Diagnostic Performance of Rapid Screening and ELISA Methods in the Detection of Hepatitis B Virus Surface Antigen among Patients Attending Tertiary Healthcare Facilities in Nasarawa State, Nigeria

**ABSTRACT**

**Background**

Hepatitis B Virus (HBV) infection is a global public health problem. It is estimated that 820,000 deaths occur every year and 3.6% of the global population including over 6 million children under the age of 5 is affected by chronic HBV infection and approximately 2 billion people suffer from HBV infection and around 280 million are carriers of HBsAg with virus harboured in their liver. In Nasarawa State Nigeria, a prevalence of 13.63% has been reported. The primary marker for diagnosis of HBV infection is hepatitis B surface antigen (HBsAg). Rapid Diagnostic Test (RDT) kits and Enzyme-Linked Immunosorbent Assay (ELISA) are the most frequently used for the detection of HBsAg.

**Aim**: The present study compared the diagnostic performance of RDT and ELISA methods in detecting Hepatitis B virus surface antigen among patients in the study area.

**Study Design**: The study was a cross-sectional study.

**Place and Duration of Study:** The study was carried out at Federal Medical Centre Keffi and Federal University Teaching Hospital Lafia in Nasarawa State, Nigeria from June 2024 to December 2024.

**Methodology:** A total of 200 samples were collected and screened for HBsAg using both DIALAB DIAQUICK HBsAg Dipstick rapid screening kits (DIALAB Austria) and DIALAB HBsAg ELISA test kit (DIALAB Austria) according to the manufacturer’s instructions. Data were analysed using the Statistical Package for Social Sciences (SPSS) Version 21.0. The differences were considered significant only when *P* ≤ 0.05.

**Results:** Out of the 200 samples screened for HBsAg using RDT and ELISA techniques, 19(9.5%) were positive while 181(90.5%) were negative using the RDT method. For the ELISA method, 23(11.5%) were positive while 177 (88.5%) were negative.

Using ELISA as a standard for the RDT method, the following were obtained: sensitivity 82.61%, specificity 100%, positive predictive value 100%, negative predictive value 97.79% and the kappa level of agreement between the two methods was 0.89 respectively.

**Conclusion**: The study highlights the high endemicity of HBV infection in Nasarawa State, Nigeria. Also, in comparing both methods, the ELISA method was found to be more sensitive than the RDT kit device. We recommend that the RDT screening kits be combined with the ELISA method to ensure timely and accurate detection of HBsAg among HBV-infected patients.

**Keywords:** Hepatitis B Virus; Hepatitis B Surface Antigen; Rapid Diagnostic Test; Enzyme-Linked Immunosorbent Assay.

**1.0 Introduction**

“Hepatitis is an inflammation of the liver and is categorised as viral and non-viral hepatitis. Viral hepatitis is the most common cause of hepatitis in the world” (WHO, 2019). “Hepatitis B is an infectious disease of global significance, with a significant health burden in Africa. The condition can be self-limiting or can progress to fibrosis, cirrhosis or liver cancer associated with the infection” (Ye *et al*., 2021; Onubi *et al*., 2023). Hepatitis B virus (HBV) is a *Hepadnavirus* that infects liver cells. Hepatitis B contributes to an estimated 820,000 deaths every year and an estimated 3.6% of the global population including over 6 million children under the age of 5 is affected by chronic HBV infection and approximately 2 billion people suffer from HBV infection and around 280 million are carriers of HBsAg with virus harboured in their liver (Ajuwon *et al*., 2021; Busayo *et al*., 2021; WHO, 2024).

