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Comparison of Four Diagnostic Tests Methods for the Detection of Dengue Virus Non-Structural-1 Antigen and IgM/IgG Antibodies

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Abstract

Objectives: Dengue virus (DENV) infection is a mosquito-borne disease that stands out as one of the major public health issues and has a wide-ranging geographical distribution throughout the tropics and subtropics. The general alarming increase in the number of cases over the last two decades can be attributed to an extent by the change in national practices to keeping records and reporting dengue to the Ministries of Health, and WHO. Dengue diagnosis is routinely carried out by detection of dengue virus (DENV) antigen NS1 (Non-structural Antigen 1) and/or anti-DENV IgM/IgG antibodies using enzyme-linked immunosorbent assays (ELISAs) and rapid diagnostic tests (RDTs). This study compared the performance of three RDTs and one ELISA used for dengue diagnosis in southeastern, Nigeria. **Design:** This study adopted a cross-sectional design that included prospective hospital-based surveillance of cases among febrile participants attending two major health facilities within the southeastern region of Nigeria. In this study, 338 HIV-infected participants from two teaching hospitals in Nigeria's southeast were systematically tested for Dengue with four methods: NS1 RDT, IgG RDT, IgM RDT, and NS1 ELISA. Their specificities and sensitivities were compared, as well as their level of concordance. Their respective performances were also evaluated using the Receivers Operational curve (ROC). **Results:** Out of the 338 patients, the dengue prevalence from the four dengue diagnostic methods Dengue

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virus NS1 ELISA, NS1 RDT, IgM and IgG seropositivity were 8.9%, 0.6%, 5.6%, and 8.0%, respectively. The Dengue IgM RDT test indicated 36.8% sensitivity, 92.8% specificity, the IgG anti-dengue specific test indicated 29.6% sensitivity, 92.9% specificity and the dengue NS1 RDT test indicated 3.3% sensitivity, 99.4% specificity when compared with the Dengue NSI Elisa test method as a reference method. **Conclusion:** The use of NSI ELISA for DENV diagnosis showed good performance and the RDTs showed, to an extent, reliable results compared with ELISA. However, diagnostic laboratories should be aware of performance variations across tests and the possibilities of cross-reactivity that may affect results.

Keywords

Dengue Virus, Diagnosis Methods, Comparison, Performance, Southeastern Nigeria

1. Introduction

Dengue virus (DENV) infection is a mosquito-borne disease that stands out as one of the major public health issues and has a wide-ranging geographical distribution throughout the tropics and subtropics [1] [2]. Dengue virus (DENV) is transmitted by mosquitoes of the Aedes genus, primarily Aedes aegypti, but also Aedes albopictus and Aedes polynesiensis [3]. DENV is a member of the family Flaviviridae [4], which also comprises other major human pathogens such as West Nile, yellow fever, Zika, Japanese encephalitis, and tick-borne encephalitis viruses. The dengue cases reported to WHO increased over 8-fold over the last two decades, from about 505,430 cases in 2000, to over 2.4 million in 2010, and 5.2 million in 2019. Reported deaths between the year 2000 and 2015 also increased from 960 to 4032, affecting mostly the younger age groups. The total number of cases was observed to have seemingly decreased during the year 2020 and 2021, as well as for reported deaths. However, the data is not yet complete and the COVID-19 pandemic might have also hampered case reporting in several countries [5] [6].

DENV infection in some cases follows a progressive scope of clinical sequelae, ranging from asymptomatic infection through to dengue fever (DF) and the more severe disease presentations of dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS).

In 2009, the World Health Organization proposed a revised classification that categorizes DENV infections as follows: 1) dengue; 2) dengue with warning signs (abdominal pain, persistent vomiting, fluid accumulation, mucosal bleeding, lethargy, liver enlargement, and increasing hematocrit with decreasing platelets); and 3) severe dengue (dengue with severe plasma leakage, severe bleeding, and/or organ failure) [7].

