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ORIGINAL ARTICLE OPEN ACCESS

Comparative Evaluation of Rapid Card Test and Enzyme Linked Immunosorbent Assay for The Detection of Hepatitis B Surface Antigen in A Tertiary Care Hospital

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ABSTRACT

Background: Hepatitis B virus (HBV) infection is one of the leading causes of death in developing countries. The most important marker for HBV diagnosis is detection of Hepatitis B surface antigen in blood.

Objective: The aim of this study was to compare the analytical sensitivity of a rapid card test in comparison to Enzyme-Linked Immunosorbent Assay (ELISA) which is considered a Gold Standard technique for the detection of HBsAg in blood samples received for HBsAg test in serology section of Microbiology Department, Central Laboratory, Sharda Hospital.

Methods: Cross-sectional observational study was conducted at the Department of Microbiology, School of Medical Sciences & Research for a period of 12 months from 1st November 2020 to 31st October 2021. The two different brands by which multiple parameters were analyzed were HEPACARD and Erba Lisa SEN HBsAg.

Results: A total of 13939 blood samples were received for HBsAg diagnosis. For the study purpose, from the total of 13939 samples, 386 were randomly selected to be included in the study with 193 positive samples and 193 negative samples. The sensitivity and specificity of Rapid card were 95.83% and 100% respectively. The Positive predictive value and Negative predictive value of Rapid card were 100% and 95.65% respectively for HBsAg detection.

Conclusion: It can be concluded that, an ideal rapid test is a boon in time-saving situations. Since HBsAg screening by a rapid test is easier, cost effective, time saving can be easily performed by any trained health care worker at any time of need. Hence, HBsAg screening can be preferable done by a Rapid card test followed by a supplemental ELISA and Polymerase chain reaction for further confirmation. Good sensitivity and specificity of Immuno-chromatography test (ICT) for early detection of HBsAg was observed.

Keywords: Hepatitis B virus, Hepatitis B surface Antigen, ELISA and Rapid Card Test.

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Introduction

Viral hepatitis is a systemic disease primarily involving the liver. Most of the cases of acute viral hepatitis are caused by Hepatitis A virus (HAV), Hepatitis B virus (HBV) or Hepatitis C virus [1]. It was discovered by Blumberg in 1963. HBV belongs to the family Hepadnaviridae, under the genus Orthohepadnavirus[2].

HBV infection is a global public health problem. It is estimated that approximately 360 million people are infected worldwide with this virus. HBV is the most widespread and the most important type among hepatitis viruses. Though it commonly produces an acute self-limiting hepatitis which may be subclinical or symptomatic, it is also capable of causing a range of hepatic complications including chronic hepatis, fulminant hepatitis, cirrhosis of liver and liver cancer. Hepatitis B virus is the only DNA virus among hepatitis viruses [3].

HBV spreads through parenteral transmission (that is, through contact with blood or other body fluids via needles, open wounds, or other portals of entry) as well as through sexual contact and perinatal (mother-to-child) transmission during delivery [3]. One of the major distinctive features of HBV infection is the risk of developing chronic liver disease varies greatly with age of acquiring the infection. For neonates and infants who acquire HBV, the risk of chronicity is almost 90%, while it decreases to 30% for children 1-5yr, and up to 2% for older children and adults [4].

HBV produces – Hepatitis B Surface antigen (HBsAg), Hepatitis B Core antigen (HBcAg) and Hepatitis B envelope antigen (HBeAg). HBsAg has been accepted as a universal and the most reliable seromarker in case of acute HBV infection due to its appearance in serum within 2-10 weeks after exposure to HBV. HBsAg particles contain common "a" antigen, linked to two sets of mutually exclusive determinants, "d" or "y" and "w" or "r" giving the four main types - adw, adr, ayw and ayr[5]).

