. Hepatitis B has also been called type B hepatitis, serum hepatitis, homologous serum hepatitis. Persistent HBV infection can lead to development of liver cirrhosis, hepatocellular carcinoma (HCC), chronic hepatic insufficiency (WHO, 2002; Butel, 2007).

The earliest record of an epidemic caused by Hepatitis B virus was made by Lurman in 1885. The virus was not discovered until 1965 when Baruch Blumberg, then working at the National Institutes of Health (NIH), discovered the Australia antigen (later known to be Hepatitis B surface antigen, or HBsAg) in the blood of Australian aboriginal people (Alter et al., 1966). Dane and colleagues detected some larger double-shelled virus-like particles 40 nm in diameter by electron microscopy (Dane et al., 1970).

### 3.2 Epidemiology

About one third of the world's population had been exposed at some time to the Hepatitis B virus. More over approximately 350 million individuals world population are chronically (long duration) infected with virus. As a result the complication of hepatitis B viral infection leads to load two million deaths annually (WHO, 2002). Transmission of hepatitis at least to 30% cases among the adults cannot be associated with an identifiable risk factor (Shapiro, 1993; Zuckerman 1996).

On a collaborative study, conducted during June - September 2006 with the objective of determining the prevalence of hepatitis B and malaria in Nepalese blood donors of Kathmandu, Nepalgunj and Biratnagar Blood Banks a total sample of the total 1200 were analyzed, 600 were collected at Kathmandu and 300 each at Nepalgunj and Biratnagar blood banks. Among the total 1200 samples, 1% (12) of the sample was found reactive for HBsAg, while only 0.33 % (4) samples were positive for malarial parasite. 1.33% (8) samples from Kathmandu and 1.33% (4) samples from Nepalgunj were positive for HBV 1% (3) samples from Nepalgunj and 0.33% (1) sample from Biratnagar were found to be positive for malarial parasite (Ghimire et al., 2006). Over the three-year period study on Nigeria, a total of 2,500 samples /donors were screened for HBsAg modal age range of the study population was 41 – 50 years, representing 43% of the study population, five hundred donors (20%) were positive for HBsAg: four hundred and fifty five males (91%) and forty five females (9%). The peak age prevalence was in the 41 – 50 year age group (24.2%) and prevalence was lowest in "31 – 40" year's age (15.3%) (Alao et al., 2009). A same type of study at Rawalpindi in India, a total number of 1428 donors who were screened for HCV and HBsAg and among them majority (97.05%) were males. 2.45% among them were seropositive for HBsAg and 2.52% for HCV (Chaudhary et al., 2005).

The coprevalence of HBV /HIV was 0.033 percent (4/11,995) among total blood donors, 4 individuals (3 males and 1 female) were found to be coinfected with HBV /HIV. The infection rate of HIV was 3.8 percent among the hepatitis B infected blood donors. The association of HBV and HIV infection was tested and the data was statistically significant ($P<0.05$) (Ghimire et al., 2002).

### 3.3. Classification

Hepatitis B virus belongs to *Hepadnaviridae* family and genus *Orthohepadnavirus.* The observation that liver carcinoma occurred in the Eastern woodchuck led to the discovery of the HBV-like woodchuck hepatitis virus (WHV) and the closely related ground squirrel

hepatitis virus (GSHV), other such viruses include Pekin ducks (DHBV), grey herons (HHBV). All these viruses are highly species specific. The great molecular and biological similarity of these viruses led to the definition of a common virus family, *Hepadnaviridae* (from *heap:* liver, and DNA: for the type of genome). The viruses infecting mammals form the genus *Orthohepadnavirus* and those infecting the birds form *Aviahepadnavirus*. Common strategy of reverse trancription places *Hepadnaviridae*, *Retroviridae* and *Caulimoviridae* of plants into one group, defined as 'retrovirales' (Kann et al., 1998).

### 3.4 Structure

It is a 42 nm enveloped viron with an icosahedral nucleocapsid core containing a partially double stranded circular DNA genome (Haword; 1996). This viron was also known as Dane particle and was named for the scientist who first published electron micrograph of virus. The envelope contains a protein called the surface antigens (HBsAg) which is important for laboratory diagnosis and immunization. HBsAg was known as Australia antigen, because it was first found in the serum of Australian aborigine. Surface antigen, core antigen, DNA polymerase and two regulatory proteins are encoded by the genome of HBV (Jawez et al., 1996).

The patient's serum who was infected with the virus particle reveals three different types of particles: a few 42 nm and many 22 nm spheres and long filaments 22 nm wide. Later are composed of surface antigens. In addition to HBsAg there are two other important antigens; the core antigen (HBcAg) and the antigen (HBeAg), both of which are located in the core but have different antigenicities (Jawez et al., 1996). This particle is the complete hepatitis B virus. Complete virions possess an isometric nucleocapsid or 'core' of 27 nm in diameter, surrounded by an outer coat approximately 4 nm thick. The protein of the virion coat is termed 'surface antigen' or HBsAg and it is sometimes extended as a tubular tail on one side of the virus particle (Butel, 2007).

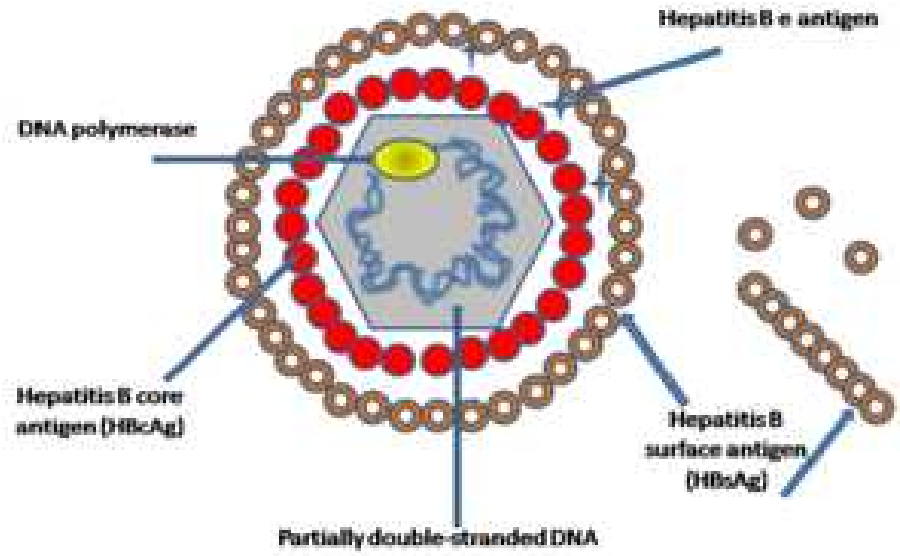


**Figure: 3.1** Structure of Hepatitis B viruses (A simplified drawing of the HBV particle and surface antigen)

Source: Harris et al., 2001

### 3.5 Pathogenesis

Hepatitis B virus infection may either be acute (self-limiting) or chronic (long-standing). Persons with self-limiting infection clear the infection spontaneously within weeks to months (Ernest, et al., 1996). The injury to the liver cells is caused by that same immune response to the hepatitis B virus in the liver cells (Zignego et al., 2006).

The incubation phase of Hepatitis B is 6 to 24 weeks. Patients may then become jaundiced although low grade fever and loss of appetite may improve. More than 95% of people who become infected as adults or older children will stage a full recovery and develop protective immunity to the virus. However, only 5% of newborns that acquire the infection from their mother at birth will clear the infection (Kerkar, 2005). This population has a 40% lifetime

risk of death from cirrhosis or hepatocellular carcinoma (Dienstag, 2008). Of those infected between the ages of one to six, 70% will clear the infection (Kerkar, 2005).

### 3.5.1 Portal of Entry

About one third of the world's population had been exposed at some time to the Hepatitis B virus. More over approximately 350 million individuals world populatoin are chronically (long duration) infected with virus. As a result, the complications of hepatitis B viral infection load to two million deaths annually (WHO, 2004). Transmission of hepatitis at least to 30% cases among the adults cannot be associated with an identifiable risk factor (Shapiro, 1993).

In the U. S. adolescents and young adults account for the majority of reported cases of hepatitis B infection. Sexual contact (intercourse) is most common means of transmission (Shaw et al., 1990). The highest concentration of hepatitis B is also found in the semen. Hepatitis B virus is spread or acquired through exposure to infected blood or infected body's secretion (Barker, 1996). Method of infection of HBV is due to blood transfusion. Addition, hepatitis B virus can be transmitted through the sharing of toothbrush and razors. Finally blood-sucking such as mosquitoes and bed bugs in the tropics has reportedly spread hepatitis B viral load (Vandelli et al., 2004; Barker, 1996).

Vertical transmission of HBV from mother to child during child birth, without into the hepatitis B surface antigen confers a 20% risk of passing the infection to her offspring at the time of birth. This is as high as 90% if mother is also positive for the hepatitis B e antigen (WHO, 2004; Martin et al., 2004)).

The use of the contaminated needles and syringes have direct modes of transmission but in Nepal and other countries screening of the donated blood for the presence of HBsAg has greatly decreased the number transfusion associated cases of hepatitis B. In developing countries the main routes of transmission are the reuse of HBV contaminated needles,

syringes lancets, and instruments including those used in tribal ceremonies (Cheesebrough, 1996).

### 3.5.2 Entry of Virus into the Cell

Entry of virus into the lost cell starts with the attachment of the virus into the specific surface receptor accomplished through the surface antigen encoded by 'S' gene. The HBsAg gene is one long open reading frame but contains three in frame "start" (ATG) codons that divide the gene into three section, pre-S1, pre-S2 and S. because of multiple start codons, polypeptide of three different sizes called large, middle and small (pre=S1+pre S2+S, pre s-2 + s or S) are produced (Beck et al., 2007; Kramvis et al., 2005). After entering the virus into the blood viruses enters into the hepatocytes and viral antigens appear on the surface of the cells. Cytotoxic T cells mediate an immune attach against the viral antiques and inflammation and necrosis occur (Bouchard et al., 2004).

