بسم الله الرحمن الرحيم

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Seroprevalence of hepatitis B and C viruses Among Ethiopians in Khartoum State

معدل الانتشار المصلي لفيروس الكبد الوبائي ب و ج لدى الاثوبيين داخل ولاية الخرطوم

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

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بسم الله الرحمن الرحيم

قال تعالي:

﴿وَوَصَّيْنَا الْإِنْسَانَ بِوَالِدَيْهِ إِحْسَانًا ۖ حَمَلَتْهُ أُمُّهُ كُرْهًا وَوَضَعَتْهُ كُرْهًا ۖ وَحَمْلُهُ وَفِصَالُهُ ثَلَاثُونَ شَهْرًا ۚ حَتَّىٰ إِذَا بَلَغَ أَشُدَّهُ وَبَلَغَ أَرْبَعِينَ سَنَةً قَالَ رَبِّ أَوْزِعْنِي أَنْ أَشْكُرَ نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَىٰ وَالِدَيَّ وَأَنْ أَعْمَلَ صَالِحًا تَرْضَاهُ وَأَصْلِحْ لِي فِي ذُرِّيَّتِي ۖ إِنِّي تُبْتُ إِلَيْكَ وَإِنِّي مِنَ الْمُسْلِمِينَ﴾

صدق الله العظيم

(سورة الاحقاف الآية 15)

Dedication

To Soul of my Mother and Father

that gave me Support and Strength to be Successful in my life

To my brothers

To my lovely sisters Tota ,Ebada ,Hadeel

To my Friends, and everyone who supported me.

Acknowlegment

Praise to ALLAH who gave me the health and strength to conduct this study, I wish to express my great thankful to my supervision Professor .Yousif Fadlalla for his continued supervision. Also I would like to thank all Staffs of Al Salam Clinic at ahead of them Dr. Ahmed Badawi for their help during samples collection, and very special thank for patients who allow me to take blood samples for this study.

And finally I would like to thank all my sisters and lovely friend Dr. Alyaa Mustafa for their support.

ABSTRACT

This study was aimed to determine the prevalence of Hepatitis B and Hepatitis C viruses among Ethiopians in Khartoum State using Enzyme-linked Immunosorbent Assay (ELISA) for detection of both HBsAg and HCV IgG. The study design was cross-sectional study which was carried out during the period from April to June 2015. A total of ninety patients attended to Al Salam Clinic Algreeif West in Khartoum State were included in the Study. The results revealed that 23(25.5%)were positive for HCV while 67(74.4%)were negative .Whereas 36(40%)were positive for HBsAg and 54(60%)were negative. According to age group there was significant relationship($p > 0.05$) on seroprevelence for both HBsAg and HCV IgG. The highest prevalence of HBsAg was(21%) in age group (26-35)yrs. While for HCV the prevalence was (14.4%) in the age group(15-25) yrs. This study revealed that HBV and HCV were more prevalence in young age and female were more susceptible to infection than male 42%,37% respectively for HBV and 28.9%,22% for HCV .Further study is required to validate these results.

ملخص **الاطروحه**

الهدف من هذه الدراسة هو تحديد مدى انتشار التهاب الكبد (ب) والتهاب الكبد (ج) بين الإثيوبيين في ولاية الخرطوم باستخدام الاليزا للكشف عن الأجسام المضادة من نمط IgG.وكان تصميم الدراسة شاملا.التي أجريت في الفترة من أبريل إلى يونيو 2015م وتم جمع 90 عينه من الإثيوبيين من الجنسين في عيادة السلام بالجريف غرب وتم تضمينها في الدراسة وكشفت النتائج أن 23 (25.5%) كانت إيجابية للنهاب الكبد نوع (ج)في حين كانت67 (74.4%) سلبي بينما ان36 (40%) .كانت إيجابية للنهاب الكبد نوع (ب)و54 (60%) كانت سلبية .حسب الفئة العمرية وكان هناك علاقة ذات دلالة إحصائية على انتشار المرض لكلا النوعي(ب و ج)وكان أعلى معدل انتشار التهاب الكبد نوع ب الفئة العمرية (26-35)٪ 21 في حين كان الانتشار الواسع لالتهاب الكبد نوع (ج)فـي الفئة العمرية (15-25) 14.4٪.وكشفت هذه الدراسة أن فيروس التهاب الكبد نوع (ب)وفيروس التهاب الكبد نوع(ج) كانوا أكثر انتشار في الأعمار الصغيرة . مطلوب مزيد من الدراسة للتحقق من صحة هذه النتائج.

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CHAPTER ONE

Chapter one

Introduction

1.1 Back ground

Hepatitis B Virus(HBV)

Hepatitis B virus (HBV) infection is a major global public health problem. Of the approximately 2 billion people who have been infected worldwide, more than 350 million are chronic carriers of HBV Approximately 15–40% of infected patients will develop cirrhosis, liver failure, or hepatocellular carcinoma (HCC). HBV infection accounts for 500 000 to 1.2 million deaths each year and is the 10th leading cause of death worldwide(Lananchy,2004)

Hepatitis B infection is acquired through contact with the blood of a person carrying the hepatitis B virus. The carrier rate of HBsAg varies world-wide. In many endemic areas, infection is largely acquired perinatally, while in other areas of high prevalence, transmission occurs during childhood, with intrafamilial spread being particularly important. Subclinical hepatitis B attacks are extremely frequent. The unusual clinical episode diagnosed in the adult tends to be more severe than that for virus A or non-A, non-B infection although the overall picture is similar.

HBsAg titres may be low or undetectable. About 10% of patients suffering an acute attack, more commonly males, will not clear the virus and will become chronic carriers. These may remain 'healthy' or suffer from various grades of chronic hepatitis and cirrhosis. Chronicity is related to impairment of humoral and cell-mediated immunity. There are two phases of hepatitis B infection, the replicative and the integrated, the former being recognized by the presence of

hepatitis B viral DNA (Double strand Nucleic Acid)in serum. Relapses of chronic hepatitis B may be related to conversion from replicative to integrated stages, to spontaneous reactivation, or to super-added virus infection, especially with delta virus.(Sherlock ,1987)

The hepadnaviridae are a family of hepatotropic DNA virus with a unique life cycle involving an RNA (Ribo Nucleic Acid) intermediate and the use of a viral polymerase enzyme with reverse trancriptase activity.

The virion of HBV is 42nm double-shelled particle known as Dane particle . The outer envelope of the virion is formed by hepatitis B surface antigen(HBsAg) the inner core,27nm in diameter ,consist of hepatitis B core antigen(HBcAg)which encloses the viral genome DNA and polymerase. The viral DNA is about 3200 nucleotides long and is circuler in configuration(Greenwood *etal*.,2012)

More than 400 million people worldwide are chronically infected by the hepatitis B virus. The virus is responsible for more than 300000 cases of liver cancer every year and for similar numbers of gastrointestinal haemorrhage and ascites. Major breakthroughs have been achieved in diagnosis and treatment of this virus. Hepatitis B vaccine reduces incidence of liver cancer. As with hepatitis C, advances have been made in molecular virology, especially for naturally occurring and treatment-induced mutant viruses. The clinical significance of low viral load and genotypes are also under investigation. Currently available monotherapies—interferon, lamivudine, and adefovir dipivoxil—very rarely eradicate the virus, but greatly reduce its replication, necroinflammatory histological activity, and progression of fibrosis. Lamivudine, and presumably other nucleoside analogues, can reverse cirrhosis of the liver.(Lai *etal*.,2003)

Hepatitis C Virus (HCV)

Chronic hepatitis C is the most common cause of chronic liver disease and cirrhosis, and the most common indication for liver transplantation in the United States (U.S.), Australia, and most of Europe. Approximately 170 million people are affected with HCV worldwide, comprising about 3% of the global population. Hepatitis C virus (HCV) is the most common chronic bloodborne infection in the U.S., and is involved in 40% of chronic liver disease. HCV was initially isolated from the serum of a person with non-A, non-B hepatitis ,Shortly after the cloning of HCV, this newfound virus was discovered to be the cause of approximately 90% of non-A, non-B hepatitis in the U.S(Chen and Morgan,2006)

The hepatitis C virus is an RNA virus that belongs to the family flaviviridae. HCV replicates in the cytoplasm of hepatocytes, but is not directly cytopathic. Persistent infection appears to rely on rapid production of virus and continuous cell-to-cell spread, along with a lack of vigorous T-cell immune response to HCV antigens. The HCV turnover rate can be quite high with replication ranging between 10^{10} to 10^{12} virions per day, and a predicted viral half-life of 2 to 3 hours. The rapid viral replication and lack of error proofreading by the viral RNA polymerase are reasons why the HCV RNA genome mutates frequently. There are six known genotypes (numbered 1 through 6) and more than 50 subtypes (e.g., 1a, 1b, 2a...). Frequent HCV mutations and numerous subtypes have made the search for an HCV vaccine challenging.

There is strong evidence demonstrating the association of chronic HCV infection to cirrhosis and hepatocellular carcinoma (HCC). HCV is a mounting global health challenge, causing a significant proportion of chronic liver disease around the world. In understanding the long-term outcomes of HCV infection, clinicians may

identify the patients at risk for HCV-related complications, and offer treatments to prevent further morbidity and mortality (Chen and Morgan,2006)

Ethiopia is endemic for many viral diseases. Serosurveys have demonstrated the high prevalence rate of hepatitis B virus. There are also indications of high transmission for hepatitis C, hepatitis E and human immunodeficiency virus (HIV)(seffa,1993)

1.2 Rationale

Hepatitis B is one of the most common infectious diseases in the world. It has been estimated that 350 million people world-wide are chronic hepatitis B virus (HBV) carriers. The global prevalence of chronic HBV infection varies widely, from high (≥8%, e.g., Africa, Asia and the Western Pacific) to intermediate (2–7% e.g., Southern and Eastern Europe) and low (<2%, e.g., Western Europe, North America and Australia (Maddrey,2000)

There are Numerous of extra hepatic manifestations have been reported in patients with both acute and chronic hepatitis B (arthralgias or arthritis, skin rashes, glomerulonephritis and neuritis), all of which are present in polyarteritis nodosa (PAN) which is the most unique and spectacular extrahepatic manifestation. In the 1970s, the frequency of PAN due to the hepatitis B (HBV) reached 30%.Clinical manifestations reflect this most classic form of PAN, Hepatic manifestations including, ALT(alanine amino trasferase) AST(Aspartate amino tranferase) elevations are mild and usually overlooked. (Trepo and Guillevin ,2001)

Hepatitis B is a disease of global importance, with > 300 million carriers of the

virus world-wide. Hepatitis B virus (HBV) is the cause of up to 80% of cases of primary liver cancer, the single most important cause of mortality globally. In countries where HBV carrier rates reach 10% HBV infection may accounts for 3% of total mortality, a level which exceeds polio-related mortality before the introduction of polio vaccine. (Maynard,1999)

Hepatitis C infection cause an indolent and slowly progressive liver disease that is asymptomatic until the development of cirrhosis and decompensated liver disease, or liver cancer.

In many cases of infected patients HCV may be responsible for extra hepatic clinical manifestations and disease. This includes certain type of vasculitis and glomerulonephritis caused by immune complex deposition .Association between HCV infection and sjogren's syndrome, essential mixed cryoglobulinaemia and membranoprolifreative glomerulonephritis type 1 have been suggested(Greenwood *etal.*,2012)

hepatitis C mortality has increased substantially since 1995. Despite small declines in recent years, rates have continued to increase among persons aged 55-64 years. Hepatitis C is an important cause of premature mortality. (Wise *etal*,2008)

1.3. objectives

1.3.1 General objectives

To Investigate the seroprevalence of HBV and HCV infection among Ethiopians in Khartoum State.

1.3.2.Specific objective:

1.To detect HBV and HCV IgG antibodies in Ethiopians .