There are many methods for diagnosing HBV infection including Rapid Card test, enzyme linked immunosorbent assay (ELISA), Enzyme Immunoassay (ELA) and polymerase chain reaction (PCR). Rapid card test is a rapid screening test for qualitative detection of HBsAg in whole blood, serum or plasma specimen. On the other hand, ELISA is enzymatic immunoassay technique of the sandwich type for the detection of HBV in human serum or plasma which antigens or antibodies are covalently bound with suitable enzymes that can catalyze the change of substrates into dyed products. It is an approved technique to investigate diverse serological markers [5]. Based on the prevalence of HBV in different areas of the world are classified as high (≥8%), Intermediate (2-7%), or low (≤2%) HBV endemicity. India has an intermediate prevalence of hepatitis B virus with a 4% to 5.4% infected population [6].

Although rapid card tests are known to have lower sensitivity and specificity than ELISA, some have similar sensitivity and specificity [7]. To reduce false positive and negative results, ideal rapid screening tests should have a high degree of sensitivity and a reasonable amount of specificity [8]. Therefore, this study was aimed to assess the sensitivity and specificity of HBsAg quick card tests compared to the industry-recognized ELISA gold standard method in a tertiary hospital.

Methods

This study was a cross-sectional observational study conducted at Department of Microbiology, School of Medical Sciences & Research for a period of 12 months from 1st November 2020 to 31st October 2021.

This study population comprised of two groups – Group A and Group B. All the Group A subjects were HBsAg seropositive whereas Group B subjects were HBsAg seronegative.

In this study, all blood samples received for HBsAg test in the serology section were included. Hemolyzed, lipemic, insufficient, unlabeled or leaked samples & samples received in wrong vacutainer were excluded.

Sample collection and Processing

Approximately, 5ml of the blood was drawn aseptically using a sterile disposable syringe and needle. The drawn blood was transferred aseptically to a sterile red capped vacutainer vial. The blood was allowed to clot at the room temperature. Subsequently the clot was centrifuged at 10,000 rpm for 10 minutes. The separated serum in this manner was pipetted and transferred into a sterile Eppendorf tube. The serum sample was appropriately labelled and stored at 2-8°c until the test was performed. The sera were subjected to rapid HBsAg test, and HBsAg ELISA test. Any sera which were found to be turbid, haemolytic or lipemic was discarded [9].

Patient Serum Samples

A total of 13939 blood samples were received for HBsAg diagnosis. Two different brands with multiple parameters were analyzed for HBsAg using HEPACARD and Erba Lisa SEN immunoassays. For the study purpose, from the total of 13939 samples, 386 were randomly selected to be included in the study. Of 386 subjects – 193 HBsAg sero-positive and 193 HBsAg sero-negative which were tested by Rapid card were included in the study. To check the sensitivity and specificity of Rapid card 46 positive (Group A) and 46 negative (Group B) samples were chosen randomly and retested by ELISA which was regarded as the Gold Standard for HBsAg detection (Table 03). Inpatient/Outpatient departments (OPD/IPD) wise distributions of samples were included in the study.

i. Determination of Hepatitis B virus surface antigen by Immunochromatographic test / lateral flow immunoassay

All the samples were analyzed for HBsAg (Hepatitis B surface antigen) using Rapid card (HEPACARD by DIAGNOSTIC ENTERPRISES).

HEPACARD is a one-step immunoassay based on the antigen capture, or 'sandwich' principle. Test procedure was performed as per the manufacturer's instructions.

Table 1. Interpretation of Results

Interpretation	Control Line	Test Line
Negative Test	Pink Line	Pink Line
Positive Test	Pink Line	Pink Line
Invalid Test	No pink Line	No pink Line

ii. Determination of Hepatitis B virus surface antigen by Enzyme linked Immuno – sorbent assay

Samples were analyzed for HBsAg (Hepatitis B surface antigen) using ELISA kit (Erba Lisa SEN HBsAg by TRANSASIA BIO-MEDICALS LTD). The results were reported qualitative based on cut-off value calculated by addition of mean value of three negative control (NC) with a factor of 0.15 (SD value provided by the manufacturer). Erba Lisa SEN HBsAg uses the sandwich ELISA technique allowing qualitative detection of as 0.1ng/mL of HBsAg in patient serum/plasma. All the samples were run along with negative control (NC) and positive control (PC) according to test procedure given by manufacturer.