Identified receptors are transferrin receptor, asialoglycoprotein receptor molecule and human liver endonexin. Mode of attachment and entry is still unknown. After the entry of the virion into the liver cell, it uncoats in the cytoplasm and the genome is delivered into the nucleus. In the nucleus, second strand DNA synthesis is completed and the gaps in both strands are repaired to form a covalently closed circular (ccc) supercoiled DNA molecule. The cccDNA serves as template for all virus transcripts. These transcripts are polyadenylated and transported to the cytoplasm, where they are translated into the viral nucleocapsid and precore antigen (C, pre-C), polymerase (P), envelope L (Large), M (Medium), S (Small), and transcriptional transactivation protein (X). The envelope proteins are inserted as integral membrane proteins into the lipid membranes of the endoplasmic reticulum. The 3.5 kb species, spanning the entire genome and pregenomic RNA, is packaged together with HBV polymerase and a protein kinase into core particles where it serves as a template for reverse transcription of negative-strand DNA. The RNA to DNA conversion takes place inside the particles (Gunther et al., 1995). The nucleocapsid can be a

source of genome amplification or they are assembled to release as virus. The nucleocapsids reach the endoplasmic reticulum, where they gain the envelope protein and bud into the lumen of the reticulum. They are secreted out of the cell via the golgi bodies (Butel, 2007; Kann et al., 1998).

### 3.5.3 Completion of Viral Genome

After entry of the viron into the cell and it's uncoating the viron DNA polymerase synthesize the missing portion of DNA is formed in the nucleus. This DNA serves as a template for mRNA synthesis by cellular RNA polymerase. The minus strand then serves as the template for the plus strand of the genome DNA. This RNA dependant DNA synthesis takes place in the newly assembled viron core in the cytoplasm. Some of the progeny DNA integrates into the host genome and this seems likely to be the DNA that maintains the carries state. Progeny HBV with its HBsAg containing involves (Beck et al., 2007; Locarnini, 2004).

### 3.5.4 Integration of the Provirus into Host Cell DNA

The provirus is transported to the nucleus of the host chromosome by the help of matrix proteins of HBV. There, the viral integrase claves the chromosomal DNA, and covalently inserts the provirus; the integrated provirus that's becomes a stable part of the cell genome. This characteristic makes about 5% patients with hepatitis B become chronic carriers of HBV. Chronic carrier is some one who has HBsAg persisting in their blood for at least 6 months (Feitelson et al., 2007).

### 3.5.5 Transcription of Viral RNA

The viral DNA polymerase synthesizes the missing part of DNA and a double stranded closed circular DNA forms in nucleus. Hepadna viruses are the only viruses that produce genome DNA by reverse transcription with MRNA as the template. Using the cellular mechanism processes the genome transcribes the mRNA and further translates to various

proteins (Beck et al., 2007; Bruss, 2007). From within the cell nucleus the hepatitis B DNA causes the liver cell to produce, via messenger RNA; surface (HBs) proteins, the core (HBc) protein, DNA polymerase, the HBe protein, HBx protein and possibly other as yet undetected proteins and enzymes (Feitelson et al., 2007).

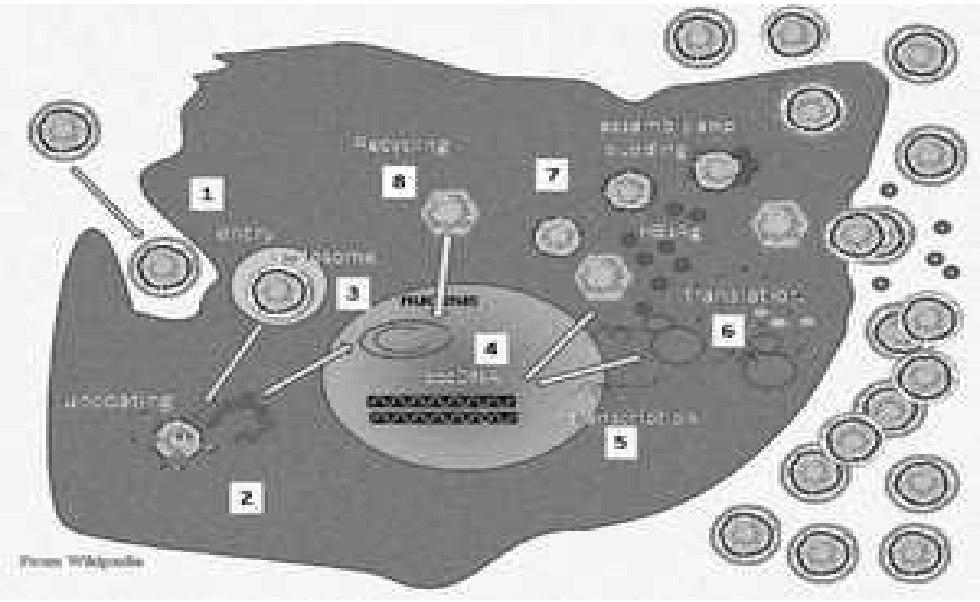


Figure: 3.2 Hepatitis B virus replication in liver.

Source: Beck et al., 2007

### 3.5.6 Translation Assembly and Maturation of Integrated Viral DNA Sequences.

The first step in the viral gene expression is mRNA synthesis. Depending on the nature of nucleic acid and part of the cell in which they replicate the virus follow the different pathway for mRNA synthesis. From within the cell nucleus the hepatitis B DNA causes the liver cell to produce, via messenger RNA; surface (HBs) proteins, the core (HBc) protein, DNA polymerase, the HBe protein, HBx protein and possibly other as yet undetected proteins and enzymes (Tang; et al,. 2001). HBV replicate in the nucleus and use the hont cell DNA-dependent RNA polymerase to synthesize its progeny DNA with full length of

mRNA as the template. DNA polymerase synthesizes the incomplete portion of viral DNA and viral mRNA synthesize by host cell RNA polymerase (Beck, 2007).

Once the viral mRNA synthesize by host cell ribosomes into the viral protein, some of which are “early proteins” i.e. enzymes required for replication of viral genome and other late proteins i.e. structural proteins of the progeny viruses. Viral mRNA is translated into precursor polypeptide that must be cleaved by the protease to produce the functional and structural proteins (Hinrichse et al., 2004; Beck, 2007).

HBV uses its mRNA as template to make progeny double strand DNA. As the replication of viral genome proceeds the structural capsid proteins to be used in the progeny virus particle are synthesized. The progeny particles are assembled by packaging the viral nucleic acid within the capsid proteins. After assembly and integration of the genome into the capsid, the virus particles are released by budding of cell membrane as all the enveloped viruses do (Beck, 2007).

### 3.5.7 Clinical Pathogenesis/ Disease /Symptoms

The hepatitis B virus primarily interferes with the functions of the liver by replicating in liver cells, known as hepatocytes. Acute infection with hepatitis B virus is associated with acute viral hepatitis. Early, non-specific symptoms are malaise, poor appetite, nausea and right upper quadrant pain which last for several days. The larger the virus dose, the shorter the incubation period and more likely that icteric hepatitis will result. The increase in aminotransferases, during acute hepatitis B varies from a moderate increase of 3 to 10 fold to > 100- fold. The enteric phase of acute viral hepatitis begins usually within 10 days of the initial symptoms with the appearance of dark urine followed by pale stools and yellowish discoloration of the mucous membranes, conjunctivae, sclerae, and skin. Jaundice becomes apparent clinically when the total bilirubin level exceeds 20 to 40 mg/l. It is accompanied by hepatomegaly and splenomegaly (Iannacone et al., 2007; WHO, 2002).

### 3.5.7.1 Acute Hepatitis

HBV virons (DANE particle) bind to the host cell via the preS domain of the viral surface antigen and are subsequently internalized by endocytosis (Dane, et al., 1970). PreS and IgA receptors are accused of this interaction. HBV-preS specific receptors are primarily expressed on hepatocytes; however, viral DNA and proteins have also been detected in extrahepatic sites, suggesting that cellular receptors for HBV may also exist on extrahepatic cells. During HBV infection, the host immune response causes both hepatocellular damage and viral clearance. Although the innate immune response does not play a significant role in these processes, the adaptive immune response, particularly virus-specific cytotoxic T lymphocytes (CTLs), contributes to most of the liver injury associated with HBV infection. By killing infected cells and by producing antiviral cytokines capable of purging HBV from viable hepatocytes, CTLs eliminate the virus (Iannacone et al., 2007).

Although liver damage is initiated and mediated by the CTLs, antigen non specific inflammatory cells can worsen CTL-induced immuno-pathology, and platelets activated at the site of infection may facilitate the accumulation of CTLs in the liver (Iannacone et al., 2007).

Acute infection with hepatitis B virus is associated with acute viral hepatitis- an illness that begins with general ill-health, loss of appetite, nausea, vomiting, body aches, mild fever, dark urine, and then progresses to development of jaundice. It has been noted that itchy skin has been an indication as a possible symptoms of all hepatitis virus types. The illness lasts for a few weeks and then gradually improves in most affected people. A few patients may have more severe liver disease (fumiliant hepatic failure), and may die as a result of it. The infection may be entirely asymptomatic and may go unrecognized (Verling, 2007).

### 3.5.7.2 Chronic Hepatitis

Chronic infection with Hepatitis B virus may be either asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a

period of several years. This type of infection dramatically increases the incidence of hepatocellular carcinoma (liver cancer). Clinical signs of chronic hepatitis are usually mild and non-specific. Chronic liver damage results from continuing, immune mediated destruction of hepatocytes expressing viral antigens. Chronic hepatitis is defined by at least six months of persistent HBV disease (HBV surface antigen (HBsAg) positive > six months). The symptoms do not correlate with the severity of disease. Serum aminotransferases may be normal, although most patients have mild to moderate elevations. During flares of disease, serum aminotransferases may be elevated 20 times normal (Brink et al., 2006). Chronic infection with Hepatitis B virus may be either asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis over a period of several years. This type of infection dramatically increases the incidence of hepatocellular carcinoma (liver cancer). Chronic carriers are encouraged to avoid consuming alcohol as it increases their risk for cirrhosis and liver cancer. Hepatitis B virus has been linked to the development of membeaneous Glomerulonephritis (MGN) (Rambaldi et al., 2007; Lai, 1991; Campbell et al., 2006). Hepatitis D infection can only occur with a concomitant infection with Hepatitis B virus because the Hepatitis D virus uses the Hepatitis B virus surface antigen to form a capsid (Taylor, 2006).). Co-infection with hepatitis D increases the risk of liver cirrhosis and liver cancer (Oliveri, 1991). *Poliarteris nodosa* is more common in people with hepatitis B infection. Co-infection with hepatitis B and hepatitis C is very uncommon. Regarding the hepatitis b, the prevalence of 2.7% in study Nepal falls in the intermediate zone of hepatitis B according to the WHO (Oliveri, 1991; Weibaum et al., 2008).

