

Original Research Article

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A Comparative Study of Screening of Hepatitis B by Two Different Immunochromatographic Methods among Patients Attending a Tertiary Care Hospital

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ABSTRACT

Hepatitis B is one of the major Global health problems affecting both developing and developed countries. Hepatitis B is caused by Hepatitis B virus which spreads parentally through blood and sexual contact. There are many markers which gives information regarding stages of hepatitis B viral infection. The HBsAg is used for detection and screening of HBV infection. Aim: The study was carried to compare different parameters viz. sensitivity, specificity, positive and negative predictive values of two immunochromatographic Rapid tests with ELISA for HBsAg. Study Design. The study was conducted in Department of Microbiology at SKIMS-MC Hospital for a period of one year. Result: Out of total of 6701 blood samples screened, 19 were positive by ELISA, 17 were positive by Test A (HepaTM Card) and 16 were positive by Test B (Alere Trueline). The sensitivity, specificity, negative and positive predictive value of test A were 89.4%, 100%, 99.9% and 100%. The sensitivity, specificity, negative and positive predictive value of test B were 84.2%, 100%, 99.5% and 100% respectively against ELISA. The Rapid tests (ICT) are not comparable to ELISA in terms of sensitivity but can be used for screening of Hepatitis B in developing countries where resources are limited as rapid tests are cost effective and easy to perform.

Keywords

Hepatitis B,
HBsAg,
Rapid tests,
ICT, ELISA

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Introduction

Hepatitis B viral (HBV) infection is a global public health problem, with 2 billion of the world's population being infected with the virus¹. An estimated 257 million people are living with hepatitis B virus infection. In developed countries of America and Europe, HBV prevalence is relatively low ($\leq 2\%$), In developing countries of Asia, Africa and the Middle East, HBV prevalence rates are much

higher, reaching 5–20% of the general population². India has approximately HBV carrier rate of 3.0% with a high prevalence rate in the tribal population. The prevalence of hepatitis B surface antigen (HBsAg) is 3–4.5% with over 40 million carriers. About 100,000 Indians die annually³. Hepatitis B is an important occupational hazard for health workers. However, it can be prevented by currently available safe and effective vaccine⁴.

In 5-10% of adult patients, the HBV infection will progress to chronic hepatitis B which can lead to cirrhosis and hepatocellular carcinoma which is life threatening⁵. In contrast, in children, 90% HBV infection will progress to chronic hepatitis and due to immune tolerance these children will not have active hepatitis at the early phase of infection. The risk for chronic HBV infection decreases to 30% of children infected between ages 1 and 4 years and to less than 5% of persons infected as adults⁶. Chronic HBV infection progresses nonlinearly through 3–4 phases, from the immune-tolerant phase to immune clearance or immune-active phase, to non-replicative inactive phase and possible reactivation⁷.

The complex serology and natural history associated with HBV infection creates challenges for the assessment of HBV prevalence and the provision of comparable global estimates. This is due to the availability of multiple laboratory markers for hepatitis B infection. Antibodies and antigens associated with this infection include hepatitis B surface antigen (HBsAg), antibody to hepatitis surface antigen (anti-HBs), antibody to hepatitis B core antigen (anti-HBc), and IgM antibody subclass of anti-HBc (IgM anti-HBc).

Some studies also report markers of high HBV replication such as hepatitis B “e” antigen (HBeAg), antibody to HBeAg (anti-HBe), and quantitative HBV-DNA⁽⁸⁾. HBsAg is the main clinical marker indicating acute or chronic infection and prevalence as well as endemicity of HBV infection, is defined by the presence of HBsAg⁽⁹⁾.

HBsAg testing is the primary way to identify persons with chronic HBV infection and several characteristics of this serological marker increase the precision of HBsAg estimates, including high specificity, long serum persistence, low possibility of chronic cases losing HBsAg⁽⁹⁾.

Materials and Methods

6701 Serum samples were included in this study from patients at SKIMS-MC Hospital, Bemina Srinagar which includes both IPD as well as OPD between the time period of 12 months from February 2017 to February 2018.

HBV serum markers (antigens and antibody) are stable at room temperature for days, at 4°C for months, and frozen at -20°C to -70°C for many years.

Because modern testing involves automated enzyme immunoassays that depend on colorimetric or chemiluminescence signal measurement, therefore samples were stored at -20°C and care was taken to avoid hemolysis of the sample because it may interfere with the ability of the assay to accurately detect this marker.

Before starting the test procedure all the samples and reagents were brought to room temperature as required by the manufacturer of kits.

Kit manual was strictly followed for each and every step of the test procedure.

The tests procedures both ELISA as well as ICT followed spontaneously.