Optical density (OD) readings were obtained with a spectrophotometer at wavelengths of 450 nm (using 620/630/650 nm as the reference wavelength). The index of each sample was calculated with the following formula: Calculation of cut-off value = 0.15 + Negative Control (COV = 0.15 + NCx). Results were interpreted in accordance with manufacturer's recommendations. [9].

Ethical Consideration

Ethical clearance was obtained from the Institutional Ethics Research Committee, SMS & R, Sharda University, before the commencement of the study.

Statistical Analysis

All the results were recorded in tabular and graphical format. Assessment of sensitivity, specificity, positive and negative predictive values and concordance were estimated.

Results

Sample distribution for ELISA

Out of 386 samples, only a total of 92 samples were selected randomly for ELISA test. In Group A and Group B, 46 positive and 46 negative samples were included for ELISA testing (table 01) by choosing 17 samples monthly.

Table 2. Interpretation of Results

Rapid card test positive	46
Rapid card test negative	46
Total	92

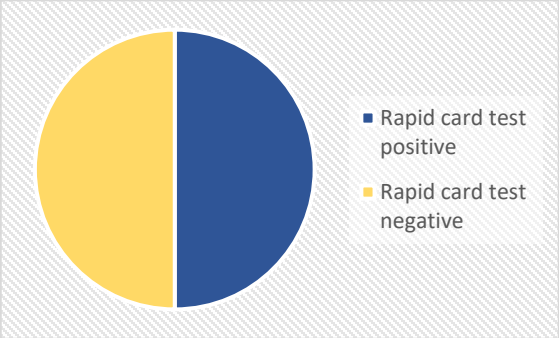

Figure 1. Sample distribution for ELISA

OPD/IPD WISE DISTRIBUTION OF SAMPLES (TOTAL NO = 92):

Out of 46 positive samples taken, 28 sample were from IPD and 18 were from OPD and out of 46 negative samples, 31 were from IPD and the remaining 15 patients were from OPD.

Table 3. Department wise distribution of Samples

Rapid card test samples	IPD	OPD
Positive samples (46)	28 (60%)	18 (39.13%)
Negative samples (46)	31 (67.39%)	15 (32.60%)

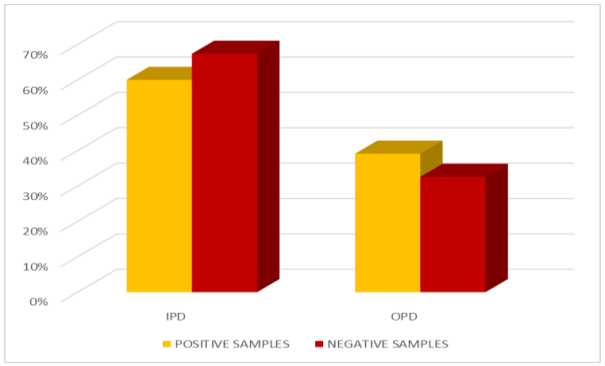

Figure 2: OPD / IPD wise distribution of samples

Comparative Evaluation Of Rapid Card Test And Elisa For Hbsag:

92 samples with 46 positive samples (Rapid card Test) and 46 negative samples (Rapid card Test) were taken for ELISA testing. Out of 46 positive samples 2 turn out to be negative by ELISA and none of the samples which were found to be negative by ICT turned out to be positive by ELISA.