#### 3.5.7.3 Hepatocellular Carcinoma

Hepatocellular Carcinoma (HCC) is one of the 10 most frequent tumors in the world, and there is considerable evidence that 80% are caused by chronic infection with HBV. Persons at increased risk of developing HCC include adult male and chronic hepatitis B patients with cirrhosis who contracted hepatitis B in early childhood (Simmonds et al., 2006). The

rate of progression to cirrhosis and HCC varies according to the age of infection and stage reached, the state of the patient's immune system, geographical factors and genetic factors (Gitlin, 1997).

### 3.5.7.4 Fulminant Hepatitis B

Fulminant hepatitis B is a rare condition that develops in about 1% of cases. It is caused by massive necrosis of liver substance and is usually fatal. A rapid fall in ALT and AST in patients with fulminant hepatic failure may be erroneously interpreted as a resolving hepatic infection, when in fact hepatocytes are being lost and the outcome is fatal (Williams, 2006).

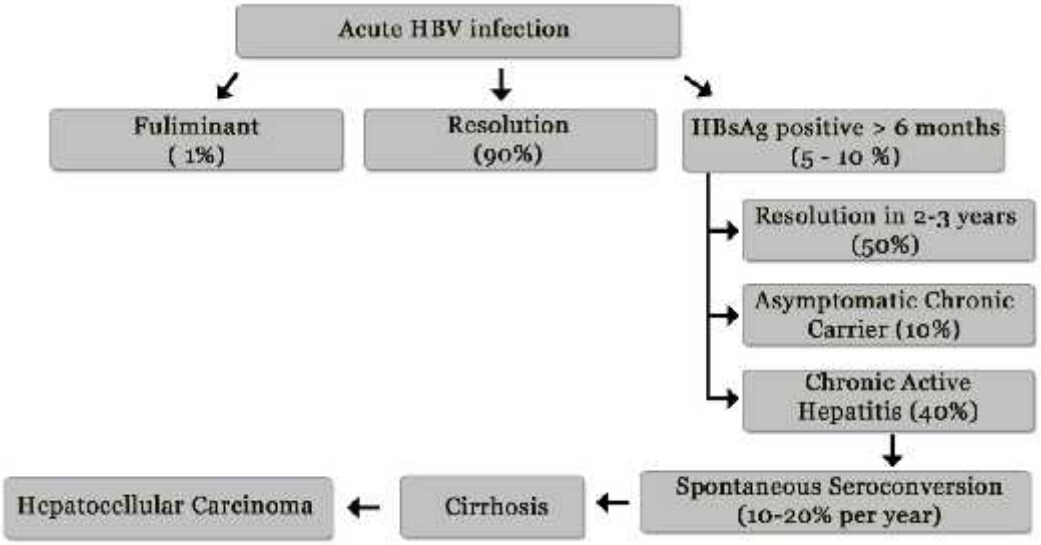


**Figure: 3.3** Progression of HBV infection acquired as an adult

Source: Brink et al., 2006

### 3.6 Lab Diagnosis

The laboratory diagnosis of HBV infections is essential to identify infected person, to identify carrier of HBV who may transmit to other (especially blood or organ donors, sex partners, pregnant women and person who comes close contact with) and to confirm the

clinical diagnosis of infection (Gitlin, 1997). Methodology used in lab diagnosis of the hepatitis B infection involves the detection of HBsAg Anti-HBe, Anti-Hbc and HBV DNA. Methodology referred according to the laboratory complexity structure (Shapiro, 1993).

Screening with hepatitis B surface antigen (HBsAg) was recommended to detect active (acute or chronic) HBV in all the blood donors and pregnant women at their first prenatal visit. Routine screening of the general population for HBV infection was not recommended. Certain persons at high risk for HBV could be screened to assess their eligibility for vaccination (Zuckerman, 2006; USPSTF, 1996). Since then, the USPSTF criteria to rate the strength of the evidence has changed. Therefore, the recommendation statement that follows has been updated and revised based on the current USPSTF methodology and rating of the strength of the evidence (Harris, 2002).

### 3.6.1 HBsAg Rapid Screening Tests

Commercially available rapid visual one step immunoassay tests card are used for the diction for HBsAg. Such cards are based on the antigen capture. Rapid card is a visual, rapid, sensitive and accurate one step immunoassay for qualitative detection of hepatitis B surface antigen is infected serum or plasma. The antigenic determinant of HBsAg protein moiety is antigenically, heterogeneous and it determines specific HBV serotypes and provides a basis for immunodetection. The principal antigenic determinant which is common to all HBV serotypes.

This method is one step immunoassay based on the antigen capture or “sandwich” principle. The method used monoclonal antibodies conjugated. To colloidal gold and polyclonal antibodies immobilized on a nitrocellulose strip in a thin line. The test sample is introduced to and flows laterally through the absorbent pad where it mixes with indicator reagent. If the donor’s serum contains HBsAg the colloidal gold-antibody conjugate binds to the antigen and forms an antigen-antibody colloidal gold complex. This complex migrates through nitro cellulose strip by capillary action and when meets the line of

immobilized antibody (test line). “T” the complex trapped forming antibody- antigen-antibody colloidal gold complex. This forms a pink band indication the sample reactive for HBsAg (Engavall et al., 1971).

### 3.6.2. Determination of HBV Antigens and Antibodies By Rapid Tests

Hepatitis B is diagnosed from the results of specific hepatitis B virus blood tests (serology) that reflect the various components of the hepatitis B virus. A discussion of each of the hepatitis B virus blood tests follows. These serological hepatitis B virus blood tests differ from the standard liver blood tests (such as the ALT and AST) that can become abnormal when the liver is damaged from whatever cause, including hepatitis B viral infection.

#### ELISA

ELISA has a high sensitivity and in an ELISA, a HBV infected person's serum is diluted 400-fold and applied to a plate to which antibodies to HBsAg are attached. If HBsAg are present in the serum, they may bind to these antigens. The plate is then washed to remove all other components of the serum. A specially prepared "secondary antibody” an antibody that binds to other antibodies is then applied to the plate, followed by another wash. This secondary antibody is chemically linked in advance to an enzyme. Thus, the plate will contain enzyme in proportion to the amount of secondary antibody bound to the plate. A substrate for the enzyme is applied, and catalysis by the enzyme leads to a change in color or fluorescence. ELISA results are reported as a number; the most controversial aspect of this test is determining the "cut-off" point between a positive and negative result (Engvall et al., 1971; Weemen et al., 1971)

#### HBsAg and Anti-HBsAg

The diagnosis of hepatitis B infection is made primarily by detecting the hepatitis B surface antigen (HBsAg) in the blood. The presence of HBsAg means that there is active hepatitis B viral infection and the absence of HBsAg means that there is no active hepatitis B viral

infection. Following an exposure to hepatitis B virus, HBsAg becomes detectable in the blood within four weeks. In individuals who recover from acute hepatitis B viral infections, the elimination, or clearance, of HBsAg occurs within four months after the onset of symptoms. Chronic hepatitis B viral infection is defined as the persistence of HBsAg for more than six months ((West et al., 1996; Alward et al., 1985).

After the HBsAg is eliminated from the body, the antibodies to HBsAg (anti-HBs) usually appear. This anti-HBsAg provides immunity to subsequent hepatitis B viral infection. Likewise, individuals who are successfully vaccinated against hepatitis B virus have measurable anti-HBsAg in the blood (Zukerman, 1996).

**Anti-HBcAg**

The hepatitis B core antigen can only be found in the liver and cannot be detected in the blood. The presence of large amounts of hepatitis B core antigen in the liver indicates an ongoing reproduction of the virus. This means that the virus is active. The antibody to hepatitis B core antigen, known as the hepatitis B core antibody (anti-HBcAg), however, is detectable in the blood. As a matter of fact, two types of anti-HBcAg antibodies (IgM and IgG) are produced ((West et al., 1996; Zukerman, 1996).

IgM anti-HBc is a marker (indicator) for acute hepatitis B infection. The IgM anti-HBcAg is found in the blood during the acute infection and lasts for up to six months after the onset of symptoms. IgG anti-HBcAg develops during the course of the acute hepatitis B viral infection and persists for life, regardless of whether the individual recovers or develops the chronic infection. Accordingly, only the IgM type of anti-HBcAg can be specifically used to diagnose an acute hepatitis B viral infection. Moreover, determining just the total anti-HBcAg (without separating its two components) is not very helpful (West et al., 1996).

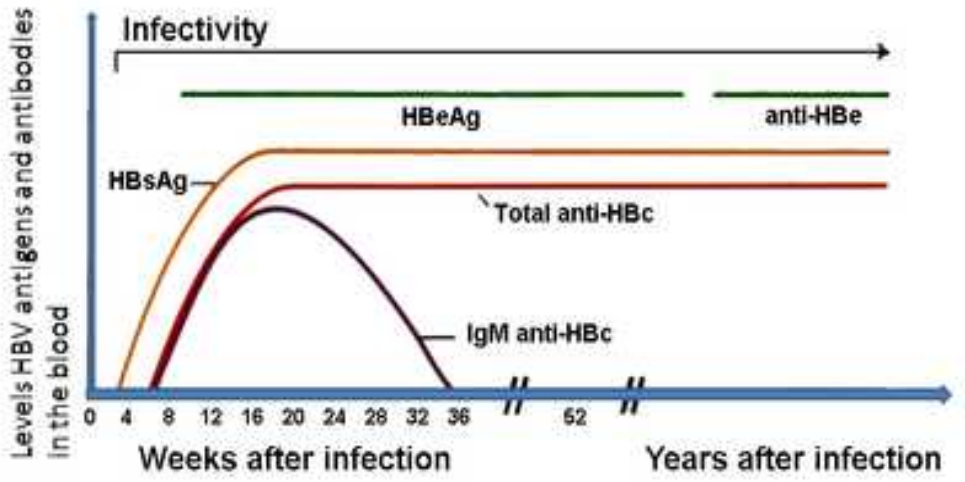


**Figure: 3.4** Timeline of detection hepatitis B infection (no late sero-conversion) hepatitis B viral antigens and antibodies detectable in the blood of a acute chronically infected person.

Source: Harris et al., 2001

**HBeAg, anti-HBe, and Pre-core Mutations**

Hepatitis B e antigen (HBeAg) and its antibody, anti-HBeAg, are useful markers to determine the likelihood of spread of the virus (transmissibility) by persons affected with chronic hepatitis B viral infection. Detecting both HBeAg and anti-HBeAg in the blood is usually mutually exclusive. Accordingly, the presence of HBeAg means ongoing viral activity and the ability to infect others, whereas the presence of anti-HBeAg signifies a more inactive state of the virus and less risk of transmission (Verling et al., 2007).

