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Comparison of ELISA & ICT Methods Determining Hepatitis B Surface in Suspected Patient Attending at Bangladesh Institute of Health Science (BIHS) General Hospital, Dhaka

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ABSTRACT

Hepatitis B virus (HBV) is a common blood borne infectious agents causing high morbidity and mortality that constitute the major global health problems. Infection with HBV results in a wide spectrum disease from subclinical to fulminant hepatitis leading to death. The most important laboratory screening test for the diagnosis of early HBV is the immunoassay for HBsAg. Different analytical methods are being used now a days for the diagnosis of hepatitis namely, ICT, ELISA, CMIA and PCR. The aim of the study to determine viral hepatitis (HBV) seromarkers in suspected patients using different methods ICT and ELISA. This cross sectional study was conducted at the Immunology laboratory, BIHS General Hospital, Dhaka. A total 240 of HBsAg test results of the patients were collected from Laboratory register book. All tests were carried out by ELISA & ICT methods and compare between them. Majority (76.3%) of the donors was within the age group of 19-30 years. Among the donors, male donors were predominant (93.75%) than female (6.25%). A total number of 240 HBsAg tests were done in ICT method and found 6 Positive (2.5%) of them. Then we done correspondence test to confirm these by ELISA method and found 100% negative results. This study shows ICT method were able to determine HBsAg negative samples reasonably well that was detected negative by ELISA. ELISA method is more specific and sensitive than that of ICT.

INTRODUCTION

Hepatitis B virus (HBV) is a common blood borne infectious agents causing high morbidity and mortality that constitute the major global health problems. Approximately one fifth of the world populations are being chronically infected with HBV. Death of 1.5 million people every year attributed to HBV related chronic liver diseases (Hayder *et al.* 2012). Infection with HBV results in a wide spectrum disease from subclinical to fulminant hepatitis leading to death. Hepatitis due to B virus often progress to chronic active hepatitis, cirrhosis of liver with development of hepatocellular carcinoma. Since these virus are mostly transmitted by transfusion of contaminated blood and blood product, however, other subjected modes of transmission like intravenous drug abuse, close personal contact, use of shared needle, razor etc cannot be ignored (Ahmed *et al.* 2009). It commonly causes asymptomatic infection but chronic infection causes scarring of the liver which is generally apparent after many years. Approximately, 75% acutely infected patients develop chronic hepatitis B infection that commonly progresses to liver cirrhosis and hepatic malignancy (Ahmed *et al.* 2009).

The HBV is found to be rapidly spreading in the

developing countries including Bangladesh. It found to be 50 to 100 times more infectious than HIV and an important occupational hazard for health workers. About 2 billion people worldwide have been infected with the virus and more than 350 million live with chronic lifelong infection (WHO, 2000). HBsAg appears during the incubation and becomes detectable in most cases during the prodrome and acute stage of the disease. It usually disappears from the blood within 5-6 months in young adults. However, prolonged persistence of the HBsAg for more than six months indicates the carrier state. These viruses are still causing the most clinically significant transfusion transmissible infections with a per unit risk of 1: 82,000 (Riskin *et al.* 2009). Looking into the data on the prevalence of the transfusion transmitted infections (TTIs), specifically HBV among blood donors permits an assessment of the occurrence of infections in the blood donor population and consequently the safety of the collected donations. It also gives an idea of the epidemiology of these diseases in the community. Most of the studies conducted in Bangladesh are limited among the professional blood donors, drug addicts, commercial sex workers or hospitalized patients (Islam *et al.* 1984). The rapid spread of HBV infection and the changed