2. To see if there is any relation between seroprevelance of the virus and age of patient.

CHAPTER TWO

CHAPTER TWO

LITERATURE REVIEW

2.1 HBV

2.1.1 Historical background

Hepatitis B virus (HBV) is a common viral pathogen that currently infects an estimated 4 million people worldwide, including 400 million who have chronic infection. Persons with chronic HBV infection are at a lifelong risk of developing hepatocellular carcinoma (HCC) or cirrhosis or both. Many persons with HBV are unaware that they carry the infection, and, of those who are chronically infected, only a minority receives routine, scheduled follow-up to monitor their disease status. Persons from high-risk populations, especially immigrants from nations where hepatitis B is highly endemic, should be tested for HBV seromarkers and should be vaccinated if they are found to be negative. The natural history of chronic HBV is a dynamic one: patients can fluctuate between periods of active liver inflammation and periods of inactive disease. Disease progression is influenced by various factors, including viral genotype and specific mutations, demographic features, concurrent viral infections, and social and environmental factors. Recent data suggest that antiviral therapy can decrease the risk of liver decomposition and liver-related death and reduce the risk of HCC in selected individuals with active liver disease and severe fibrosis. Persons identified with chronic HBV infection need lifelong, regular monitoring for the development of active liver disease and HCC(Mahon ,2005)

2.1.2 Classification and Structure

Hepatitis B virus (HBV) is the prototype member of the Hepadnaviridae (hepatotropic DNA virus) family. Hepadnaviruses have a strong preference for infecting liver cells, but small amounts of hepadnaviral DNA can be found in kidney, pancreas, and mononuclear cells. However, infection at these sites is not linked to extra hepatic disease.

HBV virions are double-shelled particles, 40 to 42 nm in diameter . with an outer lipoprotein envelope that contains three related envelope glycoprotein (or surface antigens) Within the envelope is the viral nucleocapsid, or core. The core contains the viral genome, a relaxed-circular, partially duplex DNA of 3.2 kb, and a polymerase that is responsible for the synthesis of viral DNA in infected cells. DNA sequencing of many isolates of HBV has confirmed the existence of multiple viral genotypes, each with a characteristic geographic distribution

In addition to virions, HBV-infected cells produce two distinct subviral lipoprotein particles: 20-nm spheres and filamentous forms of similar diameter These HBsAg particles contain only envelope glycoprotein's and host-derived lipids (Ganem and Princ,2004)

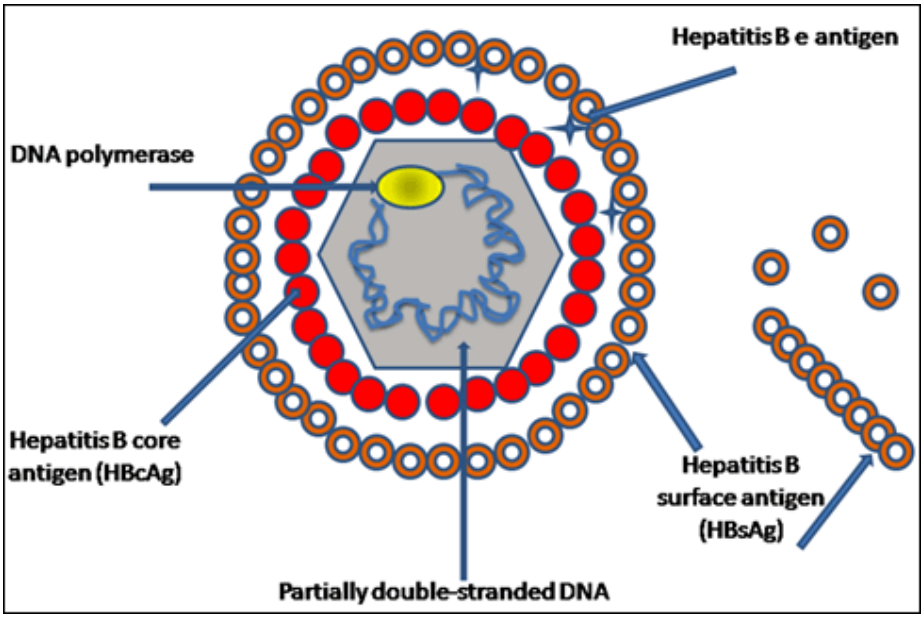


Fig I structure of HBV

2.1.3 Transmission Of HBV Infection

Hepatitis B virus is present in the blood, saliva, semen, vaginal secretions, menstrual blood, and to a lesser extent, perspiration, breast milk, tears, and urine of infected individuals. A highly resilient virus, HBV is resistant to break-down, can survive outside the body, and is easily transmitted through contact with infected body fluids .In areas of high endemicity, the most common route of transmission is perinatal or the infection is acquired during the preschool years. In areas of intermediateendemicity, transmission is either perinatal or horizontal. The route of transmission has important clinical implications , because there is a very high probability of developing chronic hepatitis B (CHB) if the infection is acquired perinatally or in the preschool years . The use of unsafe injections poses a particular public health problem in developing countries . Contaminated needles cause 8–16 million HBV infections each year, compared with 2.3–4.7 million

hepatitis C virus infections ,and 80 000–160 000 human immunodeficiency virus infections .In areas of low endemicity, most HBV infections are acquired by horizontal transmission in early adult life, i.e. .through intravenous drug use or unprotected sexual activities . Blood transfusions were once a common route of transmission, but improved diagnostic tests and progressively broader screening for HBV infection in recent years ,such as occurred in Latin American countries from 1994 to1997, has dramatically reduced the risk of acquiring HBV infection through transfusion .Other sources of infection include contaminated surgical instruments and donor organs. Health care workers, dentists, and others who have frequent contact with infected blood or blood products are at highest risk (Lavanchy,2004)

2.1.4 Stages of infection

An individual can develop hepatitis B infection that is acute and achieve complete immune clearance of virus yielding lifelong immunity, however an alternate fate of the host is the development of chronic hepatitis B. There are three stages of HBV infection based on viral-host interaction, namely, the immune tolerant phase, the immune clearance phase, and the inactive carrier phase with or without reactivation . After acute infection of HBV, some patients may remain HBeAg positive with high levels of serum HBV DNA, little or no symptoms, normal ALT levels and minimal histological activity in the liver, this phenomenon is known as the immune tolerance phase. This phase is typical of infection in children and young adults. It usually lasts for 2-4 weeks, but can last for years in those who acquired the infection during the perinatal period . Individuals in this group are highly contagious and can transmit HBV easily. When the tolerogenic effect is lost

during the immune tolerant phase, immune-mediated lysis of infected hepatocytes become active and patients enter the second stage defined as immune clearance phase, the HBV DNA level decreases and ALT level increases. The duration of clearance phase lasts from months to years. This is followed by the carrier stage, in which seroconversion of HBeAg to HBeAb occurs, HBV DNA becomes non-detectable or at low level and ALT is usually normal, reflecting very low or no replication of HBV and mild or no hepatic injury. The inactive carrier stage may last for years or even lifetime. Patients in this stage can have spontaneous resolution of hepatitis B and develop HBsAb, but a portion of them may undergo spontaneous or immunosuppression-induced reactivation of chronic hepatitis, featuring elevated ALT, high level of DNA, moderate to severe liver histological activity, and with or without HBeAg seroreversion(pan and zhang,2005)

2.1.5 Clinical finding

Many of HBV infection are asymptomatic and are detected only by presence of antibody to HBsAg. the mean incubation period for hepatitis B is 10-12 weeks. the clinical appearance of acute hepatitis B are fever, anorexia, nausea, vomiting and jaundice are typical. dark urine, pale feces and elevated transaminase level are seen they tend to be more severe than A .most chronic carrier are asymptomatic but some have chronic active hepatitis which lead to cirrhosis and death(Levinson,2004)

2.1.6 Risk groups and factors

This term applies to people who by reason of their country of birth ,way of life or type of work are higher than average risk of acquiring HBV infection or passing it on. We have already decribed one such group, babies born to carrier mothers in

high-endemic areas.table1.1 give example of high risk groups in area of low endemicity (Table I)(Collier and Oxford,2000)

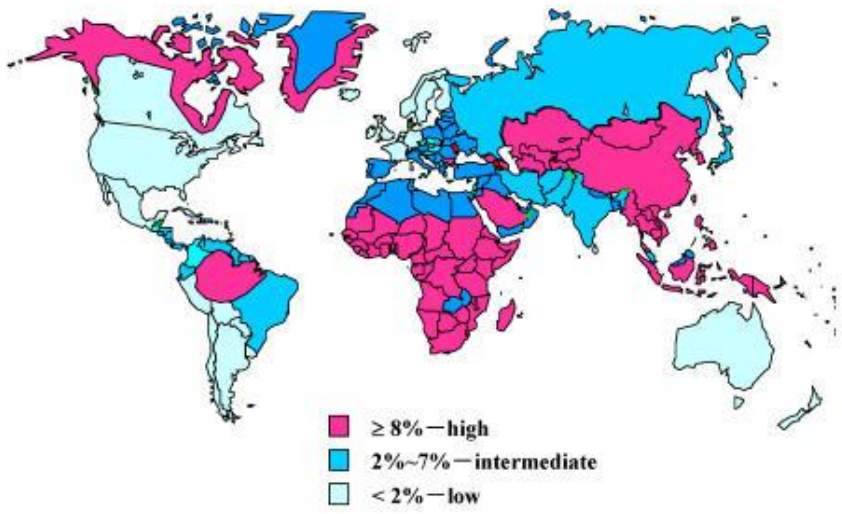
Table I: High Risk Group

category	**Risk factor or group**
General community	-Sexually promiscuous people -Intravenous drug abusers -Partners of HBsAg-positive carriers -Infants of HBsAg-positive mothers
patients	-Repeated blood transfusions -Long –term treatment with blood products e.g Haemophiliacs -Chronic renal failure
Healthcare staff	-Work in mental institutions -Tours of duty in high-endemicity areas -Surgical and dental operations -Some pathological laboratory work, including autopsies Work in std clinics Prisoners and staff in contact with them

2.1.7 Epidemiology

Worldwide, two billion people have been infected with hepatitis B virus (HBV), 360 million have chronic infection, and 600,000 die each year from HBV-related liver disease or hepatocellular carcinoma. This comprehensive review of hepatitis B epidemiology and vaccines focuses on definitive and influential studies and highlights current trends, policies, and directions. HBV can be transmitted vertically, through sexual or household contact, or by unsafe injections, but chronic infections acquired during infancy or childhood account for a disproportionately large share of worldwide morbidity and mortality. Vaccination against HBV infection can be started at birth and provides long-term protection against infection in more than 90% of healthy people. In the 1990s, many industrialized countries and a few less-developed countries implemented universal hepatitis B immunization and experienced measurable reductions in HBV-related disease. For example, in Taiwan, the prevalence of chronic infection in children declined by more than 90%. Many resource-poor nations have recently initiated universal hepatitis B immunization programs with assistance from the Global Alliance for Vaccines and Immunization. Further progress towards the elimination of HBV transmission will require sustainable vaccination programs with improved vaccination coverage, practical methods of measuring the impact of vaccination programs, and targeted vaccination efforts for communities at high risk of infection.(Fig2) (Shepard *et al;*2006)

Fig II Prevalence of HBV



2.1.8 Diagnostic method of HBV

rapid immunochromatographic assay for hepatitis B virus screening

Simple, rapid and accurate assays for hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) are helpful for clinical diagnosis and field epidemiological surveys. A commercially developed, rapid immunochromatographic test for simultaneous detection of HBsAg and HBeAg was evaluated using a total of 2463 selected samples (827 frozen sera, 1011 fresh sera, and 625 whole blood samples). Results of the rapid test were compared with standard enzyme immunoassay (EIA) methods for HBsAg and HBeAg detection. The accuracy of the rapid test was excellent and was similar for frozen sera, fresh sera and whole blood. The overall sensitivity and specificity for the detection of HBsAg were 95 and 100%, and the corresponding positive and negative predictive values were 100 and 99.7%, respectively. The sensitivity and specificity for the

detection of HBeAg were slightly less than that for HBsAg, and were 80 and 98%, with positive and negative predictive values of 91 and 94%, respectively. Thus, compared with the EIA method, the rapid test was highly sensitive and accurate for the detection of HBsAg although somewhat less sensitive and specific for detection of HBeAg. Because of its speed, simplicity and flexibility, the rapid test is ideally suited for HBsAg and HBeAg screening in population-based epidemiological studies and in low risk populations, particularly in regions of the world where hepatitis B is endemic(Lemon *et al*;2003)