Determination of Hepatitis B virus surface antigen

Enzyme linked Immuno-sorbent assay

All the samples were analysed for HBsAg (Hepatitis B surface antigen) using ELISA kit (ErbaLisa Hepatitis B by TRANSASIA BIO-MEDICALS LTD).

The results were reported qualitatively based on cut-off value calculated by addition of mean value of three NC (negative control)

with a factor of 0.15(SD value provided by manufacturer).All the samples were run in duplicates in order to increase the sensitivity of the test and to minimize the precision errors.

The test run was validated after obtaining following target values:

Interpretation	COV
Reactive	>COV
Non-Reactive	< COV

Parameter	Requirements
Blank	<0.2 OD at 450nm
Negative control	<0.1 OD at 450nm
Positive control	>1.0 OD at 450nm

All those samples with absorbance value more than cut off value were taken as positive for HBsAg as per the kit brochure. The minimum detectable concentration of HBsAg by this assay is estimated to be 0.1ng/ml.

Determination of HBsAg by Immuno chromatographic test/lateral flow immunoassay

Hepa™ Card (Reckon diagnostics pvt.LTD.)
b. AlereTrueline.

Test card were stored at 4°C as advised by the manufacturer. The test kit was kept away from direct sunlight, moisture and heat.

Results and Discussion

This study was aimed to compare the sensitivity, specificity, Negative and positive

predictive value,positive and negative Likelihood ratio and Diagnosstic accuracy of Immunochromtography technique with that of ELISA which is considered a Gold Standard technique for the detection of HBsAg. The two different brands for which multiple parameters were analyzed are HEPA™ CARD and AlereTrueline. A total of 6701 blood samples were screened for HBsAg. Out of 6701 samples, 19 (0.28%) were HBsAg positive by ELISA. Out of the 19 positive samples 17 were found positive for Brand A and 16 were positive for Brand B (Table 1).

However none of the samples which were found to be negative by ELISA turned out to be positive by ICT. When the sensitivity and specificity were calculated, the sensitivity and specificity of ICT brand A was 89.47% and 100% respectively. While as the sensitivity and specificity of Brand B was 84.20% and 100% respectively (Table 2; Fig. 1–4).

Comparison between ELISA and Brand A

The sensitivity was 89.47%, Specificity was 100%, NPV was 99.97%, PPV was 100%, positive likelihood ratio was infinity, Negative Likelihood ratio was 0.105and Diagnostic accuracy was 99%.

Comparison between ELISA and Brand B

The sensitivity was 84.20%, Specificity was 100%, NPV was 99.95%, PPV was 100%, positive likelihood ratio was infinity, Negative Likelihood ratio was 0.158 and Diagnostic accuracy was 99% (Table 3).

Table.1 Interpretation of results

Interpretation	Control line	Test line
Negative test	Pink- purple line	No Pink–Purple line
Positive test	Pink- purple line	Pink- purple line
Invalid test	No Pink–Purple line	No Pink–Purple line/Pink-purple line

Table.2 Total no. of positives by different methods

Total no. of samples	ELISA Positive	Brand A positive	Brand B Positive
6701	19	17	16

Table.3 Parameters studied by using ELISA as gold standard

Test Method	Sensitivity	Specificity	NPV	PPV	Positive likelihood ratio	Negative likelihood ratio	Diagnostic accuracy
Brand A	89.47%	100%	99.97%	100%	Infinity	0.105	99%
Brand B	84.20%	100%	99.95%	100%	Infinity	0.158	99%

Fig.1 Shows the difference in sensitivities between ELISA and two different brands of ICT

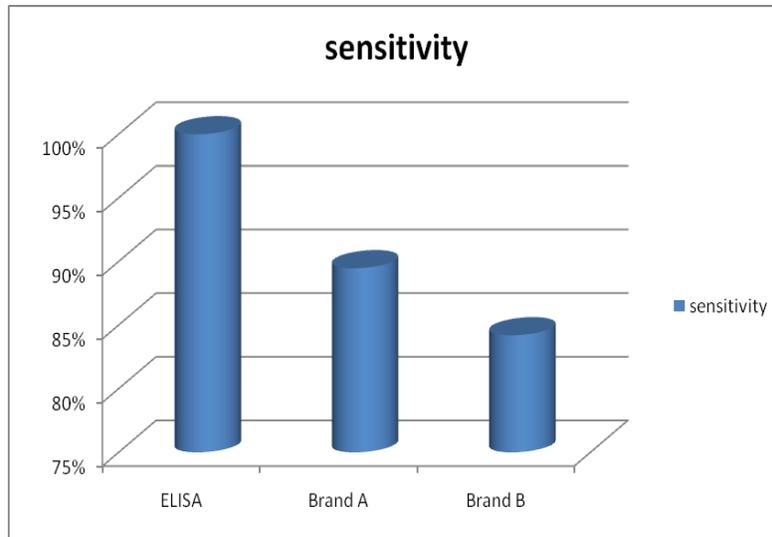


Fig.2 Shows the difference in specificities between ELISA and two different brands of ICT

