ORIGINAL ARTICLE

Performance Evaluation of Determine HBsAg 2 Rapid Card Test for the Detection of Hepatitis B Surface Antigen in Clinical Samples

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Abstract

Aim and background: Hepatitis B virus is one of the leading underlying causes of chronic liver disease. Rapid diagnostic tests with improved sensitivity and specificity for detecting hepatitis B infection could aid in large-scale community screening in resource-limited settings. This study was designed to assess the clinical performance of a rapid card test to detect HBsAg.

Materials and methods: In this study, archived once-thawed serum samples were tested on the Determine HBsAg 2 card and their performance was evaluated in reference to a chemiluminescence-based assay (HBsAg qualitative assay, Abbott Diagnostics, US).

Results: A total of 120 patient samples (46 confirmed HBsAg-positive and 74 confirmed HBsAg-negative) were used in this study. The overall median age of the study population was 44 years (IQR: 36–51 years), with a male gender predominance (90%). A specificity of 100% (74/74) and sensitivity of 84.7% (39/46) was observed for the Determine HBsAg 2 assay compared with the reference assay. The samples that showed false-negative results ($n = 7$) by the card test had HBsAg levels below the limit-of-detection of the card assay.

Conclusion: The Determine HBsAg 2 assay gives rapid results in 15 minutes with good sensitivity and specificity. This makes it a good, affordable tool for large-scale screening and public health surveillance programs.

Clinical significance: Accurate and cost-effective rapid card tests for early detection of Hepatitis B infection would enable quick isolation of infected cases, thus reducing transmission in the community.

Keywords: CLIA, Determine HBsAg 2, Hepatitis B, HBsAg, RDT.

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Introduction

Hepatitis B virus (HBV) remains a critical public health problem with notable morbidity and mortality.[1] Approximately, more than 250 million people are chronic HBV carriers,[1] with a considerable regional variation of hepatitis B surface antigen (HBsAg) positivity ranging from low (2%) to high (8%) endemicity prevalence.[2] In India, the prevalence of HBsAg positivity ranges between 3 and 4.2%, with an estimated 40 million HBV carriers.[3] The Global Health Sector Strategy (GHSS), endorsed by the World Health Organization (WHO), underscores the importance of scaling up diagnosis and testing for Hepatitis B to achieve the overall aim of elimination of viral hepatitis by 2030.[1] The focus is to reach populations and communities to improve screening, testing, and treatment for Hepatitis B. Among HBV markers, HBsAg is the most frequently used marker used to test HBV infection.[4] Rapid diagnostic testing is an effective method to screen large communities cost-effectively.[5] Despite the availability of effective vaccines and advancements in HBV diagnosis and treatment strategies, early access to testing is still a hurdle in low and middle-income countries. Although the routinely used enzyme-based immunoassays (EIA) for HBsAg detection have good sensitivity and specificity,[6] the requirement of dedicated lab infrastructure, trained personnel and high cost limits its use in resource-limited settings. Therefore, rapid diagnostic tests (RDT) for HBV may be a powerful gateway to improve patient access to diagnosis and treatment services, critical components of an effective cascade-of-care response. Point-of-care (POC) testing, which includes RDTs, performed at or near the patient site could provide timely results, resulting in rapid diagnosis and faster clinical decision-making. In this study, the clinical performance of Determine HBsAg 2 card test (Alere Medical Co. Ltd [Now Abbott]), a qualitative lateral flow immunoassay was compared with that of current routinely used ARCHITECT qualitative HBsAg assay (chemiluminescent immunoassay-based technology, Abbott Diagnostics, IL, US) for the detection of HBsAg in clinical serum samples.

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Conflict of interest: None

Materials and Methods

Study Design

This study was performed in a Liver Specialty Hospital in Delhi, India. A sum of 120 previously tested, aliquoted, thawed once serum samples were retrieved from the lab repository. Of these, 46 were true HBsAg-positive samples (HBsAg-positive and anti-hepatitis B

core antibody [anti-HBc] positive), and 74 were true HBsAg-negative samples (HBsAg-negative, anti-HBc and HBV DNA-negative). The entire set of samples was simultaneously tested on both assays in parallel. The discordant results were confirmed by a third CLIA-based assay –ARCHITECT HBsAg quantitative assay (Abbott Ireland Diagnostics Division, Sligo, Ireland).The clinical information of the samples was attained from the Hospital Information System (HIS). Samples of patients with other infection, insufficient volume and incomplete clinical information were not included. The Institutional Ethics Board (Approval No. IEC/2020/79/MA03) approved this study. It was performed according to the policy of the Declaration of Helsinki. The research involved samples that were completely deidentified and archived in the lab repository. Therefore, the patient informed consent was not required.