Asymptomatic infections are characterized by undifferentiated fever with or

without rashes. In most clinical cases, the initial presentation of dengue is not clearly known until clear signs and symptoms emerge. However, this delay in clinical presentation affects the ability to make an early and accurate clinical diagnosis. Since there are no specific therapies for dengue, such as antiviral drugs, treatments are essentially supportive, and thus, form a very important challenge in making an informed estimate of the severity of a patient's disease as early as is practicable. The preliminary stages of DENV infections are often confused with other febrile tropical diseases [8], which may lead to inappropriate therapy.

Generally, Dengue presents clinical symptoms that are quite similar to those of other febrile illnesses that are caused by other disease-causing agents. Thus, there is a general issue with disease management in routine clinical laboratories where specific dengue tests are not done to detect the presence or absence of DENV.

Since most of the patients usually are either symptomatic or present with nonspecific fever requiring differential diagnosis from malaria, laboratory confirmation using a rapid, accurate, and relatively low-cost diagnostic test is of utmost importance [9]. This differential laboratory diagnosis for DENV infection includes detection of the virus genome, non-structural (NS)-1 antigen or IgM/IgG antibodies, or a combination of these tests [10]. NS1 is a highly conserved glycoprotein produced by flaviviruses and can be detected in blood samples, usually between one and nine days after the onset of symptoms, which is very efficient for early diagnosis of DENV infection [11]. According to the WHO recommendations, confirmatory diagnosis of DENV infection includes virus detection by PCR or virus culture, detection of IgM seroconversion in paired sera, IgG seroconversion, or ≥four-fold increase in the IgG titer in paired sera [1]. ELISA-based serological tests can detect IgM, IgG, or the NS1 glycoprotein [12]. As many patients seek medical care five days after fever onset, anti-DENV IgM/IgG becomes suitable markers for diagnosing a recent DENV infection, and the anti-DENV IgG test can help differentiate primary and secondary DENV infections [13].

Non-structural gene, NS1, is a highly conserved glycoprotein that is essential for viral replication and viability produced by all flaviviruses in both membrane-associated and secreted forms [14]. Localized to cellular organelles, this antigen is secreted abundantly in sera of patients during virus replication and the early stage of infection [15]. This protein is unique in that it is secreted by mammalian cells as hexamers (dimer subunits only), while in insect cells, it is not secreted including those of mosquitoes. Identification can be done in peripheral blood prior to the formation of antibodies, and the detection rate is higher in acute primary infection from the day of onset of fever to day 9 [5] [6]. NS1 is a complement-fixing antigen that usually elicits a very strong humoral response in the host.

The general alarming increase in the number of cases over the last two decades can be attributed to an extent by the change in national practices to keeping records and reporting dengue to the Ministries of Health, and to the WHO. Also, it shows the government's recognition of the burden, and therefore the im-

portance of reporting the dengue disease burden. Also, the diagnosis of dengue infection is very important in the further management of cases. When a diagnosis cannot be successfully performed, problems can be expected. Thus, this study aims to compare the performance of four dengue test methods in the detection of dengue virus in two study sites in South Eastern Nigeria.

2. Materials and Methods

2.1. Study Design

This study adopted a cross-sectional design that included prospective hospital-based surveillance of cases among febrile participants attending two major health facilities within the southwest region of Nigeria. This study was conducted within the peak period of the dry season (January-February 2016).

2.2. Study Area

The University of Nigeria Teaching Hospital (UNTH) located in Enugu Nigeria and Nnamdi Azikiwe University Teaching Hospital situated at Nnewi were the major health facilities the study was carried out.

The University of Nigeria Teaching Hospital (UNTH) is located in Enugu Nigeria (6.44˚N 7.5˚E 192 m). Enugu State is one of the states in the eastern part of Nigeria with a population of 3,267,837 people at the census held in 2006 (estimated at over 3.8 million in 2012). The mean temperature in Enugu State in the hottest month of February is about 87.16˚F (30.64˚C), while the lowest temperatures occur in the month of November, reaching 60.54˚F (15.86˚C).

Nnamdi Azikiwe University Teaching Hospital is situated in Nnewi, which is the second largest city in Anambra State in Southeastern Nigeria. In 2006, Nnewi has an estimated population of 391,227 according to the Nigerian census. The city spans over 1076.9 square miles (2789 km^2) in Anambra State. Geographically, Nnewi falls within the tropical rainforest region of Nigeria. The city is located east of the Niger River, and about 22 kilometers southeast of Onitsha in Anambra State, Nigeria.

