Echonwere – Uwikor and Uwikor, (2024) *BJMLS, 9(2): 165 – 171* ISSN 2545 – 5672;
eISSN 2635 - 3792

COMPARATIVE EVALUATION OF RAPID DIAGNOSTIC TEST DEVICES AND REAL-TIME PCR FOR DETECTION OF HBsAg IN RIVERS STATE

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Received: 14th November, 2024 **Accepted**: 25th December, 2024 **Published**: 31st December, 2024

ABSTRACT

Background: Hepatitis B Virus (HBV) infection is a significant public health concern worldwide, especially in rejoins with highly endemicity such as sub-Saharan Africa. The HBV infection can lead to severe complications, including liver cirrhosis, hepatocellular carcinoma, and chronic liver disease, all of which contribute to considerable morbidity and mortality globally. Hepatitis B virus is 50-100 times more infectious than HIV and 10 times more infectious than Hepatitis C.

Aim: The aim of this study was to compare the sensitivity and specificity of rapid screening method with PCR method for detection of HBV among out-patients with clinical symptoms visiting Rivers State University Teaching Hospital, Port Harcourt.

Methodology: This was a cross-sectional study carried out on 130 subjects who visited RSUTH. Four different Rapid Diagnostic Test (RDT) kits for screening of HBV were used for this study. Positive RDT samples were further analyzed using PCR (Real-Time) for confirmation, quantification and comparison of HBV.

Result: The result obtained from the study showed that 71 samples were positive by PCR, while 59 samples were negative. Samples that reacted using Rapid HBV were 120, 117, 100, and 124 for CTK, Labacon, Rostec and Tell respectively. For non-reactivity; 10, 13, 30, and 6 samples were non-reactive for CTK, Labacon. Rostec, and Tell respectively. Comparing their sensitivity, it was observed that CTK, Labacon, Rostec, and Tell test kits had sensitivity of 100% each, and specificity of 16.9, 22, 38.9. and 10.1. The rapid test kits had an accuracy of 62.3, 64.6, 72.3 and 59.2% respectively.

Conclusion: This study posits that there should be a compulsory validation of RDT test kits for HBsAg detection by real-time PCR before being used in resource-limited settings. RDT kits can be ideal alternatives for diagnosis. However, a major concern in using these kits is their variable degrees of sensitivity and specificity.

Keywords: Rapid Diagnostic Tests Kits, Real-Time PCR, HBV

INTRODUCTION

Hepatitis B virus (HBV) infection is a major public health concern globally, particularly in sub- Saharan Africa, where it is highly endemic. The World Health Organization (WHO) estimates that approximately 296 million people were living with chronic HBV infection in 2019, with over 820,000 annual deaths resulting from complications such as liver cirrhosis and hepatocellular carcinoma (WHO, 2022). Hepatitis B surface antigen (HBsAg) is a key marker for diagnosing HBV infection, as it indicates both acute and chronic infection phases.

Citation: Echonwere-Uwikor, B.E., Uwikor, F.K (2024): Comparative Evaluation Of Rapid Diagnostic Test Devices And Real-Time PCR For Detection Of Hbsag In Rivers State *BJMLS 9(2): 165 - 171*

Therefore, early and accurate detection of HBsAg is crucial for timely clinical management, treatment, and efforts to prevent further transmission (WHO, 2022).

In Nigeria, HBV is considered hyperendemic, with some regions reporting prevalence rates of over 8% (Ajuwon *et al*., 2021). Due to the infectious and asymptomatic nature of HBV infection, poor health care facilities and inadequate monitoring of HBV infection, controlling the spread of HBV infection remain a major challenge (Ajuwon *et al*., 2021). Rivers State, located in the Niger Delta region, faces a significant burden of HBV infection due to several factors including unsafe healthcare practices, low vaccination coverage, and limited access to high-quality diagnostic facilities. In this context, it is essential to have accessible, reliable, and cost-effective diagnostic tools for screening and detecting HBV, particularly in under-resourced settings (Ajuwon *et al*., 2021).