2.1.8.1 Molecular Detection of HBV

Quantitation of hepatitis B virus (HBV) DNA in serum is a useful method for the monitoring of HBV replication. We attempted to develop a quantitative assay system for HBV DNA that is more sensitive, accurate, and reproducible than existing systems. We detected HBV DNA by real-time detection PCR (RTD-PCR) based on Taq Man chemistry. The efficacy of this assay was evaluated by quantitatively measuring sequential levels of synthetic DNA and DNA in clinical serum samples. The detection limit of this system was as few as 10 DNA copies/reaction. A linear standard curve was obtained between 10^1 and 10^8 DNA copies/reaction. The coefficient of variation for both intra- and interexperimental variability indicated remarkable reproducibility. This system detected HBV DNA in 100% of chronic hepatitis B patients tested and never detected HBV DNA in healthy volunteers who were negative for HBV markers. These observations suggest that RTD-PCR is an excellent candidate for a standard HBV quantification method(Abe *et al*;1999)

2.1.8.2 Advances in Molecular Diagnosis of HBV Infection and Drug Resistance

Serological markers are key elements in diagnosing acute hepatitis B virus (HBV) infection and determining its possible evolution towards chronicity. Once treatment of chronic HBV is initiated with approved anti-hepadnaviral agents, such as lamivudine, interferon-alpha, or adefovir dipivoxil, the measurement of HBV DNA in serum can not only help monitor treatment efficacy but also indicates breakthrough infection should drug resistance emerge. Advances in the molecular diagnosis of drug resistance using highly sensitive methodologies such as DNA hybridization assays can further pinpoint the type of mutation responsible and, more importantly, detect upcoming viral resistance at an early stage when the variant represents only a minor fraction of the total viral population. Such new tools are especially relevant for patients at high risk for disease progression or acute exacerbation. Recent diagnostic developments including HBV genotyping and precore/core promoter assays that could well play important future roles in HBV patient management are also reviewed(Sablon and Shapiro,2005)

2.1.8.3 Immune Fluorescence microscopy(IFM)

A few specialized laboratories use this technique for detection of antibobies.IFM detects antibodies that react against the HCV antigen semi quantitatively .Anti-HCV antibodies block the binding of Fluorescein –conjugated anti-HCV IgG to HCV antigen in frozen liver tissue .the concentration of anti-HCV antibodies is estimated semi quantitatively, This method is laborious and expensive and thus not useful for routine diagnosis. (Yarbough,1999)

2.1.8.4 Immune electron microscopy(IEM)

IEM detect VLPs in clinical specimens HCV particle are precipitated with antibody to HCV. Anti-HCV antibodies concentrations can be determined semi quantitatively by rating the antibody coating .although IEM is superior technique for specifity the sensitivity of the assay is insufficient for routine analysis IEM is difficult to perform and most clinical specimens do not contain sufficient VLP to be detected (Yarbough,1999)

2.1.8.5 Liver biopsy

Liver biopsy is usually the most specific test to assess the nature and severity of liver diseases. In addition, it can be useful in monitoring the efficacy of various treatments. There are currently several methods available for obtaining liver tissue: percutaneous biopsy, transjugular biopsy, laparoscopic biopsy, or fine-needle aspiration guided by ultrasonography or computed tomography (CT). Each of these methods has advantages and disadvantages. Liver biopsy provides an accurate diagnosis in approximately 90 percent of patients with unexplained abnormalities revealed on liver-function tests(Bravo *et al*;2001)

2.1.8.6 Virus Isolation

currently there is no reliable cell culture system for HBV

2.1.9 Immunisation against hepatitis B infection

The cornerstone of prevention for patients who are HBV seronegative is immunisation. Currently available HBV vaccines are very safe and have an efficacy of >90% in immunocompetent young individuals. Non-response is associated with a number of factors including genetically determined resistance,

advanced age, obesity, chronic liver disease, smoking, male gender and miscellaneous systemic diseases including renal failure Although universal vaccination of newborns has been implemented worldwide according to World Health Organisation recommendations, it will take several decades until the majority of the world's adult population will be immune. Unfortunately, vaccination rates are low in many countries either due to lack of funding or because of the misconception that vaccination is only necessary in high-risk groups

It is strongly recommended that all haemato-oncological patients be screened for HBV markers and immunisation against hepatitis B should be performed when appropriate (see algorithm, The conventional regimen for the HBV vaccine requires three doses at 0, 1 and 6 months. Delaying administration of the third dose in healthy individuals (up to 1 year) may increase anti-HBs antibody levels. Frequently, in haemato-oncological patients, urgent administration of chemotherapy does not allow completion of the three-dose regimen. In such cases, an effort should be made to immunise patients with at least two doses within a 3–4 week interval. The third dose can then be given a few months after chemotherapy is completed(Shepard *et al*;2006)

In most countries, immunity against HBV infection is defined as an anti-HBs titre >10 IU/l and in the UK the recommended titre is >100 IU/l. Non-response to HBV vaccines is not rare in haemato-oncological patients due to disease-associated or treatment-induced immune suppression. Thus, protection against HBV may not be achieved until all doses have been administered. Although immune suppressed patients have significantly lower response rates to vaccination, successful anti-HBs seroconversion following a three dose vaccine schedule has been reported in 57% of cancer patients, 15–68% of bone marrow transplant recipients, and in 10% in acute lymphoblastic leukemia (ALL) patients Documentation of post-vaccination

anti-HBs seroconversion is recommended. There are a number of means to augment the immune response to HBV immunization in non-responders, including adding three additional doses; doubling the vaccine dose; intradermal injection of the vaccine and use of new, more immunogenic HBV vaccines ,Finally, after allogeneic HSCT, immunity to HBV acquired through active immunization, may rarely be abolished by immune suppression and/or transplantation of HBV naïve bone marrow cells(Shepard *et al*;2006)

2.1.10 Prevention &control:

Broadly there are two approaches to the prevention of infection with HBV-modification of risk behaviour and immunization. Measures for the former include avoiding unprotected sexual contact by the use of condoms and reducing needle sharing among injecting drug users through needle exchange schemes .implementation of sensible infection control policies can reduce the risks considerably to healthcare workers and patients .it is essential that blood for transfusion and organ donors for transplantation are screened (Greenwood *et al*;2012)

2.2 HCV

2.2.1 Historical background:

Hepatitis C virus (HCV) is a leading cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma, as well as the most common indication for liver transplantation in many countries. Although the incidence of hepatitis C infection has dramatically decreased during the past decade, the worldwide reservoir of chronically infected persons is estimated at 170 million, or 3% of the global population. There is much controversy surrounding the natural history of hepatitis

C infection. The rate of chronic HCV infection is affected by a person's age, gender, race, and viral immune response. Approximately 75%-85% of HCV-infected persons will progress to chronic HCV infection, and are at risk for the development of extrahepatic manifestations, compensated and decompensated cirrhosis, and hepatocellular carcinoma (HCC). The rate of progression to cirrhosis is highly variable, and is influenced by several factors, including the amount of alcohol consumption, age of initial HCV infection, degree of inflammation and fibrosis on liver biopsy, HIV and HBV coinfection. An estimated 10%-15% of HCV-infected persons will advance to cirrhosis within the first 20 years. Persons with cirrhosis are at increased risk of developing HCC. An understanding of the natural history of hepatitis C is essential to effectively (Chen and Morgan,2006)

2.2.2 Structure of HCV:

HCV is a small, enveloped RNA virus which has been allocated to a unique genus, designated *Hepacivirus*, within the family Flaviridae. .The HCV genome is single-stranded RNA molecule of positive polarity that contains a single open reading frame (Fig3)(Thomosom and Trich,2005)

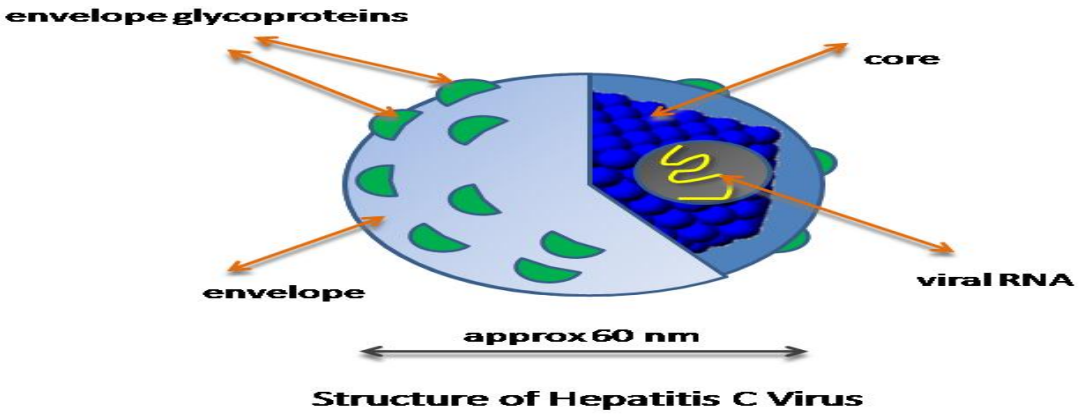


Fig 3:Structure of Hepatitis C virus

2.2.3 Transmission of hepatitis C:

The routes of HCV transmission in communities have a profound impact on the global epidemiology of HCV and therefore on the GBD related to infections acquired today. After World War II and up until the 1980s, the most important source of HCV transmission in developed countries was either by parenteral exposure to contaminated blood or blood products or by the illicit use of injectable drugs. The introduction of routine testing of donated blood has virtually eliminated transmission of HCV by blood transfusion .Illicit use of injectable drugs is today the main source of HCV infections in most developed countries (e.g. Western Europe, US) and is becoming a major source of infection in transitional economy and developing countries, accounting for 40% or more of those infected .The prevalence of chronic infection with HCV in injecting drug user populations is appreciably higher than that of either HBV or human immunodeficiency virus (HIV) (Lavanchy,2009)

In developing and transitional economy countries, the nosocomial transmission of new HCV infections is a major problem because of the re-use of contaminated or inadequately sterilized syringes and needles used in medical, paramedical and dental procedures, with an estimated 2.3–4.7 million of new infections occurring each year .In Egypt, the treatment of endemic schistosomiasis in mass programmes (discontinued in the 1980s) that frequently used unsterilized needles and syringes has led to a national HCV prevalence of more than 14%, with rates of 20–30% in young male adults .The highest reported rates of needle re-use are found in the Middle East, South-East Asia and the Western Pacific ,and the most frequently injected medications include antibiotics, vitamins and analgesics that could be taken orally most of the time, and that are usually administered for non-specific symptoms. Special attention should focus on the fact that injection solutions and

equipments are frequently purchased outside of the formal healthcare system, and that injections are dispensed by unqualified personnel in pharmacies or marketplaces.

Other modes of transmission may be less relevant from a public health perspective. In patients on chronic haemodialysis, chronic HCV infection is common, with prevalences of 10–33% (Sexual transmission of HCV occurs infrequently because HCV is far less likely than HBV to be transmitted by mucosal exposure .The risk of perinatal transmission of HCV is very low ,although there is conflicting evidence that the risk is increased by high maternal viral loads or if the mother is co-infected with HIV. The risk posed to the infant from breastfeeding is negligible and non-sexual intrafamilial transmission is very rare (Lavanchy,2009)

2.2.4 Components of the antiviral immune response.HCV

Although the hepatocyte is depicted as the target cell of hepatitis C virus (HCV) specific immune response here, other cells, including dendritic cells and macrophages, are also important in antigen presentation to the immune system. CTL = cytotoxic T cell; IL = interleukin; MHC = major histocompatibility complex; TCR = T-cell receptor; Th = T helper; Th1 = helper T cells with a type 1 cytokine profile; Th2 = helper T cells with a type 2 cytokine profile; TNF = tumor necrosis factor.