2.3. Study Population

A total of 338 HIV-infected participants attending the two university teaching hospitals within the southeast region of Nigeria were recruited for this study. The inclusion criteria, which were based on their medical history, being seropositive for HIV and malaria symptomatology, included ongoing febrile illness, suspected to be malaria and pyrexia of unknown origin at the two university teaching hospitals within the southeast region of Nigeria. This category of subjects was selected because the prodrome stage of Dengue and these diseases are similar. The study population included males and females from all age groups.

2.4. Sample Collection

The clinical manifestation of malaria and dengue are similar so blood samples

for the diagnosis of DENV infections were collected from HIV seropositive patients. A total of 338 blood samples were collected and used for differential diagnosis in the study. The blood samples were collected into EDTA bottles from each participant using a needle and syringe and were immediately transported in a cold chain to the Microbiology Laboratory. Each bottle was labeled indicating their age, sex and location.

2.5. Sample Processing and Detection of DENV

The 338 blood samples were analyzed in the laboratory by three rapid diagnostic tests; NS1 RDT, IgM and IgG (RDT) and the enzyme-linked immunosorbent assay (ELISA) method detection of dengue. DENV diagnosis was made by the detection of an IgM, IgG antibody and NS-1 antigen in blood serum by RDT using the one-step dengue ns1 antigen rapid test kit (Qingdao Hightop Biotech-co. LTD, China); One step dengue AB-IgG/IgM rapid test kit [Qingdao Hightop Biotech co.LTD, China], the human dengue virus NS1 (DV NS1) ELISA kit (Biosino Biotechnology and Science Inc.) following the manufacturer's instructions. The optical density was measured at 450 nm in the ELISA reader (Titertek Multiskan Plus, Finland, type-314).

2.6. Statistical Analysis

Data was entered into the excel sheet for review. Further analysis was done using Graphpad Prism software, version 8.0. Descriptive analysis was represented with frequencies and means. The receiver operating characteristics (ROC) curve was used to evaluate the performance of the diagnostic tool for Dengue infection.

3. Results

3.1. Prevalence of DENV across Different Diagnostics Methods

Out of the 338 patients, the dengue prevalence from the four dengue diagnostic methods Dengue virus NS1 ELISA, NS1 RDT, IgM and IgG seropositivity were 30 (8.9%), 2 (0.6%), 19 (5.6%) and 27 (8.0%,) respectively (**Figure 1**). There were no significant differences observed in dengue NS1 ELISA antigenemia vs. anti-dengue IgM seropositivity ($p = 0.439$); dengue NS1 ELISA antigenemia vs. anti-dengue IgG seropositivity ($p = 0.808$); and anti-dengue IgM vs. anti-dengue IgG seropositivity ($p = 0.593$) respectively. However, data shows that serological detection of NS1 antigen using the ELISA test with a prevalence of 8.9% ($n = 30$) was significantly greater ($p = 0.011$) compared with that of the NS1 rapid Diagnostic test indicating a prevalence of 0.6% ($n = 2$).

3.2. Performance Evaluation of Dengue IgM RDT, Dengue IgG RDT and Dengue NS1 RDT

Performance evaluation of Dengue IgM, Dengue IgG RDT and Dengue NS1 RDT was done using the Dengue NS1 ELISA test method as a standard method. The Dengue IgM RDT test indicated 36.8% sensitivity, 92.8% specificity and