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scenario in blood donation practice has inspired us to depict the trends of the prevalence of HBsAg infection among the healthy blood donors, and to describe their socio-demographic background at Hospitals in Dhaka city in Bangladesh. Such information could be a guide for planning and implementing programs for preventive measures in Bangladesh. The most important laboratory screening test for the diagnosis of early HBV is the immunoassay for HBsAg. Different analytical methods are being used now a day for the diagnosis of hepatitis namely, ICT, ELISA, CMIA and PCR. The ELISA, CMIA and PCR methods are found to be expensive and are used in advanced laboratories and major tertiary care hospitals. ICT, using by the most of the laboratories as a rapid diagnostic analytical method are now considered a good choice because of its low price, less involvement of skilled technician and infrastructure (Rahman *et al.* 2008). The rapid ICT kits are known to have less sensitivity and specificity than ELISA. The aims of this study is to determine HBsAg in patients' blood by using ICT and ELISA. ELISA is a wet lab type analytical assay that uses a solid phase enzyme immunoassay to detect the presence of an antigen or antibody in a liquid phase. Along with the enzyme-labeling of antigens or antibodies, the technique involves an immune reaction, enzymatic chemical reaction, signal detection and quantification in combination which make it one of the most specific and sensitive than other immunoassays to detect the biological molecule. Although confirmation of hepatitis B infection is based on advanced immunological, molecular and histological techniques (Mustafa *et al.* 1989) laboratory use immune-chromatographic test strips to screen hepatitis. Their mode of action is based on common principle of antibody present in the test serum or plasma reacting with the protein coated particle and migrating upward on a membrane chromatographically by capillary action to react with recombinant antigen present on the membrane thereby generating a color line in the test region. These test strips are one step rapid test. The test strips manufactures also claim that these test strips have relatively high sensitivity, specificity and accuracy but controversy often arises regarding these claims (Ahmad *et al.* 1991). Reports from various studies suggest that this rapid test method not always confirms the accuracy of the test results, increasing the risk of blood borne infection during blood transfusion. Very early stages of infection and patients in the recovering stages usually have low viral titers, reflected by low optical density (Low SOD) in the test results. These low positive cases may not be detected by rapid screening test like ICT, these can be detected by ELISA. However it should be kept in mind that method standardization is always crucial before diagnosis of an infection. A major concern in utilizing screening tests is that these tests should have a high performance in detecting infections irrespective of stages of disease. Therefore the intent of this study is to compare rapid test strip screening method with advanced immunological techniques and to recommend for a reliable, cost-effective

and less time consuming laboratory method for detection of HBV seromarkers in donors' blood.

The aim of this study is to determine hepatitis B surface antigen (HBsAg), by ELISA and ICT method among patients and compare the test results of HBsAg seromarker obtained by the above methods for evaluation in respect of sensitivity, specificity and accuracy of those methods. This study will help to evaluate the methods, comparatively better for the detection of viral hepatitis seromarkers.

MATERIALS AND METHODS

This is a cross-sectional study. The study was conducted by obtaining the blood samples from donors attending at the BIHS General Hospital Ltd, Dhaka and Bangladesh University of Health Sciences (BUHS). The study was conducted during the period of From February to October 2018. Patients qualifying criteria for the donation were included in the study. The qualifying criteria for the participants were: age between 18 to 60 years with clinical feature for hepatitis infection. Participants having low body weight, low blood pressure and anemia, professional blood donors, drug abusers, pregnant women, etc. were excluded from the study. Persons having positive history of HBV, HCV, HIV and venereal disease were also excluded from the study. A purposive sampling was followed. The entire samples collected from the laboratory record book those are fulfill the inclusion criteria of study.

Collection of Blood Sample, Processing and Preservation

Five milliliters of blood were collected by standard aseptic technique in the coded vacutainer. The blood was kept stand still to allow clotting and the serum was separated by centrifugation at 4000 rpm for 15 minutes. The serum samples were transferred to two micro centrifuge tubes. One tube for each participant was taken to the immunology laboratory of the BIHS General Hospital for detection of seromarkers for HBV by ICT methods and second was preserved for ELISA test to be done at the Immunology Laboratory, Bangladesh University of Health Sciences (BUHS).

Laboratory Methods

Screening of HBsAg was done by ICT in immunology laboratory of the BIHS General Hospital Ltd. Kits used for ICT were from Excel. ELISA 4th generation kits (Enzo diagnostics Inc, USA) were used as gold standard for comparative evaluation¹.

