

**EVALUATION OF INDETERMINATE HBsAg RESULTS
IN BLOOD DONORS AT SIRIRAJ HOSPITAL**



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**A THESIS SUBMITTED IN PARTIAL FULLFILMENT
OF THE REQUIREMENTS FOR
THE DEGREE OF MASTER OF SCIENCE (MICROBIOLOGY)
FACULTY OF GRADUATE STUDIES
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entitled
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EVALUATION OF INDETERMINATE HBsAg RESULTS IN BLOOD DONORS AT SIRIRAJ HOSPITAL

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THESIS ADVISORY COMMITTEE: KAMOL SUWANNAKARN, Ph.D.,
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Hepatitis B surface antigen (HBsAg) is one of the infectious screening markers, which is mandatory to be tested for every donated blood. The reactive result of HBsAg need to be confirmed before permanently deferred donor. The neutralization test is the one of confirmatory test for HBsAg. But this test is not feasible in every routine lab, due to additional cost consumption and extra effort resource. The aim of this study is to evaluate the neutralization confirmatory test, and create the algorithm for using this test in repeatedly reactive HBsAg blood sample in Thai blood donor with other hepatitis B virus markers such as hepatitis B core antibody (anti-HBc) and hepatitis B surface antibody (anti-HBs).

525 HBsAg repeatedly reactive samples were included in this study. All samples were retrieved from repository samples of blood donor during January 2014 to May 2016 from blood bank, Siriraj Hospital. These samples were tested by HBsAg neutralization confirmatory test, anti-HBc and anti-HBs. Sample were categorized into 3 group: low signal (S/CO 1.00-9.99), medium high signal (S/CO 10.00-299.99), and high signal (S/CO \geq 300). These groups were positive by using HBsAg neutralization confirmatory test for 27.69% (18/ 65), 90.63% (29/32) and 100 % (428/428) respectively. The other HBV markers were using to support neutralization confirmatory test. Samples with both negative anti-HBc and anti-HBs were positive neutralization confirmatory test for 10.64% (5/47). Samples with both positive anti-HBc and anti-HBs were positive neutralization confirmatory test for 66.67% (14/21). Sample with only positive anti-HBc was positive neutralization confirmatory test for 99.78% (456/457). None of sample with only positive anti-HBs was positive neutralization confirmatory test (0/9).

In conclusion, the samples with high signal (S/CO \geq 300) showed 100% positive neutralization confirmatory test, and suggested that this group may not need to use neutralization confirmatory test to confirm HBsAg result. The sample with low signal (S/CO 1.00-9.99), medium high signal (S/CO 10.00-299.99) may need to use anti-HBc to support. If anti-HBc showed negative in these groups, the neutralization confirmatory test may suggest to use as a confirm HBsAg testing.

KEY WORDS: HBsAg CONFIRMATORY TEST/NEUTRALIZATION/BLOOD SCREENING TEST/LOW REACTIVE HBsAg

69 pages

การประเมินผลการตรวจ HBsAg ที่ให้ผลไม่ชัดเจนในผู้บริจาคโลหิตของโรงพยาบาลศิริราช
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บทคัดย่อ

โปรตีนที่อยู่บนผิวของไวรัสตับอักเสบบี (HBsAg) เป็นเครื่องหมายบ่งชี้การติดเชื้อไวรัสตับอักเสบบี (HBV) จึงต้องทำการตรวจในผู้บริจาคเลือดทุกราย ในกลุ่มที่ให้ผลบวกต่อ HBsAg จำเป็นต้องได้รับการยืนยันก่อนที่จะทำการตัดสินใจปฏิเสธการรับบริจาคอย่างถาวร การตรวจ HBsAg ด้วยวิธีการ neutralization เป็น confirmatory test อย่างหนึ่งที่ถูกนำมาใช้ แต่เนื่องจากการตรวจนี้ยังไม่เหมาะที่จะใช้ในทุกห้องปฏิบัติการอันเนื่องมาจากราคา และทรัพยากรที่เพิ่มขึ้นในการตรวจ การศึกษาในครั้งนี้ จึงเป็นการศึกษาเพื่อประเมินผลการใช้ confirmatory test ในกลุ่มตัวอย่างเลือดของผู้บริจาคไทยที่ให้ผลบวกต่อ HBsAg รวมกับการใช้ marker ของไวรัสตับอักเสบบี อื่นเช่น hepatitis B core antibody (anti-HBc) และ hepatitis B surface antibody (anti-HBs)

ตัวอย่าง 525 ตัวอย่างที่ให้ผลบวกถูกใช้ในการศึกษานี้ ตัวอย่างทั้งหมดเป็นตัวอย่างที่เหลือจากงานบริการของงานบริจาคโลหิตโรงพยาบาลศิริราชในช่วง มกราคม พ.ศ. 2557 จนถึง พฤษภาคม พ.ศ. 2559 ตัวอย่างทั้งหมดถูกนำมาทดสอบด้วย HBsAg confirmatory test ตรวจหา anti-HBc และตรวจหา anti-HBs ตัวอย่างทั้งหมดถูกแบ่งออกเป็น 3 กลุ่ม ได้แก่ กลุ่มที่ให้ผลบวกต่ำ (S/CO 1.00-9.99) กลุ่มที่ให้ผลบวกปานกลางถึงสูง (S/CO 10.00-299.99) และกลุ่มที่ให้ผลบวกสูง (S/CO \geq 300) โดยตัวอย่างกลุ่มเหล่านี้พบว่าให้ผลบวกด้วยวิธี confirmatory test เป็น 27.69% (18/65), 90.63% (29/ 32) และ 100 % (428/428) ตามลำดับ เมื่อเปรียบเทียบกับ marker ของไวรัสตับอักเสบบี อื่นร่วมด้วย พบว่า ตัวอย่างที่ให้ผลบวกทั้ง anti-HBc และ anti-HBs ให้ผลบวกต่อ confirmatory test คิดเป็น 10.64% (5/47) ตัวอย่างที่ให้ผลบวกทั้ง anti-HBc และ anti-HBs ให้ผลบวกต่อ confirmatory test คิดเป็น 66.67% (14/21) ตัวอย่างที่ให้ผลบวกเฉพาะ anti-HBc ให้ผลบวกต่อ confirmatory test คิดเป็น 99.78% (456/457) และไม่มีตัวอย่างใดที่ให้ผลบวกเฉพาะ anti-HBs จะให้ผลบวกต่อ confirmatory test (0/9)

สรุปผลการศึกษา พบว่าตัวอย่างกลุ่มที่ให้ผลบวกสูง (S/CO \geq 300) แสดงผล 100% ต่อการตรวจด้วยวิธี confirmatory test แสดงให้เห็นว่า ในตัวอย่างกลุ่มนี้อาจไม่จำเป็นต้องใช้ confirmatory test ในการยืนยันผล HBsAg ในกลุ่มตัวอย่าง ที่ให้ผลบวกต่ำ (S/CO 1.00-9.99) และกลุ่มที่ให้ผลบวกปานกลางถึงสูง (S/CO 10.00-299.99) อาจจำเป็นต้องมีผล anti-HBc ในการช่วย หากพบว่า anti-HBc ให้ผลบวก อาจแนะนำให้ใช้ confirmatory test ในการช่วยยืนยัน HBsAg ทำในกลุ่มนี้

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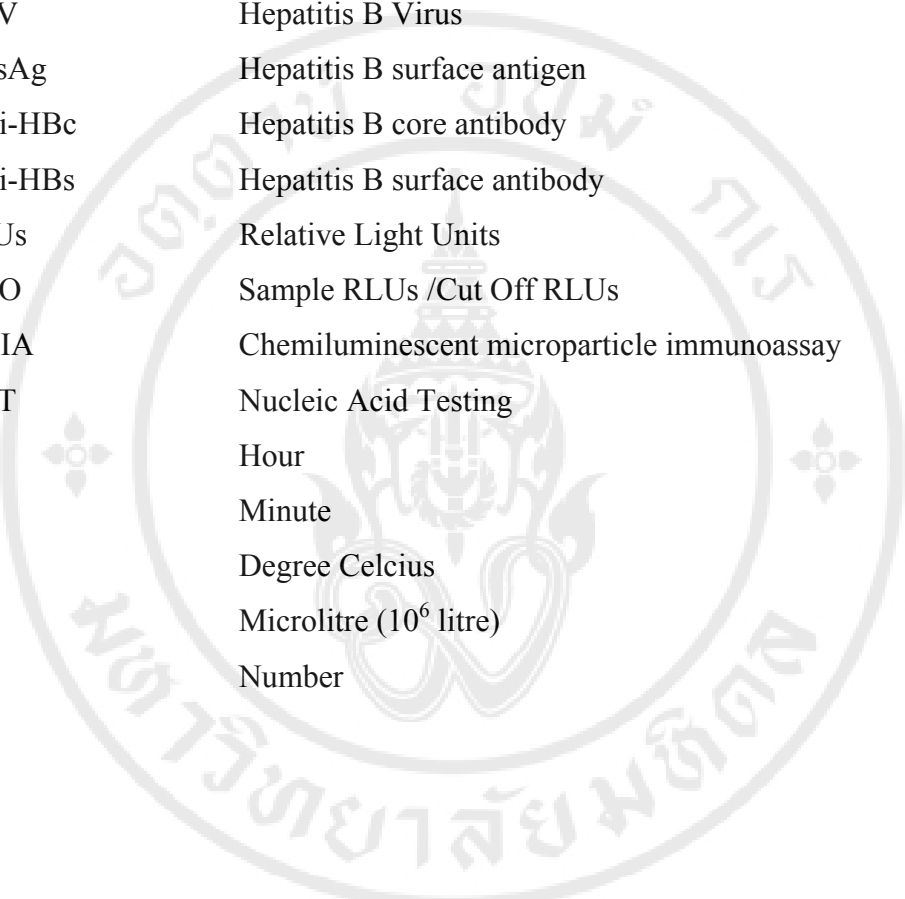
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LIST OF ABBREVIATIONS



HBV	Hepatitis B Virus
HBsAg	Hepatitis B surface antigen
Anti-HBc	Hepatitis B core antibody
Anti-HBs	Hepatitis B surface antibody
RLUs	Relative Light Units
S/CO	Sample RLUs /Cut Off RLUs
CMIA	Chemiluminescent microparticle immunoassay
NAT	Nucleic Acid Testing
hr	Hour
min	Minute
°C	Degree Celcius
ul	Microlitre (10 ⁶ litre)
no.	Number

CHAPTER I

INTRODUCTION

Hepatitis B virus (HBV) is the major etiologic agent of viral hepatitis. More than 350 million people are chronically infected worldwide (1). HBV infection can lead to progression of chronic liver disease, liver cirrhosis and hepatocellular carcinoma (2). There are 10 million new HBV infected cases per year (1) and about 1 million people annually die with HBV-related liver disease (3, 4, 5). High prevalence of HBV infection found in Southeast Asia, Asia, Africa, Latin America and Southern Europe (6,7). In 2010 HBV prevalence rate is reported as 9.23 per 100,000 in Thailand (8). However, from the study of Chimpalee N. and colleagues found that HBV prevalence rate in new Thai blood donors continuously decreased from 7.1% in 1988 to 2.6% in 2009 due to the success of expanded program on immunization (EPI) of HBV vaccine in newborn (2).

HBV belongs to family *Hepadnaviridae*, genus *Orthohepadnavirus*. Genome is a relaxed-circular partially double stranded DNA, about 3,200 base pairs (bp). It consists of 4 overlapping open reading frame (ORF) that encode the proteins namely *S* ORF for the surface protein (HBsAg), *C* ORF for nucleocapsid protein or core protein (HBcAg and HBeAg), *P* ORF for DNA polymerase/reverse transcriptase and *X* ORF for hepatitis B X protein (HBx) (3, 9, 10). Moreover, at the upstream of *S* and *C* ORF encode for pre-S and pre-C protein, respectively (3).

The structure of virion is composed of outer lipoprotein and surface protein that contains 3 parts, called the large, middle and small proteins (encode from *pre-S1*, *pre-S2* and *S* gene, respectively) (3, 5, 10). The viral nucleocapsid core contains viral DNA genome, HBcAg and DNA polymerase/reverse transcriptase (RNA-dependent DNA polymerase) (3, 10). This complete viral particle is called Dane particle (5), is a 42 nm in diameter in round shape. Moreover, there are incomplete viral particles that consist of only envelope and HBsAg (without viral genome), whose size is 22 nm in diameter (round shape) and 22 nm in width (rod

shape) (3, 10). In general, there are incomplete virions more than Dane particles by 1,000:1 to 10,000:1 ratio (10).

Transmissions of HBV are the exposures of infected blood or secretion due to HBV are found in serum and body fluids (2). Possible type of transmission are sexual contact, blood transfusion, re-use of contaminated needles and syringes, from mother to child during childbirth, and organ transplantation(2,3).

HBV can infect only humans and closely related species such as chimpanzees because the host range of HBV is very narrow and HBV is highly cell type specific (4, 11). Viral surface proteins particularly pre-S1 region bind to cellular receptors before nucleocapsid entry into target cells by using endocytotic pathway (5). Hepatocytes are the major target cells of this virus (4). The host cellular receptor is still unclear, however, there are evidences that the receptor is carboxypeptidase D (CPD) or glycoprotein 180 (gp180) by using the closely related duck hepatitis B virus (DHBV) as the model system (12).

The major interested characteristic of HBV is the replications of viral DNA come from reverse transcription of an RNA intermediate by using reverse transcriptase (10). HBV can entry and exit out of the cell without damaging cell membrane, so it is not directly cytotoxic to the cells (5, 10). Host immune response is the causative of hepatocellular injury (10).

Most of infected people are self-limiting, only 5-10% of infected adults that have incomplete clearance of virus and become chronic infection (13), which is the problem of blood transfusion (2). The screening of blood donors for HBV is HBsAg detection in sera (2) and nowadays, also detects HBV DNA by nucleic acid testing (NAT) to increase the safety of blood transfusion due to occult HBV infected cases (OBI), whose blood tests are positive for HBV DNA but negative for HBsAg (14). From the study of Louisirirothanakul S. and colleagues in 2007, rate of OBI in Thai blood donors was very high, 1 in 4232 (14). Globally rate of OBI is closely related with the HBV endemic in a region such as in low endemicity, the rate varied from about 1 in 60,000 in the United States to about 1 in 770,000 in Germany, whereas in moderately endemic areas, Japan, the rate can increase to 1 in 107,000 (14).

The classification of HBV can be divided into subtype or genotype by using the differences of protein or gene, especially in part of HBsAg (or S gene) (3).

Based on specific antigenic determinant of HBsAg, particularly “a” determinant which common to all subtype (3), there are 4 major subtypes, namely *adr*, *adw*, *ayr* and *ayw* that can be divided into 9 subtypes; *adw2*, *adw4*, *ayw1*, *ayw2*, *ayw3*, *ayw4*, *adrq-*, *adrq+* and *ayr* (15, 16). Based on the comparison of *S* gene or complete genome analysis, HBV can be classified into 8 major genotypes (A to H) (9, 17). Genotype B and C are prevalent in Asia such as China, Taiwan, Japan, Indonesia including Thailand, whereas genotype A prevail in Northwestern Europe, USA and Central Africa. Genotype D is found in Mediterranean area and India. Genotype E is restricted to Africa, genotype F to Central and South America, genotype G in France and USA, and genotype H has been identified in Northern part of Latin America, Central America and Mexico (6,18). Recently, genotype I has been found in Laos and Vietnam (17).

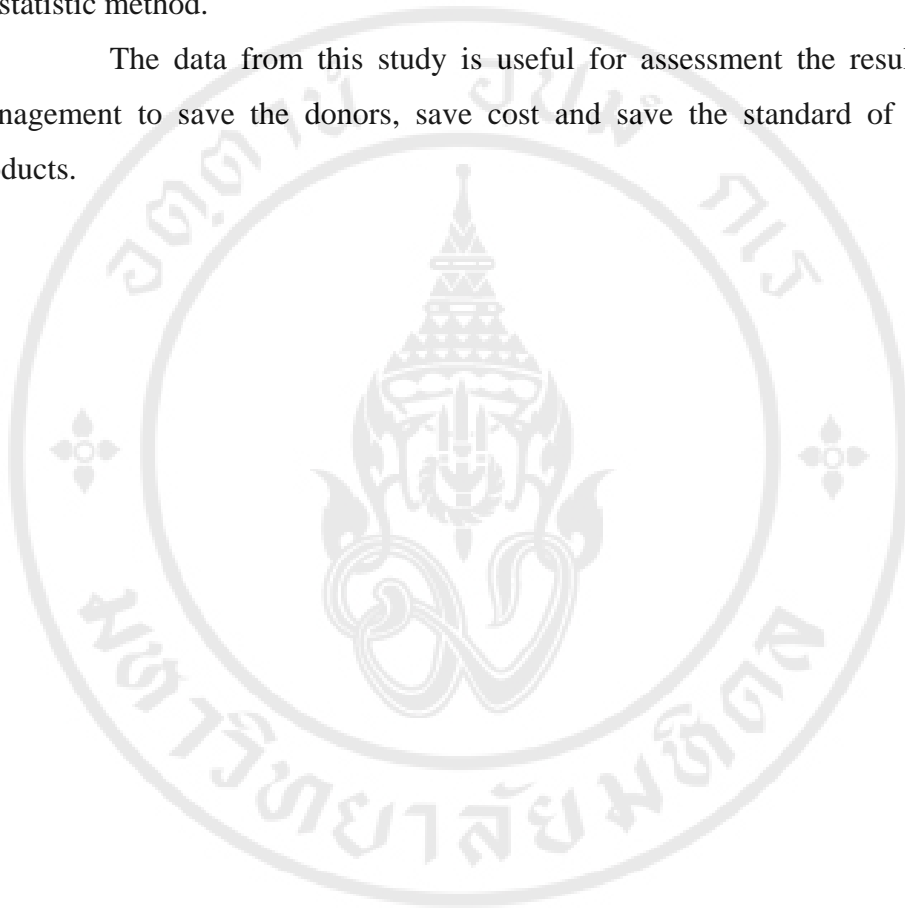
The prevalence and characteristic of HBV in Thailand was subtype *adr* (84.4%), *adw* (14.2%) and *ayw* (1.4%), while the major genotype was C (87.1%), B (11.6%) and A (1.3%) from the study of Suwannakarn K and colleagues in 2008 (9). Recently, other genotypes such as G and H have been found in Thailand but in less proportion (17).

HBV can be transmitted through blood and secretion from infected person that is the problem of transfusion medicine. For blood bank in Thailand, the routine screening test for HBV infection is HBsAg detection in sera and nowadays, also detect HBV DNA by nucleic acid test or NAT.

For the safety of blood donation, blood bank needs to use the effectiveness, reliable and cost-effective test to screen the donors. In routine screening test of blood donation at Siriraj Hospital, for serological test, HBsAg detection by Chemiluminescentmicroparticle immunoassay (CMIA). If the results are clear; strongly positive HBsAg or definitely negative HBsAg, the results can be released. But there are some indeterminate results; weakly positive HBsAg that are the problem of donor management for blood bank to accept or reject the donors. The extra test to confirm results such as detection of anti-HBc, anti-HBs or HBV DNA. HBsAg confirmatory test is the technique that using specific antibody neutralization to confirm HBsAg in sera.

This study would like to evaluate the HBsAg confirmatory test in order to use as the extra test for HBV screening in blood donors of Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital. Moreover, HBsAg positive blood donors at Siriraj Hospital, January 2014- May 2016 will be also studied and analyzed by statistic method.

The data from this study is useful for assessment the results for donor management to save the donors, save cost and save the standard of safety blood products.



CHAPTER II

OBJECTIVES

This research aim to study as followed:

1. To evaluate the HBsAg Confirmatory test in order to use as the extra test for HBV screening in blood donors at Siriraj Hospital.
2. To study and analyze HBsAg positive blood donors at Siriraj Hospital, January 2014 – May 2016

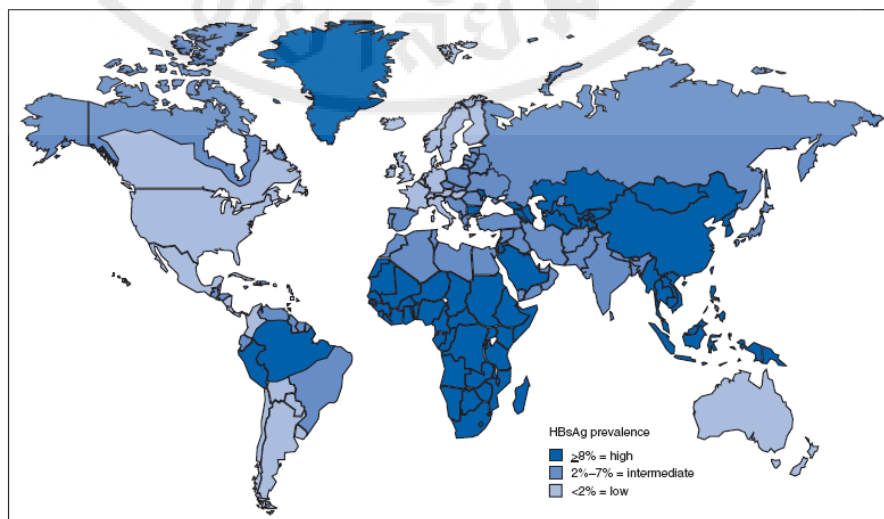
CHAPTER III

LITERATURE REVIEW

Hepatitis B virus (HBV)

Hepatitis B virus (HBV) is the major etiologic agent of viral hepatitis. It is a global public health problem. Worldwide, estimated that 2 billion people have been infected with this virus (6) and more than 350 million people are chronically infection (1). Due to HBV is non-cytopathic virus, HBV-related diseases are mainly caused by host immune response attack to the infected cells. So the range of HBV infection time course is highly variable from transient asymptomatic infection to chronic liver disease (5) lead to liver cirrhosis and hepatocellular carcinoma (2). There are 10 million people new infected cases per year (1) and about 1 million people annually die with HBV-related liver diseases (3, 4, 5, 10).

HBV infection distributes worldwide and geographic varying that can be classified into 3 groups, which are high (>8%), intermediate (2-7%) and low (<2%) endemicity (7) as shown in figure 1 (19).



* For multiple countries, estimates of prevalence of hepatitis B surface antigen (HBsAg), a marker of chronic HBV infection, are based on limited data and might not reflect current prevalence in countries that have implemented childhood hepatitis B vaccination. In addition, HBsAg prevalence might vary within countries by subpopulation and locality.
Source: CDC. Travelers' health; yellow book. Atlanta, GA: US Department of Health and Human Services, CDC; 2008. Available at <http://www.cdc.gov/travel/yellowbookch4-HepB.aspx>.

Figure 3.1 Geographic distribution of chronic hepatitis B virus (HBV) infection - worldwide, 2006* (19).

High prevalence group is in Southeast Asia, Asia, Africa, Latin America and Southern Europe (6, 7). In Thailand, HBV prevalence rate is reported as 9.23 per 100,000 in 2010 (8).

HBV was discovered in 1963 by Dr. Baruch S. Blumberg (20), subsequently received the Nobel Prize in Physiology or Medicine in 1976 from this success (3). The first time, it was called Australia antigen because these antigen found in Australian Aborigine serum that created precipitin line with serum of multiply transfused hemophilia patient (20). In 1970, David Dane and colleagues found viruses in serum of hepatitis patients and first described the complete virions those are 42 nm in diameter by using electron microscope. Moreover, they marked the smaller virus-like particles or subviral particles, which were produced more than the complete virions, are composed of only envelope and surface protein, subsequently known as HBsAg (5, 6). The complete virions are called Dane particles. In general, there are incomplete virions more than Dane particles by 1,000:1 to 10,000:1 ratio (10).