In some individuals infected with hepatitis B virus, the genetic material for the virus has undergone a particular structural change, called a pre-core mutation. This mutation results in an inability of the hepatitis B virus to produce HBeAg, even though the virus is actively reproducing. This means that even though no HBeAg is detected in the blood of people with the mutation, the hepatitis B virus is still active in these persons and they can infect others (Alward et al., 1985; Hadler et al., 1984).

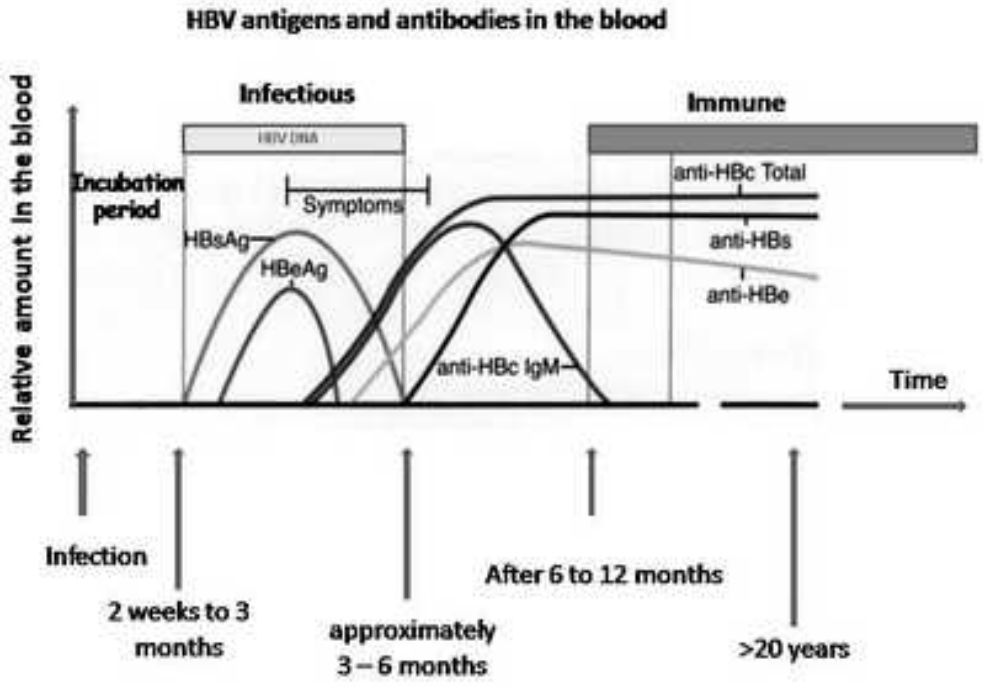


Figure: 3.5 Detectable Hepatitis B viral antigens and antibodies, in the blood following acute infection. Source: Harris et al., 2002

Table 3.1**:** Interpretation of hepatitis B virus blood (serological) tests (+ = positive and - = negative). (Source Weekly Epidemiological Records WHO, 2002)

| HBsAg | Anti-HBs | Anti-Hbc (total) | Anti-HBc IgM | HBe Ag | Anti-HBe | HBV DNA | Interpretation |
|---|---|---|---|---|---|---|---|
| + | - | + | + | + | + | + | Early phase of acute infection |
| + | - | + | + | - | + | - | Later phase of acute infection |
| - | - | + | + | - | + | - | Later phase of acute infection |
| - | + | + | - | - | - | - | Recovery with immunity |
| - | + | - | - | - | - | - | Successfully vaccinated |
| + | - | + | - | + | - | + | Chronic infection with active reproduction |
| + | - | + | - | - | + | - | Chronic infection in the inactive phase |
| + | - | + | - | - | + | + | Chronic infection with active reproduction |
| - | - | + | - | - | + or - | - | Recovery, False positive result, or Chronic infection |

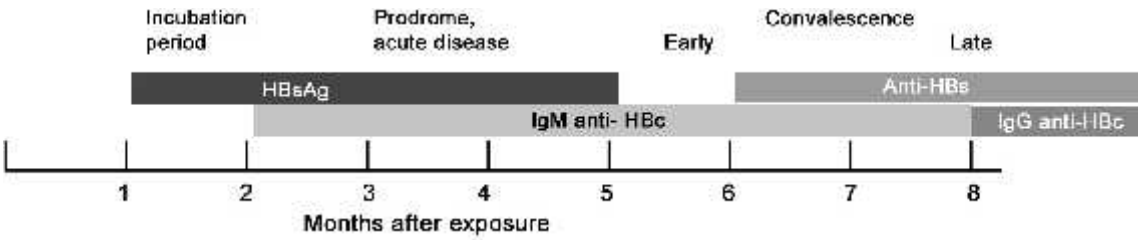


**Figure: 3.6** Important Serological diagnosis of HBV

Source: Jawetz, Melnick & Adelberg's Medical Microbiology, 1996.

### 3.6.3. Hepatitis B Virus DNA Testing

The most specific marker of hepatitis B virus reproduction is the measurement of hepatitis B virus DNA in the blood. Several different laboratory tests (assays) are available to measure hepatitis B virus DNA (Bouchard et al., 2004). The PCR (Polymerase Chain Reaction) is the most sensitive method (assay) for determining the level of hepatitis B virus DNA. This means that the PCR is the best method for detecting minute amounts of the hepatitis B virus marker. This method works by amplifying the material that is being measured up to a billion times for its detection. The PCR method, therefore, can measure as few as 50 to 100 copies (particles) of hepatitis B virus per milliliter of blood. This test, however, is actually too sensitive for practical diagnostic use (Coste et al., 2003).

For practical purposes, hepatitis B virus DNA can be measured using a so-called hybridization method (assay), which is a less sensitive test than the PCR. Unlike the PCR method, the hybridization assay measures the viral material without amplification. Accordingly, this test can detect hepatitis B virus DNA only when many viral particles are present in the blood, meaning that the infection is active. In other words, from a practical point of view, if hepatitis B virus DNA is detected with a hybridization assay, this means that the hepatitis B viral infection is active (Zoulim, 2006) .

Branched DNA technology assays, hybrid capture assays, or more sensitive polymerase chain reaction (PCR) techniques are used to detect the viral genome in the serum

(Pawlotsky et al., 2000). HBV DNA is useful to assess HBV status in patients with suspected false-positive results of serologic studies and to evaluate the level of active replication in an HBV-infected patient. Quantization of HBV DNA is helpful for predicting treatment response (Harris et al., 2002).

### 3.6.4 Other Tests of Medical Importance

Other tests are also performed in the laboratory but which are only academically importance but not performed in laboratory generally unless in special cases.

#### 3.6.4.1 Liver Biopsy Test

A liver biopsy is an important part of the work up of a patient with chromic hepatitis B virus. This test is valuable because the small core of tissue taken from the liver is generally representative of the rest of the liver. Further more a diagnosis of chronic hepatitis can usually be made from the biopsy. However the type of chronic hepatitis (or the cirrhosis it causes) whether it be hepatitis B, C or autoimmune hepatitis cannot be determined definitively from the biopsy.

#### 3.6.4.2 Biochemical Tests

Bilirubin is found in the urine and conjugated and total serum bilirubin levels are raised in most symptomatic infections (Harrison et al., 2004). A progressive decline in serum albumin concentrations and prolongation of the prothrombin time are characteristically observed after decompensate cirrhosis has developed (Harrison et al., 2004).An elevated ALT level can indicate that the liver is inflamed and there is a risk of permanent liver damage. In acute hepatitis B infection, high levels of ALT and AST are typical. ALT levels are typically higher than AST levels. Persistent elevation of serum ALT for longer than 6 months is suggestive of progression to chronic hepatitis. Alkaline phosphatase levels may be elevated, but are usually less than 3 times the upper limit of normal. Patients with

chronic active hepatitis B have mild to moderate elevation of the aminotransferases (<5 times the upper limit of normal) (Harris et al., 2002).

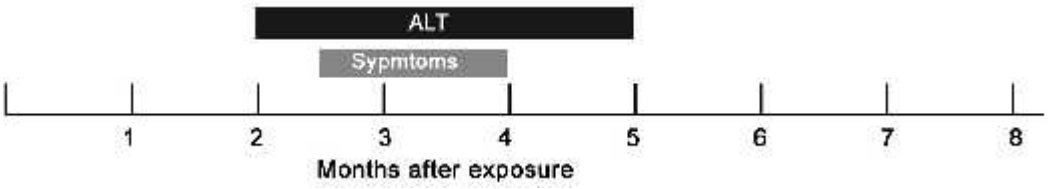


**Figure: 3.7** Symptoms and elevated level of ALT observed after months of exposure in patients with acute HBV infection

(Source: Jawetz, Melnick & Adelberg's Medical Microbiology, 1996).

### 3.7 Reactivation of HBV

Hepatitis B virus DNA persists in the body after lysogenic infection and in some people the disease re-occurs (Verling, 2007; Chu, 2007). Although rare, reactivation is seen most often in people with impaired immunity (Katz, 2008).

### 3.8 Prevention

HBV transmission can be reduced by modifying risk behaviors. The measures include following safe sexual practices such as use of condoms, avoiding sharing of needles, syringes, necessity for blood screening before transfusion, implementation of sensible control of infection policies to reduce occupational risks and avoiding saliva contact (Petersen et al., 1979; Heyward et al., 1985; Martin et al., 2004).

Passive and active immunization offers advantages in situations where prevention is difficult or not possible. A recombinant hepatitis B vaccine containing HBsAg is available and is capable of producing active immunization in 95% of normal individuals. The vaccine is ineffective in those already infected by HBV (Mast et al., 1999). Type B hepatitis can be prevented or minimized by the intramuscular injection of hyperimmune serum globulin prepared from blood containing anti-HBs. This should be given within 24

hours or at most a week, of exposure to infected blood in circumstances likely to cause infection. Vaccine can be given together with hyperimmune globulin (Hayces et al., 2002).

In general, to prevent viral hepatitis we should: (West et al., 1996).

- Follow good hygiene and avoid crowded, unhealthy living conditions.
- Take extra care, particularly when drinking and swimming, if we travel to areas of the world where sanitation is poor and water quality is uncertain.
- Never eat shellfish from waters contaminated by sewage.
- Remind everyone in our family to wash their hands thoroughly after using the toilet and before eating.
- Use antiseptic cleansers to clean any toilet, sink, potty-chair, or bedpan used by someone in the family who develops hepatitis. Avoid sharing drug needles or any other drug paraphernalia including works for injection or bills or straws.
- Avoid unsanitary tattoo methods.
- Avoid unsanitary body piercing methods.
- Avoid unsanitary acupuncture.
- Avoid needle stick injury.
- Avoid sharing personal items such as toothbrushes, razors, and nail clippers.
- Use latex condoms correctly and every time you have sex if not in a long-term monogamous relationship

Because contaminated needles and syringes are a major source of hepatitis infection, it's a good idea to encourage drug awareness programs in your community and schools. At home, speak to our child frankly and frequently about the dangers of drug use. It's also

important to encourage abstinence and safe sex for teens, in order to eliminate their risk of hepatitis infection through sexual contact (WHO, 2004).