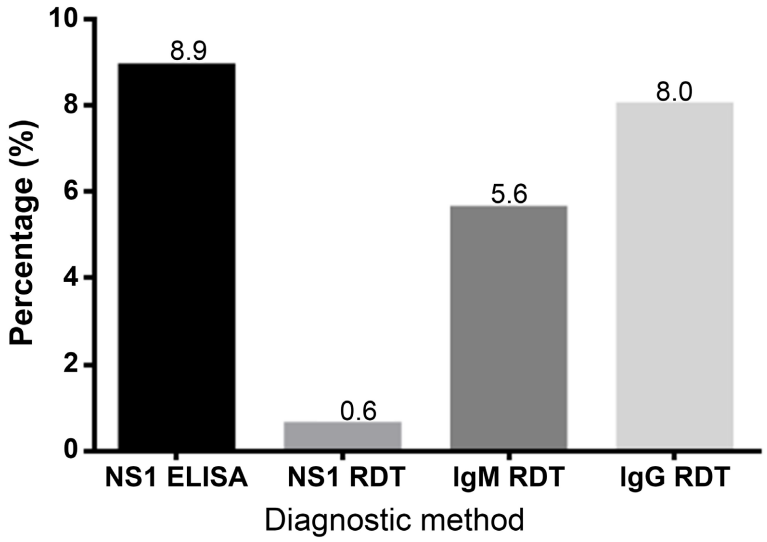

Figure 1. The performance of anti-dengue specific IgM test, IgG test and dengue NS1 RDT in HIV-infected subjects in the overall population.

89.6% accuracy when compared with the Dengue NSI Elisa antigenemia test as a standard in the overall study population. The IgG anti-dengue specific test indicated 29.6% sensitivity, 92.9% specificity and 87.9% accuracy and the dengue NS1 RDT test indicated 3.3% sensitivity, 99.4% specificity and 90.8% accuracy when compared with the Dengue NSI Elisa test method (Table 1).

3.3. Performance Evaluation Using Receivers Operational Curve

The ROC curve was used to evaluate the performance of the rapid diagnostic test for anti-dengue specific IgG antibodies, anti-dengue specific IgM antibodies and dengue NS1 rapid diagnostic test methods using the enzyme-linked immunosorbent assay (ELISA) as a standard. NS1 antigen, IgG rapid diagnostic test was found to give fewer false positives than IgM. Figure 2 shows the ROC curve and area under curve indicating the diagnostic performance of the rapid diagnostic test for anti-dengue specific IgM antibodies compared with the Standard while Figure 3 shows the ROC curve and area under curve indicating the diagnostic performance of the rapid diagnostic test for Dengue IgG antibodies compared with that of ELISA NS1. In Figure 4, the areas under the curve indicated a lack of significant difference ($p = 0.931$) in the discriminating ability of the RDT anti-dengue IgM test (AUC = 0.597) compared with the anti-dengue IgG test (AUC = 0.602). In Figure 5, the areas under the curve indicated significant difference ($p = 0.018$) in the discriminating ability of the RDT anti-dengue IgG test (AUC = 0.602) compared with the RDT dengue NS1 antigen test (AUC = 0.503). This result indicated that though the two RDT tests performed poorly when compared to the standard ELISA NS1 tests, the performance of the RDT anti-dengue IgG test was relatively better than that of RDT dengue NS1 antigen test (Figure 6). In Figure 6, the areas under the curve indicated a significant difference ($p = 0.019$) in the discriminating ability of the RDT anti-dengue IgM test

Table 1. The performances of Dengue IgM RDT, Dengue IgG RDT and Dengue NS1 RDT.

Test Performance	Dengue IgM (RDT)	Dengue IgG (RDT)	Dengue NS1 RDT
Sensitivity	0.896 (0.875 - 0.922)	0.879 (0.856 - 0.907)	0.033 (0.002 - 0.087)
Specificity	0.368 (0.179 - 0.592)	0.296 (0.151 - 0.473)	0.994 (0.990 - 0.999)
Accuracy	0.928 (0.917 - 0.941)	0.929 (0.917 - 0.945)	0.908 (0.903 - 0.918)