Virology

Genome and structure

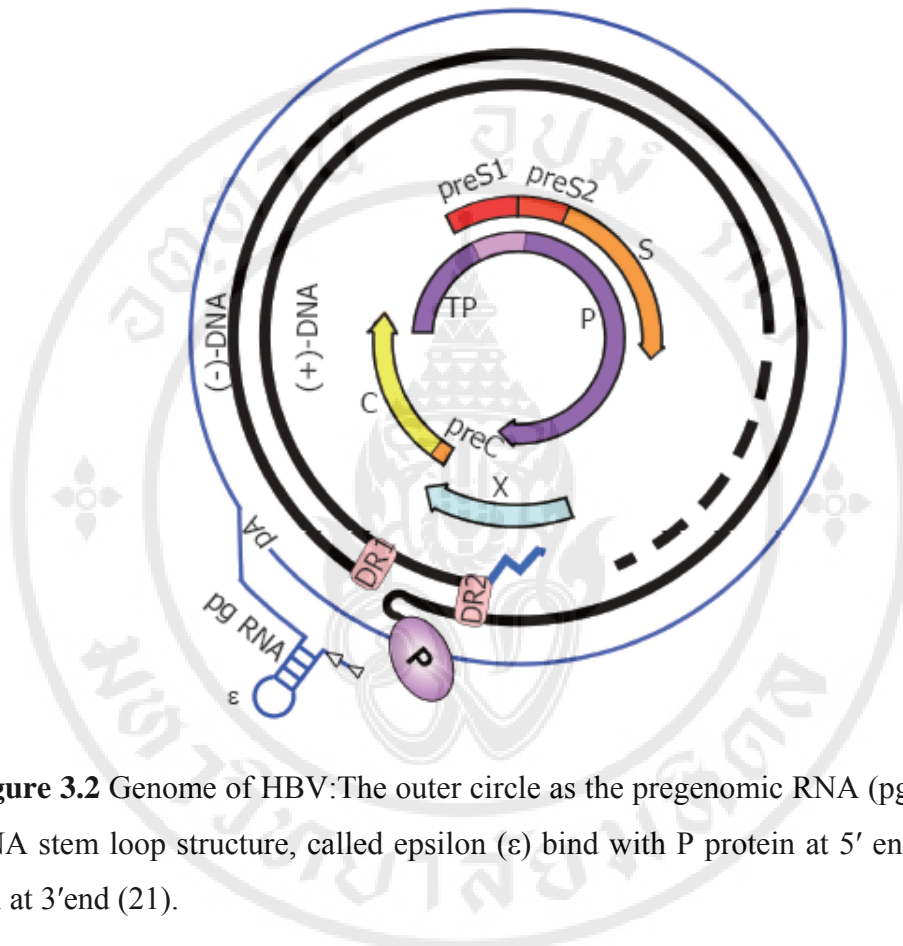


Figure 3.2 Genome of HBV: The outer circle as the pregenomic RNA (pgRNA) which RNA stem loop structure, called epsilon (ϵ) bind with P protein at 5' end and poly A tail at 3' end (21).

HBV belongs to family *Hepadnaviridae*, genus *Orthohepadnavirus*. Genome is a relaxed-circular partially double stranded DNA (rcDNA), is about 3,200 base pairs (bp). Genome of HBV have special characteristic because of it is the smallest dsDNA virus, first it consists of 4 overlapping open reading frames (ORF) that encode the proteins, namely *S* ORF for the surface protein (HBsAg), *C* ORF for nucleocapsid protein or core protein (HBcAg and HBeAg), *P* ORF for DNA polymerase/reverse transcriptase and *X* ORF for X protein (HBx) (3, 9, 10) as shown in figure 2 (21). Moreover, one ORF can create several type of one protein due to differential translation initiation such as *S* ORF encodes pre-S1, pre-S2 and S protein from 3 in-frame start codons and *C* ORF encodes pre-C and C protein from 2 in-frame start codons (24 kD and 21 kD, respectively) (3, 10). Some HBV protein is created by

modification of the initial protein such as HBeAg (16 kD) generated by cleavage of pre-C protein with cellular protease in Golgi complex (10).

Due to the positive stranded DNA are continuously synthesized from negative stranded DNA and this synthesis is stopped when completely packaging in virion, so the positive stranded DNA are shorter than negative stranded DNA. At 5' end of negative strand covalently bind with P protein (DNA polymerase/reverse transcriptase), while 5' end of positive strand link with the RNA primer (zigzag line in figure 2). There are 11 to 12 nucleotide sequence homology on both DNA strands and called direct repeats1 and 2 (DR1 and DR2), which are the starting point of replication. The negative stranded DNA is not linked itself, called nick, however genome is still circular DNA with the complimentary of DR1 and DR2 between the positive and negative stranded DNA (4, 21).

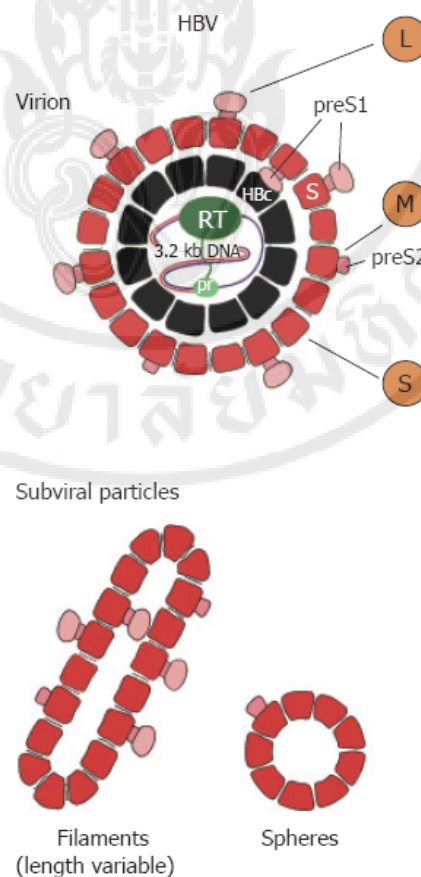


Figure 3.3 Model of HBV and subviralHBsAg particles (10).

The structure of HBV virion is composed of outer lipoprotein and surface protein that contains 3 parts, called the large (L), middle (M) and small (S) protein

(encode from *pre-S1*, *Pre-S2* and *S* gene, respectively) (3, 5, 10). The viral nucleocapsid or core contains viral DNA genome, HBcAg and DNA polymerase/reverse transcriptase (RNA-dependent DNA polymerase) (3, 10) as shown in figure 3 (10). This complete viral particle is called Dane particle as mention before. It is a 42 nm in diameter in round shape. The incomplete viral particles consist of only envelope and HBsAg (without viral genome) with 22nm in diameter (round shape) and 22 nm in width (3, 10) (rod shape, vary in length (13)). In general, there are subviral particles more than Dane particles by 1,000:1 to 10,000:1 (10).

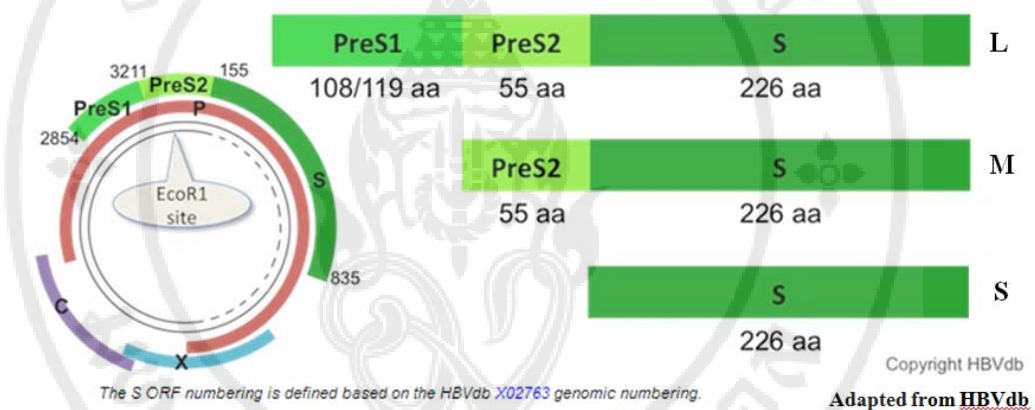


Figure 3.4 S ORF encode large (L), middle (M) and small (S) protein (22).

Hepatitis B surface antigen (HBsAg) consists of 3 co-carboxyterminal surface (glyco)-proteins, namely large (L), middle (M) and small (S) surface protein (13). S protein encode from 226 amino acid (aa) of *S* gene, adding with 55 aa of *pre-S2* gene for M protein and for L protein adding with 55 aa of *pre-S2* gene and 108 aa (for genotype D) or 119 aa (for most of genotype) of *pre-S1* gene (13) as shown in figure 4 (22). All of three proteins are glycosylated, type II transmembrane protein (4).

S gene is important to create the cell surface structure of HBV and support the virions entry to the host cells (13). Antibody to S protein is the protective immunity and use to produce the current vaccine (3, 9, 13). The function of *pre-S1* domain is attachment and entry to the host cells (5, 13). This part is necessary for infection due to contain receptor binding site (13), however, the host cell receptor is still unclear (5) and associated with host range restriction (4). In contrast to *pre-S2*

domain, its function is not clearly understood (4, 13). Although *pre-S2* contains translocation motif (TLM) which may be associated with virus entry, It has been reported that HBV lacking of TLM sequence can infect cells this suggested that *pre-S2* gene is not necessary for infection of HBV (4, 13).

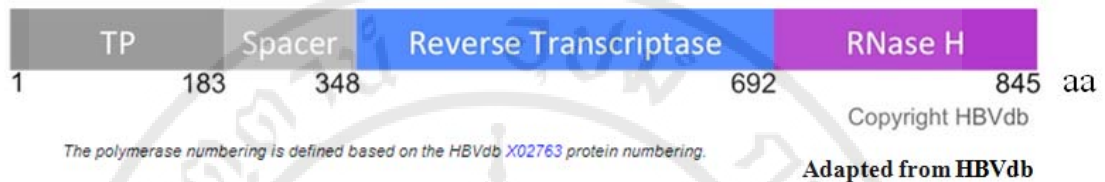


Figure 3.5 *P* ORF encode polymerase protein (23).

P ORF consists of 4 domains which are terminal protein (TP), spacer (non-conserved and unnecessary), DNA polymerase/reverse transcriptase (RT) and RNase H domain (ribonuclease H activity to degrade RNA) as shown in figure 5 (23). The two latter are conserved regions and important to viral transcription from RNA intermediate. The function of TP domain is primase activity, associated with conserve tyrosine residue, it starts negative stranded DNA synthesis (4, 21, 23, 24). *P* gene contains 832 to 845 aa depend on genotype (842 or 843 aa for almost genotype, 832 aa in genotype D and 845 aa in genotype A) (23) that is almost of all genome (29). RT enzyme has no proof-reading activity, so mutation of HBV genome can occur and produce nucleotide analogues resistant strains after long termed use of antiviral drug therapy (24).

C ORF encodes hepatitis B core antigen (HBcAg) or nucleocapsid core protein that encloses viral DNA and RT enzyme. Hepatitis B e antigen (HBeAg) is derived from pre-C/C protein. It is marker of active viral replication of HBV infection but the true function is still unknown (3, 4, 10).

X ORF encodes hepatitis B X protein (HBX) that acts as transcription transactivator and promoter protein (3).

Transmission (2, 3, 7)

Transmissions of HBV infection are the exposures of infectious blood or secretion containing HBV in large amounts (10^8 to 10^9 virions/ml) in serum and body fluids such as semen, saliva, vaginal fluids. There are no documents for respiratory transmission, food or water-borne, insects or other vectors and feces are the source of transmission.

Mode of transmission can be divided into 3 main types, sexual contact (horizontal), perinatal transmission (vertical) and parenteral/percutaneous transmission (horizontal) such as re-use needles and syringes, blood transfusion, needle stick injury, etc.

In low endemic area or developed countries such as North America, HBV infection mostly are transmitted by sexual contact or re-use needles and syringes by drug addicts. While the high endemic area such as Southeast Asia, China and Africa, the transmission occur from mother to child, particularly before the immunization program has been worldwide. Perinatal transmission of HBV can be caused by 3 possible types namely from mother to child during childbirth, from mother to infant through placenta (transplacental transmission) and postnatal infection by breast-feeding or during care. The incidence of perinatal infection to become chronic infection is 90%. The main route of parenteral transmission is injection drug usage, while the screening of HBV markers in blood donations reduces the risk of blood transfusion. Moreover, the surgery, dental care, needles stick injury, tattooing are also risk to transmission.

Host range

Hepadnaviruses can be divided into 2 genus, those are orthohepadnaviruses found in mammals and avihepadnaviruses found in birds (25).

HBV is the prototypic member of hepadnaviruses with very narrow host range and highly specific cell type. They can infect only their natural host and closely related species such as HBV can infect only humans, chimpanzees and chacma baboons but cannot infect woolly monkeys (4, 11).

The determination of host range restriction occurs at the early step of infection, which that are attachment, entry and fusion, between pre-S1 regions and cellular receptors, however, the actual cellular receptor is still unclear. But there are evidences the receptor is carboxypeptidase D (CPD) or glycoprotein 180 (gp180) by using the closely related duck hepatitis B virus (DHBV) as the model (5, 11,12, 13).

Hepadnaviruses are the hepatotropic viruses (liver-tropic viruses), so the main target cells are hepatocytes, however, the other cells such as bile ductile epithelial cells, subset of cells in pancreas, kidney or lymphoid systems may be also target cells. For examples, there are reports that use DHBV as a model, there are replication of viruses outside the liver, i.e. exocrine cells and endocrine islets of pancreas. Moreover, some reports suggested lymphocytes are the second reservoir but the immunity and response of phagocytic cells are in debate. Due to the hepatocytes are the only one confirmed of target site of replication indicated the highly cell type specific of these viruses (4).

Replication cycle

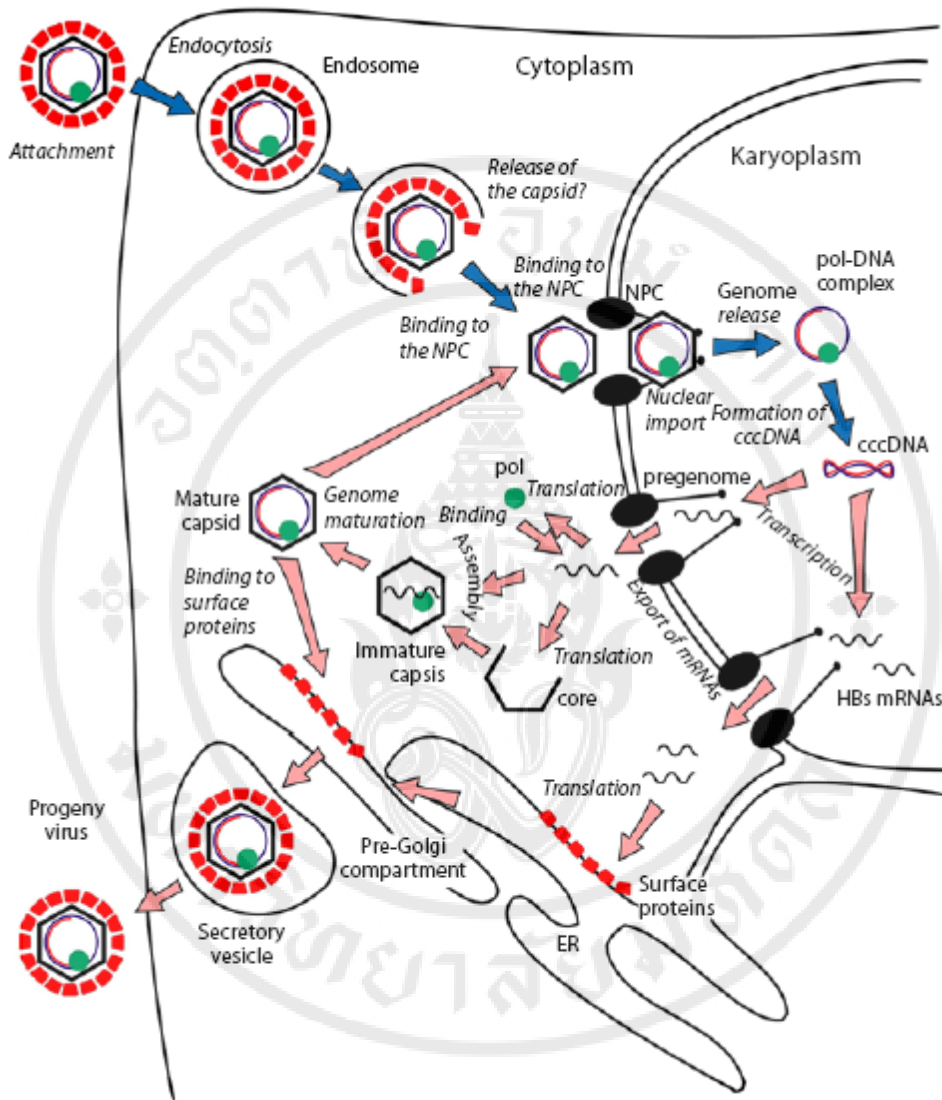


Figure 3.6 Replication cycle of HBV (5).

After transmissions, HBV spreads from blood into hepatocytes and first react between cell-bound heparan sulfate and pre-S1 region, following binding with unknown receptor (5, 10) on host cell membrane and enter to the cell by endocytosis but the actual mechanism is not understand (5).

After uncoating of viral envelope, nucleocapsid core particles released into cytoplasm and translocated through cellular microtubules and subsequently pass to the

nuclear pore complex (NPC) (as shown in figure 6 (5)) that is the cage-like structures for uncoating capsid and release genome into nucleus (5, 26).

As mention earlier, genome of HBV is a relaxed-circular partially double-stranded DNA (rcDNA), first of all rcDNA are repaired to a covalently closed circular DNA (cccDNA) by cellular DNA repair factors lead to complete positive strand and ligate the ends of two strand and subsequently remove the reverse transcriptase covalently bind with 5' end of rcDNA, however, the true mechanism is remain unclear (4).

cccDNA acts as a template for transcription by using host RNA polymerase II (10). Transcription of cccDNA generates the viral pregenomic RNA (pgRNA) that also serves as bicistronic mRNA for viral DNA polymerase/reverse transcriptase and core protein and generate 3 subgenomic mRNA including pre-S (L) mRNA and S mRNA for envelope protein and X mRNA for X protein. In additional, all RNAs have polyadenylation signal (poly-A tail) (4, 5, 21). For pgRNA, the polymerase bind with RNA stem loop structure at 5' end, is called epsilon (ϵ) and poly-A tail at 3' end as shown in figure 2 (21). The function of ϵ are associated of encapsidation of pgRNA and are the trigger for initiation step of reverse transcriptase and also serve as replication origin for viral DNA synthesis by use reverse transcriptase itself (4, 21).

Then all of viral RNAs are transported out of nucleus for translation. Core protein and polymerase protein are translated from bicistronic mRNA (pgRNA), while surface proteins are translated from pre-S (L) mRNA and S mRNA, as well as, the X and pre-C protein are also translated (4, 10).

Next, pgRNA, core protein and polymerase are assembled as immature capsid, then reverse transcription starts DNA synthesis, pgRNA acts as a template for negative stranded DNA synthesis by using reverse transcriptase itself. Subsequent degradation of RNA template by RNase H activity after that the newly synthesis of positive stranded DNA begins that use negative strand as a template. The synthesis process will stop when the assembly has been finished, so final; the positive strand is shorter than negative strand (4, 5, 10).

The mature nucleocapsid has 2 fates. For intracellular life cycle, after maturation of genome and phosphorylation of nucleocapsid protein leads to structural

change. C terminus domains of capsid show the nuclear localization signal (NLS) that interact with the adaptor protein, called importin α (that is karyophilic protein) and this complex is bound with importin β through microtubules and later pass to NPC by facilitation of importin β . At the basket of NPC (cage-like structure), mature capsid uncoating and release genome into nucleus to create viral replication again (5, 26).

Another fate, after maturation nucleocapsids exhibit the structure that bind with N-terminal domain of L surface protein lead to budding of nucleocapsid through ER membrane. The virions contain 3 parts of surface protein on the envelope, i.e. S, M and L protein. Then they are transported to Golgi complex where the glycosylation of asparagines residue in S region occurs and finally the complete virions are secreted out of cell by secretory vesicle (exocytosis) (4, 5). In addition, incomplete viral particle also budding out of the cells by secretion, too and more efficiently express than the complete virions up to 10,000 fold in serum. Both of incomplete viral particles and complete virions have the same HBsAg but difference in composition. Large amounts of L protein found in rod shaped-subviral particles and even in complete virions, whereas round shaped-subviral particles have lower quantities of L protein, may be due to the host immune response (27).

Pathogenesis

Normally, dynamic relationship between host and virus, the virus try to survive in host while the host tries to eliminate the virus infection with least damage. When virus attempt to establish in appropriate target cells, innate immune responses initiate defense mechanisms against viral infection by recognize the viral products or trigger the mechanisms by infection, namely a) virus induce apoptosis directly, b) infected cells release antiviral cytokines such as interferon type I (IFN α/β), c) stimulation of cellular innate immune response such as natural killer cells (NK) and natural killer T cells (NKT cells). NK and NKT cells can rapidly go to site of infection (before adaptive immune response) and can recognize infected cells. Function of activated NK and NKT cells are directly killing infected cells and producing cytokines that have antiviral activity and recruit inflammation cells go to site of infection. Viral specific T cells in adaptive immune responses play a major role in antiviral immunity,

both effector and regulatory roles. These T cells associate with viral pathogenesis either directly or indirectly, namely killing infected cells or producing cytokines that recruit inflammatory cells and down regulate viral replication, respectively (28).

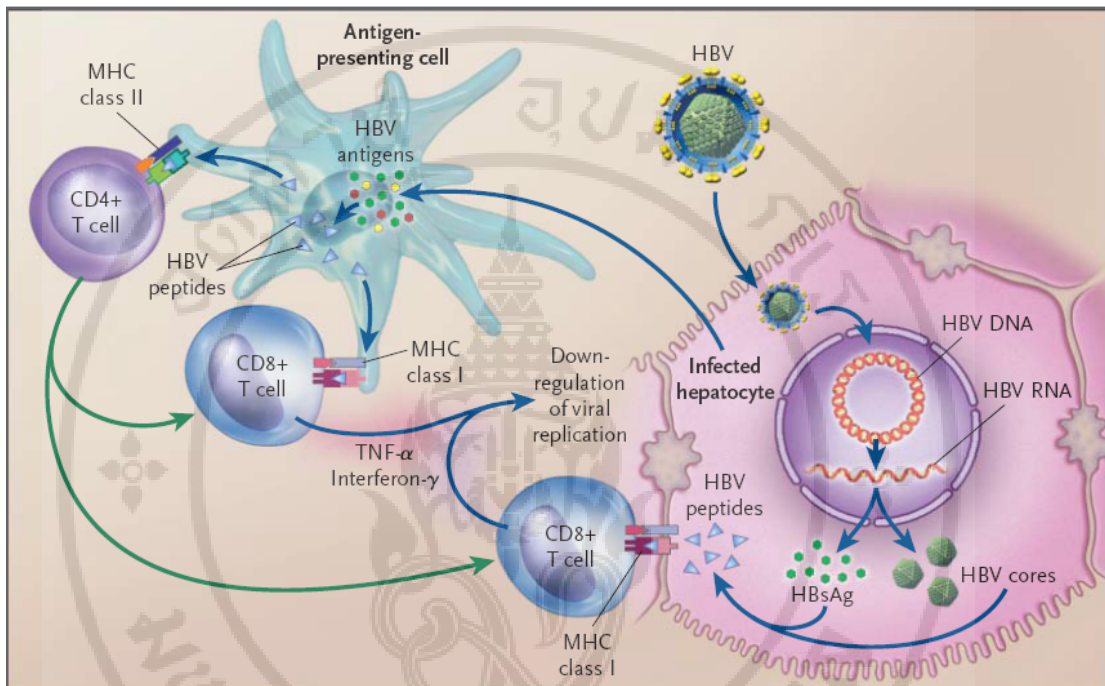


Figure 3.7 Summary of cellular immune responses to HBV (10).