Table 4. Comparison between rapid card test and ELISA

	HBsAg		
	ELISA Positive	ELISA Negative	Total cases
Rapid Card test Positive	46	0	46
Rapid Card test Negative	2	44	46
Total	48	44	92

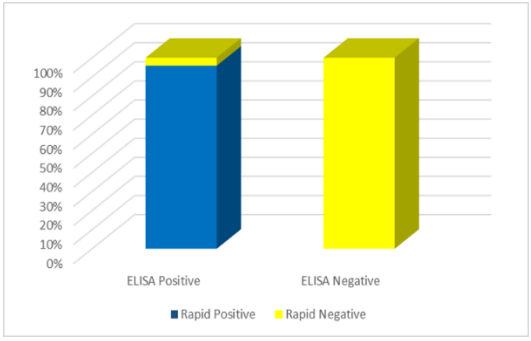

Figure 3. Comparative Evaluation of Rapid Card Test and ELISA for HBsAg

PARAMETERS STUDIED BY USING ELISA AS GOLD STANDARD:

Thus, our results confirm that the ICT could be used in the settings where ELISA is not an option. Various parameters were calculated by using following appropriate mathematical formula:

Sensitivity: TP / (TP + FN)100

Specificity: TN / (TN + FP)100

Positive predictive value: TP / (TP + FP) x100

Negative predictive value: TN / (TN + FN)

LR+= sensitivity / 1 - specificity

LR- = 1 – sensitivity / specificity

Accuracy = TP+TN / (TP+TN+FP+FN)

[TP: True Positive, TN: True Negative, FP: False Positive, FN: False Negative]

Table 5. Showing different parameters of Rapid Card test by using ELISA as gold standard.

Test Method	Rapid Card Test
Sensitivity	95.83%
Specificity	100%
NPV	95.65%
PPV	100%
Positive likelihood ratio	0.95
Negative likelihood ratio	0.05
Diagnostic accuracy	97.82%

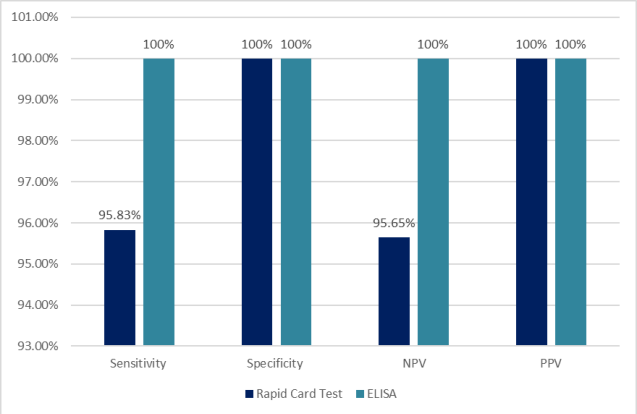

Figure 4: Shows comparison between ELISA and Rapid card Test for different parameters.

SEROPREVALENCE OF HBsAg BY RAPID CARD TEST:

A total of 13939 samples tested by Rapid card 169 turn out to be positive and 13770 were negative. Our study shows 0.01% of positive seroprevalence.

Table 6: Seroprevalence of HBsAg by rapid card test

Rapid card test	Results
Positive	169 (0.01%)
Negative	13770
Total	13939

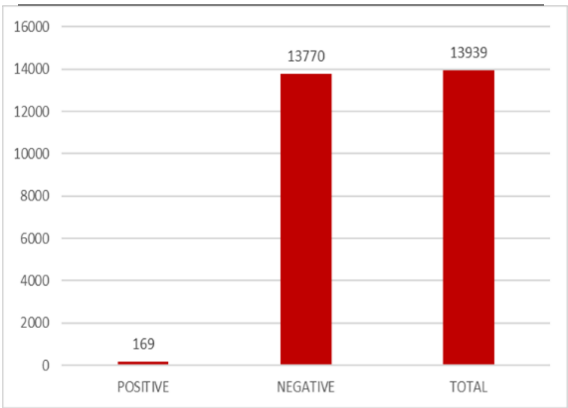

Figure 5. Showing the seroprevalence of HBsAg by Rapid Card Test

DISCUSSION

Hepatitis B is a highly infectious virus which may lead to a long-term complication. It remains a major per-cutaneous infection with grave implications for infected patients. For highly infectious viruses, 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[10].