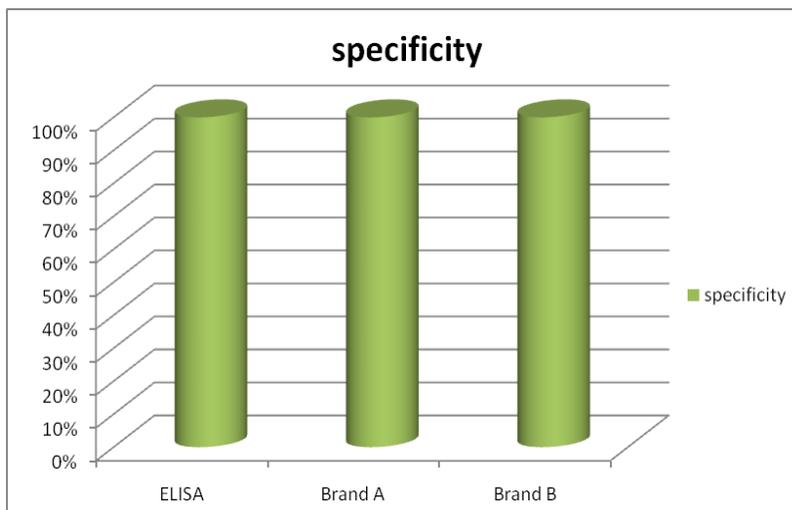


Fig.3 Shows the difference in NPV between ELISA and two different brands of ICT

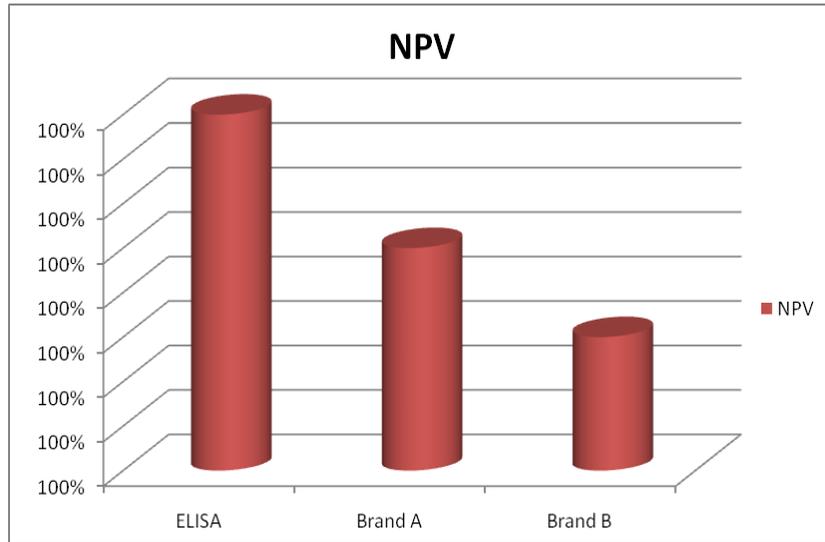
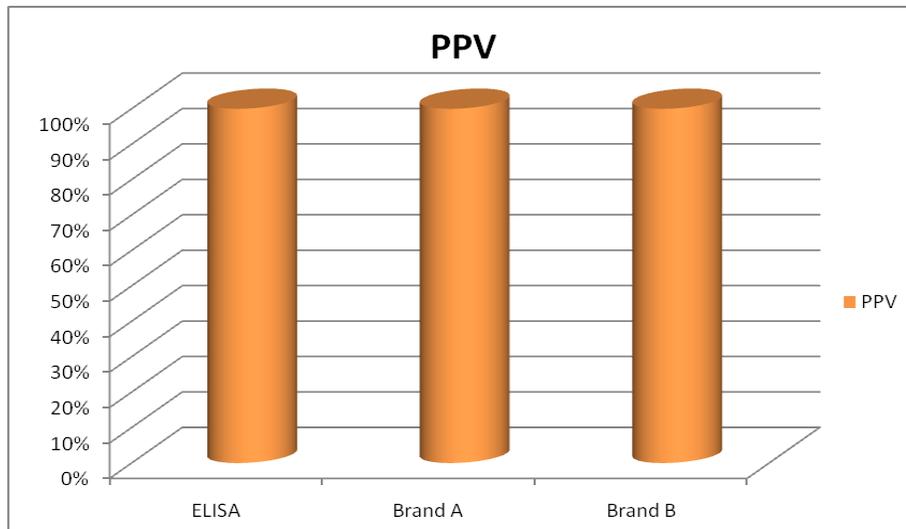


Fig.4 Shows the difference in PPV between ELISA and two different brands of ICT



There are many different markers for HBV infection but the importance of HBsAg is more than other markers because it can be detected both in early as well in late stages being secreted in higher quantities. Many different techniques have been developed to detect the hepatitis B markers like RIA, ELISA, and Chemiluminescence. Because of need of expensive equipments, expertise and large turnaround time makes these procedures unsuitable in the primary health setting.