Due to HBV entry and exit the target cells without damage so it is not directly cytopathic (not kill hepatocyte)(5). Immune response is cause of cell injury (immunopathology) (3, 10) however, innate immune response is not significant to control the virus in HBV infection (28). Pathogenesis of HBV infection are not completely understood but associate with adaptive immune response especially cellular immune response, both CD4+ helper T cells and CD8+ cytotoxic T cells (7, 28) as shown in figure 7 (10).

Infected hepatocytes produce the complete virion and protein of HBV such as HBsAg, HBcAg that are received by antigen presenting cells (APC) such as macrophages. The viral proteins are degraded through process in APC and presented on the cell surface both major histocompatibility complex (MHC) class II that specific for CD4+ T cells and MHC class I that specific for CD8+ T cells (10). Moreover

hepatocytes also present the viral proteins on the cell surface via MHC class I that specific for CD8+ T cells through intracellular processing (3).

The recognition of CD8+ T cells or cytotoxic T lymphocytes(CTL) via MHC class I on infected hepatocytes lead to directly killing infected cells via apoptosis mediated by the Fas ligand(Fas-L, a glycoprotein known to induce apoptosis), cytokines and perforin (3). Moreover, CD8+ T cells can release cytokines that induce and recruits antigen-nonspecific inflammatory cells into the liver. The mechanisms are not well understood, however may associate with the local production of proinflammatory and cytotoxic mediators including tumor necrosis factor α (TNF- α), protease such as perforin, hydrogen peroxide, superoxide anion, and nitric oxide. In addition, Fas-positive cells such as hepatocytes can be induced apoptosis by Fas-L that express on some of inflammatory cells especially NK cells, NKT cells and helper T cells (28). On the other side, the same cytokines that release from CTL such as IFN- γ and TNF- α are noncytotoxic antiviral effects that also be necessary for viral clearance due to down regulate viral replication (10, 28) summary responses of CTL to HBV infection as shown in figure 8 (28).

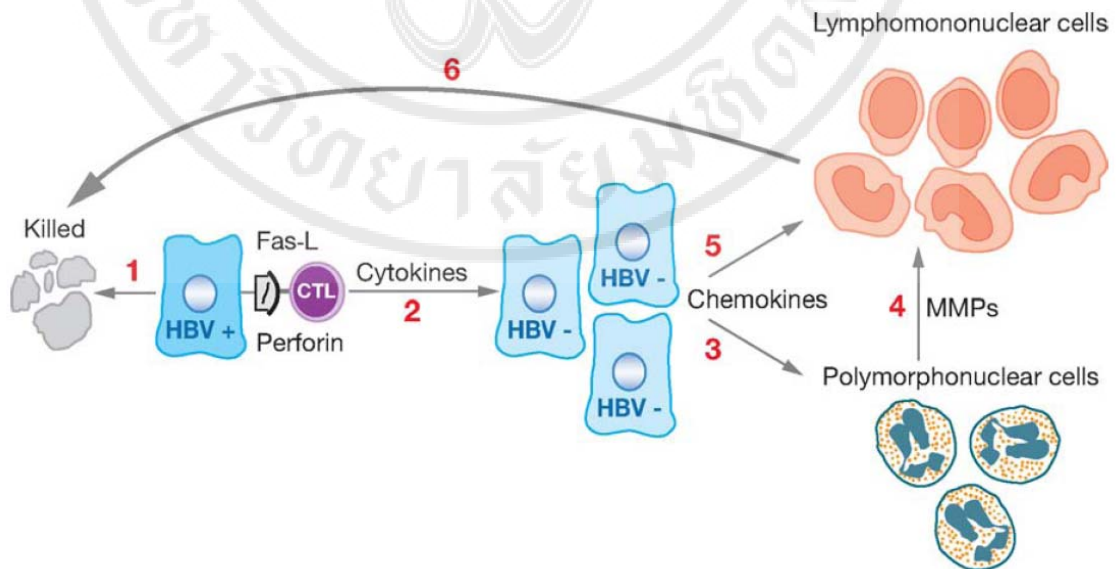


Figure 3.8 Summary responses of CTL to HBV infection (28).

The presentation of viral proteins to CD4+ T cells or helper T cells via MHC class II leads to increase the presentation of MHC class I on the hepatocytes,

decrease viral replication, release cytokines that help CTL function and B cells responses (3).

So the cause of liver injury are directly killing infected hepatocytes by CTL, products from CTL that called antigen-nonspecific inflammatory responses such as TNF- α free radicals and protease (10).

Immune responses against HBV infection are different in each person depend on the specific recognition between MHC and specific T cells. Some person have strong immune response and completely clear virus but some person such as immunodeficient patient or children that have immature immune response lead to continuable infection. So HBV infection is highly variable symptom, ranging from asymptomatic, mild symptom to severe and lead to hepatocellular carcinoma (3, 10). Most of infected people are self-limiting, only 5-10% of infected adults that have incomplete clearance of virus and become chronic infection (13). About 90% of neonates and less than 1 year old children who perinatally infect HBV from mother became asymptomatic chronic infection and HBV carrier due to immature immune system (7). Whereas, the proportion of children who infect HBV after neonate but before 6 years old is 30% that became chronic infection (3).

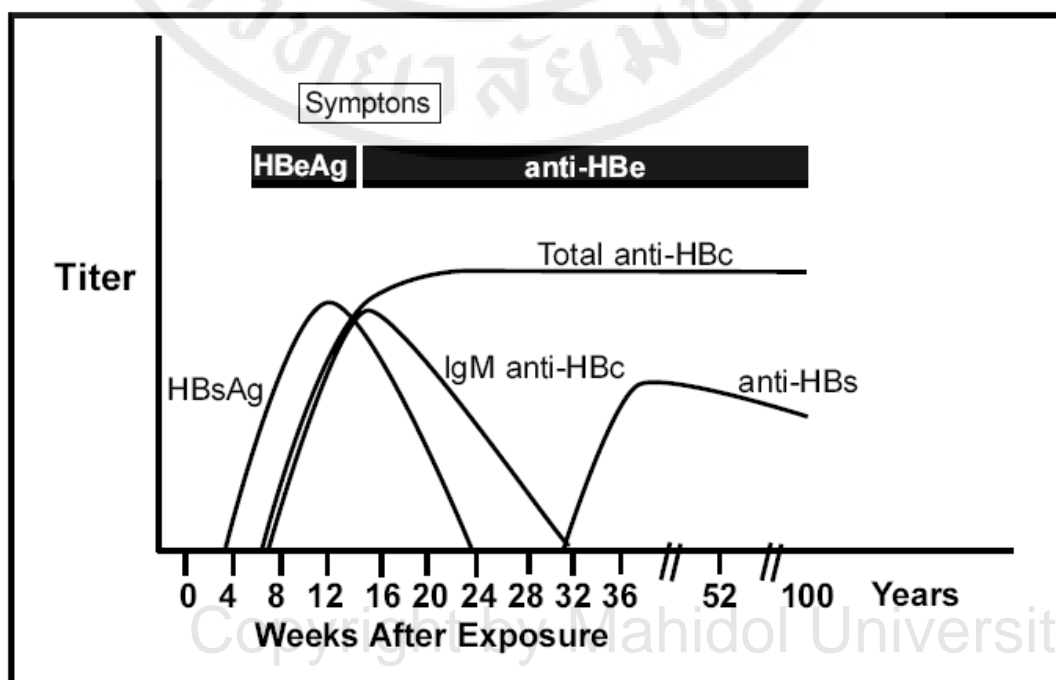


Figure 3.9 Time course of acute HBV infection with recovery (29).

Acute hepatitis B virus infection

Almost of HBV infections (90-95%) in adults are acute HBV infection (4, 13). 65% of acute HBV infection cases are asymptomatic (30) or mild symptom that are inconsecutive, the remainder are symptomatic. In pre-icteric phase, symptoms are malaise, weakness, anorexia, nausea, vomiting and right upper quadrant pain and these symptom lessen in icteric phase (jaundice) that preserve about 3 weeks (29). Incubation period of HBV infection varies from one week to 6 months (29), normally be 4 to 10 weeks (10). Figure 9 show time course of acute HBV infection with recovery (29).

HBsAg is the first serological marker that can be detected followed by antibody to nucleocapsid core antigen (anti-HBc) that are IgM isotype in the primary infection and then total anti-HBc gradually increase until constant. Viremia of acute HBV infection is very high titers, 10^9 to 10^{10} virions/ml (10). When the antibody against HBsAg that is the protective immunity occurs (anti-HBs) and HBsAg disappear that is the mark resolution of infection. Normally, acute HBV infection resolves in 6 months or less (29). In the most cases of acute HBV infection can detect HBeAg, this marker represent the viral replication and infection of HBV, the previous studies in animals such as chimpanzees show that when HBeAg occurs, 75 to 100% of hepatocytes are infected, so during acute HBV infection the rates of transmission are very high (10). When the seroconversion of HBeAg to anti-HBe (antibody against HBeAg) occurs, the replication of virus and severity of disease decrease (29).

Alanine aminotransferase levels (ALT) increase that is the marker of liver injury. It is reflection of T cells mediated immune response to eliminate infected hepatocytes (10). CTL directly recognize multiple antigens of HBV such as HBcAg, polymerase and surface protein, moreover these antigen also be recognized by helper T cells. These mechanisms are strong in acute infection whereas in chronic infection, the viral specific T cell response are greatly attenuated, however antibody responses are strong and maintain in both situations except anti-HBs are not detected in chronic infection due to the excessive HBsAg in peripheral blood. Actually, viral clearance can occurs without massive liver injury in most cases due to noncytolytic clearance mechanism by cytokine such as IFN- γ , TNF- α as described before. After viral

clearance, the definition of self-limiting is HBsAg disappear and anti-HBs can be detected (10).

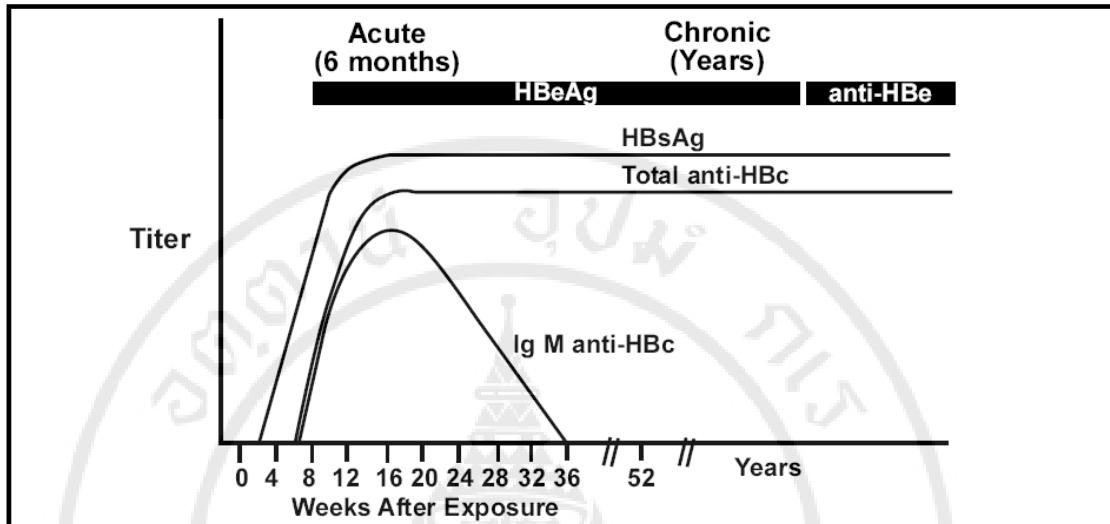


Figure 3.10 Time course of chronic HBV infection (29).

Chronic hepatitis B virus infection

Definition of chronic HBV infection is the presence of HBsAg in blood circulation for 6 months or longer due to immune response is weakly effective to clear the virus. So almost of chronic HBV infection occur in children younger than 1 year (about 90%) or in immunocompromised person whereas occurs about 5-10% in healthy adults (3, 4). The person who established with chronic HBV infection are called chronic carriers (29).

Time course of chronic HBV infection as shown in figure 10 (29). The initial steps are as same as acute HBV infection, however lack of the development of the protective antibody (anti-HBs). HBeAg is the marker of viral replication, so the presence of its in long time due to delay or failure to develop antibody to HBeAg (anti-HBe) suggested that associated with continuously replication, high viremia and more severity of the disease (29). However, the levels of viremia in chronic carriers usually are lower than during acute infection, especially carriers with anti-HBe are about 10^3 to 10^5 molecules/ml (10). In the most cases, the levels of viremia tend to lessen over time including HBeAg and followed with development of anti-HBe. The rate of this tendency is 5 to 10 % per year in chronic carriers. Normally, when HBeAg

disappear, ALT levels will arise in short time suggested that the reflection of immune cell-mediated response to attack infected hepatocytes. Followed by reduction of viremia and seroconversion of anti-HBe. However, the attack is usually insufficient to get rid of infection so there is continuous attack of immune response to infected hepatocytes. As these reasons, the dynamic diseases of chronic infection have the periods of activity and quiescence. Clinical symptoms with disease activity are fatigue, mild fever, jaundice, right upper quadrant discomfort (10, 29).

Although the viral replication decrease, HBsAg still occurs over time and sometime produced at high levels that may be due to the viral DNA integrate into host chromosomes and provide to transcript of HBsAg mRNAs. The effect of integration may disrupt viral gene especially the core and polymerase gene whereas envelope proteins still intact but the true mechanism is not clear (4).

Table 3.1 Phase of chronic HBV infection (31).

Investigation	Immune tolerant (phase 1)	Immune reactive (phase 2)	Carrier of inactive hepatitis B virus (phase 3)	Chronic hepatitis negative for hepatitis B e antigen (phase 4)
Hepatitis surface antigen	Positive	Positive	Positive	Positive
Hepatitis B e antigen	Positive	Positive	Negative	Negative
Anti-HBe	Negative	Negative	Positive	Positive
Alanine aminotransferase	Normal	High	Normal	High or fluctuating
Hepatitis B virus DNA (IU/ml)	$>2 \times 10^5$	$>2 \times 10^4$	$<2 \times 10^2$	$>2 \times 10^3$
Inflammation on histology	None or minimal	Active	None or minimal	Active

In summary, chronic HBV infection consist of 4 phase as shown in table 1 (31).The first phase is the immune tolerance phase, its lack of clinical symptoms, high levels of HBV DNA, normal ALT, and minimal histological activity. This phase is usually seen in children and young adults with perinatally acquired HBV infection. Treatment is not recommended, as response rates of current therapy are poor. The second phase is the immune clearance or immune reactive phase characterized by HBeAg to anti-HBe seroconversion (positive to negative). During this phase, immune clearance of HBV occurs and the destruction of infected hepatocytes may be manifested as an increase in ALT level. Successful HBeAg seroconversion is usually accompanied by a decrease in HBV DNA to low or undetectable values, and the

normalization of ALT levels. However, some patients fail to achieve spontaneous HBeAg seroconversion and continue to have prolonged episodes of elevated ALT levels and active disease in liver histology, with repeated hepatitis flares, leading to an increased risk of cirrhosis. During this phase, patients should be monitored closely, and if spontaneous HBeAg seroconversion does not occur after 3 to 6 months of observation, treatment should be initiated. The third phase is the inactive carrier state, characterized by the presence of anti-HBe, normal ALT, and low HBV DNA levels that are only detectable by PCR assays. Treatment is not indicated, as viral replication is already suppressed by the host immune response. Some patients go on to the fourth phase, during which there is reactivation of HBV replication, characterized by an increase in serum HBV DNA and ALT levels. HBeAg remains negative and, therefore, this phase is also known as HBeAg-negative chronic HBV infection. Spontaneous resolution is uncommon and, therefore, treatment is generally indicated (32).

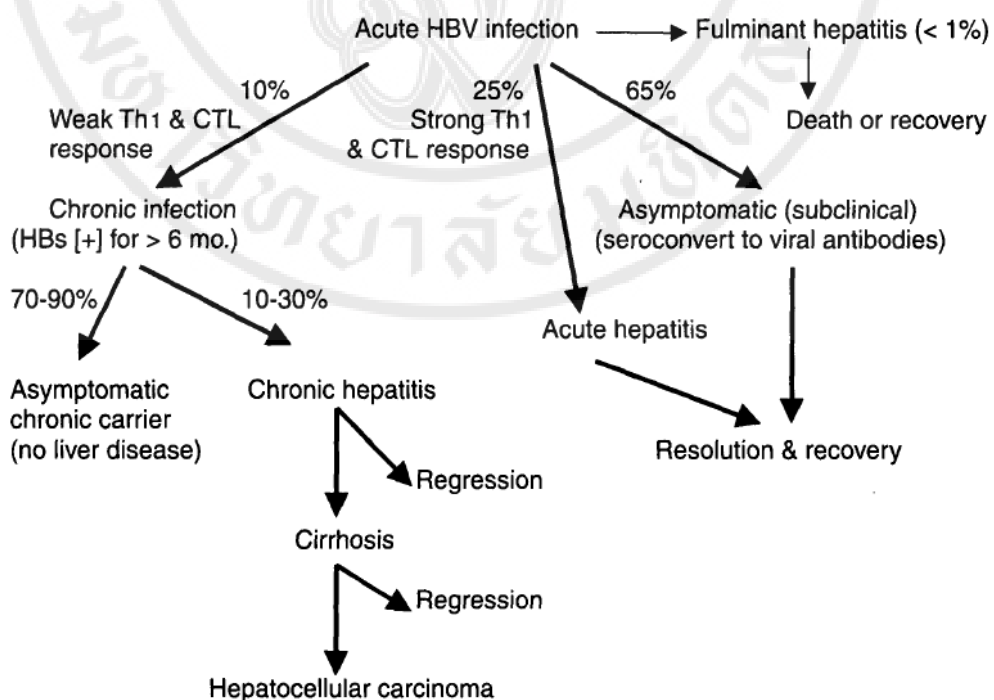


Figure 3.11 Summary of pathogenesis and outcome of HBV infection (30).

70-90% of chronic HBV infection are asymptomatic (without liver disease) while about 10 to 30% of chronic carriers who expose HBV at adulthood will

die with either cirrhosis or liver cancer, summary of pathogenesis and outcome of HBV infection as shown in figure 11(30). The prognosis of chronic hepatitis depends on many factors. One probable factor is the different of genetic background such as the history of hepatocellular carcinoma (HCC) increase the risk of HCC among HBV carriers in China. Another factor, high titers of viral replication for a long time associated with increasing risk of HCC in China study (4). Moreover there are many other risk factors to increase the development of HCC with chronic HBV infection including alcohol abuse, race (high risk in Asian or African race), genotype (high risk in genotype C), HBV DNA viral more than 10,000 IU/ml, older age, male sex presence of HBeAg, coinfection with hepatitis C and D virus infection, exposure to aflatoxin, etc (33).

Diagnosis

Due to the other viruses such as hepatitis A virus (HAV) causes acute infection and hepatitis C virus (HCV) causes both acute and chronic infection. Moreover HBV infection can cause coinfection and superinfection with other hepatitis viruses such as HCV or hepatitis D virus (HDV). The selection of diagnosis test should depend on the individual's risk factors, vaccination history, etc. Recommended screening tests for the most common viral hepatitis are HBsAg detection for HBV, anti-HCV for HCV and anti-HAV IgM for HAV (34). After exclude these viral hepatitis, the diagnosis of HBV infection can be divided into 3 types; biochemical, virological and histological tests (35).

Biochemical test, ALT (Alanine Aminotransferase) is the routine liver function test that is elevated in person with acute hepatitis or liver injury (34, 35).

Virological tests consist of serological tests and molecular techniques. Serological tests for HBV antigen detection are only HBsAg and HBeAg while HBcAg is nonsecreted protein, so not found freely in blood circulation (36) and for antibody detection are anti-HBs, anti-HBcIgM, total anti-HBc and anti-HBe. Molecular technique is useful for HBV DNA detection and quantification. Summary of HBV markers and their properties (35) showed in table 2.

Table 3.2 Summary of HBV markers and their properties (adapted from 35).

HBV markers	Properties
HBsAg	surface protein contained within the lipoprotein coat
Anti-HBs	antibody to HBsAg , indicator of recovery/immunity to HBV infection
HBeAg	viral product secreted in blood, marker of infectivity, active replication (though absent in pre-C/C mutants)
Anti-HBe	antibody to HBeAg , denoting decreased infectivity
HBcAg	core antigen (viral capsid), intracellular and not detected in serum
Anti-HBc IgM	antibody denoting recent HBV infection or exacerbation
Anti-HBc IgG	positive after HBV contact both recovery and carriers
HBV DNA	quantitative indicator of virus in blood

Histological test, liver biopsy is necessary to confirm the diagnosis, identify and grade the stage of liver disease (35). In addition, there are other investigations (31) that associated with HBV related diseases show in table 3.

Table 3.3 Investigations for HBV-related diseases (adapted from 31).

Virological tests	Biochemical tests	Histological tests
HBV serological profiles:	General liver disease investigation	Screening for liver cancer
HBsAg, HBeAg, anti-HBc	- Complete blood count	- Ultrasonography
IgM, anti-HBc, anti-HBs,	- Liver enzyme test	
anti-HBe	- Bilirubin	
	- Lipid profile	
Quantitative HBV DNA	Screening for liver cancer	Staging of liver disease
	- α -fetoprotein	- Fibroelastography
		- Liver biopsy
HBV genotype (considered for interferon therapy)		
Coinfection or superinfection with other viruses (HDV, HCV, HIV, etc.)		

ALT and HBV serological test profile including history counseling, vaccination history and risk contacts are necessary for determining status of HBV infection. The interpretation of HBV infection showed in table 4 (34).

Table 3.4 Primary and secondary tests to diagnose/monitor HBV infection (34).

	Marker	Incubation period	Acute infection	Past/resolved infection	Chronic infection	Vaccination
Primary diagnostic tests						
	HBsAg	±	+	-	+	-*
	Anti-HBs	-	-	+	-	+
	Anti-HBc-Total	-	±	+	+	-
	Anti-HBc-IgM	-	+	-	±†	-
Prognostic or monitoring tests						
	HBeAg	±	+	-	±	-
	Anti-HBe	-	-	±	±‡	-
	HBV-DNA §	±§	+	±§	±§	-

*Recent HBV vaccination within one to two weeks can lead to a false-positive test. The vaccine antigen can be detected at low levels; †May be positive in chronically infected individuals; ‡Patients with chronic HBV infection usually have detectable Hepatitis B e antigen (HBeAg) or antibody to hepatitis B e protein (anti-HBe). Rarely, both HBeAg and anti-HBe can be detected simultaneously; §Methods differ in sensitivity and standardization. Anti-HBc Antibody to hepatitis B core protein; Anti-HBs Antibody to hepatitis B surface protein; HBsAg Hepatitis B surface antigen; IgM Immunoglobulin M; + Implies positive; - Implies negative; ± May be positive or negative

HBV DNA quantification, HBeAg and anti-HBe are essential markers to prognosis and monitoring treatment (34, 35). The development of molecular techniques have great utility for diagnosis of HBV infection as well as monitoring the treatment due to HBV DNA detection is more sensitive than HBV serological profile and HBV DNA appear all the time course of both acute and chronic infection. Formerly, classical hybridization techniques can detect 10^5 viral particles per ml. However, its sensitivity is insufficient to detect too small DNA in the specimen, polymerase chain reaction (PCR) techniques is required to amplify the amount of nucleic acid. The sensitivity of PCR is about 10^4 times more than hybridization (36). For example, commercially available HBV DNA detection assays, CobasAmplicor® HBV monitor (Roche Molecular Systems, USA) have the detection range 200 to 200,000 copies/ml. (the method is semi-automated quantitative polymerase chain reaction)(34).