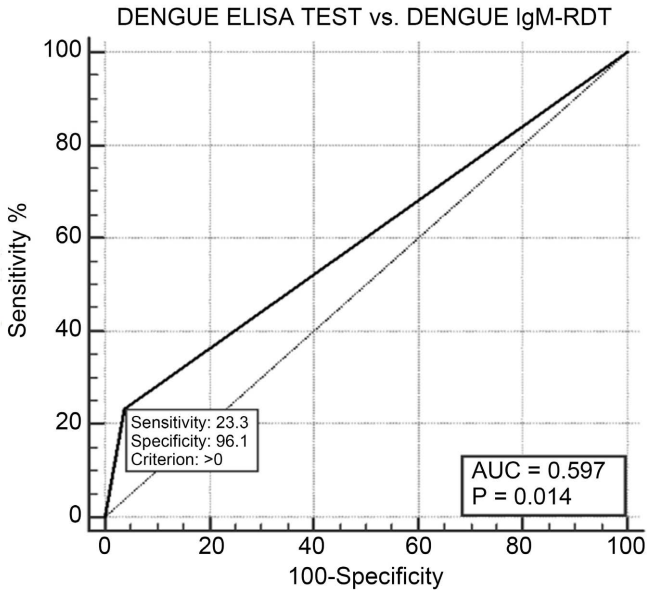

Figure 2. Receiver operating characteristics (ROC) curve and area under curve indicating the diagnostic performance of rapid diagnostic test for anti-dengue specific IgM antibodies compared with the Standard; enzyme-linked immunosorbent assay (ELISA) test for Dengue NS1 antigen.

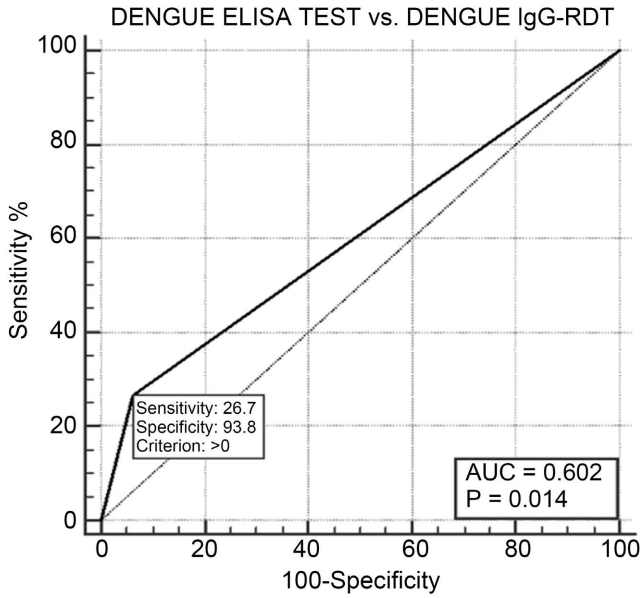

Figure 3. Receiver operating characteristics (ROC) curve and area under curve indicating the diagnostic performance of the rapid diagnostic test for Dengue IgG antibodies compared with that of ELISA NS1 test.

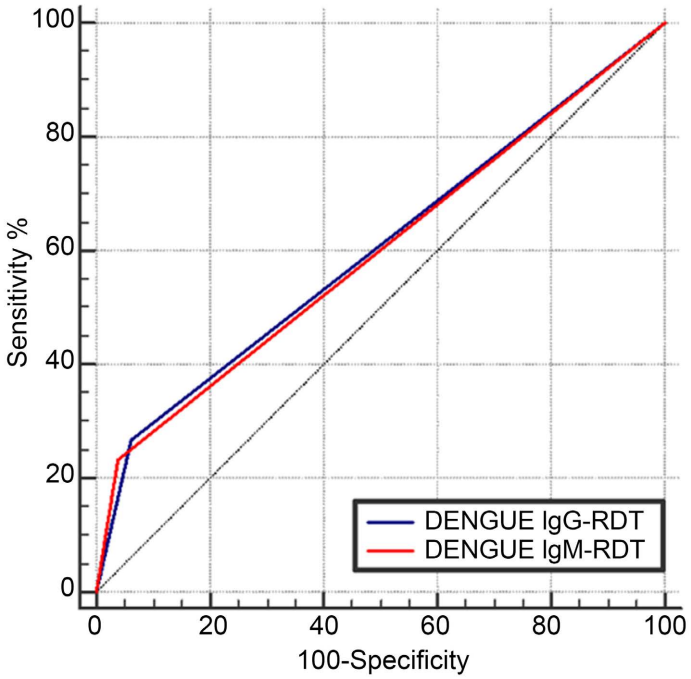

Figure 4. Receiver operating characteristics (ROC) curve and area under curve indicating the diagnostic performance of the rapid diagnostic test for Dengue IgG antibodies compared with that of anti-dengue IgM-RDT using the ELISA NS1 test as reference test.