### 3.9 Treatment of Hepatitis B

Anti-viral therapy is not warranted for acute hepatitis B because the infection will resolve on its own in most symptomatic individuals (Hollinger, et al., 2006). Acute liver failure, which, as mentioned above, may develop in less than 0.5% of adults with acute hepatitis B, however, requires prompt evaluation for a liver transplant (Castillo et al., 2006).

#### 3.9.1 Interferon

Interferon-alpha 2b (Intron A) was first shown to be effective in chronic hepatitis B virus patients in 1988. The FDA subsequently licensed it for the treatment of chronic hepatitis B viral infection. Interferon-alpha is a naturally occurring small protein that is made in the body by white blood cells to combat viral infection. In addition to its direct anti-viral effects, interferon works against hepatitis B virus by stimulating the body's immune system to clear the virus (Hollinger, et al., 2006).

For the treatment of chronic hepatitis B, a four to six month course of interferon-alpha is given. The interferon is injected under the skin either in a daily dose of 5 million units or three times a week at 10 million units. This treatment results in the long-term suppression of hepatitis B virus reproduction in about 30 to 40% of treated patients (Magnius, 1995).

Many side effects are associated with interferon therapy. Actually, even the release of endogenous (made in the body) interferon causes flu-like symptoms. Not surprisingly then, interferon injections often are accompanied by moderate to severe flu-like symptoms, including tiredness, muscle aches, fevers, chills, and loss of appetite. These flu-like symptoms are by far the most common side effects of interferon and occur in about 80% of patients undergoing treatment. Other side effects include mental depression (20%), an under-active or over-active thyroid gland (6%), and suppression of the bone marrow (40%)

(Which decreases the red and white blood cells and platelets), and variable degrees of hair loss (25%). About one fifth of patients who take interferon for hepatitis B virus discontinues treatment or requires a reduction in the dose because of the side effects (Lai et al., 2007)

### 3.9.2 Lamivudine for the Treatment of Chronic Hepatitis B

In the last five years, the focus of treatment for chronic hepatitis B virus has turned to nucleoside drugs. As mentioned previously, the hepatitis B virus has a strategy for reproducing that is similar to HIV. A number of nucleoside drugs that are used to treat HIV by slowing down the reproduction of that virus, therefore, have been tried for the treatment of hepatitis B virus. Nucleoside drugs are man-made molecules that closely resemble the biochemical units that make up genetic material (DNA and RNA). The nucleosides, therefore, work as imposters to trick hepatitis B virus genetic material and thereby slow down reproduction. Unlike interferon, the nucleoside class of compounds has no known direct effect on the immune system. Lamivudine (3TC, Epivir-hepatitis B virus) is the first nucleoside drug to receive FDA approval for the treatment of chronic hepatitis B viral infection. At 6 months, liver aminotransferase levels were still elevated (AST level, 94 IU/liter; ALT level, 121 IU/liter) and the HBV serological profile was the same as that at presentation. Treatment with lamivudine was initiated at this point, with a gradual decrease in the viral load to $10^4$ copies /ml during the 5th month after the start of treatment. This was accompanied by normalization of ALT levels (Bartholomew et al., 1997).

The clinical profile of patients who are likely to respond to lamivudine is similar to that of patients who are likely to respond to interferon. In addition, however, lamivudine can be used in patients with advanced cirrhosis. Lamivudine is taken in a twelve-month course orally at a dose of 100 mg daily. In patients with elevated liver blood tests and liver injury, lamivudine will result in the long-term suppression of viral reproduction, or activity (loss of HBeAg), in about one third of patients. In the vast majority of patients treated with

lamivudine, however, the liver blood tests will become normal (Chu et al., 2007). Moreover, hepatitis B virus DNA will be suppressed, at least temporarily, even when HBeAg (a blood marker for hepatitis B viral activity) persists. So, the response rate (loss of HBeAg) of a twelve-month course of lamivudine is about comparable to a four-month course of interferon. Also, as with interferon, the liver biopsies improve, showing less inflammation and scarring (Galibert, 1979).

### 3.9.3 Interferon and Lamivudine in Combination

No clear-cut recommendations are currently available to choose between interferon and lamivudine for the treatment of patients with chronic hepatitis B viral infection. The duration of therapy for interferon, four to six months, is well defined, whereas lamivudine requires a longer and less certain duration of therapy. In fact, for patients who do not lose HBeAg (a blood marker for viral activity) after 12 months of lamivudine, the current recommendation is for them to continue the drug indefinitely. Prolonged therapy with lamivudine, however, results in the inevitable emergence of the lamivudine-resistant variant of the virus. On the other hand, interferon has to be administered as an injection and is associated with numerous side effects, whereas lamivudine is taken orally and is well tolerated. Furthermore, unlike interferon, lamivudine can be used to treat patients with advanced cirrhosis. Indeed, lamivudine can often result in significant improvement in the symptoms of patients with advanced cirrhosis (Dienstag, 2008).

# CHAPTER IV

## 4. MATERIALS AND METHOD

During the study period, following materials were used as required in the procedures outlined in the methodology section.

### 4.1 Equipments and Others

I. Centrifuge

   (Tract Manufacturer Company, India)

II. Refrigerator

   (Hitachi, Tokyo, Japan)

III. Micropipettes

   (Sejal Plastics, India)

IV. Test tubes

   (Borosil Scientific International, India)

V. Sodium hypochlorite solution

VI. Distilled water

VII. Test tube rack, tissue paper, marker, bar codes for micro plate, adhesive seals, watch, mixing vessels, bottles (locally produced)

### 4.2 Research Site and Population.

The research site selected was Jhapa district, the Nepal Red Cross Society, Blood Transfusion Service are there at Bhadrapur (branch) and Damak (Sub-branch). The study population was the blood donor group and each blood donor was a single study sample.

### 4.3 Type of Study

The study was a descriptive cross-sectional study, carried out over a period of 10 months from July 2008 to April 2009 at NRCS, BTS Bhadrapur Branch & Damak Sub-branch.

### 4.4 Blood Collection

Blood samples were collected by authorized medical professionals (nurses and laboratory technicians) using aseptic technique. Using a sterile syringe, 350ml blood was drawn in blood bag labeled with a unique sample number. 5 ml of the same blood was dispensed in a small clean test tube labeled with the corresponding sample number (SOP, 2006). The bags taken and examined visually, discarded in the case of discoloration and blood collection was done in a sterile closed system bag with a single venepuncture. According to age limit and physical parameters volume of blood collection is either 350 ml or 450 ml.

### 4.5 Transport:

The blood bags and blood sample in test tube collected are taken to NRCS, BTS at Bhadrapur branch and Damak sub-branch laboratory keeping in ice box at 2-6 $^{0}$C.

### 4.6 Procedure for Lab Tests:

Serum separated was analyzed in the laboratory of Nepal Red Cross Society, Blood Transfusion Service, Bhadrapur Branch and Damak Sub-branch.

#### 4.6.1 Separation of Serum/ Plasma:

Blood samples collected in small test tubes were centrifuged at 3000 rpm for 4 to 5 minutes to separate serum/plasma required for screening tests as below.

#### 4.6.2 HBsAg Testing:

Rapid card test are used for the rapid card test are used for the rapid detection of hepatitis B surface antigen in human serum. HEPACARD commercially marketed by Biomed Industries India. (Details of procedure are listed in Appendices II).

**HBsAg Testing Strategy**

It is strictly recommended and defined about the process of screening of HBsAg and its confirmation by ELISA here because of the limitation of the source and economy the HBsAg diagnosis test was performed by a EIA based HEPACARD test kit.

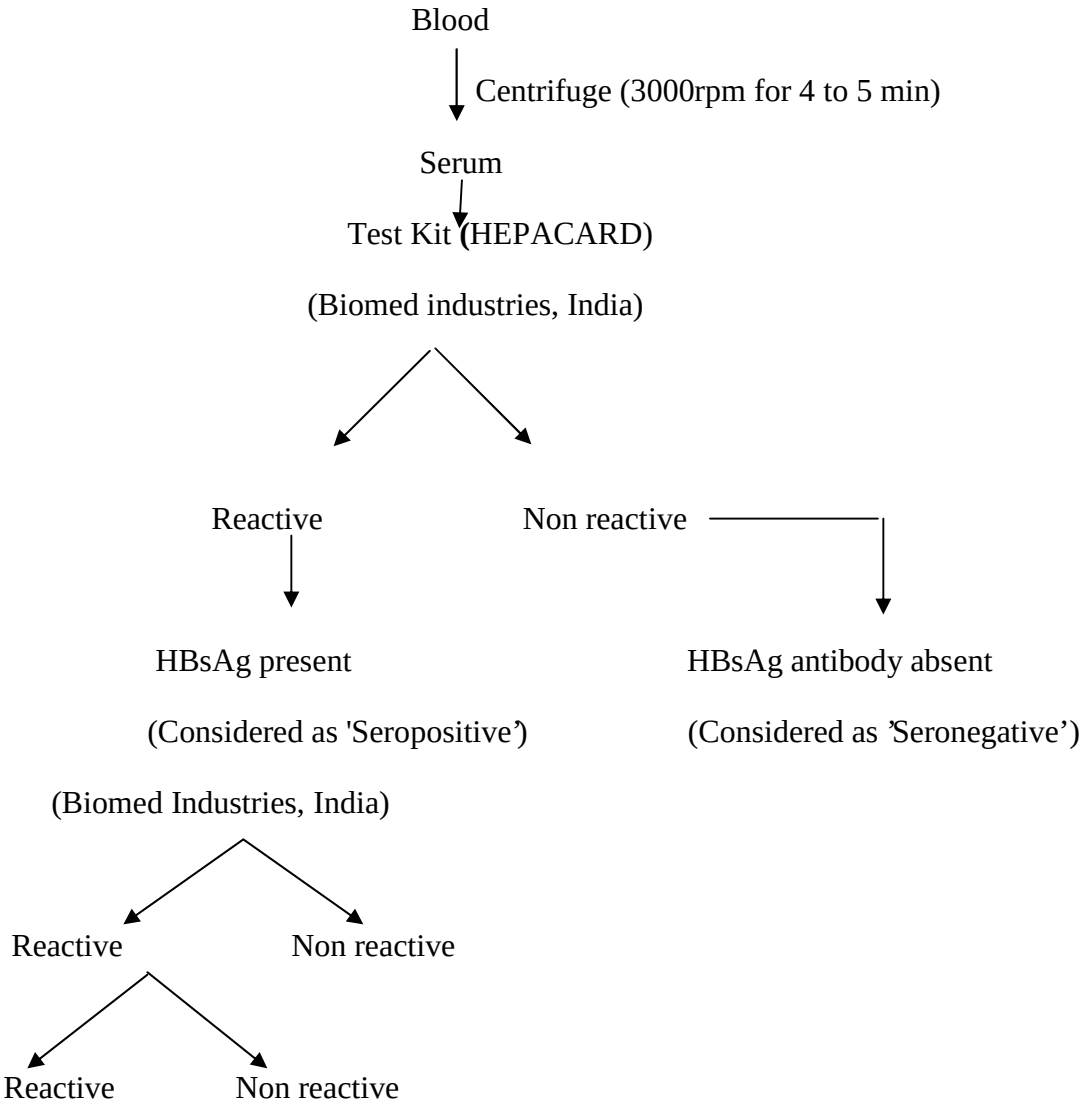


**Fig 4.1: -** Flow chart for HBsAg Testing method

### 4.6.3 Recording and Reporting of Test Results:

All the test results were collected and test result was provided to only those blood donors who were interested in knowing their serological status to the HBsAg.