In many developing countries, ICT based rapid diagnostic tests are widely used to detect HBsAg for both diagnosis and screening for HBV infections¹⁰ as these are cost effective and does not need expertise. In our study we ran the serum of the patients through two brands (A and B) of ICT methods and subjected the same sera to ELISA method. The sensitivity for Brand A and Brand B was 89.47%, 84.20% respectively with reference to ELISA. Similar

results have been shown in various other studies. A similar study shows the sensitivity of ICT can vary from 50-94%¹¹. The parameters of this study were in harmony with other such studies.^{12, 13}

The ELISA kit that was used in this study showed to have analytical sensitivity of 0.1ng/ml. A similar study showed that ELISA is known to detect the antigen concentration of less than 0.4ng/ml of HBsAg while as Rapid tests based on lateral-flow technology, which appears to be the most sensitive format, do not achieve sensitivity of 1 IU/ml for HBsAg^{14,15}.

One of the brands used can detect Hepatitis B antigen in serum or plasma in a concentration as low as 0.5ng/ml. The results of a study show that the newly developed HBsAg rapid test had an analytical detection limit between 0.2 and 0.8 IU/ml values are similar to those for HBsAg EIAs detection systems currently in use¹⁶. Some studies suggest that the diagnostic performance of RDT is comparable to ELISA¹⁷.

The diagnosis of viral infections requires the use of rapid, sensitive assays if they are to be of value in the detection or treatment of disease. Ideally, the test should be useful in the smallest laboratory, where sophisticated equipment and highly trained technical support may not be available, or for field conditions¹².

In present study the ICT reagents were stored not more than one month and venous blood was collected in clot activated tubes, then ICT was carried out in 20 min as per manufacturer's instructions. It has been seen in other studies that ICT can be carried out using small blood samples that can easily be obtained by finger pricks. The ICT reagents can be stored for as long as 3 months at room temperature (15–30°C). The rapid test can be

performed by personnel with minimal training and the results are generally available within 5 min⁽¹³⁾.

In our study Brand A was slightly more sensitive (89.47%) as compared to Brand B (84.20%), this difference is statistically insignificant.

The NPV of the two brands is 99.97% and 99.95% for Brand A and brand B respectively. Sensitivity and NPV are too more important parameters for choosing a test rather than specificity and PPV¹⁸.

There are reports with some manufacturers the sensitivity of ICT can be increased by extending the incubation period from 15 min to 60 min with respect to endpoint titer¹⁹.

Quantitative detection of HBsAg helps in evaluation of HBV DNA status of a patient, as shown in a study that a low level of HBsAg indicates a low HBV DNA burden, whereas a high HBsAg quantity does not always correspond to a high viral load. Thereby a low HBsAg level can be used as a predictor of a low HBV DNA level²⁰.

Confirmation of diagnosis in hepatitis B viral infection and assessment of prognosis is based on wide array of advanced immunological, molecular and histological assays. The immunological techniques include 2nd generation, 3rd generation and 4th generation EIA. While molecular/ genetic testing includes qualitative, quantitative and signal enhancement detection of viral genomic fragments through PCR, RT-PCR, TMA or bDNA, whereas, invasive assessment includes examination of liver biopsy. But these techniques are costly and less frequently available in economically deprived countries. On the other hand a major concern in the use of rapid ICT kit method is variable degrees of sensitivity and specificity. An ideal rapid test

should have a high degree of positive predictive value (PPV) and low degree false negative results²¹.

To summarise this was a comparative study, ICT was compared with ELISA. The two different ICT brands were studied one was HEPA™ card and other was AlereTrueline. Which showed good sensitivity and specificity. For highly infectious viruses like HBV which may cause a long term silent infection, accurate detection of the viral marker is essential for controlling the transmission of the virus. For this reason, very sensitive and specific tests are needed. The Rapid diagnostic tests like lateral flow immunoassay can be used at the point of care and do not need any expertise to perform and are cost- effective. Results from a study indicate that the ICT based HBsAg rapid test is a simple, rapid, and highly sensitive and can be powerful tool for screening and diagnostic purpose in resource- limited areas of developing countries as well as in inner-city clinics of developed countries.

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