



## Original Research Article

# Evaluation of rapid diagnostic test compared with ELISA for detection of hepatitis B surface antigen

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## ABSTRACT

**Background:** Hepatitis B is a serious type of viral infection affecting millions of people throughout the world. Some of those who become Chronic carriers may progress to develop hepatocellular carcinoma and end stage liver disease. Therefore, early diagnosis and management is the need of time. Present study was aimed to compare the relative sensitivity and specificity of rapid ICT with ELISA for the detection of HBsAg.

**Materials and Methods:** One step rapid test for detection of HBsAg was performed on samples by Meriscreen HBsAg test from Meril Diagnostics. HbsAg ELISA was done by using SD HBsAg ELISA 3.0 from SD Biostandard diagnostics Private Ltd. This is assay for qualitative detection of HBsAg from human serum and plasma. Total 526 samples were analysed.

**Results:** Out of 64 positive samples, 62 were positive by rapid card test, and 2 were negative. Out of 462 negative samples, 461 were negative by rapid card test, and one was positive. We found the sensitivity and specificity of rapid ICT was 96.8% and 99.7% as compared to ELISA.

**Conclusions:** The results are very much similar to several previous studies done in India and other parts of world. It would be wise to validate this point of care tests with a standard ELISA or other specific tests whenever feasible.

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## 1. Introduction

Hepatitis B is a serious type of viral infection affecting millions of people throughout the world. It attacks the liver and can cause both acute and chronic disease. An estimated 257 million people are living with hepatitis B virus infection (defined as hepatitis B surface antigen positive).<sup>1</sup> Some of those who become chronic carriers may progress to develop hepatocellular carcinoma and end stage liver disease. Therefore, early diagnosis and management is the need of time.

Different methods are used for the diagnosis of hepatitis B including rapid immunochromatographic technique (ICT), ELISA, EIA and PCR. Out of these methods, ELISA, EIA and PCR methods are expensive and are used in well equipped labs and major tertiary care hospitals. Rapid

diagnostic ICT kits are a good choice as they are less expensive and do not need high technical expertise or infrastructure. It has been documented that timely screening for HBV infection in marginalized populations in developed settings, and at-risk populations in endemic settings gain relevance for early detection, initiation of treatment and prevention of further transmission to infants, partners, and the community.<sup>2</sup>

However, many a times rapid and point of care tests may vary in their performance, analytical sensitivity and give false positive, and/or false negative results.

There are three possible explanations of false negative results in commercial assays. In chronic HBV carriers, the HBsAg level may be below the detection limit, that is, a high proportion of individuals with antibodies against HBV core antigen (anti-HBc) as the only serological marker of infection are low-level chronic carriers of the virus.<sup>3,4</sup> Another explanation as to why in chronic carriers, the

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HBsAg level may be below the detection limit, is that virus variants yield sequences that are not recognized by the antibodies employed in the assays.<sup>5,6</sup> A third possible explanation is that there are variants in other parts of the genome that down regulate the production of HBsAg.<sup>7</sup>

Therefore, to reduce the residual risk of false negative results in hepatitis B, the sensitivity of HBsAg screening assays has to be continuously improved.

There have been various studies to compare rapid tests and ELISA for the detection of HBsAg across the globe. However there are a handful studies from India and very few from central part.

Present study was aimed to compare the relative sensitivity and specificity of rapid ICT with ELISA for the detection of HBsAg.

## 2. Materials and Methods

A cross sectional hospital based study was planned at a tertiary care center located in central India. Informed consent was taken from all participants and study was approved by institutional ethical committee.

All the patients whose HBsAg test was sent to our lab were enrolled in the study. This was including suspected cases of hepatitis, and all antenatal women attending OPD visits. The patients with HIV, hepatitis C, and known cases of other chronic viral illness were excluded from the study. Hemolysed blood samples, inadequate samples, and patients with incomplete data or without consent were also excluded from the study.

Serum was separated from blood samples received and stored at -20°C until assayed.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of rapid card test was evaluated taking ELISA as gold standard.

### 2.1. Rapid ICT

One step rapid test for detection of HBsAg was performed on samples by Meriscreen HBsAg test from Meril Diagnostics. The test was performed according to kit instructions by trained personnel.

