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Diagnostic Performance of Rapid Test Cassette Compared to Enzyme-Linked Immunosorbent Assay in the Screening of Anti-HBs in Sokoto, Nigeria

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ABSTRACT

Background and aims: Hepatitis B virus (HBV) infection remains a major global public health challenge and is highly endemic in sub-Saharan Africa, particularly Nigeria. Detection of hepatitis B surface antibody (anti-HBs) is essential for identifying previous HBV exposure, assessing post-vaccination immunity, and guiding clinical management in individuals who test negative for hepatitis B surface antigen (HBsAg). Although enzyme-linked immunosorbent assay (ELISA) is the reference method for anti-HBs detection, its routine use is limited in resource-constrained settings because of cost, infrastructure requirements, and longer turnaround time. This study evaluated the diagnostic performance of the Egens anti-HBs rapid test cassette compared with ELISA for screening anti-HBs among selected populations in Sokoto, Nigeria.

Materials and Methods: A hospital-based cross-sectional diagnostic accuracy study was conducted between February and April 2026 among 262 participants, including individuals living with chronic HBV infection, human immunodeficiency virus (HIV) infection, Mycobacterium tuberculosis infection, and apparently healthy controls. Serum anti-HBs was detected using the Egens rapid test cassette and a commercial ELISA kit, with ELISA serving as the reference standard. Diagnostic performance indices, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), overall diagnostic accuracy, and Cohen's kappa coefficient, were calculated with corresponding 95% confidence intervals. Fisher's exact test was used to assess associations between participant characteristics and true-positive rapid test results.

Results: The mean age of participants was 29 ± 14 years, and 73.3% were male. Anti-HBs positivity was detected in 26.7% by ELISA and 27.5% by the rapid test cassette. The rapid test demonstrated a sensitivity of 95.7% (95% CI: 91.7–99.7), specificity of 97.4% (95% CI: 95.4–99.4), PPV of 93.1% (95% CI: 87.1–99.1), NPV of 98.4% (95% CI: 96.6–100.0), and an overall diagnostic accuracy of 96.9% (95% CI: 94.9–98.9). Agreement between the rapid test and

ELISA was almost perfect (Cohen's κ = 0.903, $P = 0.001$). True-positive rapid test results were significantly associated with participant group, male sex, and age category (all $P < 0.05$).
Conclusion: The Egens anti-HBs rapid test cassette demonstrated excellent diagnostic performance and almost perfect agreement with ELISA, indicating that it is a reliable alternative for anti-HBs screening, particularly in resource-limited settings where access to conventional laboratory testing is limited.

Keywords: Hepatitis B virus; Anti-HBs; Enzyme-linked immunosorbent assay; Rapid diagnostic test; Diagnostic accuracy; Nigeria.

INTRODUCTION

Viral hepatitis is the inflammation of the liver caused by viruses such as hepatitis B virus (HBV) that primarily target the liver cells. [1] Viral hepatitis poses a global health challenge and threat, and Africa is one of the regions having the highest burden. [2,3] HBV infection can present asymptomatically; however, it can turn severe [1]. This deadly infection is the leading cause of chronic liver diseases which are liver cirrhosis and hepatocellular carcinoma (HCC). [4] This virus is transmitted via percutaneous, sexual and mother to new born baby.

Hepatitis B virus infection affects hundreds of millions of people, and hundreds of thousands of people die each year globally, though there is a geographical variation. [4,5] HBV infection is hyper endemic in Nigeria. [6,7] HBV infection is preventable by a safe vaccine, given in three dosage regimen and provides protection for about 20 years. [4] This deadly infection is not curable however, it is treatable with some anti-retroviral drugs that can prevent the progression of the infection, reverse liver fibrosis, and reduce the chances of transmission. [4,8]