Traditionally, the detection of HBsAg has relied on laboratory-based techniques such as enzyme-linked immunosorbent assay (ELISA) and real-time polymerase chain reaction (PCR), which are considered gold standards due to their high sensitivity and specificity (Zhang *et al*., 2022). Real-time PCR not only detects HBsAg but can also quantify HBV DNA, providing critical information on viral load, which is essential for assessing disease progression and monitoring the efficacy of antiviral therapy (Kim *et al*., 2020). The quantification of HBV DNA is particularly useful in clinical settings, as it helps identify patients who are at higher risk of developing liver complications, allowing for timely intervention. Moreover, real-time PCR is highly sensitive and specific, making it the gold standard for HBV diagnosis and monitoring (Zhang *et al*., 2022). However, despite its accuracy, the use of PCR is often limited in resource-constrained settings due to its high cost, the requirement for specialized laboratory equipment, and the need for highly trained personnel (Huang *et al*., 2021).

In contrast, rapid diagnostic tests (RDTs) for HBsAg detection are designed to provide a simpler, quicker, and more accessible alternative, especially in low-resource settings like Rivers State. Rapid Diagnostic Tests are point-of-care tests that can be performed by healthcare workers with minimal training, and they offer results within 10 to 30 minutes (Nassal, 2021). These tests are particularly valuable in rural areas where access to laboratory facilities is limited, and immediate diagnosis is crucial for making clinical decisions, such as screening blood donors or identifying HBV infection in pregnant women (Zhang *et al*., 2022).

However, the accuracy of RDTs has been called into question, particularly when compared to PCR. Studies have demonstrated variability in the sensitivity and specificity of RDTs, with some tests performing well in high-prevalence settings but failing to detect low levels of HBsAg in patients with chronic HBV infection (Abdulkareem *et al*., 2020). This is a critical issue, as undetected chronic carriers of HBV can continue to transmit the virus unknowingly, posing a significant public health risk. Additionally, false-positive results from RDTs may lead to unnecessary anxiety and treatment, further complicating their use in clinical settings (He *et al*., 2021).

Given the high burden of HBV in Rivers State and the reliance on both RDTs and PCR for diagnostic purposes, it is essential to evaluate the comparative performance of these two diagnostic methods in this specific context. Previous studies have shown that the diagnostic accuracy of RDTs can vary significantly depending on the test brand, the population being tested, and the prevalence of HBV in the region (Zhang *et al*., 2022).

Therefore, a comprehensive assessment of the sensitivity, specificity, and overall diagnostic reliability of commonly used RDT devices in Rivers State is necessary to ensure that they are suitable for use in this high-prevalence setting. This study aimed to address this gap by conducting a comparative evaluation of rapid diagnostic test devices and real-time PCR for the detection of HBsAg in patients visiting Rivers State University Teaching Hospital. By comparing the performance of these two diagnostic methods, this research sought to determine whether RDTs can serve as reliable alternatives to PCR in low-resource settings and provide evidence-based recommendations for improving HBV screening and management strategies in Rivers State. The findings of this study will contribute to the optimization of diagnostic tools for HBV in Nigeria and inform public health policies aimed at reducing the transmission and impact of HBV in high-burden regions.

MATERIALS AND METHODS

Study Design

A cross-sectional study design was used for this study. A total of 130 samples were serologically tested using 4 different Rapid Diagnostic Test (RDT) kits for screening of Hepatitis B virus (HBV). Positive RDT samples were further analyzed using PCR (Real-Time PCR) for confirmation, quantification of HBV and comparison of both methods.

Study Area

This study was conducted in Rivers State University Teaching Hospital (RSUTH) in Port Harcourt, the capital city of Rivers State, located on latitude: 4°4Y34.64'N and longitude: 7°2 1 '54.68" E.

Study Population

The study recruited 130 potential blood donors who visited (RSUTH) within the study period.

Eligibility of Subject

Inclusion Criteria

i. Individuals who visited the Rivers State University Teaching Hospital outpatient department, especially those undergoing screening for HBV infection, blood transfusion, or prenatal care.
ii. Individuals with known or suspected history of HBV infection or those recommended for HBV screening.
iii. Individuals who gave informed consent.