### 2.2. ELISA test

HbsAg ELISA was done by using SD HBsAg ELISA 3.0 from SD Biostandard diagnostics Private Ltd. This is assay for qualitative detection of HBsAg from human serum and plasma.

Any sample that was inadequate, hemolysed, not properly labeled was excluded from the study.

## 3. Results and Discussion

A total of 532 samples were enrolled in the study, out of which 6 samples were excluded because of one or more

excluding criteria.

Thus, total 526 samples were analysed. Mean age of patients were 38 years and range 12 to 64 years. There were 269 males and 257 females .other demographic characters are shown in Table 1.

Out of 526 samples, 64 were positive by ELISA test and 462 were negative as per the cut off value calculated as per kit manual. However, out of 64 positive samples, the absorbance value for 2 samples was only slightly greater than cut off value.

Out of 64 positive samples, 62 were positive by rapid card test, and 2 were negative.

Out of 462 negative samples, 461 were negative by rapid card test, and one was positive.

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of rapid card test taking ELISA as gold standard are shown in Table 2 and Table 3.

Positive predictive value was calculated to be 98.41% and negative predictive value was 99.56%.

In our study, we compared one rapid ICT with ELISA for detection of HBsAg from patient serum. We found the sensitivity and specificity of rapid ICT was 96.8% and 99.7% as compared to ELISA. These are very much similar to several previous studies done in India and other parts of world.

In a study by Ekwi DN et al., three different ICT were evaluated as compared to SD ELISA 3.0, and had a sensitivity of 99.4, 97.7, and 98.3% respectively.<sup>8</sup> Almost similar results were shown by another study by Marcillat S at al done in Brazil.<sup>9</sup> There are several previous studies suggesting that SD ELISA 3.0 is a good standard test to compare with,<sup>10,11</sup> Currently, WHO has called for urgent action to reduce the burden of viral hepatitis and is designing HBV guidelines for screening and treatment in low- and middle-income countries.<sup>12</sup>

It has been reported that false-negative results of HBsAg POC tests are associated with a low HBsAg concentration, HBsAg mutants, low viral load, and certain viral genotypes.<sup>13–16</sup> Therefore one has to select the test wisely keeping these factors in mind.

Another study was conducted to assess the diagnostic accuracy of three point-of-care tests (Determine, Vikia, and Espline) for the detection of HBsAg in the field or a laboratory setting in the Gambia. The sensitivity and specificity of the Determine test were 88.5% and 100% in the field and 95.3% and 93.3% in the laboratory setting, respectively. The sensitivity and specificity were 90.0% and 99.8% for the Vikia test (inthefield) and 93.9% and 94.7% for the Espline test (in the laboratory).<sup>17</sup>

Another study from India has observed that immunochromatographic assays (ICAs) has a specificity of 100% but the sensitivity was 93.4%.<sup>4</sup> Study from Seoul showed 97% sensitivity and 100% specificity for detecting

**Table 1:** showing demographic characters and test outcome of all patients

Age group	Total no. tested	Positive by Rapid test	Positive by ELISA
12-24	78	5	5
25-34	125	13	13
35-44	166	20	21
45-54	104	18	18
55-64	53	6	7
Total	526	62	64

**Table 2:** Sensitivity of rapid test as compared to ELISA

Diagnostic test	No. of positive samples examined	No. positive produced by tests	No. negative produced by tests	Relative sensitivity
Meriscreen	64	62	2	96.87%

**Table 3:** Specificity of rapid test as compared to ELISA

Diagnostic test	No. of negative samples examined	No. negative produced by tests	No. positive produced by tests	Relative specificity
Meriscreen	462	461	1	99.78%

HBsAg.<sup>18</sup> In another study in healthy individuals from Karachi showed comparable sensitivity and specificity of ICT kits with ELISA technique.<sup>19</sup>

#### 4. Conclusion

There are many rapid immunochromatographic tests (ICT) available commercially claiming almost 100% sensitivity and specificity for the detection of HBsAg. Many times these are utilized by peripheral labs and hospitals without in house validation. However, there could be variations in their performance because of many factors. Therefore it would be wise to validate this point of care tests with a standard ELISA or other specific tests whenever feasible. More such studies are required to see the performance of such rapid tests in different population groups and geographical locations.

#### 5. Source of Funding

None.

#### 6. Conflict of Interest

None.

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