The HBV immune status should be determined to decide whether giving the vaccination in people who have high risk of infection such as health care workers, high risk sexual activities (multiple sexual partners, homosexual), needle stick injury, tattoos, etc. Anti-HBs occurs when resolved HBV infection or from vaccination. The protective levels are more than 10 mIU/ml. If anti-HBs decreases lower than the protective levels, a booster dose of HBV vaccine should be performed. (34).

Prevention

Since hepatitis B vaccination was first available in 1982, it is the most efficient method to prevent HBV infection (37). In Thailand HBV vaccination was started since 1988 in 2 provinces; Chiang Mai and Chon Buri as the pilot project that was expanded to nationwide in 1992 in the expanded program on immunization (EPI) and the coverage rate of national HBV vaccination increased to 98.3% in 2008 (2). From the study of Chimpalee N. and colleagues found that HBV prevalence rate in new Thai blood donors continuously decreases to 2.6% in 2009 from 7.1% in 1988 due to the successful of EPI program (2).

The Center for Disease Control and Prevention (CDC), USA recommended the extensive strategy to eradicate HBV infection. They consist of 1) give the vaccine to all infants begin at birth 2) prevent perinatal infection by routine screening of all pregnant women for HBsAg and give the immunoprophylaxis to infants who born with HBsAg-positive mothers or unknown HBsAg status mothers 3) give the vaccine to unvaccinated children and teenagers 4) give the vaccine to unvaccinated adults who have risk for HBV infection (37).

Active immunization

An inactive plasma derived vaccine was the first generation of hepatitis B (HB) vaccine that available in 1982 and a DNA recombinant HB vaccine was the secondary generation that was available in 1986. Both of them were proven to safe and efficacious to prevention HBV infection (7). DNA recombinant vaccine or recombinant subunit vaccine is commonly available for HB vaccine in nowadays, example of commercial vaccine are Recombivax HB® (Merck & Co., Inc, NJ, USA) and Engerix-B® (GlaxoSmithKline Biologicals, Rixensart, Belgium). A part of HBV gene that encode for HBsAg is cloned into common baker's yeast (*Saccharomyces cerevisiae*). HBsAg protein is produced from yeast cells and purified to prepare the vaccine (38, 39). This recombinant HBsAg contain "a" determinant at amino acid (aa) position 121 to 149 that may be the major target of protective antibody, termed anti-

HBs (9). The recommended dosing of HB vaccine for commercial vaccine showed in table 5 (33).

Table 3.5 Hepatitis B vaccines and recommended dosing schedules(33).

Vaccine	Dosing		Adult	Schedule
	Children	Schedule		
Engerix-B	10 mcg (0.5-mL vial)	Birth; one to two months, and six to 18 months of age	20 mcg (1-mL vial)	Time of first injection and then at one to two, and four to six months
Recombivax HB	5 mcg (0.5-mL vial)	Two, four, and 12 to 15 months of age	10 mcg (1-mL vial)	—
Comvax (hepatitis B and <i>Haemophilus influenzae</i> type b)*	5 mcg (0.5-mL vial)	Two, four, and six months of age	—	—
Pediarix (hepatitis B; diphtheria and tetanus toxoids and acellular pertussis; and inactivated polio)*†	10 mcg (0.5-mL vial)	—	—	—
Twinrix (hepatitis A and B)	—	—	20 mcg (1-mL vial)	Time of first injection and then at one, and six to 12 months

NOTE: Other vaccination regimens can be found at <http://cdc.gov/vaccines/recs/schedules/child-schedule.htm> and <http://cdc.gov/vaccines/recs/schedules/adult-schedule.htm>.

*—Should not be given to infants younger than six weeks.
†—Should not be given to persons older than seven years.

Normally, the intramuscular injection of HB vaccine is given in 3 times; at 0, 1-2 and 6 months. Time interval for booster injection is still undecided; however a 10-year booster has been recommended (40).

Passive immunization

Passive immunization or immunoprophylaxis is hepatitis B immune globulin (HBIG) that made from pooled serum human who have high level of antibodies against hepatitis B. HBIG is used with infants whom HBsAg-positive mothers or with person who sexual exposure, needle stick injury or after liver transplantation. Recommended doses for all neonate born to HBV infected mothers are 0.13 ml/kg HBIG, give immediately after delivery or within 12 hours after birth, followed by combination with 3 times dose of HB vaccine (7). Whereas in exposed person, the prophylaxis vary depending on the status of antibody and vaccination, vaccine or HBIG is not necessary in person who already has anti-HBs. However in unvaccinated person, HBIG 0.06 ml/kg, up to 5 ml is administered by intramuscular injection, follow with the first dose of vaccine within 24 hours after exposure (41).

Treatment

The goals of chronic HBV infection treatment are reduction the levels of viremia by suppressing HBV replication, decrease the inflammation of the liver to prevent liver failure, cirrhosis, and reduce risk of hepatocellular carcinoma (10, 33, 35). Natural histories of the disease are required for assessment of treatment such as the state of the immune system, the age of the patient, the serologic stage of infection, and genetic factors (3). In the first phase of infection, ALT levels are normal and there is no progression to cirrhosis in this phase but cirrhosis developing hastens during phase 2. Treatment is not recommended in the first phase while in the second phase, treatment should be initiated and need to virtually treat in phase 3 and 4 due to the immune clearance of infected hepatocytes that is cause of liver injury (3).

Nowadays, two therapeutic agents for the treatment of chronic HBV infection that are approved by Food and Drug Administration (FDA) and popular in many countries are interferon-alpha (IFN- α) and lamivudine (32, 33).

Interferon therapy

Patients with chronic HBV infection are unable or are insufficient to produce the endogenous interferon. Recombinant interferons are similar to naturally cytokines that produced when response to viral infection. They have immunomodulatory, antiviral effectes, inducing the display of MHC class I on cell surface of infected hepatocytes topromotecelllysis by CTL and directly inhibiting viral-protein synthesis (3). For many years, IFN- α was the mainstay of therapy. About 30% of patients who treated with this therapy had an achievement. The definitions of successful therapy are the developing of HBeseroconversion and decrease of ALT levels till to normal for more than 6 months. However, this regimen have many side effects including fever, malaise, myalgias, neutropenia, thrombocytopenia and depression that make to difficult treatment in some patients (3, 10, 42).

There are several studies reported the association between interferon alpha therapy and the differences of genotype that genotype A has a better response to short 16-week IFN α therapy than other HBV genotypes (24).More than 50 percent of patients with HBeAg-positive genotype A infections will achieveHBeseroconversion,

whereas only 30 percent of those with non-A genotypes will seroconvert(33). Treatment option of interferon therapy such as, pegylated interferon alfa-2a is subcutaneous administered drug, the dosage is once weekly for 6 to 12 months(33).

Lamivudine (3TC)

Lamivudine (3TC) was the first safe and effective oral medication for the treatment of HBV infection (24). In the first time, this drug has been successful to inhibit HIV infection by blocking reverse transcriptase and found that HBV viremia decreased in the patients who are coinfection of HIV and HBV (10). 3TC is a nucleoside analogue and phosphorylated to the triphosphate (3TC-TP) that competes with dCTP to inhibit DNA synthesis (32). Therefore this drug directly blocks the replication of HBV. Target site of all nucleoside analogues is the viral transcriptase. Lamivudine has effected in a decline of 3 to 4 log of HBV DNA in blood circulation in the first 3 months of treatment (10). At the end of treatment this drug can clear HBV DNA in up to 96% of the patient in 1 year trials however, in long term treatment escape mutant can be developed, for example at the common and highly conserved YMDD (tyrosine, methionine, aspartate, aspartate) motif in the catalytic domain of the reverse transcriptase (*P* gene), mutations from methionine to isoleucine (YIDD) or valine (YVDD) are found after treatment with lamivudine for a long time (3, 43).

Other nucleoside or nucleotide analogues

Adefovir was the second FDA-approved antiviral drug for HBV infection treatment. This drug is a nucleotide (adenosine monophosphate) analogue that inhibits the viral polymerase. Initially adefovir was developed to treat HIV infection but at the effective dose to inhibit HIV replication, it has nephrotoxic however, in lower doses (10 mg per day) it is effective to treat HBV and has less toxicity to the renal. Moreover, adefovir effectively inhibits the replication of lamivudine resistance HBV mutants (10) and the reports of the resistance mutants were less when compare with 3TC (29% and 70%) (24).

Famciclovir is another nucleoside analogue that against herpesviruses and effectively inhibits DNA polymerase of HBV however, escape mutant has been developed (3).

In addition, there are other nucleoside or nucleotide analogues that have been approved for treatment HBV infection such as tenofovir, entecavir, telbivudine, etc. Moreover, combination therapy of interferon therapy and antiviral drugs or more than one drugs treatment that acts on different target sites of replication cycle of virus have shown many advantages such as more effective inhibit replication of HBV and may decrease the drug-resistant mutants (24). The dosage of antiviral therapies for patients with chronic HBV infection and their advantages and disadvantages are shown in table 6 (33)

Table 3.6 The dosage of antiviral therapies of patients with chronic HBV infection (adapted from 33).

Drug	Adult dosage	Duration (weeks)	Estimated cost of one year of treatment	Advantages	Disadvantages
Injectable Pegylated interferon alfa-2a (Pegasys)	180 mcg per week	48	\$32,590	No resistance; highest seroconversion rate at one year; finite treatment time	Not well tolerated; expensive; subcutaneous injections; cannot use in persons with decompensated liver disease or HIV infection
Oral Adefovir (Hepsera)	10 mg per day	≥ 48	\$11,135	Oral; well tolerated	Mild effectiveness; moderate probability of resistance development; need to monitor renal function
Entecavir (Baraclude)	0.5 mg per day	≥ 48	\$9,195	Oral; well tolerated; Moderate effectiveness; low probability of resistance development	Not recommended in persons coinfecting with HIV because of possible development of HIV resistance; need to monitor renal function
Lamivudine (Epivir)	100 mg per day	48 to ≥ 52	\$4,290	Oral; well tolerated	Mild effectiveness; high probability of resistance development; need to monitor renal function
Telbivudine (Tyzeka)	600 mg per day	≥ 52	\$8,180	Oral; well tolerated; moderate effectiveness	High resistance; need to monitor renal function
Tenofovir (Viread)	300 mg per day	≥ 52	\$8,320	Oral; well tolerated; moderate effectiveness; low probability of resistance development	Need to monitor renal function

Classification

The classification of HBV can be divided into subtype or genotype by using the differences of protein or gene, especially in part of HBsAg (or *S* gene) (3). Based on specific antigenic determinant of HBsAg, particularly “*a*” determinant which common to all subtype (3), there are 4 major subtypes, namely *adr*, *adw*, *ayr* and *ayw* that can be divided into 9 subtypes; *adw2*, *adw4*, *ayw1*, *ayw2*, *ayw3*, *ayw4*, *adrq-*, *adrq+* and *ayr* (15, 16).

For “*a*” determinant (amino acids 124-147) (44), there are 2 alleles at position 126; isoleucine (I) and threonine (T). HBsAg determinants have the expression of amino acid substitutions that specify for 2 subdeterminants; *d/y* and *w/rat* position 122 and 160, respectively. Many amino acid differences have been found in different subtypes, as shown in table 7 (18).

Table 3.7 Amino acid residues specifying HBsAg determinants (18).

Position	Amino acid	Specificity
122	Lys [K]	<i>d</i>
	Arg [R]	<i>y</i>
127	Pro [P]	<i>w1^a/w2</i>
	Thr [T]	<i>w3</i>
	Leu [L]	<i>w4</i>
134	Tyr [Y]	<i>ayw2/ayw3</i>
	Phe [F]	<i>ayw1/ayw4/adw2/adw4</i>
160	Lys [K]	<i>w</i>
	Arg [R]	<i>r</i>

^a *w1* reactivity also requires Arg¹²², Phe¹³⁴ and/or Ala¹⁵⁹.

Based on a nucleotide divergence more than 8% in complete genome or exceeding 4% at the *S* gene levels, HBV can be classified into 8 major genotypes; A to H (9, 17). Genotype B and C are prevalent in Asia such as China, Taiwan, Japan, Indonesia including Thailand, whereas genotype A prevail in Northwestern Europe, USA and Central Africa. Genotype D is found in Mediterranean area and India. Genotype E is restricted to Africa, genotype F to Central and South America, genotype G in France and USA, and genotype H has been identified in Northern part of Latin America, Central America and Mexico (6,18). Recently, genotype I has been found in

Laos and Vietnam (18). The relationship between genotypes and subtypes and their geographic distribution showed in table 8 (18).

Table 3.8 Relationship between genotypes and subtypes and their geographic distribution^a (18).

Genotype	Serological Subtype	Geographic distribution
A	<i>adw2</i>	Northwestern Europe, USA, sub-Saharan Africa, East Africa [94], Japan, the Philippines [54,67]
	<i>ayw1^b</i>	Central Africa, Kenya [86]
	<i>ayw2</i>	Malawi [51], South Africa [50]
	<i>adw4^b</i>	Venezuela
B	<i>adw2</i>	Indonesia, China, Japan
	<i>ayw1</i>	South East Asia [94], Vietnam, Indonesia, Brazil, Indonesia, the Philippines [86]
	<i>adr^b</i>	Venezuela [103]
C	<i>adrq+</i>	Korea, China, Japan
	<i>adrq-</i>	Polynesia
	<i>ayr</i>	Vietnam
	<i>adw</i>	Tibet
	<i>adw2</i>	East Asia, Japan, the Philippines [86]
	<i>ayw2</i>	Tibet
	<i>ayw3</i>	Australia [Aborigines] [65]
D	<i>adwr</i>	Japan
	<i>ayw2</i>	West and Central Africa, East Africa [94], Mediterranean area, India [91], Tunisia [134], Soviet Union and eastern Europe [94]
	<i>ayw3</i>	Mediterranean, Soviet Union and eastern Europe [94], India (associated with drug addiction) [73], USA (injection drug users) [94] The Netherlands (IDUs) [130], South East European Russia [135]
	<i>ayw4^b</i>	West and Central Africa, USA [48]
	<i>adw3^b</i>	Central America [28]
E	<i>ayw4</i>	West Africa, Nigeria [69], Western and Central Africa [71]
F	<i>adw4</i>	AmerIndians of the Americas, Central American Hispanics, France, Alaska, Colombia, Brazil, Venezuela [24-26,48,136]
	<i>adw4q⁻</i>	American natives, Polynesia
	<i>adw2^b</i>	Venezuela [103]
	<i>ayw4^b</i>	Venezuela [75,103], Central America [73]
G	<i>adw2</i>	France, USA [27]
H	<i>adw4</i>	Northern part of Latin America, Central America and Mexico [28]

^a In addition to the specific references cited in the table, the data was compiled from [22-25,44,49,137].

^b Rare serological subtype.

Genotype A can be divided into 2 subgenotypes, subgenotype A1 or Aa found in South Africa and subgenotype A2 or Ae found in Europe. 2 subgenotypes of genotype B have been identified, B1 or Bj found in Japan, whereas B2 or Ba predominates in Asia. Genotype C have classified into 2 subgenotypes, subgenotype C1 or Cs is found predominantly in Vietnam, Myanmar, Thailand and subgenotype C2 or Ce is found in Japan, Korea and China (18).

The prevalence and characteristic of HBV in Thailand was subtype *adr* (84.4%), *adw* (14.2%) and *ayw* (1.4%), while the major genotype was C (87.1%), B (11.6%) and A (1.3%) from the study of Suwannakarn K and colleagues in 2008 (6). Recently, other genotypes such as G and H have been found in Thailand but in less proportion (17).

Clinical relevance between genotypes and clinical outcome of chronic HBV infection are still unclear. In Japan and China, genotype B has a good prognosis and is rarely associated with the development of HCC, however, in Taiwan more severe liver disease is associated with genotype C. Similarly, genotype D is associated with more severe liver disease than genotype A in India. Moreover, for the response to antiviral drug therapy, genotype C and D have lower response rate to interferon therapy when compared with genotype B and A, whereas, genotype B have a higher response rate to lamivudine than genotype C. In addition, there is report about subtype *adw* that are associated with greater risk of lamivudine resistance than *ayw* (6).

HBV genotype determining in patients with chronic HBV infection would help the information of etiological, clinical and virological investigations. (18).

HBsAg confirmatory test

HBsAg confirmatory test is the neutralization of HBsAg in serum or plasma. For blood donor screening test, HBsAg confirmatory test is required for confirm HBsAg positive. (45) In Thailand, HBsAg confirmatory test is not routinely perform due to high cost and not available in routine laboratory.

From our experience of blood screening in Thailand, in group of indeterminate HBsAg results (weakly reactive; 0.9-100 s/co Architect® i2000) in blood screening test we found blood donor who had repeatedly reactive HBsAg with negative neutralization and the follow up results with HBV serological markers and ID NAT indicated that the donor is not infected with HBV, while the donor who had positive HBsAg neutralization is infected with HBV. So we would like to evaluate and create algorithm for HBsAg confirmatory test for blood screening laboratory in Thailand.

CHAPTER IV

MATERIALS AND METHODS

4.1 Subjects and samples

Plasma samples were collected from EDTA tube of 525 HBsAg positive of Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, collected in January 2014 to May 2016, kept at -20°C until study.

Every donated blood samples at Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital have been screened for routine screening tests for blood born infectious pathogens including HBsAg, anti-HCV, Syphilis (antibody to *Treponemapallidum*) and HIV Ag/Ab combo test (Chemiluminescent Microparticle Immunoassay (CMIA), ARCHITECT® system, Abbott Laboratories, Germany). If negative for all markers, they will be tested for Nucleic Acid Testing (NAT) for 5 markers namely HIV-1 group M, HIV-1 group O, HIV-2, HCV, and HBV (cobas® TaqScreen MPX Test, Roche Diagnostics, USA). If positive for NAT, it will be discriminated for HIV-1 RNA, HCV RNA and HBV DNA (cobas® AmpliScreen Test, Roche Diagnostics, USA). Summary for routine screening test as shown in figure 12.

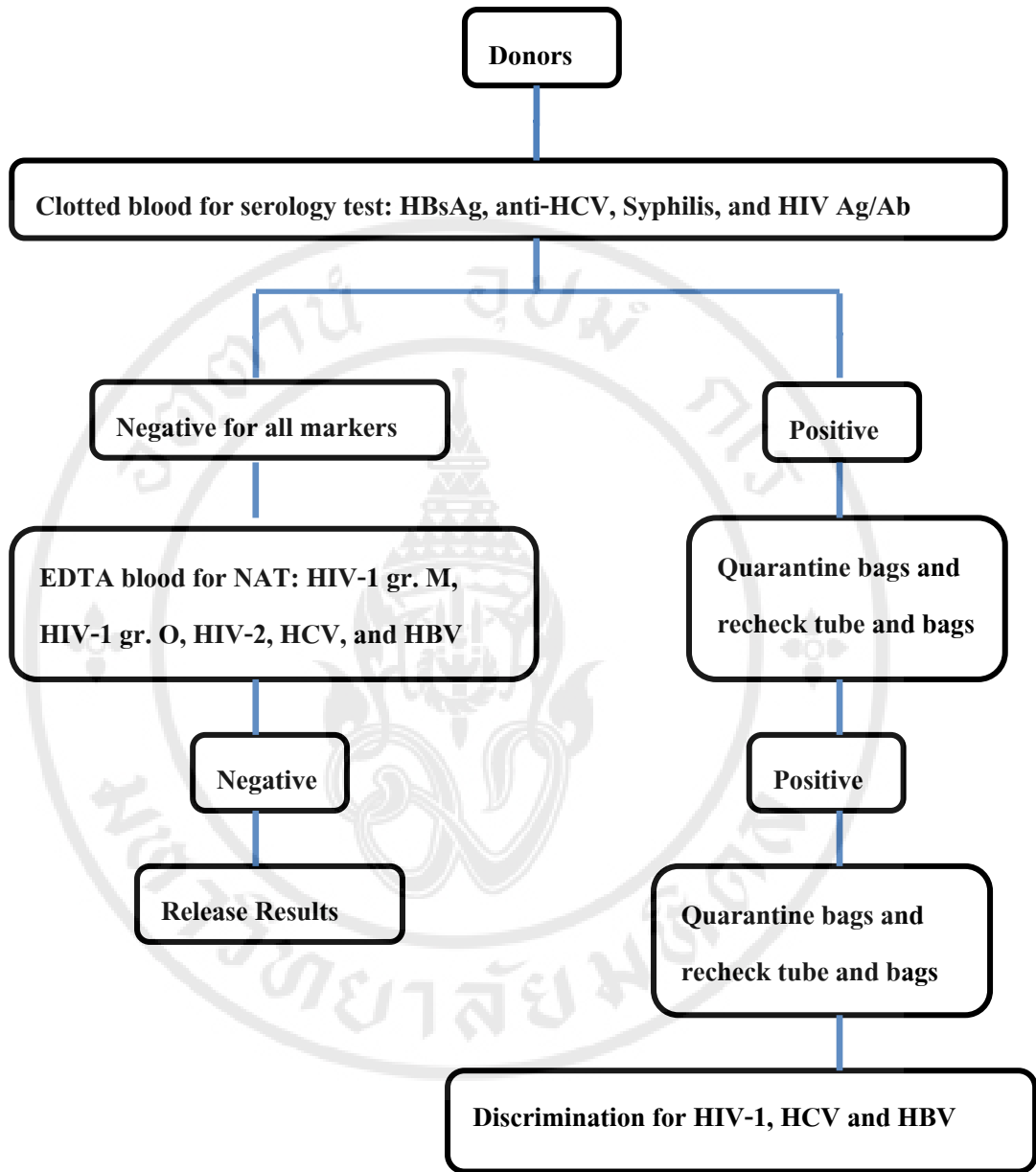


Figure 4.1 Routine screening test of Department of Transfusion Medicine, Siriraj Hospital.

The protocol of this project was approved by Siriraj Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University (Bangkok, Thailand) with COA no. Si614/2016.

4.2 Sample size calculation

This study recruited plasma samples were collected from EDTA tube of 525 HBsAg positive that kept for repository of Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital, collected in January 2014 to May 2016.

Formula for sample size calculation to estimate sensitivity.

where α = Probability of type I error = 0.05 (2-sided)

$$Z_{0.025} = 1.96$$

$$p = \text{sensitivity} = 0.95$$

$$\Delta^2 = \text{Allowable error} = 0.05$$

$$\begin{aligned} n &= \frac{z_{\alpha/2}^2 p(1-p)}{\Delta^2} \\ &= \frac{1.96^2 \times 0.95 \times 0.05}{0.05^2} \\ &= 72.9904 \\ &\approx 73 \end{aligned}$$

So estimate sensitivity for HBsAgis about (n) =73

Formula for sample size calculation

$$\text{Sample size(N)} = \frac{n}{\text{prevalence}} \times 100$$

In 2014, prevalence of 47 weakly HBsAg positive from 279 HBsAg positive

$$= (100 \times 47) / 279$$

$$= 16.85\%$$

$$\text{So, } N = (73 \times 100) / 16.85 = 433.234 \approx 434$$

In 2015, prevalence of 47 weakly HBsAg positive from 284 HBsAg positive

$$= (100 \times 47) / 284$$

$$= 16.55\%$$

$$\text{So, } N = (73 \times 100) / 16.55 = 441.087 \approx 442$$

Selected the higher number of sample size (N) = 442

$$\text{Calculate for error } 10\% = 10\% \times 442 = 44.2 \approx 45$$

$$\text{Sample size (N)} = 442 + 45 = 487$$

So, sample size (N) that need to evaluate the HBsAg confirmatory test is atleast about 487 samples. Table 9 shows Number of HBsAg positive donors at Siriraj Hospital, January 2014 to May 2016.