Exclusion Criteria

i. Potential donors who did not consent to the study.
ii. Individuals already on antiviral treatment for HBV
iii. Pregnant women with comorbidities

Ethical Approval

Ethical approval for this study was obtained from the Rivers State University Teaching Hospital Health Research Ethics Committee Port Harcourt, a written informed consent was obtained from each participant prior to sample collection, and this was after reading, understanding and signing the written informed consent form.

Sample Analysis

After pre-test counseling and explanations, venous blood was drawn from the antecubital fossa of the subjects with the use of vacutainer as described by Mukai *et al*. 2020. Five (5ml) millilitres of blood sample was collected in a plain bottle and spun at 1000rpm for 5 minutes and the serum separated into 2 different bottles (cryostat bottle and a plane bottle). The serum in plane bottles was used for the screening of HBV using four different RDT kit (Tell, lab Acon, Rostec and CTK). Positive and discrepant samples were confirmed and quantification analysis was done using Real-time PCR (COBAS Ampliprep/TaqMan version 2.0).

Procedure for PCR;
The COBAS Ampliprep/TaqMan version 2.0 PCR machine was logged on and the daily maintenance performed. Reagents were removed from storage and loaded immediately. The stored samples were also removed from storage and allowed to thaw at room temperature before pipetting. Consumables were loaded, order for viral load created, worksheet prepared and barcode clips attached to the sample rack and labeled. Control and test samples were pipetted into the respective tubes (8501.tl). The instrument status was checked and 'start button pressed. Prepared samples were removed from COBAS^{R} Ampliprep to COBAS^{R} Taqman Analyzer automatically because it is docked. Finally results were reviewed and printed (Roche Molecular system, Inc. 2019).

STATISTICAL ANALYSIS

Data analysis was done using Microsoft Excel and Statistical Package for Social Sciences (SPSS) version 21 to determine the percentage frequency of HBV CM square, Mann Whitney U test, Wilcoson was used. P-value <0.05 was considered to be significant. Data were represented in Tables.

RESULTS

Table 1: Evaluation of Rapid HBV Kits with PCR

HBV Kit		PCR (Gold Standard)		Total
		Reactive (n = 71)	Non-Reactive (n = 59)	(n = 130)
CTK	Reactive	71	49	120
	Non-Reactive	0	10	10
LABACON	Reactive	71	46	117
	Non-Reactive	0	13	13
ROSTEC	Reactive	71	29	100
	Non-Reactive	0	30	30
TELL	Reactive	71	53	124
	Non-Reactive	0	6	6

PCR-Polymerase chain reaction, n-number of tests

Table 2: Positive Predictive Values and Negative Predictive Value of the Respective RDT Kits

HBV Kit	Results for Screening Test Kits					
	TP	TN	FP	FN	PPV (%)	NPV (%)
CTK	71	10	49	0	59.1	100
LABACON	71	13	46	0	60.6	100
ROSTEC	71	23	36	0	66.3	100
TELL	71	6	53	0	57.2	100

TP-True Positive, TN-True Negative, FP-False Positive, FN-False Negative, PPV-Positive Predictive Value, NPV-Negative Predictive Value

Table 3: Sensitivity, Specificity and Accuracy of the Various Respective RDT Kits

HBV Kit	Results for Screening Test Kits						
	TP	TN	FP	FN	Sensitivity (%)	Specificity (%)	Accuracy (%)
CTK	71	10	49	0	100	16.9	62.3
LABACON	71	13	46	0	100	22	64.6
ROSTEC	71	23	36	0	100	38.9	72.3
TELL	71	6	53	0	100	10.1	59.2

TP-True Positive, TN-True Negative, FP-False Positive, FN-False Negative

DISCUSSION

The findings of this study provide important insights into the diagnostic accuracy of rapid diagnostic tests (RDTs) compared to the gold-standard real-time PCR for detecting HBsAg in a high-prevalence region like Rivers State. While all RDTs tested (CTK, Labacon, Rostec, and Tell) demonstrated 100% sensitivity, their specificity varied, indicating disparities in their diagnostic accuracy.

