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Evaluation of performance testing of different rapid diagnostic kits in comparison with EIAs to validate detection of hepatitis B virus among high risk group in Nigeria

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ABSTRACT

Background: Hepatitis B virus (HBV) causes chronic liver-associated diseases and its early detection is of high public health importance. Its diagnosis is mainly based on immunological assays among which Enzyme-Linked Immunosorbent Assay (ELISA) and rapid tests are the most common and widespread methods. However, a major challenge is the discordance of results of any two laboratory assays which cannot be easily resolved. Therefore, this study was designed to evaluate the validity and reliability of commercially available five rapid test kits in comparison with two Enzyme Immunoassays (EIAs) in Nigeria using hepatitis B surface antigen as a reference marker.

Methods: A total of 100 sera of previously diagnosed consenting HBV-positive patients from private diagnostic laboratories in Ibadan between March and August, 2011 were tested using two EIA and five rapid commercially available HBV test kits in Nigeria. Data were analyzed by SPSS version 15, while bivariate and multivariate analyses were carried out to identify associations at $P < 0.05$ considered significant.

Results: Overall, the sensitivity rates of the two EIA kits were 100% and 99.9% (95% confidence interval [CI] = 98.9–99.7) with specificity of 100% and 99.9% (95% CI = 98.9–99.7), respectively. The sensitivity of the five rapid test kits ranged from 97.5% (95% CI = 96.4–97.6) to 98.9% (95% CI = 97.9–99.9) with specificity of 80% (95% CI = 79.3–80.9) to 90% (95% CI = 89.2–91.0). Also, the positive predictive value ranged from 88% (95% CI = 88.2–89.9) to 89% (95% CI = 88.2–89.9), while the negative predictive value ranged from 80% (95% CI = 79.3–80.9) to 90% (95% CI = 89.2–91.0) for the five rapid kits. However, that of the two EIAs ranged from 99.9% (98.9–99.7) to 100%. Further analysis showed significant ($P = 0.033$) variations in the sensitivity and specificity of the EIAs and rapid test kits.

Conclusions: The results from this study have clearly revealed the challenges of diagnosis of HBV infections in Nigeria. This

KEYWORDS
HBV testing; evaluation; specificity/specificity; validity; Nigeria

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study has also demonstrated that the sensitivity of most of the rapid test kits may not be adequate when compared with EIA for early detection of HBV infections. The implications of possible misdiagnosis on the various intervention strategies that rely predominantly on correct HBV status of an individual are enormous. Therefore, there is the need to further compliment the use of rapid test kits with EIAs for HBV control in Nigeria.

Introduction

Hepatitis B virus (HBV) infection is one of the world's major unconquered diseases resulting in common serious complication of blood transfusions and liver-related conditions. It is recognized as an important viral disease in the tropical countries and has become a major global public health problem.[1] HBV is a double-stranded circular genome DNA virus of the family Hepadnaviridae and genus *Orthohepadnavirus*.[2] It is the only hepadnavirus that causes infections in human. It is a resilient virus that can exist on almost any surface for about 1 month.[3] Although several viral markers such as antibody to hepatitis B surface antigen (HBsAg), hepatitis B 'e' antigen (HBeAg), and antibody to HBeAg and antibody to hepatitis B core antigen are available for HBV diagnosis, HBsAg is the major marker used for the detection of HBV.[4] HBV has caused epidemics in parts of Asia, Africa, and also endemic in China.[5]

Medical testing is an important diagnostic procedure employed by health-care provider to proffer solutions to different health problems presented in hospitals.[6] To reduce the spread of various infections in the community, evaluation of screening test kits thus becomes of utmost public health importance.[7] Specifically, the reliability (sensitivity and specificity) of a test kit becomes particularly important when there is a need to test for the presence of transfusion transmissible infections such as hepatitis B among blood donors.[8] Rapid tests are quantitative immune-chromatographic tests for the purpose of massive screening in non-laboratory environment. It could be designed to detect antibody or antigens with a very little turnaround time for the test result to be made available for the patients.