Spontaneous clearance of HBV infection occurs in a proportion of patients living with chronic course of the disease (CHB). [9] This is evident by the appearance of hepatitis B surface antibody (anti-HBs) in the patient's blood. Anti-HBs that is produced against hepatitis B surface antigen (HBsAg) provides long-term clearance of the infection. [10] Conventionally, HBV infection is diagnosed by screening HBsAg in an individual's blood. However, screening of anti-HBs is recommended in an individual who tested negative for HBsAg. [7] This approach is important as some individuals might have been infected with HBV unaware and subsequently clear the infection in which anti-HBs appears while HBsAg disappears in the blood. Therefore, this approach identifies individuals who had previous exposure to HBV infection. Moreover, HBV vaccination is recommended in an individual who tested negative for both HBsAg and ant-HBs. [7]

The chance of HBV infection reactivation and losses of protective anti-HBs is seen in patients co-infected with some infections such as human immunodeficiency virus infection (HIV). [11,12] Therefore, screening of anti-HBs is important in such patients to ensure proper management.

Enzyme-linked immunosorbent assay (ELISA) method is very sensitive serological method used for the diagnosis of various infectious diseases. However, this method is relatively cost, needs some expertise, has high turnout time, and is not widely accessible and available especially in primary and secondary health facilities in resource-limited countries such as Nigeria. A rapid test method is cheap, accessible, does not require any special expertise, the results can be obtained in less than twenty minutes, however, ELISA is superior to it. [13] Moreover, there is paucity of data on the studies comparing ELISA with rapid test method in the screening of anti-HBs especially in our environment where HBV infection is hyper endemic. There is therefore a need to validate the rapid test kit for anti-HBs prior to using it in the clinical practice. Hence there is need to conduct this medical research.

This study aimed to determine the diagnostic performance of Egens anti-HBs rapid test

cassette by employing ELISA as a gold standard method.

MATERILAS AND METHODS

The study was cross-sectional conducted from February to April 2026 at Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto and Specialist Hospital Sokoto. The total study participants were 262, and the study populations were people living with CHB, Human immunodeficiency virus and *Mycobacterium tuberculosis* infection, as well as apparently healthy controls group.

Ethical approval was obtained and the study participants provided informed consent prior to the commencement of the study. The ethical approval Number along with the names of Institutional committee are as follows:

1. UDUTH Health Research Ethics Committee (UDUTH/HREC/2025/1502/2).
2. State Health Research Ethics Committee (SMH/1580/V. IV).
3. Hospital Ethics Committee (SHS/SU8JB/133/VOL.1).

Inclusion and Exclusion Criteria:

1. Individuals greater than or equal to 5 years of age were included into the study.
2. Individuals who were diagnosed having HIV infection or Mycobacterium tuberculosis and attending respective clinic in Specialist Hospital, Sokoto were included into the study.
3. Individuals who were diagnosed having HBV infection more than six months ago irrespective of their treatment status and attending gastroenterology clinic in UDUTH, Sokoto were included into the study.
4. Individuals who denied informed consent were excluded from the study.

Clearance of HBV infection do occur among individuals living with chronic course. Both HIV and HBV infection have common routes of transmission. Individual can be infected by HBV and show no specific signs or symptoms of HBV infection and thereafter clear the infection. This scenario can occur in both healthy and disease individuals. These formed the rationale for selecting the above study groups in the current study.

A questionnaire was used for the data collection and interviewer administered technique was employed. A **s**ystematic random sampling was employed in the selection of the study participants.

Three milliliters (3 mL) of blood was taken from each of the study participants and this was poured into a plain container, the blood was allowed to clot and centrifuged at 3000 rpm for 5 minutes to obtain the serum, and stored at -20° till assayed. The serum was used to conduct the analysis of anti-HBs among the study populations using Egens anti-HBs rapid test cassette (Nantong Egens Biotechnology CO. LTD, China), with LOT Number: 20260122. Additionally, the serum was utilized for the analysis of anti-HBs using ELISA kit (Beijing Kewei ELISA kit, China), with LOT Number: 20260202. The analysis was conducted according to the instructions of the manufacturers of the kits. The test results of Egens rapid test cassette were compared with ELISA results considered as the gold standard.