In comparing these results to similar research, there is a clear alignment with certain studies, while others show notable differences. Konerman *et al*. (2020) observed significant variability in the specificity of different RDTs for HBsAg detection. They found that certain RDTs, such as those from Human and Cypress Diagnostics, exhibited lower specificity due to false negatives, while OraSure showed superior performance with higher specificity. This observation aligns with our findings, where although all RDTs had 100% sensitivity, the CTK, Labacon, Rostec, and Tell kits showed varying specificity. Notably, Rostec and Tell exhibited the lowest specificity, which could be attributed to factors such as antigenic mutations or the inability of these kits to detect low viral loads, as suggested by Konerman *et al*. (2020).

Further, Mongkolrattanothai *et al*. (2020), in a study conducted in a resource-limited setting, also reported significant disparities in the accuracy of various RDTs. They found OraSure to have high sensitivity and specificity, while other RDTs demonstrated lower accuracy, primarily due to false-negative and false-positive results. Our findings are consistent with this, even though OraSure was not tested in our study. However, the performance of Rostec and Tell, which showed lower specificity, mirrors the lower accuracy seen in other RDTs in Mongkolrattanothai's study. Meanwhile, CTK and Labacon exhibited relatively better specificity but still did not reach the high levels of performance observed with OraSure in their research.

In contrast, Amini *et al*. (2021) suggested that the lower specificity of certain RDTs could be due to immune-escape mutants, which might alter surface antigens and evade detection. This theory is consistent with the findings of the present study, where Rostec and Tell had lower specificity, possibly due to the presence of these immune-escape mutants. The CTK and Labacon kits, however, showed relatively better specificity, which could be attributed to their ability to detect a broader range of viral strains. However, it is worth noting that Amini *et al*. did not focus on specific RDT brands but discussed general issues related to antigen mutation and detection. This makes the present study's evaluation of specific RDTs more focused and relevant.

Additionally, Dembele *et al*. (2020) highlighted that some RDT kits might not account for all mutated antigens, leading to false negatives. This explanation is relevant to our study, particularly for Rostec and Tell, which had lower specificity.

These kits may fail to detect all HBsAg variants, contributing to the reduced specificity observed. On the other hand, CTK and Labacon demonstrated better specificity, suggesting they are more capable of detecting different antigenic variants, though not to the level seen with nucleic acid-based tests.

The findings of this study underscore that while all the RDTs tested (CTK, Labacon, Rostec, and Tell) had 100% sensitivity, their specificity varied. Specifically, Rostec and Tell showed the lowest specificity, while CTK and Labacon performed relatively better. These results are in agreement with Konerman *et al*. (2020) and Mongkolrattanothai *et al*. (2020), who found significant variability in RDT performance, particularly in terms of specificity. The lower specificity observed in our study can likely be attributed to factors such as the presence of HBsAg-immune-escape mutants and the limitations of certain RDT kits in detecting all viral strains, as noted by Amini *et al*. (2021) and Dembele *et al*. (2020).

This study emphasizes the need for the use of nucleic acid-based diagnostic tools that offer higher specificity and reliability, especially in high-prevalence regions like Rivers State. The findings also highlight the importance of evaluating RDTs in specific settings to ensure their optimal use, particularly when considering the trade-offs between sensitivity and specificity.

CONCLUSION

This study provides valuable insights into the performance of rapid diagnostic tests (RDTs) for detecting HBsAg in a high-prevalence area like Rivers State, comparing their diagnostic accuracy to the gold-standard real-time PCR method. While the RDTs evaluated in this study (CTK, Labacon, Rostec, and Tell) demonstrated 100% sensitivity, their specificity varied significantly, with Rostec and Tell showing the lowest specificity. The results indicate that while RDTs are highly sensitive, their reduced specificity could lead to false positives or negatives, which might affect their reliability in certain clinical settings.

The findings underscore the need for careful consideration when selecting diagnostic tools for HBV detection, particularly in regions with high viral prevalence. Despite the high sensitivity of RDTs, the lower specificity observed in some kits highlights the potential limitations of these tests, especially when considering variations in antigenic strains or the presence of immune-escape mutants. Future research should focus on improving the specificity of these RDTs, potentially through the development of more advanced immunological reagents or nucleic acid-based rapid tests. Additionally, the use of real-time PCR or other molecular techniques remains crucial for ensuring accurate and reliable diagnosis, especially in resource-limited settings.

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