Table 4.1 Number of HBsAg positive donors at Siriraj Hospital, January 2014 to May 2016

Year	HBsAg positive	Include in this study
2014	279	229
2015	284	223
Jan-May 2016	100	73
Total	663	525

4.3 Sample Preparation

Freezed plasma from EDTA tube was thawed at room temperature. After mix plasma by vortex mixer, samples were centrifuged at 3,600 rpm. for 10 minutes.

Then samples were tested with anti-HBc, anti-HBs and HBsAg confirmatory by ARCHITECT[®] i2000 (CMIA, ARCHITECT[®] system, Abbott Laboratories, Germany) at Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital.

- The detection of anti-HBc uses 150 µl./test.
- The detection of anti-HBs uses 150 µl./test.
- The detection of HBsAg confirmatory test uses 242 µl./test.

4.4 Methods

4.4.1 HBsAg

Every donated blood samples at Department of Transfusion Medicine, Faculty of Medicine Siriraj Hospital have been screened for detection of HBsAg by using the ARCHITECT[®]HBsAg Qualitative II assay, that is a Chemiluminescent microparticle immunoassay (CMIA) by ARCHITECT[®] i2000 (Abbott Laboratories, Germany).

In the ARCHITECT HBsAg Qualitative II assay (46), sample, anti-HBs coated paramagnetic microparticles, and anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture. HBsAg present in the sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labeled conjugate. After washing, ancillary wash buffer is added to the reaction mixture. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of HBsAg in the sample and the RLUs detected by the ARCHITECT iSystem optics. The presence or absence of HBsAg in the sample is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an active calibration. If the chemiluminescent signal in the specimen is greater than or equal to the cutoff signal, the sample is considered reactive for HBsAg.

Interpretation, If $S/CO \geq 1.0$, it is reactive. If S/CO is about 0.90-0.99, it is grayzone or borderline or indeterminate. If $S/CO < 0.9$, it is non-reactive.

4.4.2 Anti-HBc

Detection of anti-HBc by using the ARCHITECT®Anti-HBcII assay, that is a Chemiluminescentmicroparticle immunoassay (CMIA) by ARCHITECT® i2000 (Abbott Laboratories, Germany).

The ARCHITECT®Anti-HBcII assay (47) is a two-step immunoassay for the qualitative determination of anti-HBc in human serum and plasma using CMIA technology. Sample and rHBcAg coated paramagnetic microparticles are combined. Anti-HBc present in the sample bind to the rHBcAg coated microparticles. The reaction mixture is washed and anti-human acridinium-labeled conjugate is added. After washed cycle, Pre-Trigger and Trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of anti-HBc in the sample and the RLUs detected by the ARCHITECT iSystem optics. The presence or absence of anti-HBc in the specimen is determined by comparing the chemiluminescent signal in the reaction to the cut off signal determined from an active calibration. If the chemiluminescent signal in the reaction is greater than or equal to the cut off signal, the specimen is considered reactive for anti-HBc.

Interpretation, If $S/CO \geq 1.0$, it is reactive. If $S/CO < 1.0$, it is non-reactive.

4.4.3 Anti-HBs

Detection of anti-HBs by using the ARCHITECT®Anti-HBs assay, that is a Chemiluminescentmicroparticle immunoassay (CMIA) by ARCHITECT® i2000 (Abbott Laboratories, Germany).

The ARCHITECT®Anti-HBs assay (48) is a two-step immunoassay for the quantitative determination of anti-HBs in human serum and plasma using CMIA technology. In the first step, sample and recombinant HBsAg (rHBsAg) coated paramagnetic microparticles are combined. Anti-HBs present in the samples bind to the rHBsAg coated microparticles. After washing, acridinium-labeled rHBsAg conjugate is added in the second step. After wash cycle, Pre-Trigger and Trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of anti-HBs in the sample and the RLUs detected by the

ARCHITECT iSystem optics. The concentration of anti-HBs in the specimen is determined using a previously generated ARCHITECT anti-HBs calibration curve.

Interpretation, Sample RLUs compared with calibration curve in machine and show the results is measured as mIU/mL. If $\text{mIU/mL} \geq 10.00$, it is protective immunity.

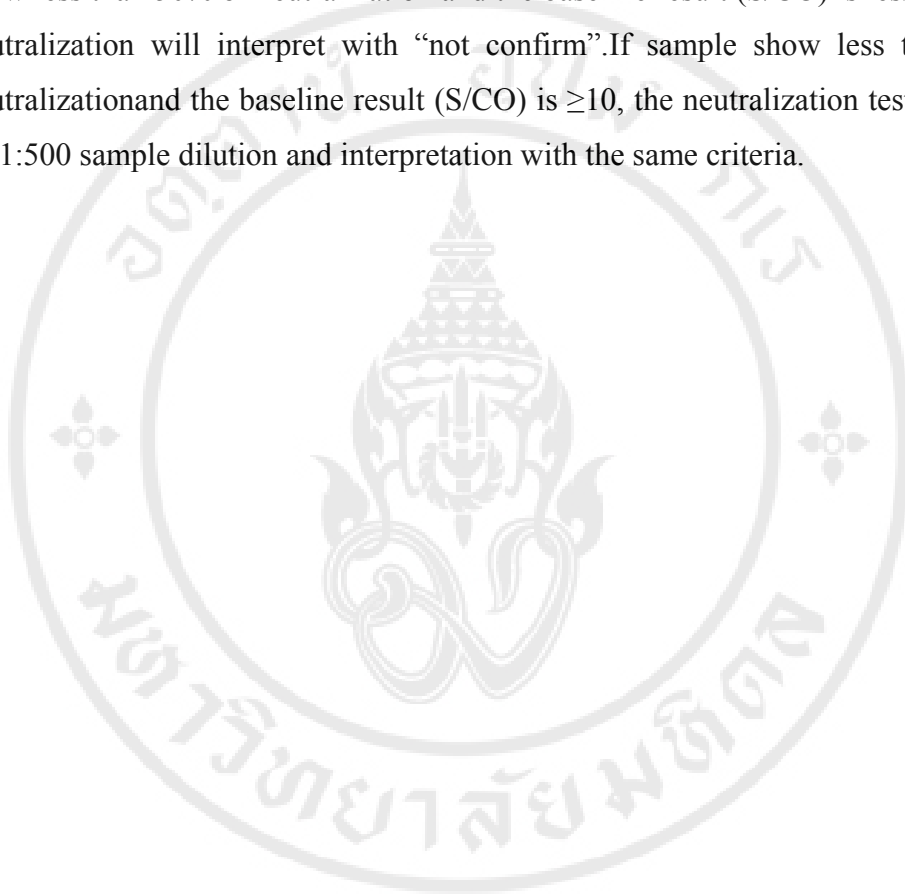
4.4.4 HBsAg neutralization confirmatory test

To confirm HBsAg in serum and plasma by means of specific antibody neutralization by using the ARCHITECT HBsAg Qualitative II confirmatory assay, that is a Chemiluminescent microparticle immunoassay (CMIA) by ARCHITECT® i2000 (Abbott Laboratories, Germany).

The ARCHITECT HBsAg Qualitative II confirmatory assay (49) consists of two single tests that are both one-step pre-treatment immunoassays. Sample and Pre-treatment 1 are combined in a reaction vessel and incubated. When HBsAg is present in the sample, it is neutralized by the antibody (anti-HBs) in Pre-Treatment 1. An aliquot of pretreated sample, anti-HBs coated paramagnetic microparticles, and anti-HBs acridinium-labeled conjugate are combined to create a reaction mixture. Any non-neutralized HBsAg present in sample binds to the anti-HBs coated microparticles and to the anti-HBs acridinium-labeled conjugate. The neutralized HBsAg is blocked from forming a sandwich with acridinium-labeled anti-HBs conjugate and anti-HBs coated microparticles. After washing, ancillary wash buffer is added to the reaction vessel and incubated. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a direct relationship between the amount of HBsAg in the sample and the RLUs detected by the ARCHITECT iSystem optics.

This sequence is repeated for the sample and Pre-Treatment 2, except Pre-Treatment 2 does not neutralize HBsAg in the sample. If the signal for non-neutralized sample (incubated with Pre-Treatment 2) result is greater than or equal to the cutoff 0.70 S/CO and the RLU of the neutralized sample (incubated with Pre-Treatment 1) is reduced by at least 50% compared to the non-neutralized sample, the sample is considered confirmed positive for HBsAg.

The interpretation result will be interpreted by 50% of neutralization. If sample show more than 50% of neutralization, the sample will be considered as “confirm positive”. If sample show less than 50%, the sample will be considered as “not confirm”. The interpretation need to consider the baseline result HBsAg. If sample show less than 50% of neutralization and the baseline result (S/CO) is less than 10, the neutralization will interpret with “not confirm”. If sample show less than 50% of neutralization and the baseline result (S/CO) is ≥ 10 , the neutralization test will be done by 1:500 sample dilution and interpretation with the same criteria.



CHAPTER V RESULTS

5.1 HBsAg neutralization confirmatory test in sample positive for CMIA

HBsAg neutralization confirmatory test applied to 525 sera sample. All samples were positive HBsAg from Chemiluminescentmicroparticle immunoassay (CMIA) by ARCHITECT® HBsAgQualitative II assay. The result of CMIA assay showed in S/CO ratio. All sample showed $S/CO \geq 1.0$, defined as positive.

475 samples were positive for HBsAg neutralization confirmatory test from 525 sample (90.47%). The sample with negative for HBsAg neutralization confirmatory test showed the low signal of CMIA test (S/CO). The median of S/CO in negative neutralization group was 1.46 while the median of S/CO in positive neutralization group was 3305.76 (Figure13).

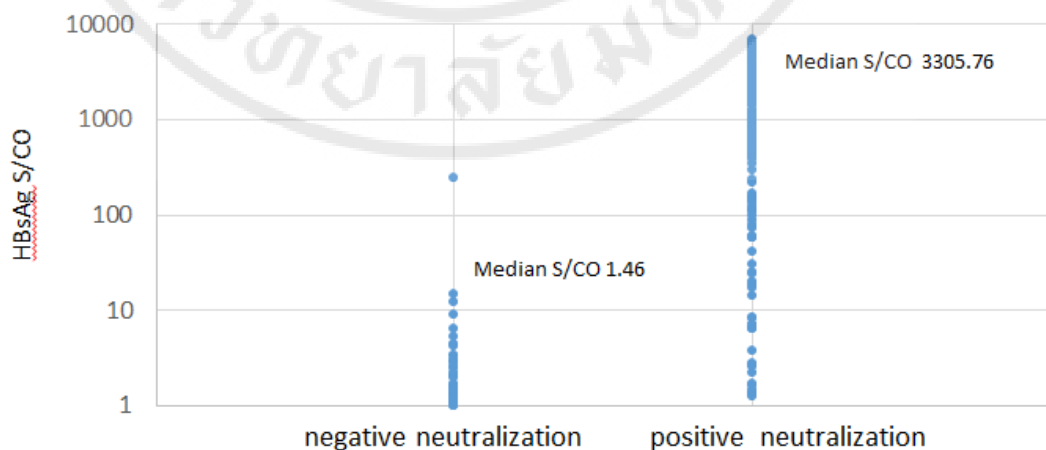


Figure 5.1 The distribution of result of HBsAg neutralization confirmatory test and CMIA (S/CO) result

5.2 HBsAg neutralization confirmatory test in different group of CMIA level

Based on CMIA result, the result showed as the ratio of S/CO. The samples were separated into 3 group based on S/CO level (**Table 10**).

Table 5.1 Group of sample based on S/CO signal.

Group	CMIA ratio (S/CO)
Low signal	1.00 - 9.99
Medium high signal	10.00 - 299.99
High signal	≥ 300

Each group was tested by HBsAg neutralization confirmatory test. The result showed that the high signal group showed 100% positive for neutralization test. The medium high signal group showed 90.62% positive for neutralization test. Meanwhile the low signal group showed 38.29% positive for neutralization test.

Table 5.2 HBsAg neutralization confirmatory test in CMIA based group

Group	Neutralization Positive	Neutralization Negative	Total	% positive
Low signal (1.00 - 9.99)	18	47	65	38.29
Medium high signal (10.00 - 299.99)	29	3	32	90.62
High signal (≥ 300)	428	0	428	100.00

The result suggest that the high signal result from CMIA test may not need to use HBsAg confirmatory test to confirm because sample with S/CO great than 300 showed 100% concordant result with neutralization test. Meanwhile the low signal group and medium high signal group may need the other criteria to concern before using HBsAg confirmatory test.

5.3 The correlation between HBsAg neutralization confirmatory test and demographic data from sample

The data of gender and age of blood donor from each sample were included in this study. 386 male samples and 139 female samples were included in this study. The correlation between gender and age with result of HBsAg neutralization confirmatory test showed in Table 12

Table 5.3 Age and gender of sample correlated with HBsAg neutralization confirmatory results.

Age	Neutralization positive	Neutralization negative	Sign. difference
Male	(n = 358)	(n=28)	$p = 0.10$
Mean	34.7	31.75	
SD	9.14	9.41	
Range	18-60	19-53	
Female	(n = 117)	(n = 22)	$p = 0.71$
Mean	34.53	30.45	
SD	9.51	10.45	
Range	18-56	18-57	
Total	(n = 475)	(n = 50)	$p = 0.01^*$
Mean	31.18	34.66	
SD	9.80	9.23	
Range	18-60	18-57	

* Significantly different by one sample t-test

There was no significantly difference between gender and neutralization test. The overall age mean showed significantly difference between positive result and negative result.

5.4 HBsAg neutralization confirmatory test with HBV marker

Anti-HBc and Anti-HBs are the other markers for HBV diagnosis. These markers can be used in routine laboratory for HBV identification. These markers were included in this study as the additional marker for supporting HBsAg test. Table 13 showed the result of HBsAg neutralization confirmatory test with individual anti-HBcor anti-HBs.

Table 5.4 Correlation between HBV marker and HBsAg neutralization confirmatory test.

HBV marker	Neutralization positive	Neutralization negative	Total	% positive
Anti-HBc				
positive	470	6	476	98.73
negative	5	44	49	10.20
Anti-HBs				
positive	14	12	26	53.84
negative	461	38	499	92.38

The sample with positive for anti-HBc would showed the high percentage of positive for neutralization test (98.73). The results suggested that anti-HBc would be the marker used for support HBsAg neutralization confirmatory test.

5.5 Correlation between HBsAg neutralization confirmatory test and HBV in each CMIA group.

Based on CMIA results that used to separate in three group with the previous criteria, the results of HBV marker were combined with CMIA group and interpreted with neutralization test (Table 14).

Table 5.5 Correlation between HBsAg neutralization confirmatory test and HBV marker in CMIA group

HBsAg neutralization	Low signal (S/CO = 1.00-9.99)	Medium High signal (S/CO = 10.00-299.99)	High signal (S/CO ≥ 300)	Total
Anti-HBc - /Anti-HBs -				
Neutralization Positive	3	0	2	5
Neutralization Negative	34	3	0	37
Anti-HBc + /Anti-HBs -				
Neutralization Positive	12	27	417	456
Neutralization Negative	1	0	0	1
Anti-HBc - /Anti-HBs +				
Neutralization Positive	0	0	0	0
Neutralization Negative	7	0	0	0
Anti-HBc + /Anti-HBs +				
Neutralization Positive	3	2	9	14
Neutralization Negative	5	0	0	5
Total	65	32	428	525

The results showed that sample with positive anti-HBc and negative anti-HBs showed the highest positive number for HBsAg neutralization confirmatory test in every CMIA group (456:1/ 99.78%). Meanwhile the other patterns of HBV markers showed inconsistency result when compared between CMIA groups. Even the sample with positive anti-HBc and positive anti-HBs showed the high percentage of positive HBsAg neutralization test, it would showed the low percentage of positive in low signal group (3:5/ 60%).

5.6 Create the algorithm for using HBsAg neutralization confirmatory test

Based on results from this study, the factor that would be effect to result of HBsAg neutralization confirmatory test was followed:

- The positive CMIA test with high signal ($S/CO \geq 300$) showed 100% positive for neutralization test. The result showed the truly positive and might not need to confirm with neutralization.
- The positive CMIA test with low signal ($S/CO = 1.00-9.99$) and medium high signal ($S/CO = 10.00-299.99$) might need to confirm with neutralization test.
- To make the decision weather confirm with neutralization or not, anti-HBc marker should be added to support the decision
- Sample with positive anti-HBc would show the high percentage positive for neutralization (almost 100%). So the neutralization might not need to confirm in this group.
- Sample with negative anti-HBc should confirm the HBsAg result with neutralization test.
- According manufacture of HBsAg neutralization confirmatory test, if sample showed the negative result of neutralization from the first test and S/CO of CMIA result was greater than 10, there was suggestion to confirm neutralization test by 1:500 sample dilution again.
- if sample showed the negative result of neutralization from the first test and S/CO of CMIA result was less than 10, the definite result would be interpreted with “not confirm”.

According to these criteria, the algorithm for using HBsAg neutralization confirmatory test showed in figure 14

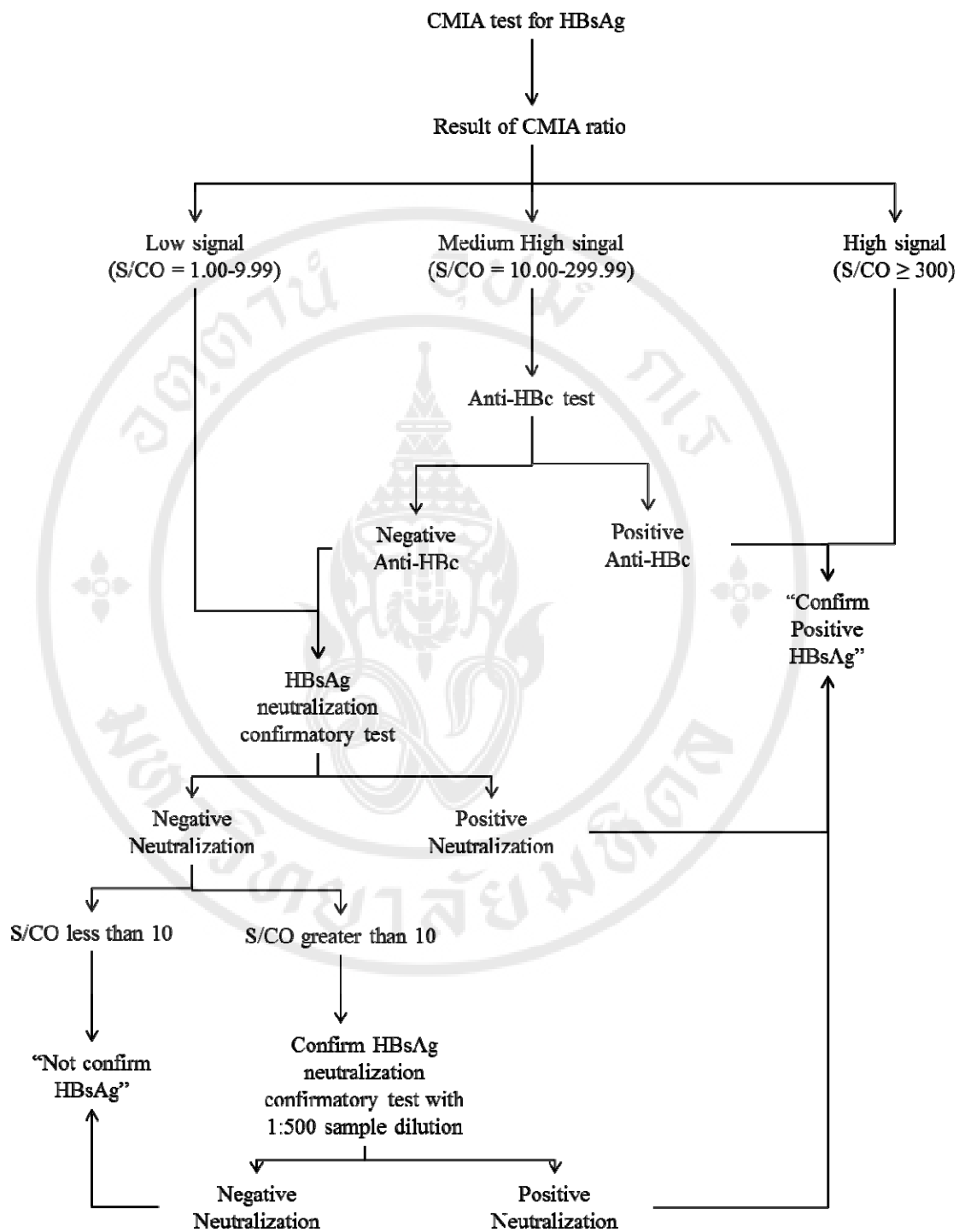


Figure 5.2 Algorithm for using HBsAg neutralization confirmatory test to confirm HBsAg for HBV infection

CHAPTER VI

DISCUSSION

HBsAg is the important marker for routine laboratory for HBV screening in blood donors. The positive HBsAg use for define the HBV positive. The sample positive for HBV would be deferred from blood donation. There is the importance to confirm this marker. CMIA test is widely used for screening HBsAg in blood donors. The clearly result from this test can be used to confirm HBV with clearly result. However, some sample may show the unclearly result and need to extra test to confirm. HBsAg neutralization confirmatory test is the one test that uses to confirm HBsAg in this case. But this test is not feasible for every laboratory. It suggests that this test may need to evaluate and has the criteria for use in order to reduce the cost and resource to invest.

This study showed the correlation between CMIA test and HBsAg neutralization confirmatory test in high signal result. Clearly positive CMIA sample with high signal (S/CO greater than 300) showed 100% positive for HBsAg neutralization confirmatory test. It suggests that HBsAg neutralization test may not need to confirm in clearly positive CMIA sample. The sample with S/CO between 1.00 and 299.99 may need to consider confirming with HBsAg neutralization test.

There was no significantly difference between gender and HBsAg neutralization confirmatory test. The overall age mean showed significantly difference between positive result and negative result. However, the demographical data was still not clear to relate with HBsAg neutralization confirmatory test.

Anti-HBc is the one of the HBV marker. This marker indicates the previous or ongoing infection with hepatitis B virus. In group of sample with S/CO lower than 300, anti-HBc may need to consider before using HBsAg neutralization confirmatory test. Anti-HBc could be used for interpretation in sample with medium high signal (S/CO = 10.00 to 299.99). If anti-HBc was positive in this group, the definite result would be "Confirm positive HBsAg". In contrast, if anti-HBc was

negative in this group, the HBsAg neutralization confirmatory would be the further confirmation test. The low signal sample with S/CO less than 10 should confirm HBsAg with neutralization test directly.

Based on manufacturer of HBsAg neutralization confirmatory test, sample with S/CO less than 10 and showed the negative for HBsAg neutralization test could be definite result as “not confirm HBsAg”. The definition of “not confirm HBsAg” result means that this sample may need to confirm HBV infection with the other test that highly sensitive, such as NAT. Even sample with S/CO greater than 10 showed the negative for HBsAg neutralization, the additional neutralization test would be done by 1:500 sample dilutions. The additional test would be defined the definite result.

The results from this study would be used to create the algorithm for using HBsAg neutralization confirmatory test in order to use in routine laboratory for blood donation. This algorithm might be helpful to save the cost and make the decision for using the confirmatory test.

CHAPTER VII

CONCLUSION

Donor management is the one important problem for Transfusion medicine, To save the false positive donors, the extra test for confirm test is need to evaluate before use.