Statistical Package for Social Science (SPSS) version 25 was used for the data analysis, and proportions were expressed as frequencies and percentages. Fisher's Exact test was done to determine the statistical association between the variables, while Cohen's Kappa test was done to find the level of agreement between the two diagnostic raters (ELISA and rapid test cassette methods). *P value* < 0.05 was considered statistically significant. The 95 % Confidence intervals for the sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy of the rapid test cassette were determined from the formula p ± 2e.s.e (p).[14] ese (p) is the standard error = square root of p (1-p)/n. p = sample proportion = rate of observation = r/n, and n = sample size.

RESULTS

Sociodemographic Characteristics of Study Participants

The total number of study participants was 262. Age of the study participants was 29 ± 14 years (Mean ± SD), and the majority, 192(73.3%) were males. The majority of the study participants were Hausa-Fulani by tribe 235(89.7%). (Table 1 shows these results).

Table 1: Sociodemographic characteristics of the study participants

Variable	Frequency n (%)
Sex	
Males	192 (73.3)
Females	70 (26.7)
Age (years)	
29 ± 14 (Mean ± SD)	
Tribe	
Hausa-Fulani	235 (89.7)
Igbo	13 (5.0)
Yoruba	7 (2.7)
Others	7 (2.7)
Marital Status	
Married	79 (30.2)
Single	165 (63.0)
Divorce	1 (0.4)
Widowed	17 (6.5)

Outcomes of ELISA and rapid test cassette in the screening of anti-HBs

Out of 262 study participants, 70 (26.7 %) were tested positive for anti-HBs with ELISA method, while 72 (27.5 %) of the study participants were tested positive for anti-HBs by employing rapid test cassette (See Table 2).

Table 2: Tests results for anti-HBs screening using ELISA and rapid test cassette methods

Assay type	Positive HBs antibody n (%)	Negative HBs antibody n (%)	Total
Anti-HBs ELISA	70 (26.7)	192 (73.3)	262
Egens anti-HBs rapid test cassette	72 (27.5)	190 (72.5)	262

Accuracy of a rapid test cassette in screening of anti-HBs by employing an ELISA method as a serological gold standard

Among the 70 study participants who tested positive for ant-HBs by ELISA method, 67 were positive for anti-HBs by Egens rapid test cassette. The false positive and false negative of the rapid test cassette were 5(6.9%) and 3(1.6%) respectively. Table 3 illustrates these results. Therefore, the sensitivity and the specificity of the rapid test cassette were 95.7% and 97.4% respectively. While the positive predictive value (PPV) and negative predictive value (NPV) of the rapid test cassette were 93.1% and 98.4%, respectively. The overall diagnostic accuracy of the rapid test cassette was 96.9%.

The 95 % confidence intervals (CI) for the sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy is given by p ± 2e.s.e (p) as earlier reported in the materials and methods section. The 95 % CI for the sensitivity = 0.957 ±2 √0.957(1-0.957)/70 = 0.957 ± 2√0.00058787143 = .0957 ± 0.04 = 0.917, 0.997 = 91.7 %, 99.7 %. 95 % CI for the specificity = 0.974 ±2 √ 0.974(1-0.974)/192 = 0.957 ± 2√0.00013189583 = 0.974 ± 0.02 = 0.954, 0.994 = 95.4 %, 99.4 %.

The 95 % CI for the PPV = 0.931 ±2 √ 0.931(1-0.931)/72 = 0.931 ± 2√0.00089220833 = 0.931 ± 0.06 = 0.871, 0.991 = 87.1%, 99.1 %. 95 % CI for the NPV = 0.984 ±2√ 0.984(1-0.984)/190 = 0.984 ± 2√0.00008286316 = 0.984 ± 0.018 = 0.966, 1.002 = 96.6 %, 100 %.

The 95 % CI for the overall diagnostic accuracy = 0.969 ±2√0.969(1-0.969)/262 =

$0.969 \pm 2\sqrt{0.00011465267} = 0.969 \pm 0.02 =$ 0.949, 0.989 = 94.9 %, 98.9 %. The Cohen's Kappa coefficient's value for ELISA and rapid test cassette was 0.903 = 90.3 %, and *P value* is 0.001.