On activation of their specific T-cell receptors, HCV-specific helper T cells assist with activation and differentiation of B cells as well as induction ,and stimulation of virus-specific cytotoxic T cells. Most of these effects are mediated by different sets of immunoregulatory Th1 (interferon-γ and interleukin-2) or Th2 (interleukin-4, interleukin-5, and interleukin-10) cytokines. In the context of class I MHC

molecules, CD8-positive cytotoxic T cells recognize HCV peptides that are synthesized and processed in infected cells ,This encounter can lead to lysis of virus-infected cells. Together with helper T cells, cytotoxic T lymphocytes may also secrete cytokines, such as interferon-γ and tumor necrosis factor-α, that inhibit replication and gene expression of several viruses, such as hepatitis B virus, cytomegalovirus, and rotavirus (Rehermann *et al*;2000)

2.2.4.1 Humoral Immune Response

Hepatitis C virus can establish persistent infection despite an active humoral and cellular immune response that is generally targeted against all viral proteins. The virus may escape from the humoral immune response if the kinetics of infection and viral replication do not allow complete neutralization of the virus by HCV-specific antibodies after primary infection. Although virus-specific antibodies may interfere with viral entry into host cells and opsonize the virus for elimination by macrophages, they cannot eliminate HCV from infected cells. In addition, HCV has a high mutation rate, especially in the hypervariable region of the envelope proteins that can be recognized by neutralizing antibodies (antibodies that can bind and eliminate virus) .Several studies have demonstrated that the humoral immune response can select HCV variants with sequence changes that allow escape from antibody recognition .However, recent studies in chimpanzees have suggested that HCV can cause persistent infection in the absence of mutations in the hypervariable region ,Thus, progression to persistent HCV infection is most likely a multifactorial process that depends on multiple aspects of virus-host interaction (Rehermann *et al*;2000).

2.2.4.2 Cellular Immune Response

The cellular immune response probably plays an important role in the outcome of HCV infection because of its ability to recognize and eliminate virus from infected cells. Most studies have concentrated on the antigen-specific immune response that is mediated by CD4-positive helper T cells and CD8-positive cytotoxic T cells.

Because chronic rather than acute infection is diagnosed in most patients, immunologic studies have been performed on patients with persistent infection who could not clear HCV. Only a few studies have analyzed the cellular immune response during the acute phase of infection. These studies suggest that the strength and quality of both helper T-cell .and cytotoxic T-cell responses differ between patients who recover and those who develop chronic infection. More important, the viral sequences that are recognized most frequently and vigorously by HCV-specific T cells vary little among all the HCV genotypes. Furthermore, several of these frequently recognized viral peptides bind with high affinity to many different class II MHC molecules, suggesting that they can be efficiently presented and recognized by patients with different MHC haplotypes ,Thus, these viral sequences could be explored for development of preventive or therapeutic vaccines against HCV (Rehermann *et al*;2000).

The cellular response against HCV could be interfered with in several ways. First, HCV elicits only a weak T-cell response in patients who develop chronic infection .In the blood of patients with chronic hepatitis C, the frequency of cytotoxic T-cell precursors that are specific for individual HCV peptides is much lower than the frequency of T cells that recognize an influenza virus peptide as a recall antigen .or peptides of other viruses that can be cleared, such as cytomegalovirus .The reasons for this relative weakness of the cellular immune response are not known.

Certainly, general immune tolerance or immunosuppression is not the cause of persistent HCV infection, because most chronically infected patients display normal immune responses against other viral agents .The emergence of viral mutants or quasi-species with sequence variations in T-cell epitopes may contribute to the apparent ineffectiveness of cell-mediated immune response .There is also increasing evidence that several HCV proteins, such as core ,E2 ,and NS5A ,interfere with the immune response. Furthermore, infected hepatocytes, which lack co-stimulatory molecules, may be relatively inefficient in priming the immune system, and the liver has been proposed as the major site where activated T cells are destroyed .Finally, the cellular immune response is a double-edged sword. An immune response that is ineffective in clearing HCV infection may be more harmful to the liver, causing chronic inflammation, hepatocellular injury, and, over several decades, liver fibrosis and cirrhosis.

Progression to persistent infection and the immunologic mechanisms of liver injury are the consequence of complicated interactions between the virus and host. Identification of immunologic correlates of viral clearance may contribute to the development of an effective vaccine and better therapy for HCV infection. (Rehermann *et al*;2000)

2.2.5 Clinical finding &complication:

Clinically the acute infection with HCV is milder than infection with HBV, fever anorexia ,nausea, vomiting and jaundice are common. dark urine ,pale feces and elevate transminase level are seen. HCV resemble hepatitis B as far as the ensuing chronic liver disease ,cirrhosis and predisposition to hepatocelluler carcinoma are concerned .many infection with HCV including both acute and chronic infection are Asymptomatic and are detected only by presence of antibody .the mean

incubation period is 8week.cirrhosis result from chronic HCV infection is most common indication for liver transplantation

HCV infection also lead to significant autoimmune reaction including vasculitis, arthragias, purpura, and membranoproliferative glomerulonephritis. HCV is the main cause of essential mixed cryoglobulinemia. the cryoprecipitates often are composed of HCV antigen and antibodies(Levinson ,2004)

2.2.6 Prevalence

Hepatitis C virus (HCV) continues to be a major disease burden on the world. In 1999, the WHO estimated a worldwide prevalence of about 3% with the virus affecting 170 million people worldwide. Generally, most studies of prevalence use blood donors to report the frequency of HCV usually by anti-HCV antibodies and do not report follow-up HCV testing. Using blood donors as a prevalence source may underestimate the real prevalence of the virus because donors are generally a highly selected population.

In the Third National Health and Nutrition Examination Survey (NHANESIII) from 1988 to 1994, an estimated HCV prevalence of 3.9 million people was found in the United States (US) with 2.7 million people found to have chronic infection with HCV (positive HCV RNA). Neither sex nor racial-ethnic group was found to be independently correlated with HCV infection. However, a majority of patients that were HCV positive were below the age of 50.

Intermediate rates of HCV have been reported out of Asia. From 1995-2000, 0.49% anti-HCV Ab were detected among 3,485,648 blood donors in Japan. This was lower than the 0.98% our of 10,905,489 blood donors reported in 1992. In China, prevalence rates were generally low with rates around 1% among donors in

Beijing and Wuhan. However, rates may be higher in certain areas such as the Hubei province (30.13%) and Inner Mongolia Autonomous Region (31.86%). Low rates have been found in Malaysia (around 1.6%) and Singapore (0.54%). [25.26] Higher rates of HCV have been found in Thailand (3.2-5.6%). Within a smaller community of 103 residents in Sherpas, Nepal, only 1 person had a borderline reaction in 2004. In New Delhi, India, 1.85% of blood donors were positive.

There have been fewer studies out of Africa, but lower rates have been reported – 1.6% among blood donors in Ethiopia and 0.9% in Kenya(Hanafiah *et al*;2013)

2.2.7 Diagnostic methods of HCV

2.2.7.1 Serological assays

Anti-HCV antibody detection

The detection of anti-HCV antibodies in plasma or serum is based on the use of third-generation EIAs, that detect mixtures of antibodies directed against various HCV epitopes. Recombinant antigens are used to capture circulating anti-HCV antibodies onto the wells of microtiter plates, microbeads, or specific holders adapted to closed automated devices. The presence of anti-HCV antibodies is revealed by anti-antibodies labeled with an enzyme that catalyzes the transformation of a substrate into a colored compound. The optical density (OD) ratio of the reaction (sample OD/internal control OD) is proportional to the amount of antibodies in the serum or plasma sample. The specificity of third-generation EIAs for anti-HCV is greater than 99%. Their sensitivity is +more difficult to determine, given the lack of a gold standard method, but it is excellent in HCV-infected immunocompetent patients. EIAs can be fully automated and are well

adapted to large volume testing. Immunoblot tests are nowadays clinically obsolete given the good performance of third-generation anti-HCV EIAs (Chevaliez and Pawlotsky,2006)

2.2.7.2 Serological determination of the HCV genotype

The HCV genotype can be determined by seeking for antibodies directed to genotype-specific HCV epitopes with a competitive EIA. The currently available assay (Murex HCV serotyping 1-6 HC02, Abbott Laboratories, North Chicago, Illinois) identifies the type (1 to 6), but does not discriminate among the subtypes, and provides interpretable results in approximately 90% of chronically infected immunocompetent patients. Mixed serological reactivities can be observed that could be related to mixed infection although cross-reactivity or recovery from one genotype infection and persistence of viremia with another genotype cannot be ruled out.

Qualitative, non-quantitative HCV RNA detection

Qualitative detection assays are based on the principle of target amplification using either “classic” polymerase chain reaction (PCR), “real-time” PCR or TMA . HCV RNA is extracted and reverse transcribed into a double stranded complementary DNA (cDNA), which is subsequently processed into a cyclic enzymatic reaction leading to the generation of a large number of detectable copies. Double-stranded DNA copies of HCV genome are synthesized in PCR-based assays, whereas single-stranded RNA copies are generated in TMA. Detection of amplified products is achieved by hybridizing the produced amplicons onto specific probes after the reaction in “classic” PCR or TMA techniques. In “real-time” PCR, each round of amplification leads to the emission of a fluorescent signal and the number

of signals per cycle is proportional to the amount of HCV RNA in the starting sample. Qualitative detection assays must detect 50 HCV RNA IU/ml or less, and have equal sensitivity for the detection of all HCV genotypes(Chevaliez and Pawlotsky,2006).

2.2.7.3 HCV RNA quantification

HCV RNA can be quantified by means of target amplification techniques (competitive PCR or real-time PCR) or signal amplification techniques (branched DNA (bDNA) assay). Five standardized assays are commercially available. Two of them are based on competitive PCR ,and two are based on real-time PCR amplification, , which can be coupled with automated extraction The most promising approach for the future is fully automated real-time PCR assays, which are faster, more sensitive than classical target amplification techniques and are not prone to carryover contamination

2.2.7.4. DIAGNOSIS OF HCV INFECTION

Acute hepatitis C

Patients with a suspicion of acute hepatitis C should be tested for both anti-HCV antibodies by EIA and HCV RNA with a sensitive technique, i.e. an HCV RNA assay with a lower limit of detection of 50 IU/ml or less. Four marker profiles can be observed according to the presence or absence of either marker. The presence of HCV RNA in the absence of anti-HCV antibodies is strongly indicative of acute HCV infection, which will be confirmed by seroconversion (i.e. the appearance of anti-HCV antibodies) a few days to weeks later. Acutely infected patients can also have both HCV RNA and anti-HCV antibodies at the time of diagnosis. It is difficult, in this case, to distinguish acute hepatitis C from an acute exacerbation of

chronic hepatitis C or an acute hepatitis of another cause in a patient with chronic hepatitis C. Acute hepatitis C is very unlikely if both anti-HCV antibodies and HCV RNA are absent. It is also unlikely if anti-HCV antibodies are present without HCV RNA. These patients should however be retested after a few weeks because HCV RNA can be temporarily undetectable, due to transient, partial control of viral replication by the immune response before replication escapes and chronic infection establishes. Apart from such cases, the presence of anti-HCV antibodies in the absence of HCV RNA is generally seen in patients who have recovered from a past HCV infection. Nevertheless, this pattern cannot be differentiated from a false positive EIA result, the exact prevalence of which is unknown.(Chevaliez and Pawlotsky,2006)

Chronic hepatitis C

In patients with clinical or biological signs of chronic liver disease, chronic hepatitis C is certain when both anti-HCV antibodies and HCV RNA (sought for with a sensitive technique, detecting 50 IU/ml or less) are present. Detectable HCV replication in the absence of anti-HCV antibodies is exceptional with the current third-generation EIAs, almost exclusively observed in profoundly immunodepressed patients, hemodialysis patients or agammaglobulinemic subjects.

In patients who have no indication for therapy or have a contra-indication to the use of antiviral drugs, virological tests have no prognostic value. Indeed, neither anti-HCV antibodies nor the HCV RNA load correlate with the severity of liver inflammation or fibrosis nor with their progression. Thus, they cannot be used to predict the natural course of infection or the onset of extrahepatic manifestations. In untreated patients, the severity of liver inflammation and fibrosis must be

evaluated every three to five years by means of a liver biopsy or non-invasive serological or ultrasound-based testing.

2.2.7.5Enzyme immunoassay(EIA):

The initial test used to diagnose HCV is an enzyme immunoassay (EIA) for anti-HCV immunoglobulin G (IgG). The HCV genome encodes a polyprotein of 3,011 to 3,033 amino acids that is processed into 10 structural and nonstructural (NS) proteins . Three generations of screening EIAs have been developed to detect antibodies against various epitopes of these proteins.