Test Procedure

Procedure of HBsAg detection (Sandwich ELISA, Wantai Biologicals, China)

A microtiter plate coated with known quantity of monoclonal antibody (solid-phase) is taken. Serum sample is added to the plate that may or may not contain HBsAg. HBsAg if present in the serum, bind to solid phase antibody in the plate during incubation (at 37°C). The plate is then washed at the end of incubation to

remove unbound antigen and any other non-specific binding. After soaking the plate on a paper towel, enzyme-linked antihuman antibodies (conjugate) which are also specific to HBsAg antigen are added and then incubated at 37°C. The plate is washed with working buffer solution to remove unbound conjugates. Substrate is added so that enzyme act on the substrate to produce a colored reaction product. Finally, blocking reagent is added to stop the enzymatic reaction and absorbance is measured using specific wavelength by Spectrophotometric plate Reader.

Procedure for HBsAg (ICT)

- Test cassette, serum or plasma specimen, and /or controls were allowed to equilibrate to room temperature (15-20 °C) prior to testing.
- The pouch was brought to room temperature before opening it. The test cassette was removed from the sealed pouch and used it as soon as possible.
- For Serum or Plasma specimen.
- The dropper was held vertically and 3 drops of serum or plasma (approximately 120 µl) was transferred to the specimen well of test cassette and the timer was started.
- After waiting for few minutes a colored line appeared. The result was read at 15-30 minutes.

Interpretation

Positive

Two distinct colored lines appear. One colored line should be in the control region (C) and another colored line should be in the test region (T).

Negative

One colored line appears in the control region (C). No apparent colored line appears in the test region (T).

Data Collection

Variables recorded at the time of enrollment of study subjects include participants' demographics, personal history, vital signs and clinical findings. Data relating to clinical information were taken from pre-designed questionnaire sheets of donors kept in the Blood bank of the BIHS General Hospital. Donors consent was obtained in the questionnaire sheets by the Blood bank personnel before donation of blood and laboratory reports.

Data Analysis

Data were analyzed with the help of the software SPSS (Statistical Package for Social Sciences) version 23 and Microsoft Excel 2016. The results were expressed as mean $\pm$ SD (standard deviation). The p-value <0.05 was considered as statistically significant.

RESULTS

Total of 240 patients were included in this study. Mean ($\pm$ SD) age was 27.45 ± 7.63 years. Age distribution of the donors was shown in table I. Majority (76.3%) of the donors were within the age group of 19-30 years, 16.3%

of the donors was within the age group of 31-40 years, and 3.8% of age group 41-50 years and >50 years.

Table 1: Distribution of patients on the basis of age groups

Age (yrs)	Frequency	Percentage (%)	Mean $\pm$ SD
19-30	183	76.3	27.45 ± 7.63
31-40	39	16.3	
41-50	9	3.8	
>50	9	3.8	
Total	240	100.0	

Results were expressed as number (percent) and mean $\pm$ SD as appropriate

Among the total 240 patients, the male was 225 (93.75%) and the female was 15 (6.25%). Among the donors, male donors were more predominant (93.75%) than females (6.25%) (Figure 1).

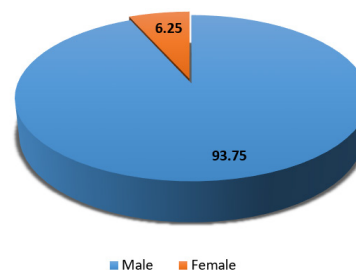


Figure 1: Gender distribution of the patients

Table 2 shows that all the specimens were also screened for HBsAg by immunochromatography (ICT) method. Out of 240 samples, 6 (2.5%) samples were positive and 234 (97.5%) samples were negative.