Table 3: Accuracy of rapid test cassette in screening ant-HBs by employing ELISA as a serological Gold standard.

Variable	Anti-HBs status (ELISA)		Total
	Positive anti-HBs (n=70)	Negative anti-HBs (n=192)	
Rapid test Cassette Positive	67	5	72
Negative	3	187	190
Total	70	192	262

True positive results for anti-HBs rapid test cassette across the variables of study participants

The prevalence of true positive results for anti-HBs rapid test cassette is significantly higher among controls 53(37.3%), males 52(27.1%), and age group 21-36 years 43 (35.8%), (*P value:* 0.0001; 0.019; 0.001, respectively). Table 4 demonstrated these results.

Table 4: Comparison of anti-HBs rapid test cassette true-positive results according to the characteristics of study participants (N = 262)

Variables	Anti-HBs rapid test cassette True Positive		P value
	Yes, n (%)	No, n (%)	
Study participants group			
HIV group (n = 62)	9 (14.5)	2 (3.2)	0.0001
HBV group (n = 15)	0 (0.0)	0 (0.0)	
TB group (n = 43)	5 (11.6)	2 (4.7)	
Controls (n = 142)	53 (37.3)	1 (0.7)	
Sex			
Male (n = 192)	52 (27.1)	1 (0.5)	0.019
Female (n = 70)	15 (21.4)	4 (5.7)	
Age group (years)			
5–20 (n = 79)	13 (16.5)	0 (0.0)	0.001
21–36 (n = 120)	43 (35.8)	2 (1.7)	
37–52 (n = 43)	7 (16.3)	2 (4.7)	
53–69 (n = 16)	3 (18.8)	0 (0.0)	
>68 (n = 4)	1 (25.0)	1 (25.0)	

Abbreviations: HIV, human immunodeficiency virus; HBV, hepatitis B virus; TB, tuberculosis; anti-HBs, antibody to hepatitis B surface antigen.

DISCUSSION

Hepatitis B virus infection is hyper endemic in Nigeria, and the Northwestern region has the highest prevalence compared to other geopolitical regions in the country. [15] The majority of studies on this deadly infection have been on the evaluation of HBsAg. HBV infection can present asymptomatically, as such a person can be infected unnoticed and can clear the infection in due course. In this scenario, the individual will have negative test results to HBsAg, but will be positive to anti-HBs. Additionally, clearance of HBV infection occurs among people living with chronic phase of the infection, and this leads to the appearance of anti-HBs. [9] However, this antibody can be lost and leads to reactivation of the infection. Moreover, anti-HBs can also be lost in certain groups of individuals such as people living with HIV. [16] Therefore, screening anti-HBs is very important for ensuring the control and proper management of HBV infection occurring with or without other co-morbidities.

The validity of a screening test method in terms of sensitivity and specificity is very important, it is the ability of the test method to measure what it sets out to measure.[14] The sensitivity of the screening test method is the ability of the test to identify the people who

have the condition, while the specificity is the ability of the screening test method to identify individuals who do not have the condition. The Positive predictive value (PPV) and Negative predictive value (NPV) measure the accuracy of a screening test method based on the prevalence. PPV reflects the proportion of individuals who tested positive for a condition and truly have it, while NPV reflects the proportion of individuals who tested negative for a condition and truly do not have it. [17]

The rapid test kits of various infectious disease conditions are widely available, affordable, and accessible. However, there is a dearth of data on comparing the rapid test method with ELISA method in screening anti-HBs in our environment having higher prevalence of HBV infection. Hence, our findings in the current project will provide the baseline information with regard to screening accuracy of anti-HBs rapid test cassette in our environment.