HCV antigens used for serologic assays. a, E, envelope; NS, nonstructural protein; a. a., amino acid sequence of recombinant protein or synthetic peptide antigen. . The sensitivities of these EIAs were low for a high-prevalence population (approximately 80%), and the fraction of positive results that were false positive was as high as 70% for a low-prevalence population (blood donors). This led to the development of more sensitive and specific second-generation EIAs (EIAs 2.0) that incorporated additional antigens from NS (c33c) and structural (c22-3) proteins that were approved for use by the Food and Drug Administration (FDA) in 1992. Second-generation assays detect HCV antibodies in 20% more patients with acute NANBH and in 10% more patients with chronic cases of infection than EIAs 1.0 do and detect HCV antibodies 30 to 90 days sooner than EIAs 1.0 do . The mean window of seroconversion was reduced from 16 weeks with EIAs 1.0 to 10 weeks with EIAs 2.0 . The sensitivities of EIAs 2.0 in a high-prevalence population are approximately 95% (based on HCV RNA detection by PCR).

In 1996, FDA approved a third-generation EIA (EIA 3.0) that added a fourth antigen (NS5) to those in EIAs 2.0. EIA 3.0 detected antibodies an average of 26

days earlier in 5 of 21 individuals with transfusion-transmitted HCV , and the sensitivity is slightly better than that of EIA 2.0 in a high-prevalence population (as high as 97%) .(Chevaliez and Pawlotsky,2006).

Use of serologic assays. Of the 25 to 35% of patients with acute infection who develop symptoms, only 50 to 70% will have detectable antibodies at that time, but 90% will have measurable antibodies after 3 months . Serologic assays detect HCV antibodies that indicate present or previous infection, but they cannot discriminate acute from chronic or resolved infection. Anti-HCV IgM antibodies can be detected in 50 to 93% of patients with acute HCV infections and 50 to 70% of chronic cases, so they are not a reliable indicator of acute infection .

Confirmation by RIBA is needed only for low-risk patients (healthy blood donors) or if a high-risk patient is HCV RNA negative . Confirmation by RIBA has not been very useful for resolving weakly positive samples (optical density ratios between 1 and 2), and molecular HCV RNA detection is recommended instead . Individuals with indeterminate RIBA results should be evaluated by a sensitive HCV RNA detection test .

Patients with acute hepatitis of uncertain origin and negative hepatitis serology panels should undergo qualitative HCV RNA testing . Occasionally, immunocompromised patients, patients undergoing hemodialysis, and patients with mixed cryoglobulinemia have false-negative serology results and may require HCV RNA testing for diagnosis . Passively transferred maternal anti-HCV antibodies may be detected in the children of HCV-infected mothers for up to 1 year; however, defined diagnostic criteria for HCV RNA detection are not available.(Chevaliez and Pawlotsky,2006)

2.2.7.6 HCV RNA Detection and Quantitation:

The presence of HCV RNA in plasma defines active infection, and HCV RNA can be detected 1 to 3 weeks post exposure . A single negative HCV RNA assay result does not exclude the possibility of active infection with a transient drop in the level of viremia below the assay's limit of detection.

Laboratories detect HCV RNA with commercially available assay kits. or by in-house home-brewed methods. Because of the limited amount of HCV RNA in infected individuals, a target or signal amplification step is needed. Reverse transcriptase (RT) PCR (RT-PCR) and transcription-mediated amplification (TMA) are target amplification methods. The branched DNA (bDNA) assay is a signal amplification technique.

For RT-PCR, an RT step converts RNA to cDNA, which is used as a template for the PCR . Primers whose sequences correspond to the 5′ untranslated region (5′ UTR) are commonly used because this is the most conserved region of the genome . The Roche AMPLICOR HCV test (Roche Diagnostics, Branchburg, N.J.) includes 37 amplification cycles followed by hybridization to an HCV-specific oligonucleotide probe. The semiautomated version of the AMPLICOR HCV test uses the COBAS instrument to reduce the hands-on time required for detection and calculation by the technologist . The qualitative AMPLICOR HCV test (version 2.0) received FDA approval in 2001 and has a lower limit of detection of 50 IU/ml . The less sensitive quantitative HCV RNA assays lack FDA approval and are available only for research purposes. (Chevaliez and Pawlotsky,2006)

TMA involves a more complex set of reactions with T7 RNA polymerase and RT under isothermal conditions to form detectable levels of RNA . TMA uses primers

that contain a T7 RNA polymerase binding site so that RT synthesizes cDNA that becomes a template from which T7 RNA polymerase can synthesize numerous copies of RNA. The RNA amplicons reenter the TMA cycle and become templates for the next replication cycle. The TMA-based VERSANT HCV RNA qualitative assay (Bayer Diagnostics, Tarrytown, N.Y.) is not approved by FDA, but it is able to detect very low levels of HCV RNA (5 IU/ml) that are undetectable with RT-PCR systems . The Procleix HIV-1/HCV assay also uses TMA technology and was approved by FDA in February 2002 for the screening of blood donations to identify HCV-positive donors who are antibody negative . A PCR-based blood screening assay (Ampliscreen HCV Test, version 2.0) is also expected to attain FDA approval.

Because of the higher sensitivities of commercially available qualitative assays in comparison to those of quantitative assays , the value of quantitative assays has been limited to pretreatment evaluations. Commercial assays no longer report results in numbers of copies of RNA per milliliter, which represent different amounts of RNA, depending on the assay. The World Health Organization international standard has provided a common unit of measure that allows comparison between results from different assays . Qualitative assays should be used to confirm viremia and assess the therapeutic response until quantitative assays with comparable sensitivities are available(Chevaliez and Pawlotsky,2006)

2.2.8 Mangement of Acute Hepatitis C:

Early identification of patients with acute HCV infection is important for their optimal management. The rate of chronic evolution is 50–90%, and the natural course of chronic hepatitis C can be associated with severe complications. Patients with chronic hepatitis C have the potential risk of developing liver cirrhosis and

hepatocellular carcinoma.[1] The social burden of HCV infection is high, including for health care workers. Extrahepatic manifestations of HCV are often troublesome and may not be reversible with viral eradication.[2] These are good reasons for the design of a prophylactic vaccine but as this has yet to be accomplished, early treatment of acute HCV infection with interferon alpha (IFN) is the only option to prevent chronicity(Manns *et al*;2006)

Immediate treatment of patients with symptomatic acute hepatitis C with recombinant IFN or pegylated IFN (PEG-IFN) monotherapy for 24 weeks can prevent the development of chronic hepatitis C in approximately 90% of cases.[3–5] Combination with ribavirin is not necessary.[6] However, symptomatic patients also have a good chance to clear HCV spontaneously.[7,8] This usually occurs in the first 12 weeks after the onset of symptoms. A wait and see strategy (that is, treatment of only those patients who remain HCV-RNA (Manns *et al*;2006)

2.2.9 HCV VACCINE:

The recent discovery of natural immunity to the hepatitis C virus and vaccine efficacy in the chimpanzee challenge model has allowed optimism about the development of at least a partly effective vaccine against this heterogeneous pathogen that is responsible for much of the chronic liver disease around the world. The immune systems of some infected individuals can spontaneously clear the virus, whereas other people need treatment with antivirals that work partly by stimulating humoral and cellular immune responses. Therefore, therapeutic vaccine strategies are also being pursued to improve treatment outcome.((Houghton and brignani,2005)

CHAPTER THREE

Chapter Three

3. Materials and Methods

3.1 Study design

The study was cross sectional study to investigate prevalence of HBV and HCV among Ethiopians in Khartoum State

3.2 Study area

This study was conducted at Al Salam Clinic in Algreef West

3.3 Study Population

Ethiopian patients attending to Al Salam Clinic

3.4 Study Period

The study was conducted during the period from April to June2015

3.5 sample size

A total of 90(45male+45 female) blood samples were collected from Ethiopians

3.6 Sample processing

All the collected blood samples were tested for presence of anti-HBV and HCV IgG antibodies using the commercially available ELISA kit(Fortress diagnostic)

3.7 Inclusion criteria

All Ethiopian attended Al salam clinic with symptoms of the diseases

3.8Exclusion criteria

Ethiopians showing no signs of infection or with diseases other than hepatitis

3.9 Data collection

Personal data were obtained by direct interviewing questionnaire (appendix)

3.10 Ethical consideration:

Permission to carry out the study was taken from the college of Graduate Studies ,Sudan University of Science & Technology .All subjects examined were informed for the purpose of the study before collection of the specimens and consent was taken from them.

3.11 laboratory work:

All serum samples were analyzed for specific anti-HBV and HCV IgG using enzyme -linked immunosorbent assay(ELISA)(Fortress Diagnostic).

Three mls of venous blood was drawn from patients after disinfection of the area using 70% alcohol, the blood sample were left for 30 min to clot and then centrifuged at 3000 r.p.m for 10 min, the serum sample were then separated into plain container and stored at -20℃ until used.

3.11.1.1 ELISA for detection HBsAg:

3.11.1.2 Principle of assay

The test is enzyme-immunoassay based on a (sandwich) principle .polystrene microtiter strip well have been coated with monoclonal anti-HBs(antibody to HBsAg).patient 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 solid phase .After washing to remove sample serum proteins second antibody conjugated to enzyme HRP and direct against a different epitope of HBsAg is added to the wells. During the second incubation step these

HRP conjugate antibodies will be bound to any anti-HBs-HBsAg complex previously formed during the first incubation and unbound HRP conjugate is then removed by washing after washing chromogen solutions containing TMB and Urea peroxidase are added to wells .in presence of the antibody-antigen-antibody HRP sandwich immune-complex, the colourless chromogen are hydrolyzed by the bound HRP conjugate to blue coloured product. The blue colour turn yellow after stopping the reaction using the stop solution the colour intensity can be measured and it proportional to amount in the sample respectively .Well containing sample negative for HBsAg remain colourless

3.11.1.3 Procedure

Step1-Reagent preparation

The reagent and sample were allowed to reach room temperature(18-30℃ for 15-30 minutes.

The stock wash buffer had been diluted **1**to**20** with distilled water.

Step2- Numbering of Wells

The strip needed were set in strip holder and sufficient number of wells including one blank(A1) three negative control(B1,C1,D1)and two well as positive control(E1) were numbered.

Step 3-Adding Sample Diluent:

Add twenty ul of sample Diluent to each well except the blank and mix by taping the plate gently

Step 4 adding sample

hundred ul of positive control, negative control and specimen were added into their respective wells except blank.

Step 5 Incubation

The plate was covered with the plate cover and incubated for 60 minutes at 37℃

Step 6 Added HRP Conjugate:

Then 50ml of HRP-conjugate were also added to each well except to blank well and mixed by tapping the plate gently.

Step 7- Incubation

The plate was covered with the plate cover and incubated for 30 minutes at 37℃

Step 8 washing

After the end of the incubation the plate cover was removed ,each well was washed 5 time with diluted washing buffer each time the well were allowed to soak for 30-60 second. after the washing end the plate turn was down onto blotting paper to remove any remainders.

Step9- Coloring

Fifty ul of chromogen A and 50ul of chromogen B were added into each well including blank and mixed by tapping plate. The plate was Incubated at 37℃ for15 minutes with avoiding light. Blue color was developed in positive control and HBV IgG positive sample wells.

Step10- stopping the reaction

Fifty ul of stop solution were added into each wells and mixed gently intensive yellow color was developed in positive control and HBV IgG positive Sample Wells.

STEP11 –Measuring the Absorbance

The absorbance was read at 450nm using the ELISA reader.

3.11.1.4 calculation of the result

The result were calculated by relating each specimen absorbance(A) value to cut-off value(C.O) of the plate.

Calculation of cut-off value

(C.O)=NC×2.1 NC(the mean absorbance value to three negative controls

Quality control range

The A value of blank well which contains only chromogen and stop solution should be less than 0.080 at 450nm

The A value of positive control must be more than or equal 0.800 at 450nm

The A value of negative control must be less than 0.100 at 450 nm

3.11.1.5 Interpretation of the results

Negative Results

Sample giving A value less than cut-off value are negative for this assay which indicate that no HBV IgG antibodies have been detected with this HBsAg ELISA kits therefore the patient is probably not infected with hepatitis B virus.

Positive Results:

Samples giving A value greater than or equal to cut-off value are considered initially reactive which indicate that HBV surface antigen has probably been detected with this HBsAg ELISA kit.