Table 2: Distribution of seromarkers status analyzed by Immunochromatography (ICT) (n= 240)

Seromarkers	ICT				P value
	Positive		Negative		
	No	%	No	%	
HBsAg	6	2.5%	234	97.5%	-

Results were expressed as number and percent

Table 3 showed that target variables hepatitis B surface antigen (HBsAg) were determined by Enzyme linked immunosorbent assay (ELISA). Out of 240 samples, 240 sample (100%) were negative. The 6 samples that were positive by ICT method were also negative by ELISA method.

Table 3: Distribution of seromarkers status by ELISA (n=240)

Seromarkers	ELISA				P value
	Positive		Negative		
	No	%	No	%	
HBsAg	0	0%	240	100%	-

Results were expressed as number and percent

DISCUSSION

Screening for common blood borne infection like HBV is crucial to ensure the safety of transfusion. The selection of screening tests depends upon a number of factors. Among them, evaluation of test performance, measured by sensitivity and specificity, is the most important factor while kit cost, equipment used, expertise, consumables and disposables are also taken into account during selection of analytical process (Saadia *et al.* 2016). This is for the first time through a structured protocol; ICT was compared with gold standard ELISA method using donors' blood for HBV seromarker HBsAg. Almost all donors were male (93.75%) which is consistent with other reports more specifically by Khan *et al.* where 96.8% of the blood donor population was male as males are the predominant donor both in developed and developing countries (Sajed *et al.* 2014; Khan *et al.* 2011). Mean ($\pm$ SD) age of the donors was 27.45 ± 7.63 and 76.3% (183 out of 240) of them were between the age group 19-30 years. This demonstrated the fact that donors belong to the younger age groups which is consistent to the study done by Sajed (Sajed *et al.* 2014). In their study 80% donors belonged to the thirties years of age. The present study compared ICT and ELISA for hepatitis B surface antigen HBsAg. Test result for HBsAg was found negative for all 240 samples in both ICT and ELISA method.

Reference centers or central blood banks found to be widely using most sensitive test methods (ELISA, CMIA and PCR) as quantitative immunoassay globally (Hayde *et al.* 2012; Clement *et al.* 2002). Rapid test, ICT is intended for qualitative detection of HBsAg in serum (Torlesse *et al.* 1997). ELISA, CMIA and other advanced methods are laboratory based, time consuming and require trained laboratory personnel. Chemiluminescence based assays are usually used for screening of blood donors in high volume blood banks owing to automation facilities, higher testing throughput and objective interpretation of results, however, expensive instrumentation is required for them thus limiting their use in resource limited settings (Saadia *et al.* 2016).

Rapid test enables early detection at sites where laboratory facilities or trained manpower are not available or there is issue of accessibility. Most rapid tests are based on immunochromatographic principles (Plitt *et al.* 2007). The rapid tests reduce the potential loss of follow up of a case when test results are on demand right away (Sato *et al.* 1996; Raj *et al.* 2001). ICT showed potentially good findings in the present study. No false positive test was observed by this method since it provided negative results of all 240 donors' sample for both HBsAg and anti-HCV that was also revealed to be negative by ELISA. Using ICT for both infections, ICT and CMIA were equally sensitive to ELISA as all the 240 samples showed negative reaction in both ICT and ELISA though 1 (1.25%) sample was positive for HBsAg. Our results showed comparable performances of the three techniques with almost 99% agreement of results. In evaluating both the seromarkers, specificity and negative predictive value of ICT were 100% that was consistent

with a study done in Pakistan where ICT and ELISA were compared for detection of HBsAg in healthy individual from Karachi that showed comparable sensitivity and specificity of ICT kits with ELISA technique (Shamsul *et al.* 2001). It is again consistent with another study done by Herring (Herring *et al.* 2006) where evaluation of nine rapid syphilis ICT kits reported 93-98% specificity. A meta-analysis reported the sensitivity of different ICT devices ranging from 85-100% and specificity 98-100% (Jafari *et al.* 2013). An Indian study reported 100% specificity and 93.4% sensitivity of rapid kits when detecting HBsAg (Kaur *et al.* 2000). ICT is suitable for use in remote and developing regions since they are simple to perform, can be transported, stored and performed at room temperature and microscopic and electrical equipment not needed. Moreover, these are cheaper and quicker as compared to other diagnostic procedures (Herring *et al.* 2006).