A substantial (96.9%) overall diagnostic accuracy (proportion of correctly classified subjects among all subjects) [18] by Egens anti-HBs rapid test cassette was observed in our study. The sensitivity (95.7%), specificity (97.4%), PPV (93.1%), and NPV (98.4%) together with the overall diagnostic accuracy (96.9%) of Egens anti-HBs rapid test cassette recorded in this study is reasonable, as in clinical practice there is no screening test method that is 100 percent perfect. [14]

The current research project found narrowed confidence limits for sensitivity, specificity, PPV, NPV, and overall diagnostic accuracy of Egens ant-HBs rapid test cassette. Additionally, almost perfect agreement (90.3%) between Egens rapid test cassette and ELISA method was observed in the screening of anti-HBs, and this agreement was not by chance as the p value was statistically significant. Therefore, these findings showed that Egens anti-HBs rapid test cassette has high precision and confidence in the results. Hence, this rapid test cassette will be found reliable in clinical practice.

The overall anti-HBs (26.7 %) obtained in the current research is lower compared to what was reported (44.2%) in Pakistan, [19] and also lower than the value (36.8%) recorded in a national survey among the general population of the six geopolitical regions in Nigeria. [20] The disparity of the overall anti-HBs might be due to the difference in the study populations. In our study, both healthy and diseased populations were included, while the National survey and the study in Pakistan enrolled apparently healthy individuals.

Furthermore, the overall anti-HBs observed in our research project is much higher than the prevalence of HBsAg among apparently healthy individuals recorded in the National survey (12.2%) and in the current study area (12.1%). [20,21] These results buttress the recommendation of screening anti-HBs in an individual who tested negative for HBs Ag.

The sensitivity (95.7 %) of the Egens anti-HBs rapid test cassette documented in the current research is higher compared to a number of anti-HBs rapid test kits, in France, 58.3% (Quick Profile rapid kit), [22] Pakistan, 70.8% (Humasis rapid test kit), [19] Brazil, 50.37% (Wama, Minas Gerais, Brazil), [23] Egypt, 64.2% (Intec Rapid test China), [24] and in Taiwan, 91.8% (General Biologicals Corporation, Hsinchu, Taiwan). [25]

However, the specificity (97.4%) of the rapid test obtained in our research is comparable to the value obtained in Pakistan (94.6%),[19] and in France 97.4 %. [22] This finding is not unexpected because the sensitivity and specificity are vice versa. High value of specificity observed in our research means that the rapid test cassette can largely discriminate those individuals who have no anti-HBs, and this is relevant in clinical practice.

The current study showed that the status, sex, and age group of the individuals influence the true positive results of the rapid test cassette, as the prevalence of anti-HBs rapid was statistically and significantly higher among controls compared to people living with HIV, *Mycobacterium tuberculosis*, and HBV infection, and also statistically and

significantly higher among males' sex, and age group 21-36 years than the other age groups. This finding together with its high specificity indicates that Egens anti-HBs rapid test cassette could be useful and reliable in the screening processes of HBV infection, as the National guidelines for the prevention, care and treatment of Viral Hepatitis B & C recommends the analysis of anti-HBs in an individual who tested negative for HBsAg.[7] This approach is critical to ensuring the control of HBV infection, as individuals with negative results to both HBsAg and anti-HBs are candidates for HBV infection vaccination.[7] Moreover, screening of anti-HBs among certain groups at risk of loss of this protective antibody is also valuable as it ensures proper management of HBV infection occurring with other comorbidities.

CONCLUSION

In conclusion, excellent screening accuracy by Egens anti-HBs rapid test cassette was observed. Narrowed confidence limits for sensitivity, specificity, PPV, NPV, and overall diagnostic accuracy documented, and almost perfect agreement not by chance observed between the ELISA method and Egens anti-HBs rapid test cassette indicate that this rapid test cassette has high precision and will be reliable in the clinical practice. Status, sex, and age group of study participants influence true positive results of rapid test cassette. We recommend further researches to build on our findings.

Declaration by Authors

Ethical Approval: Approved.
SKHREC/028/2026, State Health Research Ethics Committee. SHS/SU8JB/133/VOL.I, Hospital Ethics and Research Committee. UDUTH/HREC/2025/1502/2, Usmanu Danfodiyo University Teaching Hospital, Health Research Ethics Committee.

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