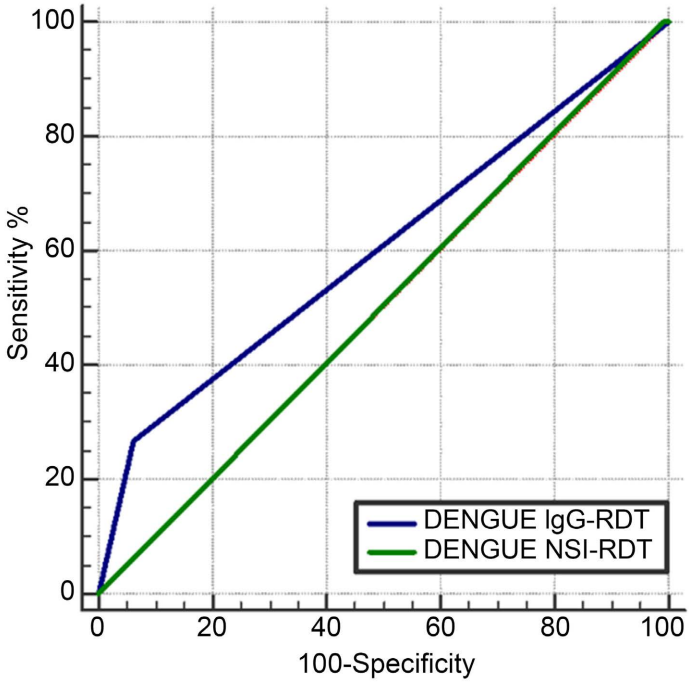

Figure 5. Receiver operating characteristics (ROC) curve and area under curve indicating the diagnostic performance of the rapid diagnostic test for Dengue IgG antibodies compared with that of dengue NS1 antigen RDT using the standard ELISA NS1 test as reference test.

(AUC = 0.597) compared with the RDT dengue NS1 antigen test (AUC = 0.503). Also, this result indicated that though the two RDT tests performed poorly when

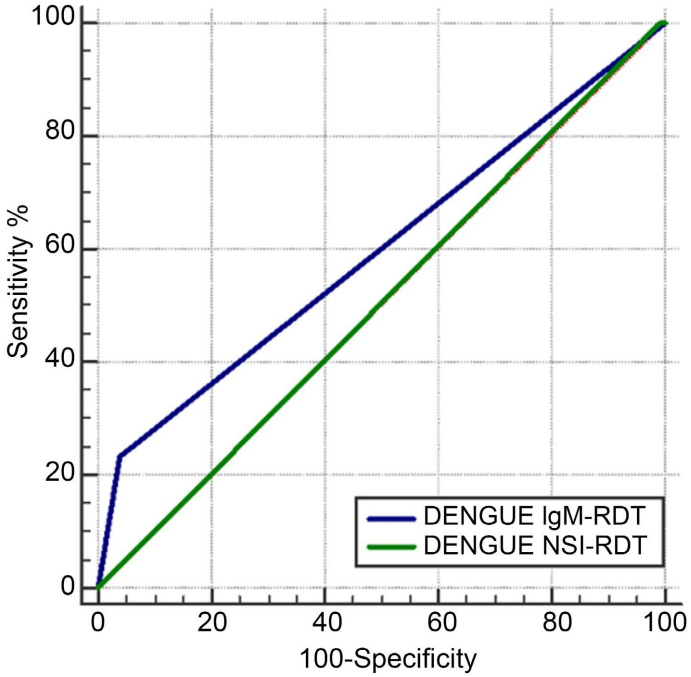

Figure 6. Receiver Operating Characteristics (ROC) curve and area under curve indicating the diagnostic performance of Rapid diagnostic test for Dengue IgM antibodies compared with that of dengue NS1 antigen RDT using the standard ELISA NS1 test as reference test.

compared to the standard ELISA NS1 tests, the performance of the RDT anti-dengue IgM test was relatively better than that of RDT dengue NS1 antigen test (**Figure 6**).

