

Al Shaban Z O<sup>1</sup>, Abdel-Hamid A Z<sup>1</sup>, Hock T T<sup>1</sup>, Hassan R<sup>2</sup>

<sup>1</sup>Infectious Disease Cluster (IDC), Advanced Medical and Dental Institute (AMDI), Universiti Sains Malaysia (USM), Penang, Malaysia.

<sup>2</sup>Haematology Department, School of Medical Sciences, USMCK, Kelantan, Malaysia.

(Received October 29<sup>th</sup>, 2009 Revised February 21<sup>st</sup>, 2010 Accepted April 28<sup>th</sup>, 2010 Published online June 8<sup>th</sup>, 2010.)

Correspondence: Zahrah Omar Al Shaban  
Email: [saqil@hotmail.com](mailto:saqil@hotmail.com)

## Comparison between RT-PCR and ELISA for the detection of HBV in blood donors

**Aim:** This study was undertaken to compare the detection of HBV in blood donors between RT-PCR and ELISA and to clarify the strengths or weaknesses of these diagnostic methods. **Methodology:** Forty-eight samples from blood donors were randomly recruited from those attending the Transfusion Medicine Unit at the Hospital University Science Malaysia, Kelantan, Malaysia. The samples were analyzed by ELISA and RT-PCR techniques for detecting the prevalence of HBV in blood donors. **Results:** The obtained results showed the significance of RT-PCR over ELISA technique for the diagnosis and monitoring of HBV in blood donors. RT-PCR recorded 14.58% of the total samples positive in respect to 6.25% of anti-HBV ELISA positive. Sensitive nucleic acid testing for the detection and accurate quantitation of hepatitis B virus (HBV) is necessary to reduce transmission through blood and blood products and for monitoring patients on antiviral therapy. **Conclusion:** RT-PCR can be used for the detection and accurate quantitation of HBV viral loads in plasma samples.

**Keywords:** HBV, ELISA, RT-PCR.

### INTRODUCTION

Hepatitis B is a serious global public health problem. Hepatitis B is preventable with safe and effective vaccines that are available in the market. In 1991, the World Health Organization (WHO) {1}, made a call for all children to receive the hepatitis B vaccine, and thus this vaccine was added to routine immunization programmes of more than one hundred countries. In Malaysia, Hepatitis B immunization programme in newborns was started in 1989 which had a dramatic effect on health of children against hepatitis B infection control. A seroprevalence study in 190,077 school children aged 7-12 years from 1997 to 2003 showed a steady decline of HBV surface antigen from 2.5% to 0.4% among children born in 1985 and 1996 respectively {2}. Later on, immunization of Hepatitis B was also offered to the healthcare workers of the Ministry of Health. In Malaysia, the prevalence rate of Hepatitis B is 5.24% {3}. It is estimated that there are about 1.3 million Hepatitis B chronic carriers and amongst haemodialysis patients, 5% are hepatitis B positive {4}. In a series of 67 cases with hepatocellular cancer at Hospital Selayang in 2003-04, 63% of cases were due to chronic Hepatitis B infection and 15% of them were due to chronic Hepatitis C infection. However, a decrease in the prevalence of HBV infection after vaccination and by taking some other precautionary measures such as the use of disposable syringes & needles, screening of HBV infection markers in blood banks, ear piercing and acupuncture, was seen in Asia Pacific countries {5}. The Ortho HBe ELISA Test System is a qualitative enzyme-linked immunosorbent assay (ELISA) for the detection of total antibody to anti-HBe in human serum or plasma. Anti-HBe appears in virtually all individuals infected with HBV and is an accurate serological marker of current and past infection. ELISA procedures provide a means for routinely detecting antibodies to specific antigens. However, PCR amplification of virus DNA is potentially a more sensitive assay than molecular hybridization. Furthermore, DNA amplification, coupled with molecular

hybridization assays, is theoretically capable of detecting attogram ( $10^{-18}$  g) levels of HBV DNA in the original sample. This technique serves as a sensitive marker for the detection of HBV DNA in certain sera which are HBsAg positive even in the absence of HBeAg. Such a serological pattern corresponds to potentially infective sera and therefore the comparison of different serological markers with molecular diagnostic tests is necessary for predicting the course of chronic liver disease. So, the current research was designed upon rational basis of molecular diagnosis for sensitive detection of HBV among blood donors in Transfusion Medicine Unit at the Hospital University Science Malaysia, Kelantan.