From this study, HBsAg neutralization confirmatory test is suitable for confirm HBV infection but some criteria need to apply. The criteria to use this test should be included the CMIA result and additional HBV marker such as anti-HBc. However, the sample with negative for HBsAg neutralization confirmatory test need to confirm with the other highly sensitivity techniques in order to make sure before making a decision to defer blood donation.

REFERENCES

1. Carey WD. The prevalence and natural history of hepatitis B in the 21st century. *Cleve Clin J Med*. 2009;76 Suppl 3:S2-5.
2. Chimparlee N, Oota S, Phikulsod S, Tangkijvanich P, Poovorawan Y. Hepatitis B and hepatitis C virus in Thai blood donors. *Southeast Asian J Trop Med Public Health*. 2011;42(3):609-15.
3. Lee WM. Hepatitis B virus infection. *N Engl J Med*. 1997;337(24):1733-45.
4. Seeger C, Mason WS. Hepatitis B Virus Biology. *Microbiol Mol Biol Rev*. 2000; 64(1): 51–68.
5. Gerlich WH, Bremer C, Saniewski M, Schüttler CG, Wend UC, Willems WR, et al. Occult hepatitis B virus infection: detection and significance. *Dig Dis*. 2010;28(1):116-25.
6. Kao JH, Chen DS. Global control of hepatitis B virus infection. *Lancet Infect Dis*. 2002;2(7):395-403.
7. Hou J, Liu Z, Gu F. Epidemiology and Prevention of Hepatitis B Virus Infection. *Int J Med Sci*. 2005;2(1):50-57. Epub 2005 Jan 5.
8. Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health. Hepatitis B[Internet].2011.[updated 2011 Jul 6;cited 2013 Mar 20]; Available from:
http://www.moph.go.th/ops/thp/images/stories/Report_pics/Thai_Report/HighLight/Y54/july/Issue_41.pdf
9. Suwannakarn K, Tangkijvanich P, Thawornsuk N, Theamboonlers A, Tharmaphornpilas P, Yoocharoen P, et al. Molecular epidemiological study of hepatitis B virus in Thailand based on the analysis of pre-S and S genes. *Hepatol Res*. 2008;38(3):244-51.
10. Ganem D, Prince AM. Hepatitis B virus infection--natural history and clinical consequences. *N Engl J Med*. 2004;350(11):1118-29.

11. Prassolov A, Hohenberg H, Kalinina T, Schneider C, Cova L, Krone O, et al. New hepatitis B virus of cranes that has an unexpected broad host range. *J Virol.* 2003; 77(3):1964-76.
12. Urban S, Schwarz C, Marx UC, Zentgraf H, Schaller H, Multhaup G. Receptor recognition by a hepatitis B virus reveals a novel mode of high affinity virus-receptor interaction. *EMBO J.* 2000;19(6):1217-27.
13. Glebe D, Urban S. Viral and cellular determinants involved in hepadnaviral entry. *World J Gastroenterol.* 2007;13(1):22-38.
14. Louisirirochanakul S, Oota S, Khuponsarb K, Chalermchan W, Phikulsod S, Chongkolwatana V, et al. Occult hepatitis B virus infection in Thai blood donors. *Transfusion.* 2011;51(7):1532-40.
15. Norder H, Couroucé AM, Magnius LO. Molecular basis of hepatitis B virus serotype variations within the four major subtypes. *J Gen Virol.* 1992;73 (Pt 12):3141-5.
16. Norder H, Hammas B, Löfdahl S, Couroucé AM, Magnius LO. Comparison of the amino acid sequences of nine different serotypes of hepatitis B surface antigen and genomic classification of the corresponding hepatitis B virus strains. *J Gen Virol.* 1992;73 (Pt 5):1201-8.
17. Louisirirochanakul S, Olinger CM, Arunkaewchaemsri P, Poovorawan Y, Kanoksinsombat C, Thongme C, et al. The distribution of hepatitis B virus genotypes in Thailand. *J Med Virol.* 2012;84(10):1541-7.
18. Kramvis A, Kew M, François G. Hepatitis B virus genotypes. *Vaccine.* 2005 Mar 31;23(19):2409-23.
19. Centers for Disease Control and Prevention. Geographic Distribution of Chronic Hepatitis B Virus Infection [Internet].2008. [updated 2012 Jan 27;cited 2013 Mar 20]; Available from:
<http://www.cdc.gov/immigrantrefugeehealth/guidelines/domestic/viral-hepatitis-figure3.html>
20. Blumberg BS, Alter HJ, Visnich S. Landmark article Feb 15, 1965: A "new" antigen in leukemia sera. By Baruch S. Blumberg, Harvey J. Alter, and Sam Visnich. *JAMA.* 1984;252(2):252-7.

21. Beck J, Nassal M. Hepatitis B virus replication. *World J Gastroenterol.* 2007;13(1):48-64.
22. Hayer J, Combet C, Deléage G, Jadeau F, Zoulim F and Kay A. The Hepatitis B virus database: Surface protein[Internet].2012.[updated 2012 Nov 29;cited 2013 Mar 25]; Available from:
<http://hbvdb.ibcp.fr/HBVdb/HBVdbProteins?protein=Surface>
23. Hayer J, Combet C, Deléage G, Jadeau F, Zoulim F and Kay A. The Hepatitis B virus database: Polymerase protein[Internet].2012.[updated 2012 Nov 29;cited 2013 Mar 25]; Available from:
<http://hbvdb.ibcp.fr/HBVdb/HBVdbProteins>
24. Michailidis E, Kirby KA, Hachiya A, Yoo W, Hong SP, Kim SO, et al. Antiviral therapies: focus on hepatitis B reverse transcriptase. *Int J Biochem Cell Biol.* 2012;44(7):1060-71.
25. Schaefer S. Hepatitis B virus taxonomy and hepatitis B virus genotypes. *World J Gastroenterol.* 2007;13(1):14-21.
26. Kann M, Schmitz A, Rabe B. Intracellular transport of hepatitis B virus. *World J Gastroenterol.* 2007;13(1):39-47.
27. Bruss V. Hepatitis B virus morphogenesis. *World J Gastroenterol.* 2007;13(1):65-73.
28. Guidotti LG, Chisari FV. Immunobiology and pathogenesis of viral hepatitis. *Annu Rev Pathol.* 2006;1:23-61.
29. Paar D. Hepatitis B Virus: Transmission, Prevention, Treatment and HIV Co-Infection. *HEPP News Brown Medical School.*2001 June;4(6-7):1-9.
30. Feitelson MA, Larkin JD. New animal models of hepatitis B and C. *ILAR J.* 2001;42(2):127-38.
31. Cooke GS, Main J, Thursz MR. Treatment for hepatitis B. *BMJ.* 2010 Jan 5;340:b5429.
32. Wai CT, Lok AS. Treatment of hepatitis B. *J Gastroenterol.* 2002;37(10):771-8.
33. Wilkins T, Zimmerman D, Schade RR. Hepatitis B: diagnosis and treatment. *Am Fam Physician.* 2010 Apr 15;81(8):965-72.
34. Krajden M, McNabb G, Petric M. The laboratory diagnosis of hepatitis B virus. *Can J Infect Dis Med Microbiol.* 2005 Mar;16(2):65-72.

35. D'Souza R, Foster GR. Diagnosis and treatment of chronic hepatitis B. *J R Soc Med.* 2004 Jul;97(7):318-21.
36. Badur S, Akgün A. Diagnosis of hepatitis B infections and monitoring of treatment. *J Clin Virol.* 2001 Jun;21(3):229-37.
37. Centers for Disease Control and Prevention. Morbidity and Mortality Weekly Report: A Comprehensive Immunization Strategy to Eliminate Transmission of Hepatitis B Virus Infection in the United States. U.S. Government Printing Office: CDC, USA; 2006 Dec. 33 p. Report No.: 16
38. Recombivax HB [package insert]. Whitehouse Station, NJ, USA: Merck & Co., Inc; 2011
39. Engerix-B [package insert]. Research Triangle Park, Rixensart, Belgium: GlaxoSmithKline Biologicals; 2012
40. Gitlin N. Hepatitis B: diagnosis, prevention, and treatment. *Clin Chem.* 1997 Aug;43(8 Pt 2):1500-6.
41. Kwon SY, Lee CH. Epidemiology and prevention of hepatitis B virus infection. *Korean J Hepatol.* 2011 Jun;17(2):87-95.
42. Raj V. Treatment of hepatitis B. *Clinical Cornerstone.* 2001;3(6):24-36.
43. Melegari M, Scaglioni PP, Wands JR. Hepatitis B virus mutants associated with 3TC and famciclovir administration are replication defective. *Hepatology.* 1998;27(2):628-33.
44. Said ZN. An overview of occult hepatitis B virus infection. *World J Gastroenterol.* 2011 Apr 21;17(15):1927-38.
45. Susan A Galel. Infectious Disease Screening. In: Mark K Fung, Brenda J Grossman, Christopher D Hillyer, Connie M Westhoff, editors. *Technical Manual.* 18th ed. Bethesda, MD: AABB; 2014. p.179-212.
46. ARCHITECT® HBsAg Qualitative II [package insert]. Abbott Ireland Diagnostics Division, Sligo, Ireland: Abbott Laboratories; 2013
47. ARCHITECT® Anti-HBcII [package insert]. Abbott GmbH & Co. KG, Wiesbaden, Germany: Abbott Laboratories; 2015
48. ARCHITECT® Anti-HBs [package insert]. Abbott Ireland Diagnostics Division, Sligo, Ireland: Abbott Laboratories; 2015

49. ARCHITECT® HBsAg Qualitative II Confirmatory [package insert]. Abbott Ireland Diagnostics Division, Sligo, Ireland: Abbott Laboratories; 2013





Table A.1 Signal of HBsAg, anti-HBc, anti-HBs, gender , age and neutralization result of this study.

No.	Study No.	Gender	Age	HBsAgQ2 (S/CO)	anti-HBc (S/CO)	anti-HBs(mIU/mL)	First HBsAgQ2 confirmatory (%)	Second HBsAgQ2 confirmatory (%) (Dilute 1:500)
1	2014-003	F	33	12.01	NR(0.11)	NR(0.42)	Not confirmed(-11.59)	Not confirmed
2	2014-005	M	38	1203.43	R(9.57)	NR(0.00)	Not confirmed(1.22)	Confirmed Positive(99.72)
3	2014-006	F	20	857.01	R(8.32)	NR(0.22)	Not confirmed(-3.80)	Confirmed Positive(98.45)
4	2014-009	F	27	3988.12	R(10.35)	NR(0.12)	Not confirmed(38.23)	Confirmed Positive(99.80)
5	2014-010	M	36	3348.58	R(10.66)	NR(0.06)	Not confirmed(20.50)	Confirmed Positive(99.83)
6	2014-011	M	32	4513.6	R(9.65)	NR(0.02)	Confirmed Positive(91.17)	
7	2014-012	M	35	729.47	R(10.22)	NR(0.00)	Not confirmed(2.33)	Confirmed Positive(99.66)
8	2014-013	M	26	3573.97	R(9.33)	NR(0.00)	Not confirmed(24.50)	Confirmed Positive(99.86)
9	2014-014	M	21	559.88	R(8.59)	NR(0.17)	Not confirmed(4.25)	Confirmed Positive(99.57)
10	2014-016	F	29	3015.39	R(10.48)	NR(0.65)	Not confirmed(7.61)	Confirmed Positive(99.84)
11	2014-017	M	23	3226.31	R(9.62)	NR(0.08)	Not confirmed(22.25)	Confirmed Positive(99.90)
12	2014-018	F	46	111.26	R(6.54)	R(79.00)	Confirmed Positive(93.85)	
13	2014-019	M	24	5078.9	R(10.16)	NR(8.12)	Confirmed Positive(89.09)	
14	2014-020	M	35	3446.75	R(10.15)	NR(0.00)	Not confirmed(20.77)	Confirmed Positive(99.86)
15	2014-021	F	30	3744.73	R(9.27)	NR(0.33)	Not confirmed(29.89)	Confirmed Positive(99.85)
16	2014-022	M	38	4146.88	R(9.67)	NR(0.00)	Not confirmed(22.49)	Confirmed Positive(99.88)
17	2014-023	M	35	4453.22	R(11.01)	NR(0.00)	Confirmed Positive(81.55)	
18	2014-024	M	29	4600.35	R(9.43)	NR(0.04)	Not confirmed(41.29)	Confirmed Positive(99.88)
19	2014-025	M	46	29.9	R(11.43)	NR(0.72)	Confirmed Positive(99.66)	
20	2014-026	M	27	2293.14	R(9.92)	NR(0.00)	Not confirmed(4.69)	Confirmed Positive(99.85)
21	2014-027	M	49	3440.75	R(10.16)	NR(1.02)	Confirmed Positive(99.21)	
22	2014-028	M	37	4608.91	R(10.31)	NR(0.00)	Confirmed Positive(84.50)	
23	2014-029	M	42	7.19	R(10.77)	NR(0.21)	Confirmed Positive(100.61)	
24	2014-030	M	26	657.15	R(7.65)	NR(0.00)	Not confirmed(2.76)	Confirmed Positive(99.58)
25	2014-031	M	29	2882.04	R(9.89)	NR(1.19)	Not confirmed(10.00)	Confirmed Positive(99.84)
26	2014-032	M	21	4612.72	R(9.96)	NR(0.13)	Not confirmed(33.46)	Confirmed Positive(99.88)
27	2014-033	M	29	2172.96	R(9.65)	NR(0.21)	Not confirmed(1.11)	Confirmed Positive(99.83)
28	2014-034	M	44	5917.1	R(9.73)	NR(0.13)	Confirmed Positive(96.01)	
29	2014-035	M	30	3953.97	R(10.30)	NR(0.09)	Not confirmed(35.52)	Confirmed Positive(99.89)
30	2014-036	M	43	2399.45	R(9.88)	NR(0.00)	Not confirmed(1.37)	Confirmed Positive(99.86)
31	2014-038	M	29	5781.56	R(11.25)	NR(0.02)	Confirmed Positive(90.37)	
32	2014-039	M	26	5452.09	R(10.11)	NR(0.68)	Confirmed Positive(89.32)	
33	2014-040	F	28	3738.5	R(9.79)	NR(0.26)	Not confirmed(52.60)	Confirmed Positive(99.95)
34	2014-041	M	42	1059.31	R(9.57)	NR(6.72)	Confirmed Positive(99.19)	
35	2014-042	F	32	967.02	R(9.88)	NR(0.00)	Not confirmed(1.06)	Confirmed Positive(99.70)
36	2014-043	M	27	2598.11	R(9.81)	NR(0.19)	Not confirmed(5.65)	Confirmed Positive(99.84)
37	2014-044	M	30	1.25	NR(0.07)	NR(0.04)	Not confirmed(0.00)	
38	2014-045	F	27	726.39	R(8.30)	NR(0.20)	Not confirmed(1.36)	Confirmed Positive(99.70)
39	2014-046	F	30	2.86	NR(0.12)	NR(1.47)	Not confirmed(5.62)	
40	2014-048	M	38	4041.02	R(9.72)	NR(0.00)	Not confirmed(29.84)	Confirmed Positive(99.88)
41	2014-049	F	23	4298.61	R(10.32)	NR(0.08)	Not confirmed(31.45)	Confirmed Positive(99.87)
42	2014-050	F	46	5203.6	R(9.89)	NR(0.04)	Confirmed Positive(83.43)	
43	2014-051	M	46	584.66	R(10.20)	R(10.21)	Confirmed Positive(98.87)	
44	2014-052	M	33	2267.72	R(10.49)	NR(0.33)	Not confirmed(2.72)	Confirmed Positive(99.85)
45	2014-053	M	25	5218.1	R(9.70)	NR(0.09)	Confirmed Positive(95.87)	
46	2014-054	F	30	5874.83	R(9.66)	NR(0.10)	Confirmed Positive(66.09)	
47	2014-055	F	40	6317.68	R(9.56)	NR(0.29)	Confirmed Positive(80.56)	
48	2014-056	M	29	5472.33	R(9.87)	NR(0.05)	Not confirmed(41.72)	Confirmed Positive(99.84)
49	2014-057	M	44	4985.9	R(10.48)	NR(2.57)	Confirmed Positive(83.37)	
50	2014-058	M	24	2331.34	R(9.79)	NR(0.00)	Not confirmed(-4.99)	Confirmed Positive(99.83)

Table A.1 Signal of HBsAg, anti-HBc, anti-HBs, gender , age and neutralization result of this study. (cont.)

No.	Study No.	Gender	Age	HBsAgQ2 (S/CO)	anti-HBc (S/CO)	anti-HBs(mIU/mL)	First HBsAgQ2 confirmatory (%)	Second HBsAgQ2 confirmatory (%) (Dilute 1:500)
101	2014-115	F	29	149.49	R(8.94)	NR(3.23)	Confirmed Positive(99.03)	
102	2014-116	M	42	5008.32	R(7.37)	NR(0.00)	Confirmed Positive(98.23)	
103	2014-117	M	24	781.57	R(9.14)	NR(0.00)	Not confirmed(-0.21)	Confirmed Positive(99.39)
104	2014-118	F	35	5533.67	R(9.35)	NR(0.00)	Confirmed Positive(79.22)	
105	2014-119	M	35	5018.87	R(9.83)	NR(0.00)	Confirmed Positive(85.48)	
106	2014-120	M	48	668.84	R(9.40)	NR(0.40)	Confirmed Positive(99.11)	
107	2014-121	F	42	4655.53	R(8.73)	NR(0.00)	Confirmed Positive(99.48)	
108	2014-122	M	39	571.62	R(9.45)	NR(6.34)	Confirmed Positive(99.12)	
109	2014-123	M	32	4511.92	R(8.98)	NR(0.00)	Confirmed Positive(72.88)	
110	2014-124	M	43	4197.04	R(10.14)	NR(0.00)	Confirmed Positive(94.42)	
111	2014-125	M	34	1855.4	R(8.50)	NR(0.00)	Not confirmed(-1.80)	Confirmed Positive(99.89)
112	2014-126	M	24	3805.31	R(9.69)	NR(0.00)	Confirmed Positive(80.64)	
113	2014-127	M	52	3056.31	R(9.98)	NR(1.92)	Confirmed Positive(98.60)	
114	2014-128	F	49	3372.02	R(9.61)	NR(0.00)	Not confirmed(18.21)	Confirmed Positive(99.90)
115	2014-129	M	33	2839.71	R(9.49)	NR(0.00)	Not confirmed(12.55)	Confirmed Positive(99.89)
116	2014-130	F	37	5205.15	R(9.73)	NR(0.00)	Confirmed Positive(89.67)	
117	2014-131	M	40	4933.69	R(10.58)	NR(1.09)	Confirmed Positive(86.64)	
118	2014-132	M	25	3596.07	R(10.12)	NR(0.00)	Not confirmed(18.70)	Confirmed Positive(99.88)
119	2014-133	M	34	526.62	R(7.13)	NR(0.00)	Not confirmed(-0.09)	Confirmed Positive(99.35)
120	2014-134	M	25	2686.69	R(9.55)	NR(0.00)	Not confirmed(2.91)	Confirmed Positive(99.84)
121	2014-135	M	24	6026.44	R(9.62)	NR(0.00)	Confirmed Positive(90.46)	
122	2014-136	M	26	4485.17	R(10.14)	NR(0.31)	Confirmed Positive(50.34)	
123	2014-137	M	40	2018.2	R(9.03)	NR(0.00)	Not confirmed(-0.99)	Confirmed Positive(99.82)
124	2014-138	M	33	4719.21	R(9.64)	NR(0.00)	Confirmed Positive(76.55)	
125	2014-139	M	30	4711.44	R(10.00)	NR(0.00)	Not confirmed(37.35)	Confirmed Positive(99.93)
126	2014-140	M	31	4835.64	R(9.84)	NR(0.88)	Confirmed Positive(87.34)	
127	2014-141	M	24	3652.56	R(9.87)	NR(0.00)	Not confirmed(17.51)	Confirmed Positive(99.88)
128	2014-142	F	37	940.57	R(8.88)	NR(0.00)	Not confirmed(0.95)	Confirmed Positive(99.64)
129	2014-143	F	41	1907.45	R(8.54)	NR(0.04)	Not confirmed(-4.18)	Confirmed Positive(99.84)
130	2014-144	F	37	4699.54	R(9.45)	NR(0.00)	Not confirmed(33.51)	Confirmed Positive(99.82)
131	2014-145	M	27	2338.25	R(9.94)	NR(0.00)	Not confirmed(-4.12)	Confirmed Positive(99.87)
132	2014-146	M	44	5124.35	R(9.90)	NR(0.00)	Confirmed Positive(74.84)	
133	2014-147	M	31	2407.97	R(9.88)	NR(0.00)	Confirmed Positive(98.86)	
134	2014-148	M	33	6256.61	R(9.27)	NR(3.01)	Confirmed Positive(67.32)	
135	2014-149	M	34	71.67	R(12.49)	NR(2.19)	Confirmed Positive (99.54)	
136	2014-150	M	25	2.81	R(9.89)	NR(2.21)	Confirmed Positive (97.36)	
137	2014-151	F	25	2735.11	R(9.84)	NR(0.13)	Not confirmed (3.43)	Confirmed Positive(99.85)
138	2014-152	F	40	6225.78	R(9.04)	NR(0.00)	Confirmed Positive (91.61)	
139	2014-153	F	18	2370.76	R(9.54)	NR(0.00)	Not confirmed (-3.50)	Confirmed Positive(99.30)
140	2014-154	M	22	3744.53	R(9.20)	NR(0.00)	Not confirmed (14.30)	Confirmed Positive(99.85)
141	2014-155	M	22	5711.24	R(9.61)	NR(0.22)	Confirmed Positive (97.94)	
142	2014-156	M	28	4909.89	R(9.81)	NR(0.00)	Not confirmed (31.70)	Confirmed Positive(99.87)
143	2014-157	M	39	2944.04	R(9.62)	NR(0.30)	Not confirmed (15.60)	Confirmed Positive(99.88)
144	2014-158	M	34	2508.21	R(10.26)	NR(0.60)	Not confirmed (1.37)	Confirmed Positive(99.84)
145	2014-159	F	19	431.61	R(6.85)	NR(0.00)	Not confirmed(3.80)	Confirmed Positive(99.07)
146	2014-160	M	26	5497.79	R(9.51)	NR(0.01)	Confirmed Positive (81.22)	
147	2014-167	M	21	499.34	R(8.73)	NR(0.10)	Confirmed Positive (97.16)	
148	2014-169	M	28	1.28	NR(0.11)	NR(0.02)	Not confirmed(-1.03)	
149	2014-170	M	21	661.2	R(7.17)	NR(0.27)	Not confirmed(4.85)	Confirmed Positive(99.66)
150	2014-177	M	44	2.58	R(10.73)	NR(0.00)	Confirmed Positive(91.91)	

Table A.1 Signal of HBsAg, anti-HBc, anti-HBs, gender , age and neutralization result of this study. (cont.)