### 4.6.4 Statistical Analysis

Statistical analysis was done using Chi Square Test formula (Appendix V) wherever applicable (Singh ML, Statistical Methods).

# CHAPTER V

## 5. RESULTS

### 5.1 Study Population

During the study period from July 2008 to April 2009 screening of Hepatitis B surface antigen (HBsAg) was done from sera of 1340 and 937 blood donors in laboratory of Blood Transfusion Service, NRCS, at Bhadrapur branch and Damak Sub-branch of Jhapa district respectively.

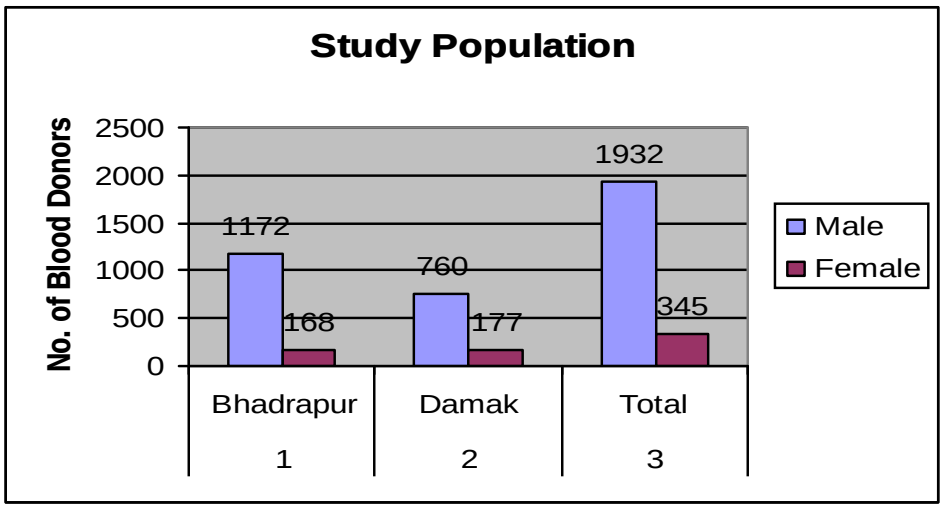


**Fig 5.1: -** Group of blood donors as studied Population

### 5.2 Seroprevalence of HBsAg:

#### 5.2.1 Overall Seroprevalence

Seroprevalence of HBsAg on Jhapa overall blood donors (2277) were screened on 13 (0.57%) samples at Bhadrapur donors 7 (0.52%) and at Damak 6 (0.64%) were as the hepatitis B infected.

**Table: 5.2.1** HBsAg prevalence and Blood Donors of Bhadrapur and Damak.

| | Total Blood Donors | HBsAg +ve | % Prevalence |
|---|---|---|---|
| Bhadrapur | 1340 | 7 | 0.522% |
| Damak | 937 | 6 | 0.64% |
| Total | 2277 | 13 | 0.57% |

### 5.2.2 Gender wise Overall Seropravalence

During the study period the result revealed that, male blood donors of Damak had overcome the prevalence rate 6 (0.79%) to the blood donors of Bhadrapur by 6 (0.51%). Out of 168 female blood donors at Bhadrapur single donor is screened for infection by hepatitis with prevalence of 1 (0.59%) but no single female donors are screened as infected at Damak.

**Table 5.2.2** Gender wise seroprevalence of HBsAg at Bhadrapur and Damak

| S.N. | Site | Male | | Female | | Overall % Prevalence |
|---|---|---|---|---|---|---|
| | | No. | % prevalence | No. | % prevalence | |
| 1 | Bhadrapur | 6/1172 | 0.51% | 1/168 | 0.59% | 0.522% |
| 2 | Damak | 6/760 | 0.79% | - | - | 0.64% |
| 3 | Total | 12/1932 | 0.62% | 1/345 | 0.29% | 0.57% |

### 5.2.3 Age Group and Gender Wise Seroprevalence of Blood Donors of Jhapa

On the male blood donors at Bhadrapur the donors of age group 51-60 years were seem to be highest seroprevalence (14.2%). The least seroprevalence was observed on the age group of 21-30 years (0.25%). Seroprevalence of age group less than 20 years is 0.43% is observed on female. On the male blood donors at Damak the donors of age group 31-40 Years were seem to be highest seroprevalence (1.51%). The no seroprevalence was observed on the age group of 21-30 years (0.25%). On female only the age group of less than 20 years and more than 40 years had screened for HBsAg at Damak and seroprevalence of all age group for female was not screened.

**Table 5.2.3** Distribution of HBsAg on Blood donors of Jhapa Age group wise and Gender Wise

| S.N | Age Group | Gender | % Prevalence | | Overall |
|---|---|---|---|---|---|
| | | | Bhadrapur | Damak | |
| 1 | > 20 | Male | - | - | - |
| | | Female | 1/30 (3.3%) | - | 1/70 (1.42%) |
| 2 | 21-30 | Male | 1/395 (0.25%) | 3/374 (0.80%) | 4/769 (0.52%) |
| | | Female | - | - | - |
| 3 | 31-40 | Male | 2/462 (0.40%) | 3/198 (1.51%) | 5/660 (0.75%) |
| | | Female | - | - | - |
| 4 | 41-50 | Male | 2/106 (1.91%) | - | 2/286 (0.7%) |
| | | Female | - | - | - |
| 5 | 51-60 | Male | 1/7 (14.2%) | - | 1/31 (3.22%) |
| | | Female | - | - | - |
| 6 | Total | | 7/1340 (0.52%) | 6/937 (0.64) | 13/2277((0.57%) |

# CHAPTER - VI

## 6. DISCUSSION AND CONCLUSION

### 6.1 HBsAg Seroprevalence

Comparing this study with similar investigation conducted among blood donors in Jhapa indicate that HBV prevalence seems to be similarity with slightly higher, prevalence of HBV determined (0.46%) was less marked than the findings in the Kathmandu valley by Joshi et al., (2002) as 1.26%, Ghimire et al., (2006) as 0.88%. Current prevalence was similar to the reporting of Chander et al., (2003) in Bhairahawa, Nepal (0.45%) and Karki et al., (2008) in Kathmandu (0.53%). The fluctuation trend of prevalence can be attributed to various factors such as availability of vaccine and mass vaccination programmes, increasing awareness of HBV as well as self exclusion of high risk groups from blood donation, strict donor selection by health officers or even specificity of the test kit used.

The percent positive of HBsAg was found to increase steadily from Eastern (2%) to Far Western (6.2%) development regions. Among the districts studied, Kailali district gave characteristically high prevalence followed by Rukum and Kaski than Jhapa (0.57%). Other districts representing for the prevalence are Sankhuwasabha, Jhapa, Ramechhap, Sarlahi, Dhanusa, Baglung, Gulmi, Palpa and Dang. None of the samples represented from Kathmandu valley were positive for HBsAg. The age groups of 16-20 years and 36-40 years were found to be associated with the hepatitis B infection. Furthermore, various percent prevalence of the infection was encountered from 16 to 40 years subjects and a single case was also observed from 63 years old man (Manandhar et al., 1997)and also in Jhapa Single donor of age higher than 51 was screened positive. Comparing this data history Jhapa was observed lower prevalence towards to hepatitis B. HBV seroprevalence in the present study (0.57%) was lower than the prevalence reported at different part of

Asia by Luksamijarulkul et al., (2002) in Thailand (4.61%), Chattoraj et al., (2008) in Calcutta, India (0.99%), Tserenpuntsag et al., (2008) in Mongolia (8.1%), Chaudhary et al., (2007) in Rawalpindi (2.45%), Bhattacharya et al., (2007) in West Bengal (1.28% - 1.66%).

Overview of the similar investigation shows that the HBV prevalence in Jhapa is lower than the other countries. These variations might be due to geographical variations, risk behaviors of the population under study, kits and strategy used for tests, period of study and donor selection criteria or even awareness status of the people in particular area. The figure determined here is the prevalence among healthy donors and the findings do differ from the observations of Shrestha (2002) as 0.93%, Manandhar et al., (2000) as 4.0%, Sawayama et al., (1999) as 1.1%, Shrestha (1989) as 0.9%, due to differences in population selected for study.

Various prevention and intervention techniques are implemented to prevent the disease hepatitis B but still new cases are found. Transfusion associated cases of hepatitis B are although reduced due to detection by serological tests, the danger of occurrence on transfusion not minimized to zero. This is due to the appearance of disease in blood after approximately months later and the test performed by commercial test kit which have not sensitivity 100%. Beside this, the process of direct participation of risk group to the blood donation should be minimized and have an effort to prevent hepatitis B.

### 6.2 Conclusion

The study was integrated on the blood donors of Blood Transfusion Service of NRCS Jhapa. On the two sites, the donors identified as infected by hepatitis B (0.522%) & (0.64%) at Bhadrapur and Damak respectively.From this study, it is concluded that the hepatitis B infection has the Risk group of age 51-60 years (14.28%) at Bhadrapur and 21-30 years (1.51%) at Damak, this may be due to the immunologically inactive age and active towards various activities respectively.