“In Nigeria an overall prevalence of 13.6% has been reported and is considered a high prevalence country. Though the estimates of HBV cases are inconsistent, additional clarity is required to manage HBV-associated public health challenges” (Busayo *et. al*., 2021; Akabuike *et al*., 2024). Hepatitis B virus infection is among the common infectious diseases with global public health importance with Nigeria among the identified hyper-endemic countries for hepatitis B virus infection (Onubi *et al.*, 2023). In Nasarawa State, Nigeria, the prevalence of HBsAg ranging from 7.8% to 13.63% has been previously reported (‌Innocent *et al*., 2022; Ndubuisi *et al*., 2022; Egbe *et al*., 2023; Abel *et al*., 2024). Di Filippo & Navas. (2023) “recognized three epidemiological patterns of HBV infection on the basis of prevalence rate; high (> 8%), intermediate (approximately 2-8%) and low (< 2%) endemicity regions. For HBV infection diagnosis, patients are screened both on serological and molecular levels” (Nagpal *et al*., 2021).

“Since 1990, rapid screening kits have been the most commonly neither used method of diagnosing HBV infection due to their low cost, ease of handling, robust working, and not requiring any complex instrumentation or trained personnel” (Nagpal *et al*., 2021). “The kits are extensively used for emergency, field survey diagnosis, laboratory, and home testing” (Hayder *et al*., 2012; Xiao *et al*., 2020). “Rapid test employs monoclonal or polyclonal antibody immobilized lateral flow immunoassay (LFIA) for the determination of HBsAg in blood, serum, and plasma samples” (Hayder *et al*., 2012). “The use of RDT for Point of Care (POC) testing was found to be 73.7% sensitive and 97.8% specific for HBsAg. Although the POC testing strips demonstrated sufficiently high sensitivity for varied genotypes, frequent false negatives were observed. Despite the several economic advantages, RDT kits exhibit less sensitivity and specificity as compared to NAT resulting in false positive and false negative results” (Nagpal *et al*., 2021). “Another frequently used technique for the diagnosis of HBV infection is ELISA, which is a sandwich type of enzymatic immunoassay employing monoclonal antibodies. ELISA is highly sensitive and specific due to enzymatic amplification of the signal and monoclonal antibodies recognized by WHO for most parts of varied HBV strains, respectively” (Kim, 2017; Nagpal *et al*., 2021).

The present work compares the diagnostic performance of rapid screening and Elisa methods in the detection of Hepatitis B Virus Surface Antigen (HBsAg) in blood samples among patients attending tertiary healthcare facilities in Nasarawa State, Nigeria, in terms of analytical sensitivity, specificity, negative predicted value, positive predicted value and the kappa level of agreement.

**2.0 Materials and Methods**

**2.1 Study Design:** The study was a cross-sectional study which included the comparison of RDT screening and ELISA techniques for the diagnosis of Hepatitis B Surface Antigen (HBsAg) in the blood of patients.

**2.2 Study Area and Population:** The study areas for this research work were the Federal Medical Centre (FMC) Keffi and Federal University Teaching Hospital (FUTH) Lafia, Nasarawa State, Nigeria. Nasarawa State is located in the North Central geopolitical zone also referred to as the Middle Belt region of Nigeria. It is located between latitude 7° 45′ and 9° 25’ N of the equator and between longitude 7° and 9° 37′ E of the Greenwich meridian. It has boundaries with Kaduna state in the North, Plateau State in the East, Taraba and Benue states in the South, Kogi State and the Federal Capital Territory to the West. The 2006 population Census estimated a population of about 1,826,883 and a total land area of 26,875.59 square kilometres, with a density of about 67 persons per square kilometre comprising 13 Local Government Areas (Figure 1) (About Nasarawa State, 2025).



Figure 1: Map of Nasarawa State showing the study areas highlighted (Adebambo *et al.*, 2023).

**2.3 Study Participants:** The study participants were patients attending the various selected health facilities, who consented to participate in the study by signing the consent form (after adequate information concerning the study).

**2.4 Inclusion Criteria:** Participants who were above 5 years and had not been immunized in the last month with HBV Vaccine

**2.5 Exclusion Criteria:** Participants who were below 5 years old and those who recently took HBV vaccines were excluded from the study.