4. Discussion

Dengue diagnosis is routinely carried out by detection of dengue virus (DENV) antigen NS1 and/or anti-DENV IgM/IgG antibodies using enzyme-linked immunosorbent assays (ELISAs) and rapid diagnostic tests (RDTs). This study compared the performance of three RDTs and one ELISA used for dengue diagnosis in southeastern, Nigeria.

The findings in this study show that all the dengue assay methods used were all useful and correlated well with each other but varied significantly with the standard (dengue NS1 ELISA). It was found that IgM was more accurate than IgG as judged by the sensitivity and specificity results. These results can be justified as the infections were at different stages with NS1 antigen found from day 1 to day 9 after the onset of fever. The detection of anti-NS1 is inhibited if anti-NS1 antibodies are produced; IgM is detectable by Day 3 to Day 5 after the onset of illness in primary dengue, IgM can appear as early as day 3 - 5 in primary infection, peaking several weeks after recovery and remaining at detectable levels for several months [16]. Antibody titers do not increase immediately, meaning IgM RDT are <50% sensitive for at least 4 days after symptom onset in primary infection, reducing their usefulness in clinical management.

Commercial dengue NS1 antigen-capture ELISA and immunochromatographic (Rapid) strip tests have been designed to be highly specific, with no demonstrable cross-reactivity with other flavivirus NS1 species. Hence, the use of multiple dengue diagnostic tools is advocated at all times because each method is ideal for a particular scenario, not all scenarios.

NS1 rapid diagnostic tests were found to show a very high tendency toward giving higher false-negative results than the other dengue test methods; hence, may not be suitable as an alternative to others in every situation. Not so much is known about the probable causes of false-positive NS1 tests, except for possible cross-reactivity with other flaviviruses and possibly, cytomegalovirus (CMV) and possible improper storage conditions of the kit which might affect the performance [17].

The anti-Dengue virus NS1 RDT quantitation showed the least performance, indicating a poor performance in discriminating or distinguishing between normal and abnormal viral loads in Dengue fever when compared with the serum NS1 ELISA antigen test. This suggests that Dengue NS1 RDT may not be an effective alternative to serum ELISA quantification in Dengue virus-infected patients at every point in time except at the earliest days of infection.

The accuracy of the NS1 antigen ELISA test is considered high with sensitivity 55% - 82% and specificity 97% - 100%. The NS1 protein is unique in that it is secreted by mammalian cells as hexamers (dimer subunits only), while in insect cells, it is not secreted including those of mosquitoes. Identification can be done in peripheral blood prior to the formation of antibodies, and the detection rate is higher in acute primary infection from the day of onset of fever to day 9 [5] [6]. Unlike IgM and IgG, it is present during the acute viraemic phase of infection, consistent with its postulated role in viral replication.

Since the NS1 rapid test aims to detect dengue NS1 antigen, it should be performed within 5 days of onset of fever. The anti-Dengue IgG-RDT quantitation showed a lesser performance when compared with the serum NS1 ELISA antigen test, indicating a poor performance in discriminating or distinguishing between normal and abnormal viral loads in Dengue fever. This suggests that anti-dengue IgG-RDT may not be an effective alternative to serum ELISA quantification in Dengue virus-infected patients at every point in time except at the earliest days of infection. There is therefore the need to use more than one dengue test method to validate results. Although all of the rapid diagnostic methods are easy to operate, IgG RDT, however, shows more accuracy in the detection of antibodies responsible for Dengue compared to other rapid test methods.

However, virus isolation and nucleic acid detection have been seen to be more accurate than antigen detection. However, antigen detection is still preferable as the use of nucleic acid tests is not widely available due to greater cost.

5. Conclusion

The use of ELISA for DENV diagnosis showed good performance and the RDTs

showed to an extent, reliable results compared with ELISA. However, diagnostic laboratories should be aware of performance variations across tests and the possibilities of cross-reactivity that may affect results. More than one dengue test method to validate results is recommended for laboratories that could afford it.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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