### MATERIALS AND METHODS

Forty-eight sera were collected from randomly recruited blood donors who attended the Transfusion Medicine Unit at the Hospital University Science Malaysia, Kelantan. All sera were collected aseptically and stored at -20 °C prior to testing. ELISA test data was collected from Transfusion Medicine Unit at the Hospital University Science Malaysia, Kelantan. For RT-PCR, the viral nucleic acid extraction kit (DNA/ RNA) by Yeastern biotech was used to extract DNA from sera whereas for preparation for RT-PCR, Geno-Sen's HBV Real Time PCR Kit Quantitative with the Rotor Gene™ 2000/3000/6000 was used.

### RESULTS

Forty-eight sera samples were collected from blood donors and tested by Enzyme-linked immunosorbent assay (ELISA) for detection of HBV in Hospital University Science Malaysia, Kelantan. The results pointed out that 42/48 samples were negatives for HBV, 3/48 samples were indeterminate and 3/48 samples were ELISA positives HBV. While in the same

forty-eight samples, RT- PCR technique recorded 41/48 samples negatives and 7/48 samples positives for HBV- RDA. The results are listed in Table I.

**Table I:** Investigation of HBV in blood donor samples using ELISA and RT-PCR.

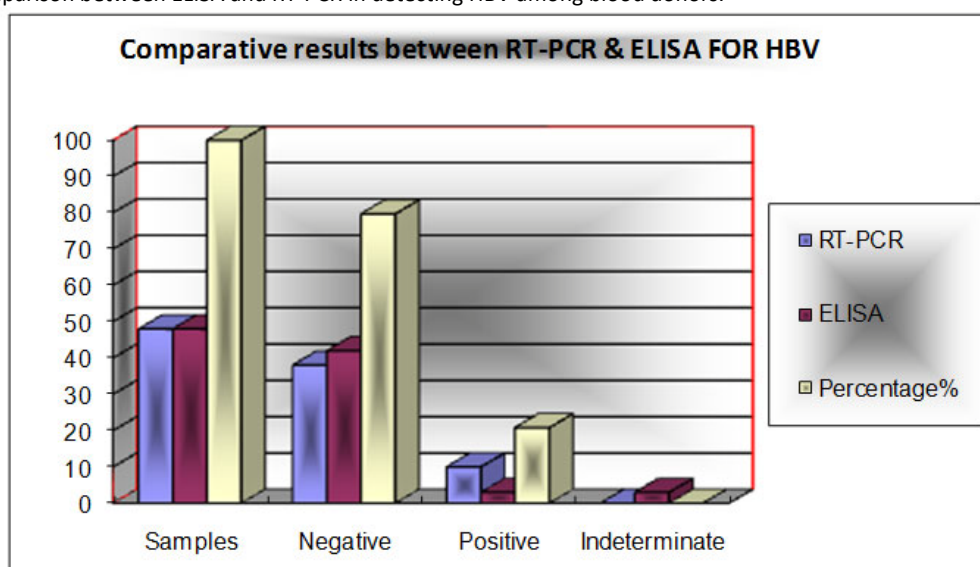
Case of sample	Technique	
	HBV-ELISA	RT-PCR
- ve	42 (87.50%)	41 (85.42 %)
+ve	3 (06.25%)	7 (14.58 %)
Indeterminate	3 (06.25%)	0 (00.00%)

Percentages of false negativity of HBV- ELISA test compared to RT-PCR techniques are listed in **Table II**.

**Table II:** Percentage of false negativity of HBV- ELISA test compared to RT-PCR techniques in detection of HBV among blood donors.

Case of sample	HBV-ELISA	HBV-RT-PCR	Percentage of false negativity
- ve	42	41	2.4%
+ve	3	7	40.0%
Indeterminate	3	00	0.00%

**Fig. 1:** Comparison between ELISA and RT-PCR in detecting HBV among blood donors.



**DISCUSSION**

Serological markers are indispensable in the diagnosis of HBV infection. HBsAg, in spite of being a common diagnostic marker of HBV infection, does not provide information about active virus replication. The detection and quantification of hepatitis B virus (HBV)'s DNA play an important role in diagnosing and monitoring HBV infection as well as assessing therapeutic response. Despite the wide availability of good hepatitis B surface antigen (HBsAg)-based detection systems for hepatitis B virus (HBV), there is evidence that transmission of HBV through blood and blood products occurs during the serological window period and more importantly during the later stages of infection due to occult hepatitis B infection as reported by Chu and Lok {6}. Individuals negative for HBsAg but positive for HBV DNA in blood or tissues with or without the presence of HBV antibodies are categorized as occult HBV infection {7}.

Several epidemiologic studies of hepatitis B infection in South East Asia reveal a high incidence of the disease in China (5-18%) and Taiwan (15-20%) {8}. Muddier & Canapati {9} reported the highest incidence with a carrier rate of 23.35% among the Nicobarese tribe of the Nicobar islands which are situated in the Bay of Bengal, India.