Determine™ HBsAg 2 Assay (Test Assay)

It is a qualitative, visually read lateral flow immunoassay that utilizes a volume of 50 µL of plasma or serum or whole blood (venous or fingerstick) to detect HBsAg with a calculated analytical sensitivity of 0.1 IU/mL, as mentioned by the manufacturer.[7] The assay kit contains single-use test strips and a buffer bottle; both stored at room temperature and require no specific temperature storage. The assay result is expected to be evaluated for a minimum of 15 minutes and a maximum of 30 minutes after adding the sample.

ARCHITECT HBsAg Qualitative II Reagent Kit Test (Reference Assay)

This assay was taken as the study's reference assay, a chemiluminescent immunoassay (CLIA). The assay was performed on the ARCHITECT i2000 SR (Abbott Ireland Diagnostics Division, Sligo, Ireland) system. The subsequent chemiluminescent reaction is monitored as relative Light Units (RLUs).

ARCHITECT HBsAg Assay (HBsAg Quantitative)

It was used to quantify the HBsAg antigen levels in samples, showing discordant results in both assays. It is also a CLIA-based test, where the following chemiluminescent reaction is measured as RLU by the ARCHITECT i2000 SR (Abbott Healthcare Pvt. Ltd, Illinois, US) instrument. The test results are generated as IU/mL by comparing them with the calibrator curve.

Statistical Analysis

Continuous and categorical variables were analyzed using the mean ± standard deviation or median with interquartile range (IQR), or percentages (%), as appropriate. The statistical analysis was achieved with MedCalc software (https://www.medcalc.org/calc/diagnostic_test.php, Version 22.014; accessed October 11, 2023).

Results

Out of 120 patient serum samples used in the study: 46 were confirmed HBsAg-positives and 74 were confirmed HBsAg-negatives (Flowchart 1). The population's median age was 44 years (IQR: 36–51 years). The male-to-female ratio was 9:1. The baseline demographics of the study population are depicted in Table 1. Out of 46 confirmed HBsAg-positive samples, 39 (84.7%) samples were tested positive by Determine HBsAg 2 test. All the samples with discordant results were further subjected to HBsAg Quantitative assay. Among the 7 HBsAg confirmed positive samples, which showed as "false-negative" by Determine HBsAg 2 assay, were tested positive by HBsAg quantitative assay. Among the HBsAg-negative samples ($n = 74$), all samples showed 100% concordance with the Determine HBsAg 2 test. So, overall, a sensitivity of 84.7% (95% CI: 71.13–93.66%) and specificity of 100% (95% CI: 95.14–100%) was seen for the Determine HBsAg 2 card test in comparison to the reference assay (Table 2).

Discussion

The present study intended to evaluate the clinical performance of the Determine HBsAg 2 card test for the qualitative detection of HBsAg compared with ARCHITECT HBsAg Qualitative II as the reference assay. Determine HBsAg 2 card test is a simple, rapid lateral flow immunoassay which provides test results within