**2.6 Sample Size:** The sample size for this study was determined using the formula by Busayo *et al*. (2021).

N= Z2pq/d2

Where N= Minimum sample size

Z= Standard normal distribution at 95% confidence interval = 1.96

P= prevalence of HBV in previous studies = 13.6% =0.136

q= (1-p) = 0.864

d= precision or margin of error= 5% = 0.05

Substituting the values in the formula;

N= Z2pq/d2 will mean

N = 1.962 × 0.125 × (1-0.136) / 0.052

N = 3.8416 × 0.136 × 0.864 / 0.0025

N = 0.4514 / 0.0025

N = 180. 56

However, 10% attrition of 180.56 = 18.10

Therefore N = 180.56 + 18.10 = 198.66

It was rounded up to 200 to increase the chances of detection

N = 200

**2.7 Sensitivity, Specificity, PPV and NPV:** The ability of the screening test to give a positive finding when the person tested has the disease is known as sensitivity. It is expressed as a percentage.

Sensitivity = Persons with the disease detected by the screening test ×100

 Total number of people tested with the disease

The ability of the screening test to give a negative finding when the person tested is free of the disease is known as specificity. It is also expressed as a percentage.

Specificity = Total number without disease detected negative by the screening test x 100

 Total number of people tested with the disease

The percentage of true positives among total positives is the PPV while the NPV is the percentage of true negatives among the total negatives (Prabha *et al*., 2022).

**2.8 Data Analysis:** The Chi-square (X2) test was performed for all the data to check for a relationship in detecting HBV Infection using the statistical package for Social Sciences (SPSS) Version 21.0. The differences were considered significant only when *P* ≤ 0.05.

**2.9 Specimen Collection:** A total of 200 blood specimens were collected between June and December 2024 using the venepuncture method described by Cheesbrough (2006). The arm of the individual was tied with a tourniquet and the position of a vein was disinfected with cotton wool soaked in 70% alcohol before inserting a needle and drawing two millilitres (2ml) of blood specimen from each consenting participant into labelled sterile vacutainer. All specimens collected were transported to the laboratory in an ice box for analysis.

**2.10** **Test Procedure:** Testing was done for the presence of HBsAg using the “DIALAB DIAQUICK” HBsAg Dipstick rapid screening kits following the manufacturer’s instruction. Both HBsAg positive and negative samples were properly labelled and marked. Re-testing of the samples was done using the “DIALAB” HBsAg Sensitive ELISA following the manufacturer’s instruction. Furthermore, sensitivity, specificity, negative predicted value, positive predicted value and the kappa level of agreement were calculated for both the employed methods.

**3.0 Results**

**3.1 Comparison of HBV Serological Results between RDT and ELISA Methods**

Out of the 200 samples screened for HBsAg, 19(9.5%) tested positive while 181(90.5%) were negative using the RDT method, while the ELISA method, 23(11.5%) tested positive while 177(88.5%) tested negative though there was no statistical significance (*P*> .05) (Table1).

**3.2True positivity and False negativity Results for HBsAg**

True positivity and false negativity were also determined where the 19 samples positive using RDT were also positive using ELISA giving a true positive value. Furthermore, 4 out of the 181 negative samples for RDT were positive using ELISA giving a false negative value (Table 2).

**3.3 Sensitivity, Specificity, NPV, PPV and KLA**

Using ELISA as a standard for the RDT method, the RDT kit gave the following values: sensitivity 82.61%, specificity 100%, positive predictive value 100%, and negative predictive value 97.79%. The kappa level of agreement between the two methods was 0.89 respectively (Table 3).

Table 1: Comparison of HBVSerological Results between RDT and ELISA Methods among Patients Attending Selected Tertiary Healthcare Facilities in Nasarawa State

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Methods | Total samples examined | No. Positive/percentage | No. Negative/percentage | *P*-Value |
| RDT | 200 | 19 (9.50%) | 181 (90.50%) | 0.625 |
| ELISA | 200 | 23 (11.50%) | 177 (88.50%) |  |

*P > 0.05* Shows no statistical difference.