The study indicates that, male blood donors are more susceptible to the incidence of Hepatitis B infection. Female blood donors of Bhadrapur are not screened for infection of Hepatitis B at Damak NRCS, BTS. But Bhadrapur observed as (3.33%) at the age group of below 20 years on volunteer donors.

# CHAPTER - VII

## 7. SUMMARY AND RECOMMENDATIONS

### 7.1. Summary

The study was carried out to determine the seroprevalence of HBsAg in blood donors at NRCS, BTS Jhapa and the infections under study was hepatitis B. Seroprevalence of HBsAg infection was determined and its seroprevalence was estimated. The prevalence was studied on the basis of sex, age groups. Major findings of the study can be summarized as follows:

1. The study included a total of 2277 blood donors, during the 10 months period. Of total 1340 blood donors included at Bhadrapur 1172 (87.5%) were male and 168 (12.5%) were female where as Damak total 937 blood donors 760 (81.1%) were male and 177 (18.9%) were female.

2. On male blood donors age group of 21-30 and 31-40 at Bhadrapur and Damak, Damak have higher prevalence than at Bhadrapur i.e. 0.25% (1/395), 0.4% (2/462)(Bhadrapur) and 0.80% (3/374), 1.51% (3/198) (Damak) respectively.

3. Higher seroprevalence observed on age group of 51-60 i.e. 14.2% (1/7).

## 7.2. Recommendation

The study was focused on the blood donors of Jhapa district but the study should have deviation to whole the district and nation. The risk group still on danger should be studied and other risk group like international travelers, trekkers, factory workers, commercial sex workers etc should not be neglected. Based on the findings of the study, following recommendations are put forward for concerned:

1. Mandatory screening of all the HBsAg concerned should be continued following standard algorithms developed by WHO/ Government.

2. Donor notification and counseling should be immediately implemented to make the regular blood donors as the safe source of blood.

3. Healthy young donors may be encouraged for decreasing the chances of transmission of HBsAg.

4. Government policy and protocols for selection of Kits/ Reagents and algorithms may be helpful in quality improvement for HBsAg screening.

5. Further studies on HBsAg infection should be carried out through the country with larger sample size for better understanding.

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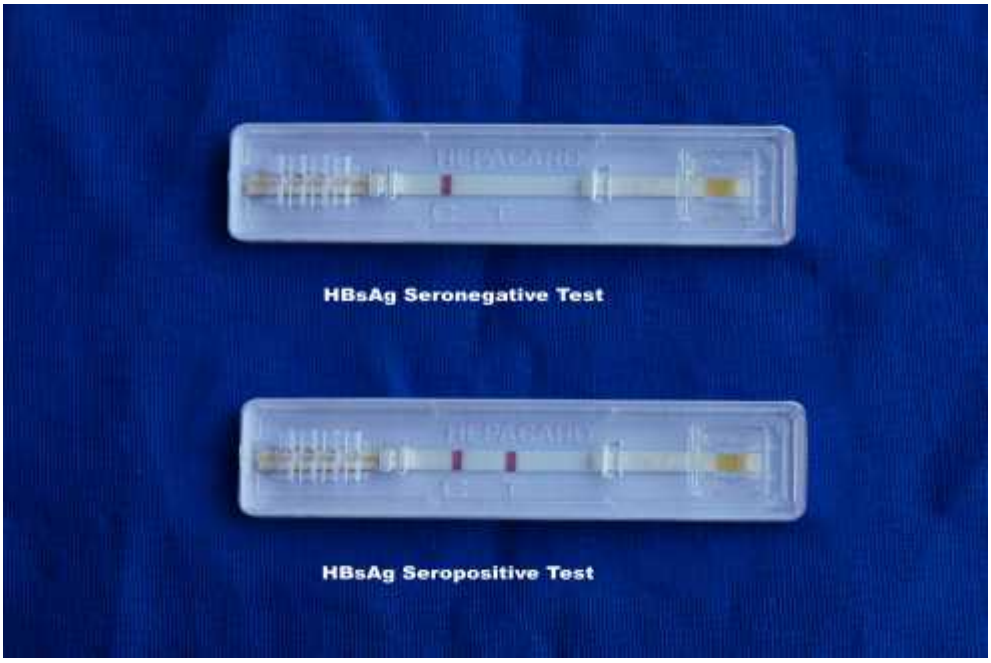
# PHOTOGRAPHS




**Photograph 1:** HEPACARD Immunochromatographic Test Kits


**Photograph 2:** Test Kit and Its Cover

## Appendix-I

## Donors Screening Form

# Appendix-II

**Manufacturers Introduction**

# Appendix-III

**HBsAg Test (For Research purpose, not for Laboratory Test).**

1. The kit should not be used beyond the expiration date on the kit label.

2. Do not mix or substitute reagents with those from other lots or sources.

3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluents, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

4. This assay is designed to eliminate interference by soluble receptors, ligands, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

Sample collection and storage

Cell culture supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum and assay immediately or aliquot and store samples at -20° C.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for

15 minutes at 1000 x g at 2 - 8° C within 30 minutes of collection. Store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Note: Citrate plasma has not been validated for use in this assay.

Storage done at 2-8$^0$C, HEPACARD has shelf Life 18 months from the date of manufactured.

- Done in only human serum or plasma immediately after collection either not stored at 2-8$^0$C up to 3 days following the collection, or if not possible froze at -20$^0$C.
- Heamolysed blood sample or the sample with microbial contamination should be discarded.
- Cloudy or visible sample produce the unreliable results so the sample is centrifuged at 10,000 rpm for 10 minutes.

**Assay procedure**

- Allow all reagents to reach room temperature. Arrange and label required number of strips. Prepare all reagents, working standards and samples as directed in the previous sections.
- Add 100 ul of **Standard**, Control, or sample per well. Cover with the adhesive strip. Incubate for 2 hours at 37° C.
- Remove the liquid of each well, don't wash.
- Add 100 ul of **Detection Reagent A** to each well. Incubate for 1 hour at 37°C. **Detection Reagent A** may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.
- Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (350 ul) using a squirt bottle, multi-channel pipette, manifold dispenser or auto washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove

any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

- Add 100 ul of **Detection Reagent B** to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37° C.
- Repeat the aspiration/wash as in step 5.
- Add 90 ul of **Substrate Solution** to each well. Incubate for 30 minutes at room temperature. Protect from light.
- Add 50 ul of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
- Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

**Specificity**

This assay recognizes recombinant and natural human HBsAg. No significant cross-reactivity or interference was observed.

**Sensitivity**

The minimum detectable dose of human HBsAg is typically less than 39 pg/ml. The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest detectable concentration that could be differentiated from zero.

**Detection Range**

156-10,000 pg /ml. The assay range was estimated by calculating the coefficient of variation (CV) of each standard constructing five independent standard curves. The standard curve concentrations used for the ELISA's were 10,000 pg/ml, 5,000 pg/ml, 2,500 pg/ml, 1,250 pg/ml, 625 pg/ml, 312 pg/ml, 156 pg/ml.

**Important Note:**

1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

2. It is recommended that no more than 32 wells be used for each assay run if manual pipetting is used since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipetting is available.

3. Duplication of all standards and specimens, although not required, is recommended.

4. When mixing or reconstituting protein solutions, always avoid foaming.

5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

6. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

**Calculation of results**

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the HBsAg concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

**Storage of test kits and instrumentation**

1. Unopened test kits should be stored at 2-8⌈C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Refer to the package label for the expiration date.

2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

3. A micro titer plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

**Precaution**

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

**Procedure for Test**

- Specimen, buffer and the HEPACARD is brought to room temperature before testing, and the pouch of the card was opened.
- Labeling of the card was done according to the name of the donor.
- 2-4 drops of buffer was added to the well.
- 2 drops of (70 µl) of the serum was added to the well of the card with the help of the micro tip.
- For 20 minutes the kit was kept to allow the reaction.
- Results read.
- Kit was discarded immediately.

# Appendix – IV

**Evaluation of tests Kits**

Infection with the Hepatitis B virus is characterized by the appearance of certain viral markers including Hepatitis B surface Antigen (HBsAg) in the blood. It is recommended that all blood donations are tested for this marker to avoid transmission to recipients.

Laboratory diagnosis of Hepatitis B infection centers on the detection of HBsAg, as the most important means for ensuring blood safety. Positive test reactions are then confirmed by retesting with a neutralization assay.

The performance of currently available hepatitis B surface antigen (HBsAg) commercial kits was analyzed by using a panel of 212 well-characterized plasma donors all over the country and a panel of nine recombinant HBsAg mutants containing single point or combinations of mutations between amino acid residues 124 and 147 of the "a" determinant. HBsAg commercial kits in this study were machine-based immunoassays with a one-step sandwich ELISA method using either an automatic closed system or manual system. The sensitivity of all machine-based assays evaluated with 105 HBsAg plasma panels was 100% (95% CL = 95.6–99.9%), whereas the specificity with 107 HBsAg negative plasma ranged from 99.07% to 100% (95%CL = 94.2–99.9%). The relative performance of these kits to detect the hepatitis B virus (HBV) mutant panel members of the "a" determinant was found to differ. Interestingly, any commercial kits with monoclonal antibody capture and polyclonal antibody detection (mono/poly), but not mono/mono Ab capture and detection, could pick up the common HBsAg Gly145Arg mutant either solely or in combination with other mutations within the "a" determinant. New versions of HBsAg test kits should recognize multiple HBsAg epitopes in order to detect mutant HBsAg, together with providing good analytical sensitivity and specificity,

because of the importance of these assays in HBV diagnosis and in protecting the safety of the blood supply (WHO, 1999).