The intention of screening is to ensure early detection of infection in a community and achieve prompt intervention and management to reduce mortality and suffering from the disease. Although screening may lead to early diagnosis, not all screening tests have been shown to benefit the persons being screened. For example, overdiagnosis and misdiagnosis bring about a false sense of security.[9] In Nigeria, HBV has been of a major public health importance.[1] From the earlier studies[10,11] done to recent studies[12,13] carried out in different populations in Nigeria, the prevalence rates have not declined; therefore, to achieve intervention, there is the need for an

effective surveillance for the infection, prompt and accurate diagnosis of infection, and use of appropriate test kits for blood screening to facilitate safe blood transfusion and to achieve prevention of the spread of the virus.[7,14]

The first marker to appear and become detectable during HBV infection is usually HBsAg, and this is evident in the nonspecific prodrome stage and in the early phase of the acute infection but becomes undetectable in convalescence phase.[15] The HBsAg persistence of >6 months signifies chronic hepatitis B (CHB).[16] As a result, HBsAg is used as the marker of infection for both HBV screening and to detect suspected acute cases or CHB in any community.[17] Several test kits have been circulated and used for hepatitis B screening in Nigeria without policy on quality of acceptable test kit.[18] Therefore, the need to evaluate available rapid test kits and adoption of policy on screening the kits becomes necessary. The major challenge for HBsAg rapid tests is to detect the low levels of the target antigen that are present in a relatively high proportion of asymptomatic carriers.[19,20] In order to achieve prevention of the spread of HBV, this study was designed to evaluate some selected rapid test kits commonly used for detection of HBsAg in Nigeria and compare the results with Enzyme Immunoassays (EIAs) for test validity using high risk population.

Methodology

Participants and sample collection

A total of 100 sera of previously diagnosed consenting HBV-positive patients from private diagnostic laboratories in Ibadan were tested using five rapid commercially available HBV test kits in Nigeria, and their results were compared with two EIAs for test validity. The consenting participants were referred to the Department of Hematology, University College Hospital, Ibadan, between March and August 2011. This high-risk population included spouses of HBV-infected persons who volunteered to participate in the study. HBsAg was used as a reference marker of infection for HBV testing. The choice of these patients was borne out of the sociodemographic information obtained from them which included gender, marital status, their age brackets, occupation, and cohabitation with larger society. They also represented different segments of the community with dearth of knowledge about HBV transmission and were of low educational background. The private diagnostic laboratories which referred them to our facility claimed that they were previously tested with both rapid and EIA test kits but did not specify the exact kits used for the tests.

Test kits used for assay procedures

The five commercially available rapid kits included Diaspot™ Rapid Diagnostic kit, Miami, FL, USA; IND *in vitro* diagnosticum, Canada; EXACT Diagnostic devices, NY, USA; ACON, ACON Biotech, China; Global strips reagents, USA, while two commonly used EIA kits were Monolisa® Ag HBs PLUS ELISA (Bio-Rad), France and Diagnostic Automation/Cortez Diagnostic, CA, USA. Each procedure was followed according to manufacturer's protocol including the interpretations of the results. Both the sensitivity and specificity of each assay and those of negative (NPV) and positive predictive values (PPV) were calculated accordingly.

Ethical approval: Ethical approval for the study was obtained from Oyo State Ethical Review Board committee of the Ministry of Health (AD3/479/349).

Results

The performance of each of the five commercially available rapid kits and two EIA kits used for evaluation of HBsAg in this study is shown in Table 1. Overall results show that the specificity and sensitivity of the two EIA test kits were 99.9% and 100% (95% confidence interval [CI] = 98.9–99.7) with specificity of 99.9% and 100% (95% CI = 98.9–99.7) for Monolisa® Ag HBs PLUS ELISA (BIO-RAD) and Diagnostic Automation/Cortez Diagnostic, respectively. However, for the five rapid test kits evaluated, the sensitivity ranged from 97.5% (95% CI = 96.4–97.6) to 98.9% (95% CI = 97.9–99.9) with specificity of 80% (95% CI = 79.3–80.9) to 90% (95% CI = 89.2–91.0) (Table 2). Further analysis of the results also revealed that the PPV ranged from 88% (95% CI = 88.2–89.9) to 89% (95% CI = 88.2–89.9), while NPV ranged from 80% (95% CI = 79.3–80.9) to 90% (95% CI = 89.2–91.0) for the five rapid kits. However, that of the two EIAs ranged from 99.9% (98.9–99.7) to 100%. Further analysis showed significant (P = 0.033) variation in the sensitivity and specificity of the EIA and rapid test kits (Table 2).

Table 1. Comparison of the results of different rapid test kits for hepatitis B surface antigen (HBsAg) detection with EIA test.