Table 2: True positivity and False negativity Results for HBsAg using RDT and ELISA Methods

|  |  |  |  |
| --- | --- | --- | --- |
| HBsAg Test Results | Positive | Negative | Total |
| Positive | 19A | 0.00B | 19 |
| Negative | 4C | 177D | 181 |
| Total  | 23 | 177 | 200 |

KEY: A = true positive C = false negative B = false positive D = true negative

Table 3: Sensitivity, Specificity, Negative Predicted Value (NPV), Positive Predicted Value (PPV) and Kappa Level of Agreement (KLA) of RDT in Comparison with ELISA Technique

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Variable | Sensitivity | Specificity | PositivePredictive Value | NegativePredictive Value | Kappa Level of Agreement |
| Values | 82.61% | 100% | 100% | 97.79% | 0.89 |

**Discussion: “**Hepatitis B virus (HBV) remains a common cause of viral hepatitis, with possible long-term complications of fibrosis, cirrhosis and hepatocellular carcinoma in patients with chronic infection. Immunoassays are the most convenient and reliable detection platforms and their improvement for the detection of viral antigens is an active research area” (WHO, 2019; Nguyen *et al*., 2020). The present work compared the diagnostic performance of Rapid screening and ELISA methods in the detection of Hepatitis B Virus Surface Antigen (HBsAg) regarding the analytical sensitivity, specificity, negative predicted value, positive predicted value and the kappa level of agreement for the detection of HBsAg in blood samples among patients attending selected tertiary healthcare facilities in Nasarawa State, Nigeria using the DIALAB RDT kit and the DIALAB ELISA test kit respectively.

Out of the 200 samples tested, an overall prevalence of 9.5% was observed with the RDT method and 11.5% with the ELISA method respectively. It was observed in the present study that 4(2%) of the total samples which tested negative with the RDT kit were positive for HBsAg when tested with the ELISA technique indicating that ELISA was more sensitive.This is in agreement with the report of Fasola *et al*. (2022) who reported a prevalence of 5.5% by RDT while a prevalence of 13.1% was reported for ELISA among blood donors in Nigeria. Adeleke *et al*. (2021) also reported a prevalence of 5.7% for RDT and 14.6% for ELISA in Nigeria. Furthermore, an overall prevalence of 4.8 % using the RDT kit was reported by Famoni *et al*. (2024). Adding that while the ELISA test was more sensitive, the RDT was more specific. On the contrary, a higher prevalence of 19.9 % using RDT and 22.4% using ELISA was reported by Uche *et al*. (2022) in Lagos Nigeria. Interestingly, Ezeonu *et al*. (2019) reported a prevalence of 8.9% for HBsAg with the RDT while it was 2.8% with ELISA among blood donors in the Federal Capital Territory, Abuja, Nigeria. The reason for the difference in prevalence between HBsAg RDT and the ELISA test could be that the rapid cards used for HBsAg detection which are immunochromatographic based might not have the same accuracy in all regions. This is because differences exist in the prevalence of HBV infection in a given population (Sharma *et al*., 2013; Prabha *et al*., 2022). In addition, the rapid test strips make use of recombinant proteins from the prototype virus only, especially for HBV. Currently, eight types of HBV genotypes have been reported in different parts of the world. Furthermore, the subtypes and genotypes of HBV in circulation show different distribution patterns regarding geography and epidemiology (Gerlich, 2013). Because the RDT does not cover all subtypes it does not detect it when tested. As a result, samples that tested negative using the RDT may turn out to be positive using ELISA. It could also be a result of insufficient coating of the HBsAg-specific antibodies; or the nature of the antibody (Kwenti *et al*., 2017; Nagpal *et al*., 2021).