**Predictive Values**

The positive predictive value (PPV) is the probability that when the test is reactive, the specimen does contain antibody to HBV. Results were expressed as mean ± SD. Statistical Analysis was carried out for finding P values. $P<0.05$ was considered statistically significant. This may be calculated in two ways:

1. Using the simple formula a/ (a+b) which will give an approximate value.

2. Using the more precise formula which takes the prevalence of HBV in the population into account.

$$PPV = \frac{(\text{Prevalence})\,(\text{Sensitivity})}{(\text{Prevalence})\,(\text{Sensitivity}) + (1 - \text{prevalence})\,(1 - \text{specificity})}$$

The negative predictive value (NPV) is the probability that when the test is negative, a specimen does not have antibody to HBV. This may be calculated using:

1. The simple formula d/ (c+d) which will give an approximate value.

2. The more precise formulas which takes the prevalence of HBV in the population into account:

$$NPV = \frac{(1 - \text{Prevalence})\,(\text{Specificity})}{(1 - \text{Prevalence})(\text{Specificity}) + (\text{Prevalence})(1 - \text{Sensitivity})}$$

The probability that a test will accurately determine the true infection status of a person being tested varies with the prevalence of HBV infection in the population from which the person comes. In general, the higher the prevalence of HBV infection in the population is greater the probability that a person testing positive is truly infected (i.e., the greater the Positive Predictive Value (PPV)). Thus, with increasing prevalence, the proportion of false-

positive decreases conversely, the likelihood that a person showing negative test results is truly uninfected (i.e. the negative predictive value (NPV) decreases as prevalence increases). Therefore, as prevalence increases, so it does the proportion of samples testing false-negative. However, this effect only becomes apparent at prevalence of 80% and above. For calculating the positive and negative predictive values recorded in this report, the more precise formula at option 2 was used (WHO, 1999).

# Appendix V

**Co-relation between the different attributes among the blood donors**

**1. Seroprevalence of HBsAg in Blood donors at Bhadrapur and Damak**

| | Bhadrapur | Damak | Total |
|---|---|---|---|
| HBsAg Positive | 7 | 6 | (a+b) = 13 |
| HBsAg Negative | 1333 | 931 | (c+d) = 2264 |
| Total | (a+c) = 1340 | (b+d) = 937 | N = 2277 |

*($H_0$):* There is no difference in seroprevalence rate of HBsAg among Bhadrapur and Damak blood donors in total.

*($H_1$):* There is a significant difference in seroprevalence rate of HBsAg among Bhadrapur and Damak blood donors in total.

Test Statistic: $\chi 2 = \dfrac{(ad- bc)^2.N}{(a+b)(c+d)(a+c)(b+d)}$

**$\chi 2$ cal =0.135**

From table of $\chi 2$, we have $\chi 2$ tab at (2-1) (2-1) degree of freedom =1, 95% confidence level, is equal to 3.841.

**Conclusion:** Since $\chi 2$ calculated (0.135) < $\chi 2$ tabulated (3.841), the null hypothesis is accepted, which means there is no significant difference in seroprevalence of HBsAg among blood donors of Bhadrapur and Damak.

**2. Seroprevalence of HBsAg in male and female blood donors in Overall**

| | Males | Females | Total |
|---|---|---|---|
| HBsAg Positive | 12 | 1 | (a+b) = 13 |
| HBsAg Negative | 1920 | 344 | (c+d) = 2264 |
| Total | (a+c) = 1932 | (b+d) = 345 | N = 2277 |

*($H_0$):* There is no difference in seroprevalence rate of HBsAg among male and female blood donors in total.

*($H_1$):* There is a significant difference in seroprevalence rate of HBsAg among male and female blood donors in total.

Test Statistic: $\chi 2 = \dfrac{(ad- bc)^2.N}{(a+b)(c+d)(a+c)(b+d)}$

**$\chi 2$ cal =0.565**

From table of $\chi 2$, we have $\chi 2$ tab at (2-1) (2-1) degree of freedom =1, 95% confidence level, is equal to 3.841

**Conclusion:** Since $\chi 2$ calculated (0.585) < $\chi 2$ (3.841) tabulated, the null hypothesis is accepted, which means there is no significant difference in seroprevalence of HBsAg among male and female donors in total.

**3. Seroprevalence of HBsAg in blood donors of age group below 20 years age groups of Bhadrapur donors.**

| | 18-20 yrs | (other age groups) | Total |
|---|---|---|---|
| HBsAg Positive | 1 | 6 | (a+b) = 7 |
| HBsAg Negative | 199 | 1134 | (c+d) = 1333 |
| Total | (a+c) = 200 | (b+d) = 1140 | N = 1340 |

*($H_0$):* There is no significant difference in seroprevalence rate of HBsAg among the blood donors of age group below 20 years and among other age groups.

*($H_1$):* There is a significant difference in seroprevalence rate of HBsAg among the blood donors of age group below 20 years and among other age groups.

Test Statistic: $\chi 2 = \frac{(ad- bc)^2.N}{(a+b)(c+d)(a+c)(b+d)}$

**$\chi 2$ cal =0.03**

From table of $\chi 2$ we have

$\chi 2$ tab at (2-1)(2-1) degree of freedom = 1, 95% confidence level, is equal to 3.841.

**Conclusion:** Since $\chi 2$ calculated (0.03) < $\chi 2$ tabulated (3.841), the null hypothesis is accepted which means that the seroprevalence of HBsAg in the donors of age group below 20 years is significantly similar than other age group donors.

**4. Seroprevalence of HBsAg in blood donors of age group 21-30 years and of other age groups as a whole of Bhadrapur.**

| | (21-30)yrs | (other age groups) | Total |
|---|---|---|---|
| HBsAg Positive | 1 | 6 | (a+b) = 7 |
| HBsAg Negative | 354 | 979 | (c+d) = 1333 |
| Total | (a+c) = 355 | (b+d) = 985 | N = 1340 |

*($H_0$):* There is no significant difference in seroprevalence rate of HBsAg among male blood donors of age group 21-30 years and among other age groups.

*($H_1$):* There is a significant difference in seroprevalence rate of HBsAg among the male blood donors of age group 21-30 years and among other age groups.

Test Statistic: $\chi 2 = \frac{(ad- bc)^2.N}{(a+b)(c+d)(a+c)(b+d)}$

**$\chi 2$ cal = 0.538**

From table of $\chi 2$ we have

$\chi 2$ tab at (2-1)(2-1) degree of freedom =1,95% confidence level, is equal to 3.841.

**Conclusion:** Since $\chi 2$ calculated (0.538) < $\chi 2$ tabulated (3.841), the null hypothesis is accepted which means that the seroprevalence of HBsAg in the donors of age group 21-30 years is significantly similar with blood donors of other age groups.

**5. Seroprevalence of HBsAg in blood donors of age group 31-40 years and in whole blood donors of other age groups as a whole of Bhadrapur.**

| | (31-40)yrs | (other age groups) | Total |
|---|---|---|---|
| HBsAg Positive | 2 | 5 | (a+b) = 7 |
| HBsAg Negative | 536 | 797 | (c+d) = 1333 |
| Total | (a+c) = 538 | (b+d) = 802 | N = 1340 |

($H_0$): There is no significant difference in seroprevalence rate of HBsAg among blood donors of age group 31-40 years and among other age groups of Bhadrapur.

($H_1$): There is a significant difference in seroprevalence rate of HBsAg among the female blood donors of age group 31-40 years and among other age groups Of Bhadrapur.

Test Statistic: $\chi 2 = \dfrac{(ad- bc)^{2.}N}{(a+b)(c+d)(a+c)(b+d)}$

**$\chi 2$ cal =0.392**

From table of $\chi 2$, we have, $\chi 2$ tab at (2-1) (2-1) degree of freedom =1, 95% confidence level, is equal to 3.841.

**Conclusion:** Since $\chi 2$ calculated (0.392) < $\chi 2$ tabulated (3.841), the null hypothesis is accepted which means that the seroprevalence of HBsAg in the donors of age group 31-40 years is not significantly different than blood donors of other age groups.

**6. Seroprevalence of HBsAg in blood donors of age group 41-50 years and in whole blood donors of other age groups as a whole of Bhadrapur.**

| | (41-50) Years | (other age groups) | Total |
|---|---|---|---|
| HBsAg Positive | 2 | 5 | (a+b) = 7 |
| HBsAg Negative | 105 | 1227 | (c+d) = 1333 |
| Total | (a+c) = 107 | (b+d) = 1233 | N = 1340 |

($H_0$): There is no significant difference in seroprevalence rate of HBsAg among blood donors of age group 31-40 years and among other age groups of Bhadrapur.

($H_1$): There is a significant difference in seroprevalence rate of HBsAg among the female blood donors of age group 31-40 years and among other age groups Of Bhadrapur.

Test Statistic: $\chi 2 = \dfrac{(ad- bc)^2.N}{(a+b)(c+d)(a+c)(b+d)}$

**$\chi 2$ cal =2.463**

From table of $\chi 2$, we have

$\chi 2$ tab at (2-1)(2-1) degree of freedom =1,95% confidence level, is equal to 3.841

**Conclusion:** Since $\chi 2$ calculated (2.463) < $\chi 2$ tabulated (3.841), the null hypothesis is accepted, which means that the seroprevalence of HBsAg among the blood donors of age group 41-50 years to the others age group of Bhadrapur donors.

**7. Seroprevalence of HBsAg in blood donors of age group 51-60 years and in whole blood donors of other age groups as a whole of Bhadrapur.**

| | (51-60) Years | Others age group | Total |
|---|---|---|---|
| HBsAg Positive | 1 | 6 | (a+b) = 7 |
| HBsAg Negative | 6 | 1327 | (c+d) = 1333 |
| Total | (a+c) = 7 | (b+d) = 1333 | N = 1340 |

($H_0$): There is no significant difference in seroprevalence rate of HBsAg among blood donors of age group 51-60 years and among other age groups of Bhadrapur.

($H_1$): There is a significant difference in seroprevalence rate of HBsAg among the female blood donors of age group 51-60 years and among other age groups of Bhadrapur.

Test Statistic: $\chi 2 = \dfrac{(ad- bc)^2 N}{(a+b)(c+d)(a+c)(b+d)}$

**$\chi 2$ cal =25.65**

From table of $\chi 2$, we have, $\chi^2$ tab at (2-1)(2-1) degree of freedom =1,95% confidence level, is equal to 3.841

**Conclusion:** Since $\chi 2$ calculated (25.65) > $\chi 2$ tabulated (3.841), the null hypothesis is rejected, which means that the seroprevalence of HBsAg among the blood donors of age group 51-60 have a significant different with others age groups.

# Appendix – VI

Seropositivity rate of HBsAg (Nation wide and in Kathmandu)

| **Years** | **2007/2008** | | **2006/2007** | | **2005/2006** | | **2004/2005** | | **2003/2004** | |
|---|---|---|---|---|---|---|---|---|---|---|
| Site | **Nationwide** | **Kathmandu** | **Nationwide** | **Kathmandu** | **Nationwide** | **Kathmandu** | **Nationwide** | **Kathmandu** | **Nationwide** | **Kathmandu** |
| Hepatitis B | 0.56 % | 0.62 % | 0.37 % | 0.76 % | 0.9% | 0.8% | 0.85 % | 0.86 % | 0.88 % | 1.02 % |

Source: Nepal Red Cross Society, Central Blood Transfusion Service, Kathmandu, Nepal.