Findings of the present study is also consistent with study conducted in Iran where 6 rapid strips/devices were compared with gold standard method (Khadem *et al.* 2007). In another study from Seoul for detecting HBsAg, rapid technique showed 97% sensitivity and 100% specificity (Irwig *et al.* 2002). In our study, overall specificity results for both HBsAg was high i.e. 97-100%. These results are different to an study conducted in Lahore, Pakistan by Khan (Khan *et al.* 2010) who demonstrated 93% to 100% specificity for HBsAg by ICT method but the sensitivity was 50% for both HBsAg. In the present study sensitivity was 100% for HBsAg which is higher than the just mentioned study. The present study was carried out as a pilot basis to compare two methods ICT with ELISA for HBsAg. Although the number of samples tested was limited yet we could infer that the two methods had performed equally well and in limited resource settings, the ICT could be used as an alternative for HBV seromarker screening.

A total number of 240 HBsAg tests were done in ICT method and found 6 Positive (2.5%) of them. Then we done correspondence test to confirm these by ELISA method and found 100% negative results. We know, ELISA method is more specific and sensitive than that of ICT. We conclude that, those 6 positive results found in ICT method was false positive.

CONCLUSION

This study shows ICT method were able to determine HBsAg negative samples reasonably well that was detected negative by ELISA. The rapid test was not only compatible with currently established and advanced diagnostic methods but also cheaper. It can be recommended that ELISA comparable rapid devices may be allowed to be used for initial screening of hepatitis B, in remote areas where cost is an issue. The present study concludes that our findings demonstrate comparable performances of ICT and ELISA for screening of HBV seromarker. We suggest using the ICT assay in situation i) where the laboratory cannot afford to have a more advanced system for blood donors ii) as a backup and iii) in life-threatening situations where time saving may be life-saving.

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Conflicts of Interest

The author hereby declares that there are no conflicts of interest concerning this paper.

Informed Consent

Informed consent was obtained from all individuals included in this study.