Regarding the analytical sensitivity, specificity, negative predicted value (NPV), positive predicted value (PPV) and the kappa level of agreement for the detection of HBsAg using ELISA as a standard, the following values were recorded; Sensitivity of 82.61%, Specificity 100%, PPV of 100%, NPV of 97.79% and the Cohen’s Kappa agreement was 0.89. This means that the ability of the RDT to correctly identify true positives (patients with HBV) or true negatives (patients without HBV) was 82.61 and 100 respectively. Similarly, the results indicate the likelihood that positive or negative tests from the RDT method accurately reflect the actual presence or absence of HBV among patients is 100 and 97.79% respectively. This is in agreement with the findings of Imtiaz *et al*. (2017) who recorded 97.10%, 99.76 %, 98.52% and 99.54% for sensitivity, specificity, PPV and NPV of Hepacard one-step rapid test respectively; while the sensitivity, specificity, PPV and NPV of ELISA were 97.87%, 99.75%, 98.92% and 99.51% respectively. Their study noted that in comparing both ELISA and RDT methods for assessing the presence of HBsAg, the ELISA test method was found to be more sensitive than the rapid test strip device.

In a similar study carried out by Fowotade *et al*. (2021) among pregnant women visiting the University College Hospital, Ibadan, Nigeria, the sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of the rapid test kit were 72.2%, 97.4%, 94.8%, 76.5% and 96.8% respectively. Similarly, Adeleke *et al*. (2021) reported that using ELISA as a reference, the sensitivity and specificity of RDT were 31.4% and 98.7% respectively, while the positive predictive value and negative predictive value for RDT were 80.0% and 89.4% respectively. Lemma *et al*. (2021) reported a sensitivity of 70.7% and specificity of 95% with its kappa value of 0.69 from Gondar, North West Ethiopia indicating a good agreement with ELISA. In a study carried out by Eko Mba *et al*. (2019) in Libreville, Gabon, the sensitivity of HBsAg compared to ELISA tests for the detection of HBsAg was 78.0% while the specificity of RDT for the detection of HBsAg was 100%.Also, Prabha *et al.* (2022) in India, reported the sensitivity of the RDT was 83.4%, specificity of 100%, Positive Predictive Value (PPV) of 100% and Negative Predictive Value (NPV) of 99.4% and ELISA the sensitivity, specificity, PPV and NPV were all 100%. Chaurasia & Shrivastava. (2020) on the other hand reported asensitivity and specificity of rapid RDT of 96.8% and 99.7% as compared to ELISA also in India.Al-Matary & Al Gashaa. (2022) in their study carried out in Yemen observed that the rate of sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV), in addition to determining the diagnostic accuracy rate and error rate for all rapid diagnostic kits in detecting HBV compared to the ELISA technique, are less accurate and associated with more false negatives. Several reports have attributed the differences in specificity, sensitivity, PPV and NPV for HBsAg using RDT and ELISA to reasons such as low levels of HBsAg below detection limits especially in chronic HBV carriers. It has also been reported that false-negative results of HBsAg RDT tests are associated with the presence of HBsAg mutants, low viral load, and certain viral genotypes (Jargalsaikhan *et al*., 2020; Magnus*et al*., 2021; Al-Matary & Al Gashaa, 2022).

**Conclusion:** Although the HBsAg seropositivity was high among the study population, the efficacy of rapid test kits compared to ELISA was low. Validating the point-of-care tests with a standard ELISA or other specific tests whenever feasible is highly recommended to curb the transmission of the virus as RDTs are the commonly used screening methods, especially in remote and resource-limited areas.

**Consent**

Written consent was obtained from all subjects after explaining in detail the entire research protocol.

**Ethical Approval**

Institutional ethical approval was obtained from the Health Research Ethics Committee of the Federal Medical Centre Keffi, Nasarawa State (FMC/KF/HREC/02644/24).

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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