No.	Study No.	Gender	Age	HBsAgQ2 (S/CO)	anti-HBc (S/CO)	anti-HBs(mIU/mL)	First HBsAgQ2 confirmatory (%)	Second HBsAgQ2 confirmatory (%) (Dilute 1:500)
151	2014-180	F	44	5973.86	R(9.23)	NR(0.00)	Confirmed Positive (86.31)	
152	2014-181	F	18	1.57	NR(0.10)	R(12.49)	Not confirmed (-5.53)	
153	2014-182	M	21	939.48	R(9.20)	NR(0.00)	Not confirmed (0.63)	Confirmed Positive(99.64)
154	2014-183	F	22	250.04	NR(0.13)	NR(0.00)	Not confirmed (-0.25)	Not confirmed
155	2014-184	M	25	5457.37	R(9.26)	NR(0.00)	Confirmed Positive (92.84)	
156	2014-185	F	21	1198.06	R(9.43)	NR(0.00)	Not confirmed (1.35)	Confirmed Positive(99.68)
157	2014-186	M	42	4285.71	R(10.15)	R(21.67)	Not confirmed (37.41)	Confirmed Positive(99.88)
158	2014-187	M	44	4362.75	R(9.41)	NR(0.00)	Confirmed Positive (94.35)	
159	2014-188	M	45	5352.49	R(8.67)	NR(0.00)	Confirmed Positive (85.34)	
160	2014-189	M	28	429.89	R(4.33)	NR(0.00)	Not confirmed (0.08)	Confirmed Positive(99.29)
161	2014-190	F	44	4355.05	R(9.93)	NR(1.49)	Confirmed Positive (98.29)	
162	2014-191	M	38	1.03	NR(0.13)	NR(5.93)	Not confirmed(10.27)	
163	2014-192	M	28	5745.97	R(9.49)	NR(0.00)	Confirmed Positive (84.04)	
164	2014-193	F	20	5.23	NR(0.14)	NR(0.48)	Not confirmed(3.70)	
165	2014-194	M	22	516.32	R(6.68)	NR(0.09)	Not confirmed (1.33)	Confirmed Positive(99.50)
166	2014-196	M	21	216.87	R(9.65)	NR(0.52)	Confirmed Positive (99.24)	
167	2014-197	M	37	4743.63	R(10.13)	NR(3.02)	Confirmed Positive (82.21)	
168	2014-201	M	26	478.9	R(8.26)	NR(0.01)	Not confirmed (1.30)	Confirmed Positive(99.57)
169	2014-202	M	27	454.05	R(9.61)	NR(0.00)	Not confirmed (0.81)	Confirmed Positive(99.33)
170	2014-203	F	33	707.49	R(8.94)	NR(0.00)	Not confirmed (2.01)	Confirmed Positive(99.57)
171	2014-205	M	32	4090.29	R(10.03)	NR(0.27)	Not confirmed (47.24)	Confirmed Positive(99.85)
172	2014-206	M	23	3054.88	R(10.12)	NR(0.00)	Not confirmed (14.16)	Confirmed Positive(99.85)
173	2014-207	M	32	5623.46	R(9.92)	R(30.88)	Confirmed Positive(91.69)	
174	2014-208	M	26	57.39	R(10.92)	NR(2.60)	Confirmed Positive(98.91)	
175	2014-209	M	27	1531.75	R(8.51)	NR(0.19)	Confirmed Positive(98.22)	
176	2014-210	M	38	4723.12	R(11.17)	NR(0.33)	Confirmed Positive(82.87)	
177	2014-211	M	41	6.41	R(11.35)	NR(0.17)	Confirmed Positive(101.41)	
178	2014-212	M	39	4729.21	R(9.85)	NR(0.00)	Confirmed Positive(93.48)	
179	2014-214	F	34	5930.3	R(10.23)	NR(0.12)	Confirmed Positive(88.35)	
180	2014-216	F	43	4354.23	R(10.17)	NR(0.56)	Confirmed Positive(59.01)	
181	2014-217	M	28	3895.61	R(10.00)	NR(0.90)	Not confirmed(30.55)	Confirmed Positive(99.88)
182	2014-218	F	31	123.26	R(9.95)	R(10.01)	Confirmed Positive(99.14)	
183	2014-219	F	29	1.04	NR(0.14)	NR(6.44)	Not confirmed(4.23)	
184	2014-220	M	26	3234.13	R(9.51)	NR(0.15)	Confirmed Positive(99.08)	
185	2014-221	M	51	5181.63	R(10.10)	NR(0.20)	Confirmed Positive(88.59)	
186	2014-222	M	31	3264.02	R(9.89)	NR(0.00)	Not confirmed(8.26)	Confirmed Positive(99.86)
187	2014-223	M	21	3656.34	R(9.89)	NR(0.08)	Not confirmed(24.95)	Confirmed Positive(99.87)
188	2014-224	F	30	2867.38	R(10.23)	NR(0.11)	Not confirmed(1.14)	Confirmed Positive(99.89)
189	2014-225	M	23	4.39	NR(0.010)	NR(2.83)	Not confirmed(0.36)	
190	2014-226	M	41	4964.63	R(9.73)	NR(2.45)	Confirmed Positive(87.07)	
191	2014-227	M	22	457.01	R(7.53)	NR(0.00)	Not confirmed(-2.84)	Confirmed Positive(99.31)
192	2014-228	M	22	3696.93	R(10.24)	NR(0.09)	Not confirmed(20.25)	Confirmed Positive(99.86)
193	2014-229	M	25	5137.24	R(9.94)	NR(0.00)	Confirmed Positive(93.82)	
194	2014-230	F	28	4109.29	R(9.65)	NR(0.30)	Confirmed Positive(92.45)	
195	2014-231	M	28	3817.94	R(9.40)	NR(0.00)	Not confirmed(26.91)	Confirmed Positive(99.84)
196	2014-233	M	56	5757.09	R(11.63)	NR(0.00)	Confirmed Positive(97.52)	
197	2014-234	M	30	4157.17	R(9.77)	NR(0.06)	Confirmed Positive(54.55)	
198	2014-235	M	32	4249.24	R(10.17)	NR(0.00)	Not confirmed(48.07)	Confirmed Positive(99.92)
199	2014-236	M	37	685.16	R(9.53)	NR(1.49)	Not confirmed(6.44)	Confirmed Positive(98.45)
200	2014-237	M	29	2636.03	R(9.74)	NR(0.06)	Not confirmed(4.54)	Confirmed Positive(99.81)

Table A.1 Signal of HBsAg, anti-HBc, anti-HBs, gender, age and neutralization result of this study. (cont.)

No.	Study No.	Gender	Age	HBsAgQ2 (S/CO)	anti-HBc (S/CO)	anti-HBs(mIU/mL)	First HBsAgQ2 confirmatory (%)	Second HBsAgQ2 confirmatory (%) (Dilute 1:500)
201	2014-238	M	26	3520.43	R(10.06)	NR(0.20)	Not confirmed(17.82)	Confirmed Positive(99.86)
202	2014-239	M	32	2556.04	R(9.71)	NR(0.11)	Not confirmed(-4.17)	Confirmed Positive(99.85)
203	2014-240	M	29	2929.9	R(9.70)	NR(0.22)	Not confirmed(12.77)	Confirmed Positive(99.88)
204	2014-241	M	41	57.07	R(9.53)	NR(2.53)	Confirmed Positive(99.89)	
205	2014-242	F	34	4699.11	R(9.88)	NR(0.15)	Confirmed Positive(77.69)	
206	2014-243	M	31	1276.18	R(9.98)	NR(0.10)	Not confirmed(-1.70)	Confirmed Positive(99.78)
207	2014-244	M	47	4873.88	R(9.78)	NR(0.27)	Confirmed Positive(87.76)	
208	2014-245	F	44	4041.86	R(9.90)	NR(0.95)	Confirmed Positive(98.94)	
209	2014-246	F	48	24.2	R(10.86)	NR(0.15)	Confirmed Positive(99.93)	
210	2014-247	M	37	446.16	R(8.49)	NR(0.02)	Not confirmed(1.64)	Confirmed Positive(99.42)
211	2014-248	M	27	146	R(8.75)	NR(1.20)	Confirmed Positive(98.14)	
212	2014-249	M	42	4526.25	R(10.51)	NR(0.69)	Confirmed Positive(89.24)	
213	2014-250	M	51	3712.93	R(9.66)	NR(1.24)	Confirmed Positive(97.22)	
214	2014-251	M	26	443.24	R(7.06)	NR(0.83)	Not confirmed(4.63)	Confirmed Positive(99.42)
215	2014-252	F	30	5484.87	R(10.12)	NR(0.08)	Confirmed Positive(94.47)	
216	2014-253	F	29	4051	R(10.09)	NR(0.12)	Confirmed Positive(97.87)	
217	2014-254	F	23	932.07	R(8.09)	NR(1.30)	Not confirmed(-1.22)	Confirmed Positive(99.73)
218	2014-255	F	24	3924.53	R(9.34)	NR(0.00)	Not confirmed(25.16)	Confirmed Positive(99.86)
219	2014-256	M	36	4462.41	R(10.42)	NR(0.04)	Confirmed Positive(73.79)	
220	2014-257	F	35	3260.28	R(9.84)	NR(0.10)	Not confirmed(15.92)	Confirmed Positive(99.86)
221	2014-258	M	29	3839.82	R(11.02)	NR(0.04)	Not confirmed(34.62)	Confirmed Positive(99.85)
222	2014-259	M	49	510.82	R(9.77)	R(129.39)	Confirmed Positive(95.83)	
223	2014-260	M	37	2290.39	R(10.02)	NR(0.02)	Not confirmed(0.68)	Confirmed Positive(99.84)
224	2014-261	F	23	1.15	NR(0.29)	NR(0.00)	Not confirmed(-21-21)	
225	2014-262	M	26	4276.55	R(9.35)	NR(0.09)	Confirmed Positive(72.41)	
226	2014-263	M	36	5196.12	R(10.59)	NR(0.00)	Confirmed Positive(89.00)	
227	2014-264	M	49	4923.49	R(9.89)	NR(0.00)	Confirmed Positive(67.22)	
228	2014-265	M	36	1.95	NR(0.20)	R(76.83)	Not confirmed(-11.53)	
229	2014-266	M	32	2816.46	R(9.52)	NR(0.16)	Not confirmed(14.30)	Confirmed Positive(99.86)
230	2015-003	M	30	1852.04	R(11.14)	NR(0.00)	Not confirmed(0.87)	Confirmed Positive(99.79)
231	2015-005	M	38	2466.53	R(11.26)	NR(0.00)	Not confirmed(2.84)	Confirmed Positive(99.91)
232	2015-006	M	29	2088.97	R(9.34)	NR(1.12)	Confirmed Positive(98.44)	
233	2015-009	M	22	5773.44	R(9.58)	NR(0.00)	Confirmed Positive(93.42)	
234	2015-011	M	37	3741.98	R(10.07)	NR(0.07)	Confirmed Positive(81.46)	
235	2015-012	F	19	3219.93	R(9.53)	NR(0.32)	Not confirmed(5.54)	Confirmed Positive(99.37)
236	2015-014	F	20	5264.5	R(9.33)	NR(0.00)	Confirmed Positive(66.41)	
237	2015-018	M	25	687	R(10.11)	NR(0.12)	Not confirmed(2.17)	Confirmed Positive(99.70)
238	2015-019	M	32	4401.48	R(10.20)	NR(0.01)	Confirmed Positive(82.60)	
239	2015-020	M	30	3312.28	R(9.62)	NR(0.24)	Not confirmed(9.68)	Confirmed Positive(99.87)
240	2015-023	F	28	1.09	NR(0.22)	NR(0.00)	Not confirmed(5.81)	
241	2015-025	M	34	813.87	R(8.31)	NR(0.290)	Not confirmed(-1.27)	Confirmed Positive(99.66)
242	2015-027	M	29	1.1	NR(0.07)	NR(0.00)	Not confirmed(5.97)	
243	2015-028	M	22	1	NR(0.11)	NR(4.03)	Not confirmed(-15.08)	
244	2015-030	M	31	4878.31	R(10.66)	NR(0.11)	Confirmed Positive(80.40)	
245	2015-032	F	18	727.31	R(8.01)	NR(0.00)	Not confirmed(4.12)	Confirmed Positive(99.48)
246	2015-033	M	50	17.45	R(12.60)	NR(1.88)	Confirmed Positive(100.03)	
247	2015-034	M	38	1808.39	R(11.25)	NR(0.20)	Not confirmed(-0.42)	Confirmed Positive(99.53)
248	2015-035	M	40	4689.43	R(10.61)	NR(0.00)	Confirmed Positive(71.83)	
249	2015-037	F	46	1.15	NR(0.07)	NR(0.00)	Not confirmed(-16.74)	
250	2015-038	M	33	3917.72	R(11.04)	NR(4.12)	Confirmed Positive(58.97)	

Table A.1 Signal of HBsAg, anti-HBc, anti-HBs, gender , age and neutralization result of this study. (cont.)

No.	Study No.	Gender	Age	HBsAgQ2 (S/CO)	anti-HBc (S/CO)	anti-HBs(mIU/mL)	First HBsAgQ2 confirmatory (%)	Second HBsAgQ2 confirmatory (%) (Dilute 1:500)
251	2015-039	M	43	297.55	R(11.00)	NR(0.00)	Confirmed Positive(99.55)	
252	2015-040	M	40	5186.83	R(10.99)	NR(0.00)	Confirmed Positive(93.03)	
253	2015-041	M	38	4762.1	R(10.92)	NR(0.18)	Confirmed Positive(83.80)	
254	2015-042	M	31	5348.11	R(10.54)	NR(0.00)	Confirmed Positive(92.28)	
255	2015-043	M	24	1.12	NR(0.07)	NR(0.24)	Not confirmed(-1.92)	
256	2015-045	F	37	1853.79	R(10.31)	NR(0.64)	Confirmed Positive(98.98)	
257	2015-046	M	47	4134.87	R(10.06)	NR(0.19)	Not confirmed(32.73)	Confirmed Positive(99.68)
258	2015-047	F	30	1.34	R(11.79)	NR(2.57)	Confirmed Positive(106.25)	
259	2015-048	M	51	3560.98	R(6.92)	NR(0.02)	Not confirmed(0.99)	Confirmed Positive(99.78)
260	2015-049	M	30	3.36	NR(0.12)	NR(0.33)	Neg(C1=0.33, C2=0.25)	
261	2015-051	M	24	1450.92	R(10.82)	NR(0.16)	Not confirmed(-4.31)	Confirmed Positive(99.71)
262	2015-053	M	27	4624.77	R(9.75)	NR(0.03)	Confirmed Positive(79.54)	
263	2015-054	M	29	718.53	R(9.76)	NR(0.46)	Confirmed Positive(99.24)	
264	2015-055	M	26	2982.5	R(9.46)	NR(0.00)	Not confirmed(12.58)	Confirmed Positive(99.67)
265	2015-056	M	33	4146.32	R(9.87)	NR(0.00)	Confirmed Positive(65.28)	
266	2015-057	M	37	4684.99	R(9.93)	NR(0.00)	Confirmed Positive(73.45)	
267	2015-058	M	24	805.92	R(6.52)	NR(0.00)	Not confirmed(1.08)	Confirmed Positive(99.30)
268	2015-059	F	57	2.08	R(2.85)	HR(>1000.00)	Not confirmed(-0.48)	
269	2015-060	F	29	492.26	R(8.73)	NR(0.00)	Not confirmed(1.45)	Confirmed Positive(99.68)
270	2015-061	F	31	3112.03	R(10.45)	NR(0.00)	Not confirmed(7.35)	Confirmed Positive(99.67)
271	2015-062	M	35	4555.12	R(11.07)	NR(2.32)	Confirmed Positive(63.98)	
272	2015-064	M	36	502.77	R(8.38)	NR(0.00)	Not confirmed(4.21)	Confirmed Positive(98.52)
273	2015-065	F	23	1650.08	R(11.73)	NR(0.00)	Not confirmed(0.24)	Confirmed Positive(99.63)
274	2015-066	M	47	4541.29	R(12.96)	NR(1.30)	Confirmed Positive (97.95)	
275	2015-067	M	25	517.56	R(9.73)	NR(0.00)	Not confirmed(3.39)	Confirmed Positive(99.75)
276	2015-069	M	60	975.51	R(7.78)	NR(0.00)	Confirmed Positive (99.09)	
277	2015-070	M	19	1.31	NR(0.10)	NR(0.47)	Not confirmed(-9.22)	
278	2015-071	M	23	4022.98	R(11.42)	NR(0.04)	Not confirmed(46.10)	Confirmed Positive(99.69)
279	2015-072	M	32	1759.81	R(10.05)	NR(1.40)	Confirmed Positive (96.48)	
280	2015-073	M	38	560.35	R(9.05)	NR(0.05)	Not confirmed(0.91)	Confirmed Positive(99.08)
281	2015-074	F	31	1972.67	R(9.35)	NR(0.00)	Confirmed Positive (98.86)	
282	2015-075	F	23	698.82	R(10.54)	NR(0.00)	Not confirmed(6.58)	Confirmed Positive(99.64)
283	2015-077	M	50	1510.66	R(10.72)	NR(0.000)	Confirmed Positive (99.09)	
284	2015-078	F	42	3640.08	R(8.82)	NR(0.75)	Not confirmed (23.85)	Confirmed Positive(99.88)
285	2015-079	M	29	2896.73	R(7.33)	NR(0.00)	Not confirmed (3.00)	Confirmed Positive(99.93)
286	2015-080	M	36	4464.39	R(9.81)	NR(0.03)	Confirmed Positive (77.68)	
287	2015-081	M	45	3183.6	R(9.67)	NR(0.00)	Not confirmed (5.51)	Confirmed Positive(99.87)
288	2015-084	M	36	5413.54	R(9.95)	NR(0.00)	Confirmed Positive (95.86)	
289	2015-087	F	38	14.92	NR(0.08)	NR(0.03)	Not confirmed (0.93)	Not confirmed
290	2015-088	M	27	1.47	NR(0.09)	NR(0.00)	Not confirmed (-4.56)	
291	2015-089	M	20	1.01	NR(0.14)	NR(1.48)	Not confirmed (1.13)	
292	2015-090	M	38	3748.73	R(9.33)	NR(0.00)	Not confirmed (17.15)	Confirmed Positive(99.89)
293	2015-091	F	35	3130.38	R(8.18)	NR(0.28)	Not confirmed (20.95)	Confirmed Positive(99.87)
294	2015-092	F	53	18.48	R(12.06)	NR(1.14)	Confirmed Positive(99.93)	
295	2015-093	M	18	1075.51	R(9.59)	NR(0.00)	Not confirmed (-7.73)	Confirmed Positive(99.89)
296	2015-094	M	39	5615.55	R(10.85)	NR(0.08)	Confirmed Positive(93.82)	
297	2015-095	M	32	3333.81	R(10.27)	NR(0.00)	Not confirmed (14.87)	Confirmed Positive(99.87)
298	2015-096	M	35	3981.45	R(9.31)	NR(0.21)	Not confirmed (25.09)	Confirmed Positive(99.89)
299	2015-097	M	25	3564.66	R(9.46)	NR(0.19)	Not confirmed (16.63)	Confirmed Positive(99.84)
300	2015-098	M	50	1418.45	R(10.39)	NR(0.90)	Confirmed Positive(98.00)	

Table A.1 Signal of HBsAg, anti-HBc, anti-HBs, gender , age and neutralization result of this study. (cont.)

No.	Study No.	Gender	Age	HBsAgQ2 (S/CO)	anti-HBc (S/CO)	anti-HBs(mIU/mL)	First HBsAgQ2 confirmatory (%)	Second HBsAgQ2 confirmatory (%) (Dilute 1:500)
301	2015-099	M	38	2.63	R(10.13)	NR(4.974)	Confirmed Positive(98.44)	
302	2015-100	F	30	3377.72	R(9.41)	NR(0.34)	Confirmed Positive(86.61)	
303	2015-101	M	36	4553.43	R(10.83)	NR(0.84)	Confirmed Positive(63.21)	
304	2015-105	M	51	4061.55	R(10.97)	NR(0.61)	Confirmed Positive(98.35)	
305	2015-106	M	36	4.15	NR(0.08)	NR(0.00)	Not confirmed(-4.85)	
306	2015-107	M	40	4041.88	R(10.95)	NR(0.00)	Not confirmed(40.22)	Confirmed Positive(99.88)
307	2015-109	M	38	3930.27	R(10.52)	NR(0.46)	Confirmed Positive(70.62)	
308	2015-112	M	47	578.68	R(10.45)	NR(0.27)	Confirmed Positive(99.33)	
309	2015-114	M	40	3513.7	R(10.96)	NR(0.21)	Not confirmed(23.33)	Confirmed Positive(99.88)
310	2015-115	F	30	869.85	R(10.88)	NR(0.00)	Not confirmed(0.84)	Confirmed Positive(99.69)
311	2015-117	M	30	1.2	NR(0.47)	NR(0.00)	Not confirmed(-6.69)	
312	2015-118	M	40	1455.12	R(10.09)	NR(1.05)	Confirmed Positive(96.85)	
313	2015-119	M	24	2.99	NR(0.09)	NR(0.00)	Not confirmed(9.93)	
314	2015-120	M	39	5344.05	R(10.24)	NR(0.17)	Confirmed Positive(92.64)	
315	2015-122	M	34	706.2	R(7.94)	NR(0.07)	Not confirmed(-1.76)	Confirmed Positive(99.64)
316	2015-123	F	41	1059.55	R(9.75)	NR(4.01)	Confirmed Positive(99.21)	
317	2015-124	M	24	764.98	R(9.10)	NR(0.18)	Confirmed Positive(99.15)	
318	2015-126	M	22	1.07	NR(0.17)	NR(4.77)	Not confirmed(-3.89)	
319	2015-127	M	32	488.98	R(10.70)	NR(0.00)	Not confirmed(2.68)	Confirmed Positive(99.38)
320	2015-129	F	31	98.53	R(10.29)	NR(0.00)	Confirmed Positive(99.88)	
321	2015-130	M	40	2.94	R(1.69)	R(67.54)	Not confirmed(0.32)	
322	2015-133	M	35	398.06	R(8.69)	NR(0.15)	Not confirmed(2.11)	Confirmed Positive(99.23)
323	2015-134	F	32	4371.29	R(10.66)	NR(0.00)	Confirmed Positive(91.01)	
324	2015-138	M	33	3430.4	R(10.00)	NR(0.04)	Not confirmed(20.18)	Confirmed Positive(99.89)
325	2015-139	M	53	4848.01	R(9.57)	NR(0.00)	Not confirmed(38.15)	Confirmed Positive(99.92)
326	2015-140	M	51	3595.74	R(11.33)	NR(0.00)	Not confirmed(12.91)	Confirmed Positive(99.89)
327	2015-141	F	52	4362.23	R(10.03)	NR(0.00)	Not confirmed(25.59)	Confirmed Positive(99.83)
328	2015-142	F	21	2239.26	R(10.42)	NR(0.23)	Confirmed Positive(99.37)	
329	2015-144	M	20	1029.1	R(9.51)	NR(0.00)	Not confirmed(-0.47)	Confirmed Positive(99.68)
330	2015-145	M	31	2034.24	R(10.58)	NR(0.00)	Not confirmed(-3.39)	Confirmed Positive(99.87)
331	2015-146	F	22	3864.49	R(10.13)	NR(0.00)	Confirmed Positive(81.46)	
332	2015-147	M	23	613.55	R(2.41)	NR(0.09)	Not confirmed(-1.67)	Confirmed Positive(99.60)
333	2015-148	F	54	5419.45	R(9.76)	NR(0.00)	Confirmed Positive(91.79)	
334	2015-149	M	28	2085.62	R(9.29)	NR(0.00)	Not confirmed(4.28)	Confirmed Positive(99.84)
335	2015-150	F	18	1634.7	R(9.86)	NR(0.73)	Confirmed Positive(75.19)	
336	2015-151	M	27	724.73	R(9.08)	NR(0.04)	Not confirmed(2.19)	Confirmed Positive(99.72)
337	2015-152	M	35	2855.02	R(10.97)	NR(0.00)	Not confirmed(3.49)	Confirmed Positive(99.87)
338	2015-153	M	59	5571.92	R(9.01)	NR(0.17)	Confirmed Positive(90.29)	
339	2015-154	M	37	3365.86	R(10.34)	NR(0.22)	Not confirmed(21.06)	Confirmed Positive(99.86)
340	2015-155	M	41	1.44	NR(0.09)	NR(0.00)	Not confirmed(-8.36)	
341	2015-156	M	54	1683.74	R(9.61)	NR(0.06)	Not confirmed(0.56)	Confirmed Positive(99.84)
342	2015-157	M	40	4614.3	R(9.85)	NR(0.16)	Confirmed Positive(85.87)	
343	2015-158	M	40	3683.62	R(9.57)	NR(0.23)	Not confirmed(26.27)	Confirmed Positive(99.89)
344	2015-159	F	45	2940.77	R(9.69)	NR(0.00)	Not confirmed(9.08)	Confirmed Positive(99.87)
345	2015-160	F	24	431.7	R(5.73)	NR(0.13)	Not confirmed(-2.09)	Confirmed Positive(99.45)
346	2015-161	M	49	58.63	R(10.75)	NR(0.06)	Confirmed Positive(98.89)	
347	2015-162	F	19	1.1	NR(0.10)	NR(9.23)	Not confirmed(-7.62)	
348	2015-163	M	32	2799.93	R(9.85)	NR(0.12)	Not confirmed(8.35)	Confirmed Positive(99.27)
349	2015-164	M	38	5311.61	R(11.20)	NR(0.00)	Confirmed Positive(83.12)	
350	2015-167	M	24	2773.54	R(9.56)	NR(0.41)	Not confirmed(6.45)	Confirmed Positive(99.67)

Table A.1 Signal of HBsAg, anti-HBc, anti-HBs, gender , age and neutralization result of this study. (cont.)