REFERENCES

- Ahmad, Q., Chowdhury, S. G., Islam, M. N., Khan, F. D., Alam, M. R., & Miah, A. H. (1991). HBsAg amongst unscreened operated patients. *Bangladesh Medical Research Council Bulletin*, 17(1), 11-16.
- Ahmed, M. U., Begum, H. A., Hossain, T., & Chakraborty, P. (2009). Incidence of common transfusion transmitted diseases among blood donors. *Journal of Armed Forces Medical College, Bangladesh*, 5(1), 4-6.
- Clement, F., Dewint, P., & Leroux-Roels, G. (2002). Evaluation of a new rapid test for the combined detection of hepatitis B virus surface antigen and hepatitis B virus e antigen. *Journal of clinical microbiology*, 40(12), 4603-4606.
- Hayder, I., Ahmed, W., & Alam, S. E. (2012). Comparison of Different ICT Kits for HBsAg and Anti HCV Using Gold Standard ELISA. *Pakistan Journal of Medical Research*, 51(3).
- Herring, A. J., Ballard, R. C., Pope, V., Adegbola, R. A., Chagalucha, J., Fitzgerald, D. W., ... & Peeling, R. W. (2006). A multi-centre evaluation of nine rapid, point-of-care syphilis tests using archived sera. *Sexually transmitted infections*, 82(suppl 5).
- Irwig, L., Bossuyt, P., Glasziou, P., Gatsonis, C., & Lijmer, J. (2002). Designing studies to ensure that estimates of test accuracy are transferable. *Bmj*, 324(7338), 669-671.
- Islam, M. N., Islam, K. M., & Islam, N. (1984). Hepatitis-B virus infection in Dhaka, Bangladesh. *Bangladesh Medical Research Council bulletin*, 10(1), 1-6.
- Jafari, Y., Peeling, R. W., Shivkumar, S., Claessens, C., Joseph, L., & Pai, N. P. (2013). Are Treponema pallidum specific rapid and point-of-care tests for syphilis accurate enough for screening in resource limited settings? Evidence from a meta-analysis. *PLoS one*, 8(2), e54695.
- Kaur, H., Dhanao, J., & Oberoi, A. (2000). Evaluation of rapid kits for detection of HIV, HBsAg and HCV infections. *Indian journal of medical sciences*, 54(10), 432-434.
- Khadem, A. M., Omrani, M. D., & Movahedi, V. (2007). Comparative evaluation of immunochromatographic rapid diagnostic tests (Strip and Device) and PCR methods for detection of human hepatitis B surface antigens.
- Khan, J. K., Lone, D. S., Hameed, A., Munim, R., Bhatti, M., Khattak, A. A., ... & Munir, M. (2010). Evaluation of the performance of two rapid immunochromatographic tests for detection of hepatitis B surface antigen and anti HCV antibodies using ELISA tested samples. *Annals of King Edward Medical University*, 16(1 SI).
- Mustafa, M., Islam, M. N., Rahman, M., & Salauddin, A. K. (1989). Prevalence of hepatitis B surface antigen (HBsAg) among parenteral drug abusers at Dhaka. *Bangladesh Medical Research Council Bulletin*, 15(1), 1-7.
- Plitt, S. S., Somily, A. M., & Singh, A. E. (2007). Outcomes from a Canadian public health prenatal screening program for hepatitis B: 1997-2004. *Canadian journal of public health*, 98, 194-197.
- Rahman, M., Khan, S. A., & Lodhi, Y. (2008). Unconfirmed reactive screening tests and their impact on donor management. *Pak J Med Sci*, 24(4), 517-9.
- Raj, A. A., Subramaniam, T., Raghuraman, S., & Abraham, P. (2001). Evaluation of an indigenously manufactured rapid immunochromatographic test for detection of HBsAg. *Indian journal of pathology & microbiology*, 44(4), 413-414.
- Riskin, D. J., Tsai, T. C., Riskin, L., Hernandez-Boussard, T., Purtill, M., Maggio, P. M., ... & Brundage, S. I. (2009). Massive transfusion protocols: the role of aggressive resuscitation versus product ratio in mortality reduction. *Journal of the American College of Surgeons*, 209(2), 198-205.
- Saadia, A., Tarar, M. R., Naeem, T., Alvi, N., & Qureshi, M. (2016). Comparison Of Chemiluminescence Immunoassay And Immunochromatographic Test For Syphilis Screening In Blood Donors Attending A Tertiary Care Hospital Of Lahore. *Biomedica*, 32(2).
- Sajed, A. N., Iram, S., Haidar, S., Sarwar, A., Ahmad, I., Imran, S., ... & Akbar, M. (2014). Prevalence of HCV in apparently healthy voluntary blood donors. *IOSR-JDMS*.
- Sato, K., Ichiyama, S., Iinuma, Y., Nada, T., Shimokata, K., & Nakashima, N. (1996). Evaluation of immunochromatographic assay systems for rapid detection of hepatitis B surface antigen and antibody, Dainascreen HBsAg and Dainascreen Ausab. *Journal of clinical microbiology*, 34(6), 1420-1422.
- Shahtaj, K., Farah, F., Noor, R., & Fazle, R. (2011). Reducing the risk of transfusion-transmitted infections through predonation screening of blood donors. *International Journal of Pathology*, 9(2), 59-62.
- Shamsul, A. Q., Shamsa, A., Muhammad, A., Syed, I. A., & Aqeel, A. (2000). Detection of hepatitis B viruses in normal individuals of Karachi. *JCPSP, Journal of the College of Physicians and Surgeons Pakistan*, 10(12), 467-469.
- Torlesse, H., Wurie, I. M., & Hodges, M. (1997). The use of immunochromatography test cards in the diagnosis of hepatitis B surface antigen among pregnant women in West Africa. *British journal of biomedical science*, 54(4), 256-259.
- World Health Organization (2000). The world health report 2000: health systems: improving performance.