Rapid test kits	Number Positive	Number Negative	Monolisa HBsAg ELISA Positive	Monolisa HBsAg ELISA Negative	HBsAg diagnostic automation/Cortez diagnostic Positive	HBsAg diagnostic automation/Cortez diagnostic Negative
Global	88	12	89	11	90	10
Diaspot	89	11	90	10	90	10
IND	89	11	90	10	90	10
ACON	88	12	89	11	90	10
EXACT	89	11	90	10	90	10

Table 2. Comparison of the sensitivities of five selected rapid test kits for hepatitis B surface antigen (HBsAg) detection with EIA test assays.

Name of test kit	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (PPV) (95% CI)	Negative predictive value (NPV) (95% CI)
Global	97.5% (96.4–97.6)	80% (79.3–80.9)	88% (87.1–88.7)	80% (79.3–80.9)
Diaspot	98.9% (97.9–99.9)	90% (89.2–91.0)	89% (88.2–89.9)	90% (89.2–91.0)
IND	98.9% (97.9–99.9)	80% (79.3–80.9)	89% (88.2–89.9)	80% (79.3–80.9)
ACON	98.9% (97.9–99.9)	80% (79.3–80.9)	88% (88.2–89.9)	80% (79.3–80.9)
EXACT	98.9% (97.9–99.9)	80% (79.3–80.9)	89% (88.2–89.9)	80% (79.3–80.9)
Monolisa HBsAg ELISA	99.9% (98.9–99.7)	99.9% (98.9–99.7)	99.9% (98.9–99.7)	99.9% (98.9–99.7)
Diagnostic automation/ Cortez diagnostic,	100%	100%	100%	100%

Discussion

HBV is an important viral disease in the tropical countries and has become a major global public health problem.[1,21] The control of HBV in any country depends largely on the availability of effective and accurate diagnosis using immunological test assays to detect cases of infection with very low pathogens in blood. The overall results of this study show that the performance of most of the assays is less than optimal in the country even under a tertiary health facility laboratory where the study was carried out (Table 1). Overall, only EIA test kits designed to detect HBV could be reliably recommended for screening HBsAg in Nigeria. On the other hand, such assays with very high sensitivity will have lower specificity as observed in this study. False-negative results may lead to wrong decision, thus making the infected person to cohabit with other members of the community who are not HBV positive and infect them, considering the long effect of the asymptomatic nature of HBV infection.[15] This is of greater concern in controlling the spread of the virus in an endemic nation like Nigeria with grave consequences.[18,22]

The detection of HBV infection and its diagnosis are mainly based on immunological assays among which Enzyme-Linked Immunoassay (ELISA) and rapid tests are the most common and widespread diagnostic methods.[18,19] An important problem mainly encountered at this point is the discordance results of two assays,[22,23] which can be resolved depending on the availability of suitable kits. Hence, kits evaluation gains importance for performance. Though ELISA shows a high degree of sensitivity, it is a costly and time-consuming job, so rapid tests become a good alternative for ELISA in blood banks and other testing laboratories in course of time.[1] Moreover, uses of synthetic antigens in some rapid kits have increased the specificity.[18] Performance of rapid test would be satisfactory with a lower degree of false negatives and high predictive value.

This study revealed an efficiency of 97% and 100% among all the five selected rapid test kits (Table 2). It was found that kit efficiency did not vary significantly among the five rapid test kits evaluated. It is worth mentioning in this study that serum samples were used which therefore affected the performance of rapid tests if whole blood samples were used.[14] However, ELISA proved to be more superior to rapid tests in terms of sensitivity as this did not have any effect on the performance.[19] As for the case of diagnosis of infectious disease such as HBV with discordant results, this may have serious consequences among the patients as it causes unnecessary mental stress and tension. For proper diagnosis of infection as well as disease management and prevention, identification of appropriate test kit is necessary.[1,19,24] Specifically, the reliability (sensitivity and specificity) of a test kit becomes particularly important when there is a need to test for the presence of transfusion transmissible infections such as hepatitis B among blood donors.[8]

The five selected rapid diagnostic tests evaluated were not as expensive as those of EIA test kits evaluated in this study, easier to complete at a shortest time, and impose little discomfort to the patients, since a very small specimen quantity is required; this makes it an easy test of choice in the private diagnostic laboratories and blood banks (Figures 1 and 2). The commonly used rapid test kits evaluated have high sensitivity, and the specificity observed was almost the same in comparison with ELISA. Although the specificity and sensitivity of performance of these kits were high when compared with EIAs,[19] the packaging of these kits was

Figure 1. Detection of hepatitis B surface antigen detection using five rapid test assays.