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[11]. Rapid tests are equally sensitive to ELISA and yet they are cheaper and quicker. ELISA, EIA, PCR and other advanced methods are laboratory based, time consuming and require trained personnel. Rapid test enables early detection at sites where laboratory facilities or trained manpower are not

available or there is issue of accessibility. The rapid tests reduce the potential for loss of follow up of a case when results are not given straight away. The high laboratory cost is another factor that reduces the willingness to screen the general population. Ideally rapid devices should have a high degree of sensitivity and a reasonable specificity so as to minimize false positive and false negative results[11].

In our study, ELISA was compared with the rapid card test for the screening of HBsAg. ELISA was taken as the gold standard for serological testing of HBsAg. The study population comprised of two groups – Group A (HBsAg sero - positive) and Group B (HBsAg sero - negative) subjects. Samples, which were tested by ELISA maximum were from IPD. The sensitivity of Rapid card test was 95.83%, specificity 100%, PPV and NPV were 100% and 95.65% respectively with references to ELISA.

We observed a sensitivity and specificity of 95.83% and 100% respectively for HBsAg detection. The positive predictive value of rapid ICT was 100%. Raj et al.[12] reported that sensitivity was 79% and specificity was 98.9%. Another study showed 100% sensitivity of rapid test kit with a specificity of 91.7% for HBsAg[13]. Kaur et al.[14] reported 100% specificity and 93.4% sensitivity of ELISA to pick up all false negative. A study reported by Ansari et al.[15] showed that rapid assays with strip or device had sensitivity between 97.5% and 99.2% and specificity between 97.5% and 99.2%.

This study finding corroborates with other studies, which have shown the PPV of Rapid ICTs to be more than 80%. So, the ICT based RDTs had a major advantage due to easy perform, once less technical effort and rapid result generation. Whereas ELISA is more costly as lab needs to be equipped with instruments like ELISA reader and washer.

In comparison to ELISA, main advantage of the ICT based RDT is that a single sample can be run without waiting for the samples to be gathered and processed. Lacking of lab infrastructure in rural and remote areas, ICT based RDT can play a major role in diagnostic and in patient management of acute hepatitis infection. The sensitivity and specificity of various kits may vary and this needs to be kept in mind while performing tests. But initial validation requires with ELISA will help to make proper diagnosis.

India is designed as an intermediate endemic country with 3% – 4.2% prevalence[2]. In our study prevalence was found to be 0.01%, which was lower when compared with other study conducted by Sharma RK et al in 2019, showed 10.6% of prevalence in Himachal Pradesh[16]. However, a study from South India showed 5.16% HBV prevalence[17]. The lower prevalence was found in our study might be due to the pandemic scenario which lessened the sample to be tested for HBsAg.

The limitation of this study is that out of the 386 sample only 92 samples were tested by ELISA. For more specific results all the sample should have been tested. In our study, sex, age group, risk factors, the socio economy and high-risk group were not mentioned. If it was mentioned then these would have been more helpful for the clinicians to corelate with the treatment.

CONCLUSION

Rapid card testing is inexpensive, less time-consuming (it takes no more than 30 minutes to get a result), and does not require a skilled specialist to screen for HBsAg in an emergency. Therefore, we can draw the conclusion that an ideal swift test is a blessing in time-saving circumstances. Because HBsAg screening by rapid card test is less time consuming, it can be conducted at any time by any health care worker. It is unquestionably preferable as a screening test not only prior to hemodialysis, but also for any other emergency surgery followed by a supplementary ELISA and Polymerase chain reaction for confirmation is recommended.

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