No.	Study No.	Gender	Age	HBsAgQ2 (S/CO)	anti-HBc (S/CO)	anti-HBs(mIU/mL)	First HBsAgQ2 confirmatory (%)	Second HBsAgQ2 confirmatory (%) (Dilute 1:500)
351	2015-168	M	37	4537.91	R(9.43)	NR(0.10)	Confirmed Positive(84.76)	
352	2015-169	M	33	4522.92	R(10.05)	NR(0.05)	Confirmed Positive(96.01)	
353	2015-171	M	39	4699.1	R(10.63)	NR(0.06)	Confirmed Positive(50.12)	
354	2015-172	M	25	4980.29	R(11.01)	NR(0.00)	Confirmed Positive(78.02)	
355	2015-173	M	54	111	R(10.96)	NR(2.27)	Confirmed Positive(98.73)	
356	2015-174	M	34	1202.52	R(8.44)	NR(0.00)	Not confirmed(0.85)	Confirmed Positive(99.45)
357	2015-175	M	38	1.31	R(10.35)	NR(0.47)	Confirmed Positive (100.00)	
358	2015-176	M	28	4543.12	R(9.20)	NR(0.00)	Confirmed Positive (88.40)	
359	2015-177	M	50	3173.74	R(10.63)	NR(0.63)	Confirmed Positive (97.19)	
360	2015-178	F	24	3695.01	R(9.82)	NR(0.20)	Not confirmed(31.06)	Confirmed Positive(99.68)
361	2015-179	M	26	3778.33	R(8.97)	R(11.02)	Not confirmed(47.73)	Confirmed Positive(99.71)
362	2015-180	M	26	4528.12	R(9.75)	NR(0.11)	Confirmed Positive (95.11)	
363	2015-181	M	58	873.76	R(8.27)	R(12.72)	Confirmed Positive (96.81)	
364	2015-182	F	46	4727.34	R(9.42)	NR(0.00)	Confirmed Positive (87.31)	
365	2015-183	M	45	2385.07	R(9.76)	NR(0.06)	Confirmed Positive (98.57)	
366	2015-184	M	44	3891.56	R(10.99)	NR(0.09)	Not confirmed (22.61)	Confirmed Positive(99.68)
367	2015-185	M	46	4162.71	R(9.67)	NR(0.71)	Confirmed Positive (71.13)	
368	2015-186	M	39	2972.82	R(10.79)	NR(0.74)	Not confirmed (3.20)	Confirmed Positive(99.67)
369	2015-187	M	53	88.45	R(11.60)	NR(0.66)	Confirmed Positive (98.72)	
370	2015-188	F	25	4196.21	R(9.32)	NR(0.09)	Confirmed Positive (85.25)	
371	2015-189	M	31	3770.77	R(10.14)	NR(0.01)	Not confirmed (20.57)	Confirmed Positive(99.71)
372	2015-190	M	52	3239.39	R(11.29)	NR(0.49)	Confirmed Positive (91.52)	
373	2015-191	M	43	3810.27	R(9.41)	NR(0.57)	Confirmed Positive (98.06)	
374	2015-192	F	24	417.92	R(7.23)	NR(0.00)	Not confirmed (0.75)	Confirmed Positive(98.20)
375	2015-193	F	52	4705.13	R(9.94)	NR(0.11)	Confirmed Positive (88.75)	
376	2015-194	M	35	4106.06	R(9.60)	NR(0.00)	Not confirmed (44.95)	Confirmed Positive(99.71)
377	2015-195	M	43	5312.08	R(11.00)	NR(2.60)	Confirmed Positive (91.98)	
378	2015-198	M	44	5038.66	R(10.77)	NR(0.14)	Confirmed Positive (88.43)	
379	2015-200	M	23	8.21	NR(0.17)	NR(0.00)	Confirmed Positive(98.95)	
380	2015-205	F	43	1641.28	R(9.56)	NR(0.00)	Not confirmed(3.62)	Confirmed Positive(99.51)
381	2015-206	M	49	20.3	R(11.85)	NR(1.34)	Confirmed Positive(99.37)	
382	2015-207	M	44	1.5	R(9.70)	NR(2.29)	Not confirmed(-14.86)	
383	2015-208	M	33	3971.91	R(10.87)	NR(0.00)	Confirmed Positive(96.36)	
384	2015-209	M	26	527.51	R(10.31)	NR(0.00)	Not confirmed(4.54)	Confirmed Positive(98.96)
385	2015-211	M	52	4173.9	R(11.09)	NR(0.13)	Confirmed Positive(63.84)	
386	2015-212	F	34	5275.39	R(10.22)	NR(0.04)	Confirmed Positive(83.42)	
387	2015-215	F	21	1.18	NR(0.12)	NR(0.00)	Neg(C1=0.68,C2=0.60)	
388	2015-216	F	40	235.29	R(9.52)	NR(0.65)	Confirmed Positive(99.06)	
389	2015-217	M	26	2313.22	R(9.72)	NR(3.66)	Not confirmed(4.73)	Confirmed Positive(99.61)
390	2015-219	M	30	1.07	NR(0.09)	NR(0.00)	Not confirmed(-8.00)	
391	2015-221	M	37	5050.04	R(9.60)	NR(0.00)	Confirmed Positive(91.88)	
392	2015-222	M	27	4889.71	R(9.56)	NR(0.00)	Confirmed Positive(91.07)	
393	2015-223	M	37	893.3	R(10.53)	NR(7.15)	Confirmed Positive(98.14)	
394	2015-225	M	47	5257.2	R(9.98)	NR(0.08)	Confirmed Positive(92.06)	
395	2015-226	F	26	3393.28	R(9.52)	NR(0.13)	Not confirmed(40.52)	Confirmed Positive(99.76)
396	2015-228	F	47	1078.04	R(8.95)	NR(0.12)	Not confirmed(0.40)	Confirmed Positive(99.39)
397	2015-229	M	45	3221.98	R(9.66)	NR(0.00)	Not confirmed(13.76)	Confirmed Positive(99.71)
398	2015-230	M	36	561.8	R(8.29)	NR(0.07)	Not confirmed(3.04)	Confirmed Positive(99.16)
399	2015-231	M	52	4101.31	R(10.12)	NR(0.10)	Confirmed Positive(90.43)	
400	2015-232	M	19	4744.98	R(10.12)	NR(0.12)	Confirmed Positive(52.09)	

Table A.1 Signal of HBsAg, anti-HBc, anti-HBs, gender , age and neutralization result of this study. (cont.)

No.	Study No.	Gender	Age	HBsAgQ2 (S/CO)	anti-HBc (S/CO)	anti-HBs(mIU/mL)	First HBsAgQ2 confirmatory (%)	Second HBsAgQ2 confirmatory (%) (Dilute 1:500)
401	2015-234	F	46	1.23	R(8.04)	R(318.09)	Not confirmed(-10.70)	
402	2015-235	M	25	5403.81	R(9.14)	NR(0.48)	Confirmed Positive(90.35)	
403	2015-236	M	47	339.38	R(8.91)	NR(0.00)	Confirmed Positive(69.00)	
404	2015-237	M	34	3869.75	R(10.45)	NR(0.01)	Confirmed Positive(99.35)	
405	2015-238	M	54	2036.97	R(10.22)	NR(1.17)	Confirmed Positive(99.10)	
406	2015-239	M	26	4290.34	R(9.79)	NR(3.13)	Confirmed Positive(79.14)	
407	2015-240	F	29	3291.64	R(9.71)	NR(0.16)	Not confirmed(18.53)	Confirmed Positive(99.61)
408	2015-241	M	55	1.34	NR(0.016)	NR(0.00)	Confirmed Positive(100.07)	
409	2015-242	M	33	724.66	NR(0.33)	NR(0.00)	Confirmed Positive(99.27)	
410	2015-243	M	39	136.3	R(9.87)	NR(0.00)	Confirmed Positive(99.36)	
411	2015-244	M	36	5022.08	R(10.01)	NR(0.09)	Confirmed Positive(76.50)	
412	2015-245	M	39	3147.46	R(9.94)	NR(0.00)	Not confirmed(9.32)	Confirmed Positive(99.56)
413	2015-246	F	30	5107.5	R(9.83)	NR(0.05)	Confirmed Positive(84.50)	
414	2015-247	M	39	3646.72	R(10.43)	NR(0.03)	Not confirmed(15.06)	Confirmed Positive(99.66)
415	2015-248	M	45	1554.84	R(11.34)	NR(1.13)	Confirmed Positive(98.31)	
416	2015-251	F	37	2.22	NR(0.09)	NR(0.00)	Not confirmed(-22.65)	
417	2015-254	M	39	4938.27	R(10.24)	NR(0.11)	Confirmed Positive(86.48)	
418	2015-255	F	50	77.7	R(8.74)	NR(0.14)	Confirmed Positive(99.68)	
419	2015-256	F	35	2.04	NR(0.61)	R(31.69)	Not confirmed(-10.38)	
420	2015-257	F	26	1954.34	R(9.67)	NR(0.63)	Not confirmed(-2.79)	Confirmed Positive(99.47)
421	2015-258	M	39	1.04	NR(0.12)	R(15.74)	Not confirmed(12.94)	
422	2015-259	F	35	2996.36	R(9.41)	NR(0.01)	Not confirmed(8.68)	Confirmed Positive(99.66)
423	2015-260	M	36	1.72	R(10.92)	NR(2.05)	Confirmed Positive(98.56)	
424	2015-261	M	29	3624.46	R(10.01)	NR(0.16)	Confirmed Positive(98.25)	
425	2015-262	F	39	5299.49	R(9.62)	NR(0.09)	Confirmed Positive(92.78)	
426	2015-264	M	24	3294.8	R(9.80)	NR(0.00)	Not confirmed(20.35)	Confirmed Positive(99.71)
427	2015-265	F	25	3234.69	R(8.84)	NR(0.59)	Confirmed Positive(93.23)	
428	2015-266	M	33	1633.38	R(7.67)	NR(0.00)	Confirmed Positive(99.21)	
429	2015-267	M	28	3234.34	R(10.64)	NR(0.26)	Not confirmed(7.85)	Confirmed Positive(99.63)
430	2015-268	M	28	2268.81	R(10.01)	NR(0.04)	Not confirmed(2.49)	Confirmed Positive(99.64)
431	2015-269	M	52	1.44	R(11.82)	NR(0.00)	Confirmed Positive(107.61)	
432	2015-270	F	22	2.56	NR(0.11)	R(180.07)	Not confirmed(-24.53)	
433	2015-271	M	32	2893.9	R(11.02)	NR(0.15)	Not confirmed(3.68)	Confirmed Positive(99.69)
434	2015-272	M	37	4522.05	R(9.37)	NR(0.11)	Confirmed Positive(77.69)	
435	2015-273	M	37	4400.92	R(9.55)	NR(0.50)	Confirmed Positive(78.99)	
436	2015-274	F	56	14.12	R(10.07)	NR(3.89)	Confirmed Positive(101.96)	
437	2015-275	M	53	3.35	R(7.01)	R(29.47)	Not confirmed(-6.29)	
438	2015-276	M	40	153.1	R(9.79)	NR(1.38)	Confirmed Positive(99.01)	
439	2015-277	F	45	3994.01	R(9.63)	NR(0.00)	Confirmed Positive(67.10)	
440	2015-278	M	32	533.4	R(8.99)	NR(0.12)	Not confirmed(-0.54)	Confirmed Positive(98.98)
441	2015-279	M	37	5022.19	R(9.83)	NR(0.29)	Confirmed Positive(93.98)	
442	2015-280	M	29	2094.08	R(10.09)	NR(0.00)	Confirmed Positive(98.90)	
443	2015-281	F	53	2632.49	R(10.34)	NR(7.86)	Confirmed Positive(83.49)	
444	2015-282	M	19	1.53	NR(0.08)	R(31.67)	Not confirmed(4.92)	
445	2015-283	M	22	626.84	R(7.54)	NR(0.00)	Not confirmed(-3.38)	Confirmed Positive(99.04)
446	2015-284	M	42	1.04	R(9.39)	HR(>1000)	Not confirmed(1.61)	
447	2015-285	F	29	3673.34	R(9.62)	NR(1.43)	Confirmed Positive(92.91)	
448	2015-286	F	26	724.62	R(9.07)	NR(0.00)	Not confirmed(-2.19)	Confirmed Positive(99.27)
449	2015-287	M	39	4104.68	R(9.62)	NR(6.27)	Confirmed Positive(91.29)	
450	2015-288	M	47	3186.42	R(11.07)	NR(0.00)	Not confirmed(14.29)	Confirmed Positive(99.67)

Table A.1 Signal of HBsAg, anti-HBc, anti-HBs, gender , age and neutralization result of this study. (cont.)

No.	Study No.	Gender	Age	HBsAgQ2 (S/CO)	anti-HBc (S/CO)	anti-HBs(mIU/mL)	First HBsAgQ2 confirmatory (%)	Second HBsAgQ2 confirmatory (%) (Dilute 1:500)
451	2015-289	F	43	1.47	NR(0.19)	R(24.53)	Not confirmed(9.62)	
452	2015-290	M	32	5181.08	R(9.19)	NR(0.01)	Confirmed Positive(75.35)	
453	2016-001	M	21	1497.12	R(8.90)	NR(0.02)	Not confirmed(30.87)	Confirmed Positive(95.04)
454	2016-003	M	54	5769.12	R(9.34)	NR(0.20)	Confirmed Positive(81.88)	
455	2016-004	M	42	5535.95	R(9.90)	NR(0.23)	Confirmed Positive(96.66)	
456	2016-005	F	29	5559.36	R(9.19)	NR(0.00)	Confirmed Positive(63.33)	
457	2016-006	M	54	5023.71	R(9.74)	NR(0.01)	Confirmed Positive(90.31)	
458	2016-007	M	51	3321.56	R(9.62)	NR(0.08)	Confirmed Positive(98.93)	
459	2016-008	F	48	4759.34	R(10.31)	NR(0.12)	Confirmed Positive(95.75)	
460	2016-009	F	29	5403.71	R(9.75)	NR(0.12)	Confirmed Positive(90.63)	
461	2016-010	F	26	2164.44	R(9.44)	R(35.33)	Confirmed Positive(97.08)	
462	2016-011	M	23	6.78	R(8.83)	NR(0.06)	Confirmed Positive(96.18)	
463	2016-012	M	30	623.33	R(9.16)	NR(0.00)	Not confirmed(-2.46)	Confirmed Positive(99.09)
464	2016-013	F	32	2316	R(9.59)	NR(0.00)	Not confirmed(-0.94)	Confirmed Positive(99.55)
465	2016-014	F	39	61.15	R(9.55)	NR(0.00)	Confirmed Positive(98.94)	
466	2016-015	F	23	1.33	NR(0.10)	NR(0.00)	Not confirmed(-14.94)	
467	2016-016	M	22	5014.74	R(9.87)	NR(0.16)	Confirmed Positive(57.63)	
468	2016-017	M	25	1172.74	R(9.59)	NR(0.08)	Not confirmed(-0.26)	Confirmed Positive(99.40)
469	2016-018	M	27	5687.59	R(9.70)	NR(0.11)	Confirmed Positive(89.55)	
470	2016-019	M	42	2578.48	R(10.24)	NR(0.32)	Not confirmed(30.17)	Confirmed Positive(99.74)
471	2016-020	M	35	1.7	NR(0.16)	NR(0.04)	Not confirmed(-20.06)	
472	2016-021	M	34	3040.42	R(10.39)	NR(0.00)	Not confirmed(10.73)	Confirmed Positive(99.72)
473	2016-022	F	31	4844.76	R(9.30)	NR(0.01)	Confirmed Positive(53.66)	
474	2016-023	M	37	3268.55	R(10.27)	NR(0.08)	Not confirmed(5.26)	Confirmed Positive(99.65)
475	2016-024	M	32	1.62	NR(0.17)	NR(0.11)	Confirmed Positive(99.56)	
476	2016-025	M	36	4206.1	R(10.62)	NR(0.00)	Confirmed Positive(54.08)	
477	2016-026	M	19	1.25	R(9.01)	R(27.96)	Confirmed Positive(104.79)	
478	2016-027	M	31	4899.94	R(10.91)	NR(0.03)	Confirmed Positive(51.07)	
479	2016-028	F	27	4458.43	R(9.69)	NR(0.09)	Confirmed Positive(78.84)	
480	2016-030	M	24	3.84	R(9.67)	NR(2.91)	Confirmed Positive(96.94)	
481	2016-031	M	37	498.35	R(8.04)	NR(0.00)	Not confirmed(-2.58)	Confirmed Positive(98.73)
482	2016-032	M	25	873.9	R(8.77)	NR(0.000)	Not confirmed(3.73)	Confirmed Positive(99.45)
483	2016-034	F	45	140.27	R(9.10)	NR(0.26)	Confirmed Positive(98.77)	
484	2016-035	M	52	9.05	NR(0.11)	NR(0.00)	Not confirmed(0.48)	
485	2016-036	F	32	2805.21	R(10.46)	NR(0.44)	Not confirmed(8.50)	Confirmed Positive(99.61)
486	2016-037	M	20	857.32	R(6.34)	NR(0.02)	Not confirmed(3.26)	Confirmed Positive(99.21)
487	2016-039	M	33	2.48	NR(0.16)	NR(0.00)	Not confirmed(-34.16)	
488	2016-040	M	39	4576.95	R(11.12)	NR(0.33)	Not confirmed(20.18)	Confirmed Positive(99.69)
489	2016-041	M	26	555.87	R(11.66)	NR(0.00)	Not confirmed(2.46)	Confirmed Positive(99.10)
490	2016-042	M	29	4711.57	R(11.05)	NR(0.03)	Confirmed Positive(87.44)	
491	2016-043	M	34	2350.55	R(10.88)	NR(0.00)	Not confirmed(6.26)	Confirmed Positive(99.57)
492	2016-048	F	34	763.09	R(10.80)	NR(0.00)	Not confirmed(4.31)	Confirmed Positive(99.16)
493	2016-049	M	51	40.58	R(8.70)	NR(3.90)	Confirmed Positive(99.98)	
494	2016-050	M	23	4.47	NR(0.22)	NR(0.23)	Not confirmed(-4.19)	
495	2016-051	F	39	2801.38	R(11.14)	NR(3.33)	Confirmed Positive(88.59)	
496	2016-055	F	33	2.22	R(10.25)	R(29.21)	Confirmed Positive(96.11)	
497	2016-056	M	39	2598.06	R(10.17)	NR(7.05)	Confirmed Positive(96.58)	
498	2016-058	M	28	5176.44	R(10.86)	NR(0.00)	Not confirmed(27.02)	Confirmed Positive(99.68)
499	2016-059	M	27	2503.69	R(11.02)	NR(0.02)	Not confirmed(7.49)	Confirmed Positive(99.63)
500	2016-061	M	45	4443.84	R(13.00)	NR(1.07)	Confirmed Positive(75.71)	

Table A.1 Signal of HBsAg, anti-HBc, anti-HBs, gender , age and neutralization result of this study. (cont.)

No.	Study No.	Gender	Age	HBsAgQ2 (S/CO)	anti-HBc (S/CO)	anti-HBs(mIU/mL)	First HBsAgQ2 confirmatory (%)	Second HBsAgQ2 confirmatory (%) (Dilute 1:500)
501	2016-062	M	35	3631.83	R(11.30)	NR(0.16)	Confirmed Positive(98.07)	
502	2016-063	M	32	3305.76	R(10.96)	NR(0.70)	Not confirmed(7.44)	Confirmed Positive(99.60)
503	2016-066	F	24	1.15	NR(0.63)	NR(0.00)	Not confirmed(-13.41)	
504	2016-067	F	38	643.86	R(9.03)	NR(0.75)	Confirmed Positive(98.87)	
505	2016-068	F	53	165.61	R(9.95)	NR(0.59)	Confirmed Positive(98.90)	
506	2016-069	F	25	6.53	NR(0.098)	NR(0.00)	Not confirmed(-12.67)	
507	2016-070	F	44	648.3	R(9.68)	NR(0.00)	Confirmed Positive(99.08)	
508	2016-071	M	31	2918.43	R(10.41)	NR(0.45)	Confirmed Positive(93.77)	
509	2016-074	M	26	25.45	R(9.72)	NR(3.01)	Confirmed Positive(99.71)	
510	2016-075	M	38	2532.18	R(10.98)	NR(0.00)	Not confirmed(12.98)	Confirmed Positive(99.60)
511	2016-076	M	34	3917.14	R(10.96)	NR(0.00)	Not confirmed(22.77)	Confirmed Positive(99.73)
512	2016-079	M	22	3879.69	R(10.69)	NR(0.00)	Not confirmed(17.54)	Confirmed Positive(98.61)
513	2016-080	M	48	6347.64	R(12.01)	NR(0.26)	Confirmed Positive(96.99)	
514	2016-083	M	25	703.46	R(10.74)	NR(0.00)	Not confirmed(2.12)	Confirmed Positive(99.05)
515	2016-084	M	37	3615.61	R(11.17)	NR(0.07)	Not confirmed(6.95)	Confirmed Positive(99.66)
516	2016-086	F	35	4798.58	R(11.08)	NR(0.00)	Confirmed Positive(65.07)	
517	2016-088	M	26	850.76	R(10.79)	NR(0.00)	Not confirmed(2.90)	Confirmed Positive(99.17)
518	2016-089	M	39	3005.07	R(9.71)	NR(0.15)	Not confirmed(7.82)	Confirmed Positive(99.67)
519	2016-090	M	39	4078.42	R(9.28)	NR(0.21)	Confirmed Positive(91.45)	
520	2016-091	M	36	768.99	R(9.98)	NR(0.03)	Not confirmed(2.20)	Confirmed Positive(99.78)
521	2016-092	M	39	5007.87	R(9.91)	NR(0.06)	Confirmed Positive(84.13)	
522	2016-094	M	42	1728.51	R(10.20)	NR(0.00)	Not confirmed(1.04)	Confirmed Positive(99.61)
523	2016-095	F	48	8.22	R(10.29)	NR(1.56)	Confirmed Positive(99.59)	
524	2016-096	M	32	3501.7	R(9.06)	NR(0.23)	Not confirmed(11.53)	Confirmed Positive(99.69)
525	2016-097	M	55	4834.76	R(7.44)	NR(0.09)	Confirmed Positive(80.54)	

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