Borderline

Sample with A value to cut-off ratio between 0.9 and 1.00 are considered borderline samples And retest is recommended. repeatedly positive sample can be considered positive for HBsAg.

3.11.2.1 ELISA for detection of anti HCV IgG

3.11.2.2 Principle

This Kit employs solid phase, indirect ELISA method for detection of antibodies to HCV in two step incubation procedure.Polystrene microwell strips are pre-coated with recombinant,highly immunoreacctive antigens corresponding to the core and the nonstrual region of HCV (fourth generation ELISA) .During the first incubation step,anti-HCV specific antibodies if present ,will be bound to solid phase pre-coated HCV antigens.The well are washed to remove unbound serum proteins,and rabbit anti-human IgG antibodies(anti-IgG) conjugated to horseradish peroxidase(HRP-conjugate)is added.During the second incubation step,these HRP-conjugate antibodies will be bound to any antigen-antibody(IgG) complex previously formed and the unbound HRP-conjugateis then removed by washing. Chromogen solutions containing Tetramethylbenzidine(TMB)and urea peroxidase are added to the wells and in presence of the antigen-antibody-anti-IgG(HRP) immunocomplex the colorlees chromogen are hydrolyzed by bound HRP conjugate

to blue –colour product .The blue colour turn yellow after stopping the reaction with sulphuric acid .The amount of colour intensity can be measured and is proportional to amount of antibody captured in the wells and the sample respectively. Wells containing samples negative for anti-HCV remain colourless.

3.11.2.3 Procedure

Step1-Reagent preparation

the reagent and sample were allowed to reach room temperature(18-30°C for 15-30 minutes.

The stock wash buffer had been diluted **1**to**20** with distilled water.

Step2- Numbering of wells

The strip needed were set in strip holder and sufficient number of wells including one blank(A1) three negative control(B1,C1,D1)and two well as positive control(E1) were numbered

Step 3-Adding diluent

Hundred ml of specimen diluents were added into each well except blank well

Step 4-Adding sample

Ten ml of samples, positive and negative control were added into their respective well except blank well.

Step 5- Incubation

The plate was cover with the plate cover and incubated for 30 minutes at 37℃

Step 6- washing

After the end of the incubation the plate cover was removed ,each well was washed Five times with diluted washing buffer each time and the wells were allowed to soak for 30-60 second. After the washing end the plate turn down onto blotting paper to remove any remainders

Step 7- Adding conjugate

Hundred ul of HRP-conjugate were added to each well except to blank well.

Step 8- Incubation

The plate was covered with the plate cover and incubated for 30 minutes at 37℃

Step 9- washing

After the end of the incubation the plate cover was removed ,each well was washed 5 times with diluted washing buffer each time the well were allowed to soak for 30-60 second. after the washing end the plate was turn down onto blotting paper to remove any remainders

Step10- Coloring

Fifty ml of chromogen A and 50ml of chromogen B were added into each well including blank and mixed by tapping plate.

Step 11 Incubation

the plate was incubated at 37℃ for 15 minutes, avoiding light. Blue colour was developed in positive control and HBV IgG positive sample wells.

Step12- stopping the reaction

Fifty ul of stop solution were added into each well and mixed gently intensive yellow color was developed in positive control and HBV IgG positive sample well

STEP13 –Measuring the Absorbance

The absorbance was read at 450nm using the ELISA reader.

3.11.2.4 Calculation of the results

The result were calculated by relating each specimen absorbance(A) value to cut-off value(C.O) of the plate.

Calculation of cut-off value:

(C.O)=NC+0.12 NC(the mean absorbance value to three negative controls

Quality control range:

The A value of blank well which contains only chromogen and stop solution should be less than 0.080 at 450nm

The A value of positive control must be more than or equal 0.800 at 450nm

The A value of negative control must be less than 0.100 at 450 nm

3.11.2.5 Interpretation of the results

Negative Results:

Sample giving A value less than cut-off value are negative for this assay which indicate that no HBV IgG antibodies have been detected with this HBsAg ELISA kits therefore the patient is probably not infected with hepatitis B virus.

Positive Results:

Samples giving A value greater than or equal to cut-off value are considered initially reactive which indicates that HBV surface antigen has probably been detected with this HBsAg ELISA kit.

Borderline

Sample with absorbance o.d≤cut-off×2 are considered borderline and retesting of those samle in duplicates is recommended .Repeatedly positive samples could be considered positive for HCV infection

3.11.3.Data Analysis

Collected data were analysis by a computer system using statistic package for social sciences(SPSS) program using the chi-square test statistical significance was set at p. values<0.05.

CHAPTER FOUR

Chapter Four

4. Results

4.1.Seroprevelance of HBsAg and HCV among Ethiopians

As shown in Fig.4.1. 36/90 of Ethiopian patient were HBV positive

In Fig 4.2. 23/90 of them were HCV positive

4.2. Distribution of gender group among positive results

The result revealed that out of 45 females 13/45(28.9%)were positive and out of 45 males10/45(22.2%) were positive of HCV .For HBsAg 19/45(42.2%) were positive of females and 17/45(37.7%) males were positive

4.3.Distibution of age group among positive results

Table4.3 displays that distribution of age group among positive results ,it was found that 15(16.6%)were positive for HBsAg and 13(14.4%) for HCV among age group(15-25)years. 19(21.1%) were positive for HBsAg and 9(10%)for HCV among age group (26-35)years and 2(2.2%) positive for HBsAg and 1(1.1%) for HCV among age group (36-45)years

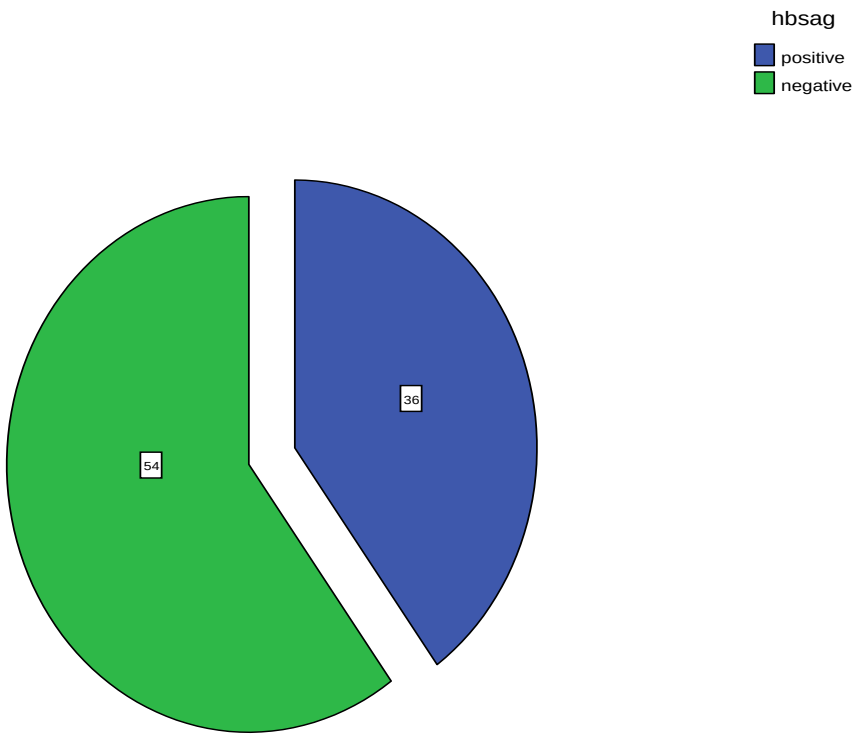


Fig4.1..Frequency of HBsAg among Ethiopian patients

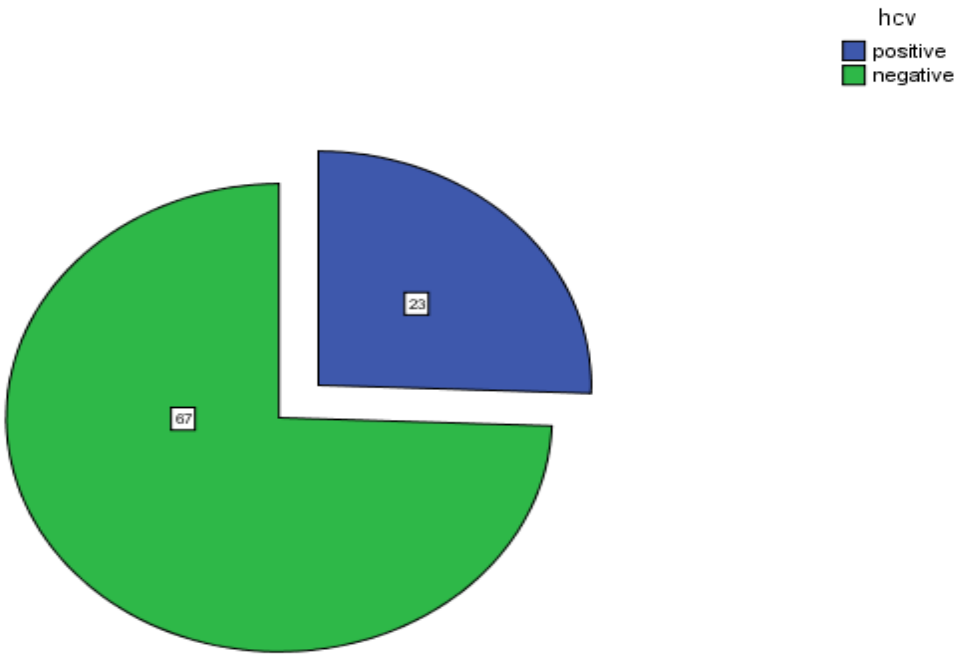


Fig 4.2.Freqency of HCV Among Ethiopian Patients

Table4.3 Frequency of HBV and HCV Among Ethiopian According

Age group	HBV-IgG				HCV-IgG			
	POSITIVE		NEGATIVE		POSITIVE		NEGATINE	
	NO	%	NO	%	NO	%	NO	%
15-25	15	16.6	37	41.1	13	14.4	36	40
26-35	19	21.1	15	16.6	9	10	28	31.1
35-45	2	2.2	2	2.2	1	1.1	3	3.3
TOTAL	36	40	54	60	23	25.5	67	74.4

CHAPTER FIVE

Chapter five

Discussion

5.1 Discussion

The result revealed that there was high prevalence of HBV and HCV 36(40%)and 23(25%) respectively and also there was association between this high seroprevalence rate and age group.

The present result also reveals lower prevalence of HBsAg than that reported in Sudan by Mudawi(2008) who found that hepatitis B virus seroprevelence ranges from 47%-78% .With HBsAg prevalence varying from 6.8% in central Sudan to 26% in Southern Sudan. Studies pointed to infection in early childhood in Southern Sudan while there was a trend of increasing age in Northern Sudan. Hepatitis B virus was the commonest cause of chronic liver disease and hepatocelluler carcinoma and was the second commonest cause of acute liver failure in Sudan.

This result disagreed with that result reported by Taye and Abdulkerim(2014) who found that the overall prevalence of HBV and HCV among Ethiopian patients in Ethiopia were 22.3% and 3.6% respectively. The result of this study revealed that prevalence of HBV and HCV was higher in females19(21.1) 13(14.4) respectively and this again disagree with result of Taye and Abdulkerim(2014) which shows that the prevalence of HBV and HCV among males from the total HBV and HCV screening was 52/358(14.5%) and 6/220(2.7%)respectively which is higher than female . However among age group it was found that HBsAg prevalence was high in age group26-35(21%) and for HCV in age group 15-25(14,4) which is disagree to Taye and Abdulkerim(2014) result

This result also differ from Gasim (2011) result which was conducted to investigate the seroprevelance of HBV(HBsAg) and anti-HCV among hemodialysis patient at Ahmed Gasim hemodialysis unit .Of 353 patient enrolled in study ,HBsAg and anti-HCV were detected in 16(4.5%) and 30(8.5%) patients respectively. None of patient were co-infected with HBV and HCV.

Also differ from that result of Tessema(2010) in which a total of 6361 blood doner were screened at Gonder University Technical Hospital blood bank for HBV and HCV.The results showed that 769(12.1%) were HBV positive and 480(7.5%) were HCV positive.

These differences may be attributed to their high sample size and difference in environment , localities and social factors.