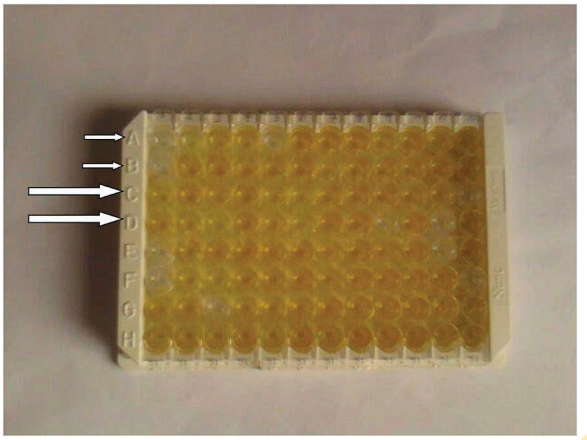

Figure 2. ELISA plate showing results of the test assays. Key: Microwell A1 = NC 1 (Negative control 1)Microwell B2 = NC 2 (Negative control 2)Microwell C1 = LPC (Low positive control) Microwell D1 = HPC (High positive control)

below international standard. Although the results of this study supported the choice of the use of the rapid test kits evaluated, however, it is pertinent to note that some false-negative results were documented. This could be as a result of rough packaging, thereby exposing the reagents to a harsh temperature.[24,25] Poor packaging could interfere with the quality of the test kits and hamper the outcome of the test results.

In this study, the PPV and NPV for HBV testing using EIAs are very high and at optimal when compared with those of the rapid test assays (Table 2). According to kit evaluation for HBV detection in this study, the number of false-positive and false-negative results obtained is really a matter of concern.[19] Again false-negative results have a threat of silent transmission and spreading of diseases among people and also create more interest for sensitive assays like ELISA. Therefore, in a resource-poor setting where ELISA is unavailable, practice of using rapid test kits for blood banks both in private and government owned may lead to spread of deadly infection, especially for kits that detect only IgG and thus miss the IgM which is a critical marker of early infection[7,26] Testing with these kits might lead to false negatives for samples of recent infection. Therefore, tests kits capable of capturing both IgM and IgG will be needed to reduce the chance of false negatives. An individual with false-positive results may have suffered psychological stress attached to such result. This could be avoided if there is a sensor board responsible for ensuring good packaging and condition of the test kits at purchase. Such board should ensure that the manufacturer's detail address and the expiry dates are well stated on the test kit.[7,19,22]

The specificities and sensitivities observed in this study can be improved upon, if testing and diagnostic issues such as false negative, rough packaging, false positive, and expiring date, with the manufacturer detailed address are attended to by the concerned authorities like the Federal Ministry of Health and Ministry of Science Laboratory Technology, involving the Nigeria Institute of Science laboratory Technology. Commercially available ELISA kit is good for screening of HBV infections as against the use of rapid tests kits which needs their performance (sensitivity and specificity) to be improved upon and this can be achievable as evident in most of the kits evaluated and validated considering a resource-poor setting like Nigeria.[22] A regular mechanism of kits evaluation will help in ensuring availability of quality commercial test kits for HBV diagnosis in Nigeria. Also, accurate detection of the viral marker is essential for controlling the transmission of the pathogen when handling a highly infectious virus such as HBV which causes a long-term asymptomatic infection.[27] In lieu of this, it is highly desirable to validate detection assays before approving their use for laboratory diagnosis.

Conclusion

The results from this study have clearly revealed the challenges of diagnosis of HBV infections in Nigeria. This study has also demonstrated that the sensitivity of most of the rapid test kits may not be adequate when compared with EIA for early detection of HBV infections. The implications of possible misdiagnosis on the various intervention strategies that rely predominantly on correct HBV status of an individual are enormous. Also, regular evaluation of test kits for both EIAs and rapid test kits with proper validation of one assay with another may avert misdiagnosis of HBV.[28] Therefore, there is the need necessary to further compliment the use of rapid test kits with EIAs for HBV control in Nigeria.

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Declaration of interest

All authors declare no conflicts of interest for whatsoever.

Authors' contributions

(a) Study design (All authors)

(b) Sample collection (AOY)
(c) Reagent acquisition, laboratory, and data analysis (All authors)
(d) Wrote first draft of the manuscript (BAS)
(e) Revised the manuscript (All authors)
(f) Read and approved the final draft (All authors)

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