Flowchart 1: A schematic flow diagram

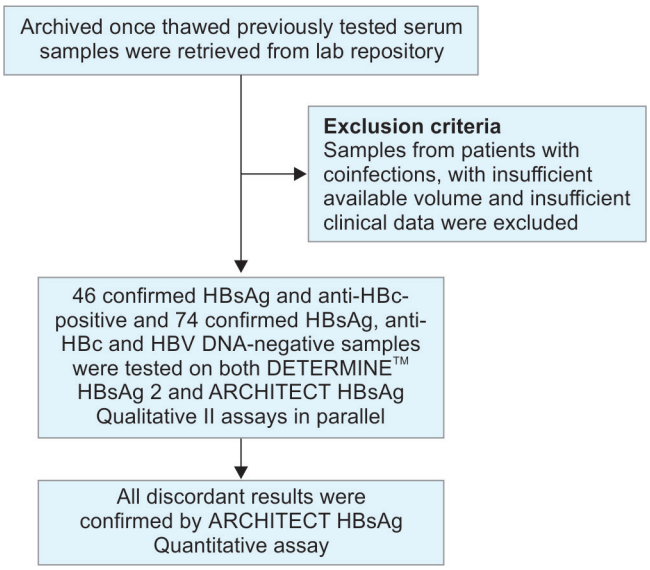

Table 1: Baseline characteristics of the study population

Baseline characteristics	*All samples (n = 120)*	*HBsAg-positive samples (n = 46)*	*HBsAg-negative samples (n = 74)*
Age			
Median(IQR) years	44 (36–51.2)	43.5 (36–49.7)	44 (36–52.7)
Gender			
Male (*n*, %)	108 (90%)	43 (93.4%)	65 (87.8%)
Female (*n*, %)	12 (10%)	3 (6.6%)	9 (12.2%)
ALT levels (IU/mL), median (IQR)	34 (23.3–66)	34.8 (26.7–67.9)	33.8 (22.7–62.7)
AST levels (IU/mL), median (IQR)	34.1 (26–76.2)	34.2 (28.4–88.2)	34.1 (24.7–59.9)
Total bilirubin levels (mg/dL), median (IQR)	0.82 (0.5–1.3)	0.91 (0.59–1.33)	0.73 (0.56–1.81)

ALT, alanine transaminase; AST, aspartate transferase; IQR, interquartile range

Table 2: The statistical analysis of Determine HBsAg 2 test

Statistic parameter	*Value*	*95% CI*
Sensitivity	84.78%	71.13–93.66%
Specificity	100%	95.14–100.00%
Positive predictive value*	100%	90.97–100%
Negative predictive value*	99.53%	99.08–99.76%
Accuracy*	99.54%	96.10–100%
Disease prevalence*	3.00%	

*These parameters are dependent on disease prevalence, which is taken as 3% for HBV infection in India as per the previous literature[3]

30 minutes. The test results did not vary at 15 minutes and 30 minutes. Of 74 confirmed HBsAg-negative samples, a 100% concordance was seen between the test and reference assay. Among 46 true HBsAg-positive samples, 39 (84.7%) samples were positive in the Determine HBsAg 2 test. All the 7 positive samples that showed negative results by Determine HBsAg 2 card test were found to have low (≥0.05 IU/mL but ≤0.1 IU/mL) HBsAg levels as detected by a third test, HBsAg quantitative assay. As claimed by the manufacturer, the analytical sensitivity of the Determine HBsAg 2 assay is 0.1 IU/mL. This explains the disparity in results among those 7 samples, as the antigen levels were beyond the detection limit of the Determine HBsAg 2 assay. Therefore, overall, a sensitivity of 84.7%, a specificity of 100%, and a diagnostic accuracy of 99.5% were observed for the Determine HBsAg 2 test. Despite advances in diagnostics and treatment for HBV, low access to diagnostic services remains a significant challenge. Accurate, reliable, and affordable POC assays are needed for mass testing of the general public. Currently, in-use Rapid HBsAg card tests show good clinical sensitivity and specificity[5,8] but show low accuracy due to poor analytical sensitivity (2–10 IU/mL).[5,6] This leads to "missed" HBV cases and increased transmission rates. HBV can remain "silent" inside the liver for years and with increasing age, may cause liver-related complications, leading to decreased quality of life and healthcare costs. Therefore, the need of the hour is to screen as many individuals as possible, especially in endemic and hyperendemic zones, which are "hotspots" for hepatitis B infection. Although the current chemiluminescent-based qualitative and quantitative HBsAg assays with good sensitivity and specificity are available,[9,10] cost and technical expertise make them less accessible in low and middle-income countries. Therefore, accurate and affordable card tests with lower limit-of-detection (LoD) are good alternatives for rapid diagnosis of HBV infection. One such card test evaluated by a previous study[11] and this current study showed that Determine HBsAg 2 assay could improve the detection of HBV-infected individuals with lower HBsAg levels, preventing the spread of infection to other contacts. This would enable more and more individuals to enter the cascade-of-care process. In addition, this Determine HBsAg card test can be helpful for public health epidemiological and surveillance programs where good and inexpensive assays are indispensable. A limitation of this study includes the retrospective nature of the study design, so, large sample size studies with patient categories covering different genotypes and mutants would give a better scenario to assess the diagnostic performance of this assay.

Conclusion

The Determine HBsAg 2 assay has the probability to render a quick and reliable diagnosis at the point-of-care for early testing of HBV-infected individuals with low levels of HBsAg, which allows them to receive timely care and treatment, further mitigating the chances of progression to severe liver disease.

Clinical Significance

Rapid diagnostic tests with an improved sensitivity for hepatitis B screening would aid to fill in the gaps in the current HBV diagnostics and would enable more individuals to access to care and treatment.

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