5.2 Conclusion

The study concluded that 25%,40% of 90 Ethiopian patient were HCV , HBsAg positive respectively. Which reveals that HBV and HCV have high prevalence rate in Ethiopians and the infections had high rate among young age.

5.3 Recommendations

1. HBsAg & HCV testing should be performed by health authority to Ethiopian people when arrive Sudan and prevent positive one from inter to reduce spread of infection

2. Increase awareness of HBsAg and HBV infection among peoples

3. The use of advanced techniques for diagnosis of virus like PCR.

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Appendix

Appendix

QUESTIONNAIRE

Name:…….

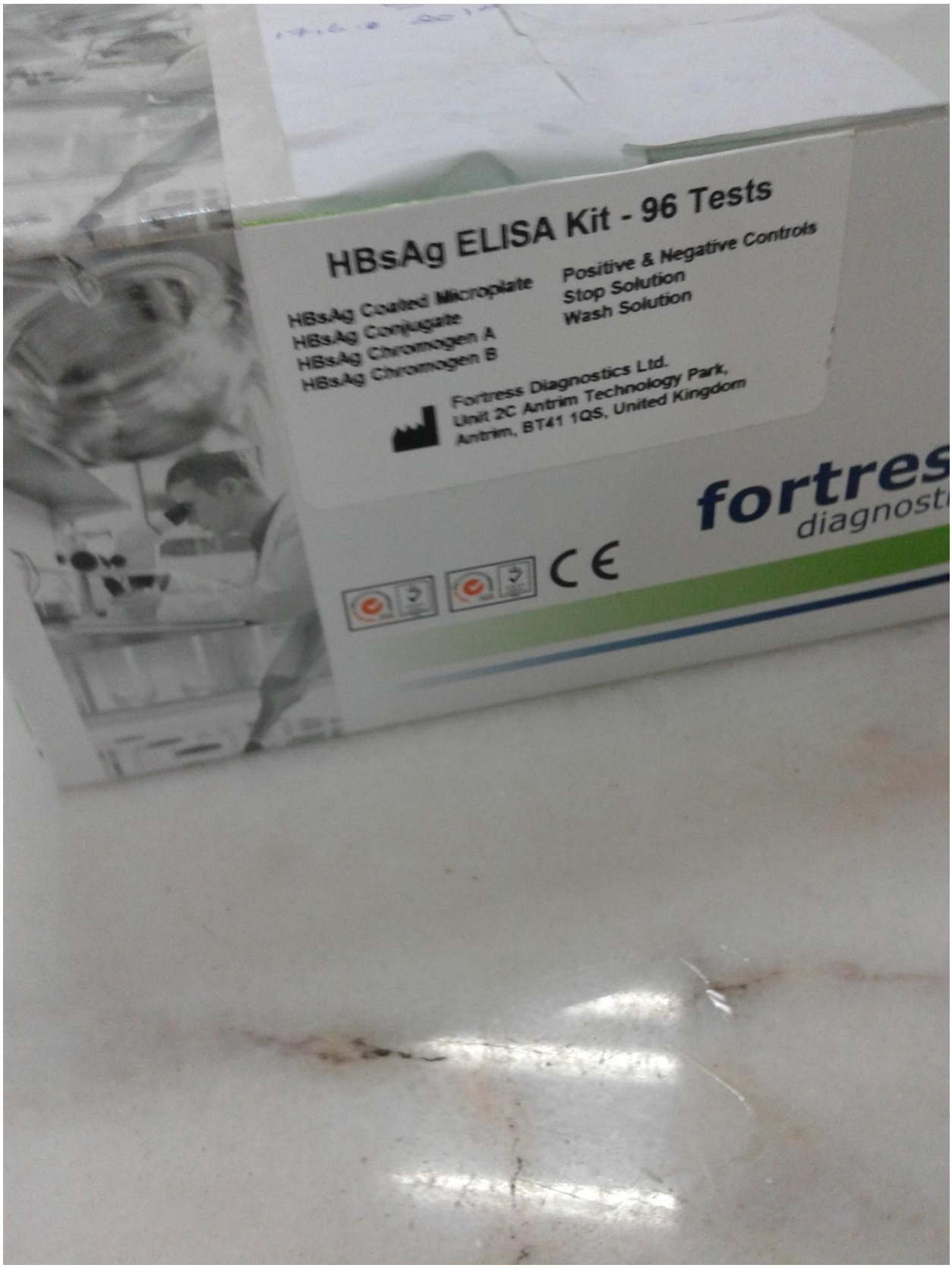
Age:……..

gender:……..

Occupation:……..

Previous infection:

Yes……No…….


HBsAg ELISA Kit - 96 Tests
HBsAg Coated Microplate
HBsAg Conjugate
HBsAg Chromogen A
HBsAg Chromogen B
Positive & Negative Controls
Stop Solution
Wash Solution
Fortress Diagnostics Ltd.
Unit 2C Antrim Technology Park,
Antrim, BT41 1QS, United Kingdom
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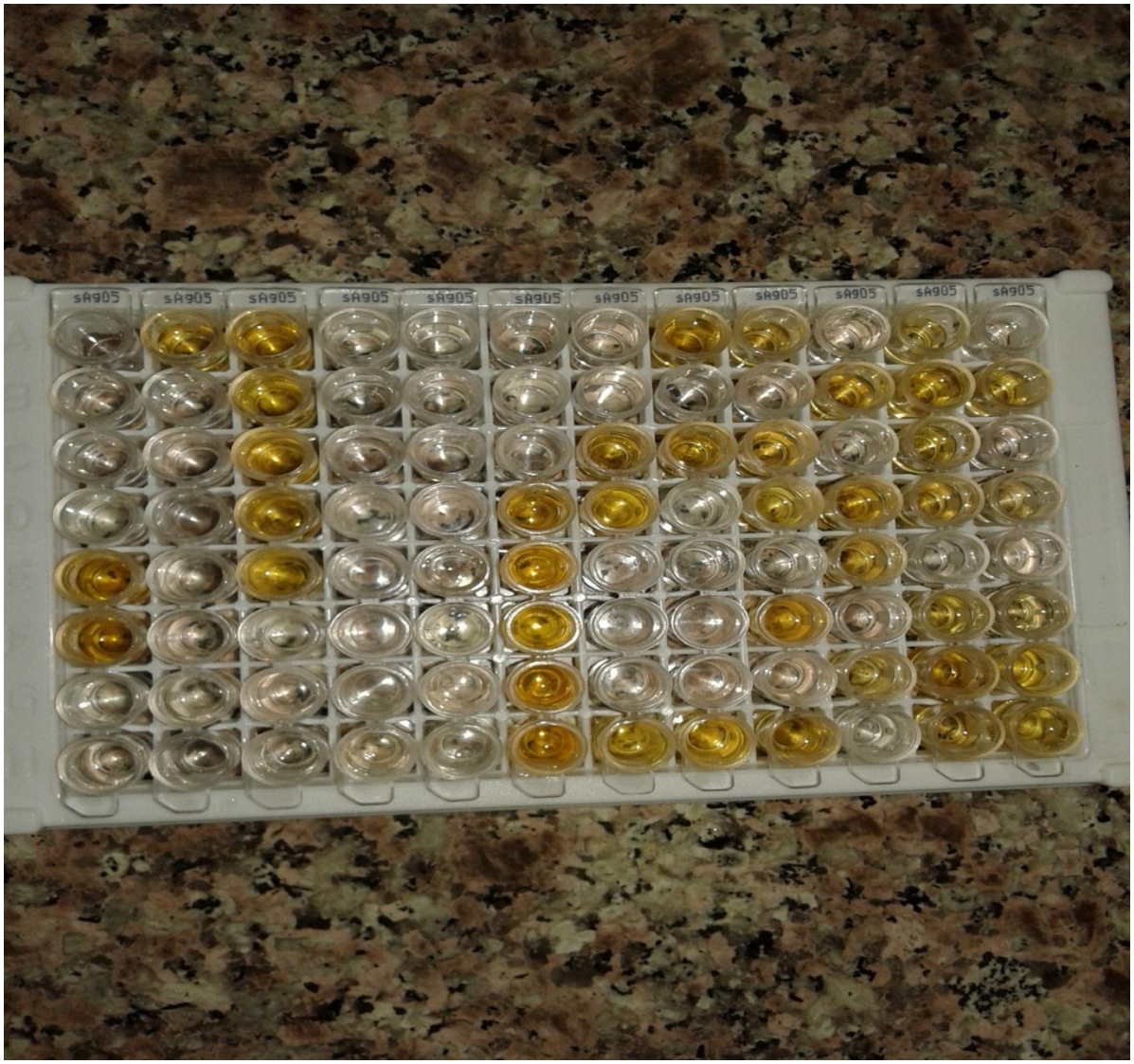
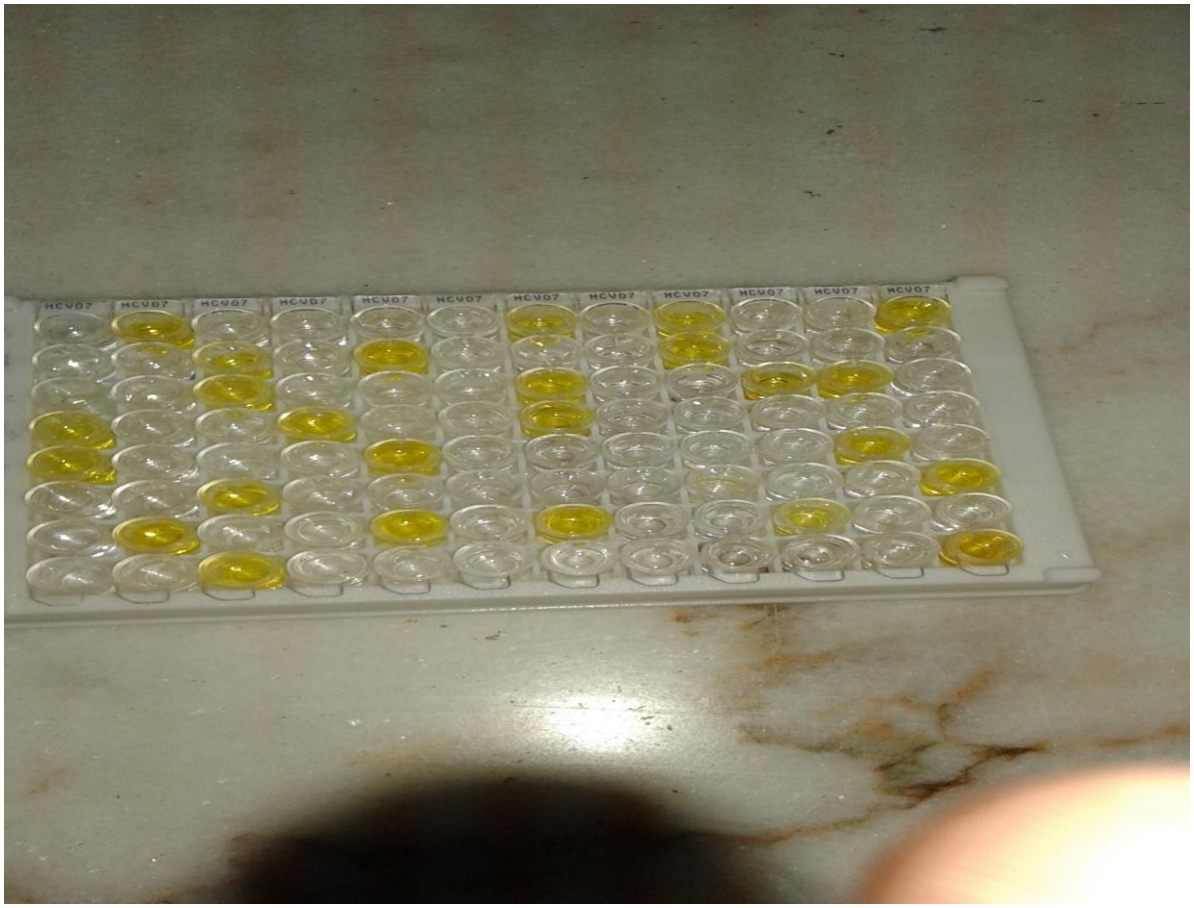
HBsAg ELISA Kits

h 

HBsAg IgG Color Plate



HCV IGg Color Plate

fortress diagnostics

ISO 13485 accredited company

BXEO781A

96 TESTS

STORE AT 2-8°C

FOR IN-VIRTO DIAGNOSTICS USE ONLY

ANTI-HCV (4th Generation)

ELISA

Intended Use:

Fortress HCV kit is an enzyme-linked immunosorbent assay for qualitative detection of antibodies to hepatitis C virus in human serum or plasma. It is intended for screening blood donors and diagnosing patients related to infection with hepatitis C virus.