Various laboratory markers are used which include liver enzymes and detection of antibodies for hepatitis B virus. The serological method of Enzyme-Linked Immunosorbent Assay (ELISA) gives the quantitative value in titres of antibodies detected in the serum. Levels of HBV- DNA in the blood serve as an important marker in monitoring the disease progression and treatment efficacy of chronic HBV infection. Several commercial assays are available for accurate measurement of HBV genomic DNA, but many of them are hampered by relatively low sensitivity and limited dynamic range. The high sensitivity, wide linear range, good reproducibility, and genotype inclusivity of this HBV real-

time PCR assay, combined with a small sample volume requirement and low cost, make it particularly, well suited for application to large clinical and epidemiological studies. The quantitation of the HBV DNA level is very useful in monitoring the progression of the disease and the efficacy of treatment in chronic HBV infection [10]. As the clinical significance of low HBV DNA levels in chronic hepatitis B patients is recognized increasingly [11], an assay which can detect a lower level of HBV DNA becomes an important tool for such research. Comparing data obtained from ELISA and RT-PCR in diagnosis of HBV, there was a marked discrepancy in detection percentage within the two techniques. The percentage of false negativity of HBV- ELISA screening compared to RT-PCR test among blood donors was 2.4% (1 out of 42). This false negativity may be due to the presence of low levels of antibodies in blood during the HBV-window period. This has been documented by McCoy et al., [12], who reported that 90% of infected people produce antibodies within three months of exposure and 10% of them take a longer time to produce the antibodies and thus, the test needs to be repeated six months after exposure.

Also, the increased false negativity for antibody tests (ELISA) compared with RT-PCR-DNA detection in HBV blood samples may be due to weak and delayed response of these immunocompromized volunteers to produce antibodies.

The present study showed an interesting and important data on comparing results for false negativity among blood donors with HBV. This may be due to the high prevalence of HBV infection among blood donors. This suggests a higher specificity of RT-PCR than ELISA technique. The high degree of false negativity associated with ELISA screening for HBV among blood donors is a big problem and leads to increased risk of transfusing contaminated blood.

## CONCLUSION

Sensitive nucleic acid testing for the detection and accurate quantitation of hepatitis B virus (HBV) is necessary to reduce transmission through blood and blood products and for monitoring patients on antiviral therapy. RT-PCR can be used for the detection and accurate quantitation of HBV viral loads in plasma samples. This method is reliable, accurate, and reproducible.

## REFERENCES

- 1- *Global surveillance and control of hepatitis B. WHO report, 2000.*
- 2- *Ng KP, Saw TL, Baki A, Rozainah K, Pang KW, et al. Impact of the Expanded Program of Immunization against hepatitis B infection in school children in Malaysia. Med. Microbiol. Immunol.2005; 194:pp.163-168.*
- 3- *Loh KY, Kew ST. Hepatitis B infection: What the primary care doctors should know. Malaysian Family Physician.2006; 1:pp.8-10.*
- 4- *Teles SA, Martins RM, Lopes CL, Santos C MA, Yoshida CF. Immunogenicity of a recombinant hepatitis B vaccine (Euvax-B) in haemodialysis patients and staff. Eur. J. Epidemiol.2004; 17:pp.145-149.*
- 5- *Chen CJ, Wang LY, Yu MW. Epidemiology of hepatitis B virus infection in the Asia-Pacific region. J. Gastroenterol. Hepatol.2000; 15:pp.3-6.*

- 6- *Chu CJ, Lok AS. Clinical utility in quantifying serum HBV DNA levels using PCR assays. J. Hepatol.2002; 36:pp.549-551.*
- 7- *Hu KQ. Occult hepatitis B virus infection and its clinical implications. Viral Hepat.2002; 9:pp.243-57.*
- 8- *Chen WN, Oon CJ, Toh I. Altered antigenicities of hepatitis B virus surface antigen carrying mutations outside the common 'a' determinant. Am. J. Gastroenterol.2000; 95; pp.1098-1099.*
- 9- *Mudur G. Research finds high hepatitis B rate in Indian island tribes. Br. Med. J.2000; 321:pp.404.*
- 10- *Paraskevis D, Pult C, Haid A N, Tassopoulos M, Raptopoulo D, et al. Development and assessment of a novel real-time PCR assay for quantitation of HBV DNA. J Virol Methods.2002; 103:pp.201-212.*
- 11- *Lok AS, Heathcote EJ, Hollinger JH. Management of hepatitis B. Gastroenterology.2001; 120:pp.1828-1853.*
- 12- *McCoy CB, Shapshak P, Metsch LR, Rivers JE, McCoy HV, et al. HIV-1 prevention: interdisciplinary studies on the efficacy of bleach and development of prevention protocols. Arch. Immunol. Ther. Exp. (Warsz).1995; 43:pp.1-9.*