Summary:

Hepatitis C virus (HCV) is an envelope, single stranded positive sense RNA (9.5 kb) virus belonging to the family of Flaviviridae. Six major genotypes and series of subtypes of HCV have been identified. Isolated in 1989, HCV is now recognized as the major cause for transfusion associated non-A, non-B hepatitis. The disease is characterized with acute and chronic form although more than 50% of the infected individuals develop severe, life threatening chronic hepatitis with liver cirrhosis and hepatocellular carcinomas. Since the introduction in 1990 of anti-HCV screening of blood donations, the incidence of this infection in transfusion recipients has been significantly reduced. The first generation of HCV ELISAs showed limited sensitivity and specificity and was produced using recombinant proteins complementary to the NS4 (c100-3) region of the HCV genome as antigens. Earlier generation tests, which included recombinant / synthetic antigens from the Core (c22) and nonstructural regions NS3 (c33c, c100-3) and NS4 (c100-3, c200) resulted in a remarked improvement in sensitivity and specificity. Clinical studies show that significant amount of HCV infected individuals develop antibodies to NS5 non-structural protein of the virus. For this, the forth generation tests include antigens from the NS5 region of the viral genome in addition to NS3 (c200), NS4 (c200) and the Core (c22). Forth generation tests have improved sensitivity and shorten the time between infection with HCV and the appearance of detectable antibodies.

Principle of the Assay:

This kit employs solid phase, indirect ELISA method for detection of antibodies to HCV in two-step incubation procedure. Polystyrene microwell strips are pre-coated with recombinant, highly immunoreactive antigens corresponding to the core and the non-structural regions of HCV (Fourth generation HCV ELISA). During the first incubation step, anti-HCV specific antibodies, if present, will be bound to the solid phase pre-coated HCV antigens.

The wells are washed to remove unbound serum proteins, and rabbit anti-human IgG antibodies (anti-IgG) conjugated to horseradish peroxidase (HRP-Conjugate) is added. During the second incubation step, these HRP-conjugated antibodies will be bound to any antigen-antibody (IgG) complexes previously formed and the unbound HRP-conjugate is then removed by washing. Chromogen solutions containing Tetramethylbenzidine (TMB) and urea peroxide are added to the wells and in presence of the antigen-antibody-anti-IgG (HRP) immunocomplex: the colorless Chromogens are hydrolyzed by the bound HRP conjugate to a blue-colour product. The blue colour turns yellow after stopping the reaction with sulphuric acid. The amount of colour intensity can be measured and is proportional to the amount of antibody captured in the wells, and to the sample respectively. Wells containing samples negative for anti-HCV remain colourless.

Assay principle scheme: Indirect ELISA

Ag(p)+Ab(s)→[Ag(p)–Ab(s)+ENZ]→ [Ag(p)–Ab(s)–ENZ] →blue→yellow (+) Ag (p)+→ [Ag (p) +ENZ] → [Ag (p)] – no colour (-)

Incubation 1 Incubation 2 Immobilized Complex Colouring results

30min. 30min. 15min

Ag (p) – pre-coated HCV antigens(core, NS3/4,NS5);

Ab (s) – HCV antibodies in sample (IgG);

ENZ – HRP conjugated rabbit anti-human IgG;

Kit Contents	Volume
Microwell Plate 96 Tests	1 plate (8x12/12x8 well strips per plate)
Negative Control	1 x 0.2ml
Positive Control Serum 1	1 x 0.2ml
Specimen Diluent	1 x 13ml
HRP – Conjugate Reagent	1 x 13ml
Stock Wash Buffer	1x 50ml (Dilute 1 to 20 with distilled water before use. Once diluted, stable for two weeks at 2-8°C)
Chromogen Solution A	1 x 7ml (Ready to use and once open, stable for one month at 2-8°C)
Chromogen Solution B	1 x 7ml (Ready to use, once open, stable for one month at 2-8°C)
Stop Solution	1 x 7ml
Plastic Sealable Bag	1 Unit
Plate Cover	1 Sheet
Package Inserts	1 Copy

Additional Materials And Instruments Required But Not Provided:

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

Specimen Collection, Transportation And Storage:

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 haemolysis 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 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, 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:

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 5cycles, dispensing 350-400µl/well and aspirating the liquid for 5times. If poor results (high background) 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 50 ml of the concentrate with 950ml of water for a final volume of 1000ml 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 anti-HCV ELISA kit, during storage protect the reagents from contamination with microorganism or chemicals.

Precautions and Safety:

The 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°C 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 1 hour 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 by dust, reactive chemicals, 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:

Step 1 - 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, re-solubilize 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 Wash buffer.

Step 2 - Numbering Wells:

Set the strips needed in strip-holder and number sufficient number of wells including three Negative control **(e.g. B1, C1, D1)**, two Positive control **(e.g. E1, F1)** and one Blank **(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.

Step 3 - Adding Diluent:

Add **100µl** Specimen Diluent into each well except the blank.

Step 4 - Adding Sample:

Add **10µl** of Positive control, Negative control, and Specimen into their respective wells. **Note: Use a separate disposal pipette tip for each specimen, Negative and Positive Control as to avoid cross-contamination.** Mix by tapping the plate gently.

Step 5 - Incubating (1):

Cover the plate with the plate cover and incubate for **30 minutes at 37°C.** It is recommended to use thermostat-controlled water tank to assure the temperature stability and humidity during the incubation. If dry incubator is used, do not open the door frequently.

Step 6 - Washing (1):

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

Step 7 - Adding HRP-Conjugate:

Add **100µl** HRP-Conjugate to each well except the Blank.

Step 8 - HRP-Conjugate Incubating (2):

Cover the plate with the plate cover and incubate for **30 minutes at 37°C.**

Step 9 - Washing (2):

At the end of the incubation, remove and discard the plate cover. Wash each well **5**times with diluted Wash buffer as in **Step6.**

Step 10 - 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 15 minutes avoiding light.** The enzymatic reaction between the Chromogen A/B solutions produces blue colour in Positive control and anti-HCV positive sample wells.

Step 11 - Stopping Reaction:

Using a multichannel pipette or manually, add **50µl** Stop Solution into each well and mix by tapping the plate gently. Intensive yellow colour develops in Positive control and anti-HCV positive sample wells.

Step 12 - 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 and Quality Control:

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 relating 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 controls. 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 controls

1. Calculation of Cut-off value (C.O.) = *Nc + 0.12

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

Important: If the mean OD value of the negative control is lower than 0.02, take it as 0.02. If higher than 0.02 see the Quality control range.

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 at 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 absorbance less than the Cut-off value are negative for this assay, which indicates that no antibodies to hepatitis C virus have been detected with this anti-HCV ELISA kit. Therefore, the patient is probably not infected with HCV.

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 antibodies to hepatitis C virus have probably been detected using this anti-HCV ELISA kit. Retesting in duplicates of any initially reactive sample is recommended. Repeatedly reactive samples could be considered positive for antibodies to HCV and therefore the patient is probably infected with hepatitis C virus. Blood unit positive for HCV antibodies should be immediately discarded.

Borderline: Samples with absorbance **O.D.≤Cut-off ×2** are considered borderline and retesting of those samples in duplicates is recommended. Repeatedly positive samples could be considered positive for hepatitis C virus infection.

Follow-up and supplementary testing of any anti-HCV positive samples with other analytical system (e.g. RIBA, WB) is required to confirm the diagnosis.

Test Performance And Expected Results:

Clinical Specificity: A blood donor population of 2948 individuals was tested with 3 different kits from different manufacturers. The specificity of this anti-HCV ELISA kit was 99.55%.

Manufacturer	-ve	+ve	False Pos	Specificity
Manufacturer 1	2896	38	14	99.52
Manufacturer 2	2895	38	15	99.48
Anti HCV Elisa Fortress	2897	38	13	99.55

Clinical Sensitivity: Among 480 clinical hepatitis C patients confirmed positive 3.0 of 479 were positive when tested with this anti-HCV EISA kit. The sensitivity was 99.79%.

Analytical Specificity:

- No cross reactivity observed with samples from patients infected with HAV, HIV, HBV, CMV, and TP. No interference was observed from rheumatoid factors up to 2000U/ml.
- This assay performance characteristics are unaffected from elevated concentrations of bilirubin, haemoglobin, and triolein.
- Frozen specimens have been tested to check for interferences due to collection and storage.

Reproducibility:

Reproducibility		Within Run		Between Run	
Sample	No	Mean OD	CV%	Mean OD	CV%
Weak Positive	10	0.436	9.1	0.401	9.5
Moderate Positive	10	0.946	7.0	0.916	7.5
Strong Positive 1	10	1.917	4.4	1.895	4.2
Strong Positive 2	10	2.372	3.8	2.309	4.0

HCV Genotype Antibody Testing:

1. Non-repeatable positive results may occur due to the general biological characteristics of ELISA assays. The assay is design to achieve very high performance characteristics of sensitivity and specificity and the "indirect model" minimizes the unspecific reactions, which can occur due to interference between unknown meters in sample and the rabbit anti-human IgG used as a conjugate. Antibodies may be undetectable during the early stages of the disease and in some immunosuppresed individuals.

GENOTYPE	SAMPLES	POSITIVE
1 a-b	15	15
2 a-b	13	13
3 a-b	10	10
4th	6	6
5	12	12
6	18	18
TOTAL	74	74

2. Positive results must be confirmed with another available method. Any positive result must be interpreted together with the patient clinical information and other laboratory results.
3. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, volumes, sample nature and quality.
4. 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 the several reasons, most of which are related but not limited to inadequate washing step. The prevalence of the marker will affect the assay's predictive values.
5. 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.

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. Alter H.J. (1978) You will wander where the yellow went: A 15-year retrospective of post transfusion hepatitis. In: Moore SB. ed. Transfusion-Transmitted Viral Diseases. Alington, VA. Am. Assoc. Blood Banks, pp. 53-38.
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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 peroxidise are added to the wells. In presence of the antibody-antigen-antibody HRP sandwich immune-complex, the colourless 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 0.22u filters. Plasma samples collected into EDTA, sodium citrate or heparin may be tested, but highly lipaemic, 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 5cycles, dispensing 350-400µl/well and aspirating the liquid for 5times. If poor results (high background) 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

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distilled or deionized water. Use only clean vessels to dilute the buffer.

Step2 Numbering Wells:

Set the strips needed in strip-holder and sufficient number of wells including three Negative controls(e.g.B1,C1,D1), two Positive Control(e.g.E1,F1) and one Blank(e.g>E1,F1) 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 taping 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 tank 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 plate 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. Intensive 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 relating 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 controls. 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 controls.

1. Calculation of Cut-off value

Cut-off value (C.O.) = *Nc x 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.

Exaample:

1.Calculation of Nc:

Well No	B1	C1	D1
Negative conttrols DD value	0.02	0.012	0.016

Nc= 0.016 (the Nc Value is lower than 0.05 so take it as(0.05

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 at 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
	18	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 2000U/ml observed.
3. No high dose hook effect up to HBsAg concentrations of 200000ng/ml observed during clinical testing.
4. Frozen specimens have been tested too to check for interferences 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-repeatable positive result may occur due to the general biological and biochemical characteristics of ELISA assays. The test is design to achieve 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 the several reasons, most of which are related but not limited to inadequate washing step.
3. .Any positive results must be interpreted in conjunction with patient clinical information and other laboratory testing results.
4. Common sources for mistakes: kits beyond the expiry date, bad washing procedures, contaminated reagents, incorrect assay procedure steps, insufficient aspiration during washing, failure to add samples or reagents, equipment, timing, 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. Riss, New York, N.Y. 142. Stevens, C. E., P. E. Taylor, M. J. Tong, P. T. Toy, G. N. Vyas, P. V. Nair,

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