ABSTRACT

Background: Detection of specific antibodies in the blood/body fluids is the primary testing method for HIV infection. A rapid test is one of the assays used in detecting HIV-specific antibodies. This study was conducted to evaluate the laboratory performance of the Wantai HIV 1/2 Rapid Test Kit.

Study Design: Cross-sectional laboratory-based performance characteristics study.

Place of Study: The study was conducted across all the six geopolitical zones in Nigeria (North Central, North East, North West, South West, South East, and South-South).

Methodology: This study was a cross-sectional laboratory-based performance evaluation with specimens obtained from Nigeria's six geopolitical zones, characterised in the reference laboratory, and used to evaluate the Wantai test-kit. Ten millilitres of whole blood were collected from all the study sites in EDTA vacutainer tubes through venous puncture from participants, coded, centrifuged, and plasma harvested into cryovials in three aliquots of 2mls each. All specimens were retested using a combination of determine and Unigold rapid test kits. Samples that tested positive or negative concordantly with these kits were used to evaluate the Wantai HIV test kit. The data were entered in Excel and analysed with IBM-SPSS-version 25.
Result: Of the 1353 samples, 697 were HIV positive, and 656 were negative. Wantai RTK accurately detected 694(99.6%) positive and 649(98.9%) negative samples. Its sensitivity was 99.57% [95% CI:98.75%-99.91%], and the specificity was 98.93%[95%CI:97.81%-99.57%], with 99.26%[95%CI:98.64%-99.64%] accuracy. Collecting the correct plasma volume was at a 90.0% rate, reading the test result within the time limit, line visibility, interpreting the result, learning the procedure, design of the kit and waste generated were all at the rate of 100.0%.

Conclusion: With a sensitivity of 99.6% and a specificity of 98.9%, the Wantai HIV rapid test is suitable for inclusion as a first-line (screening) test in Nigeria’s HIV testing algorithm.

Keywords: Rapid test kits; HIV/AIDS; specificity; sensitivity; accuracy.

1. INTRODUCTION

Human immunodeficiency virus and Acquired immune deficiency syndrome (HIV/AIDS) interventions such as prevention of mother-to-child transmission (PMTCT), Antiretroviral Therapy (ART), HIV testing services (HTS), Blood safety, and HIV Surveillance established by the Government of Nigeria and Partners for the control of HIV/AIDS infection largely depend on the establishment and provision of accurate and reliable diagnosis [1]. Detection of specific antibodies in the blood or other body fluids is the primary testing method for HIV and the standard procedure for diagnosing HIV infection [2]. A rapid test is one of the assays used in detecting HIV-specific antibodies. Rapid test kits (RTKs) are evaluated in the laboratory and placed in appropriate combinations (Testing Algorithm) for reliable diagnosis of HIV infection.

HIV Diagnostics started in Nigeria in the mid-1980s and involved using ELISA for screening and Western Blot for confirmation [3]. Testing was facility-based and restricted to centers of high virological competence, mainly in the University Teaching Hospitals and Research Institutes. The procedure required highly skilled laboratory scientists, and clients had to endure a long waiting for results to be released. All the test kits and reagents required a cold chain. The researchers did evaluations in collaboration with Teaching Hospitals, and National AIDS and STDs Control Program (NASCP) coordinated the process. Back then, the procedure was simple, and National Agency for Food and Drug Administration and Control (NAFDAC) issued approvals for using test kits in Nigeria based on recommendations of the evaluation team [3].

The 2003 sentinel survey adopted a serial algorithm: Capillus (Trinity Biotech) was used for screening; Genie II (Organics) for confirmation and Determine (Abbott Laboratories) as the tiebreaker in cases of discordance. The first non-cold chain-dependent laboratory-based interim algorithm was constructed by Prof. Olaleye of UCH Ibadan, commissioned by NASCP in 2005. The Federal Government of Nigeria (FGoN), in collaboration with the President’s Emergency Plan for AIDS Relief (PEPFAR) Program, implemented and completed the Phase 1 laboratory-based evaluation with a specific focus on non-cold chain dependent HIV RTKs to suit both infrastructure and the varied national skill level in 2006 [4]. Before 2007, there was no formal evaluation of HIV RTKs (non-cold chain dependent) for developing a national algorithm in Nigeria. However, the scale-up of HIV testing to increase access to HIV support services and tailoring prevention programs to meet country-specific needs made it necessary to develop national guidelines and directions for testing procedures.

A total of nine HIV rapid test kits were evaluated and rated: six of them (Determine, double-check gold, Sure check, Bundi, Statpak, and Unigold) met all the evaluation criteria. An interim algorithm was generated, consisting of five of the six rapid test kits. Following the approval of the protocol, the Federal government of Nigeria (FMoH), in collaboration with the US Government and development partners, proceeded to implement the Phase 2 evaluation of the algorithms [5]. The Phase 2 evaluation of the algorithms was carried out at the point of service to ascertain the ability of test kits to maintain their performance in the actual testing environment conducted by staff of varying skill levels. All the five HIV RTKs that performed well in the phase I evaluation were evaluated under field conditions, and their sensitivities and specificities were determined as single and combination tests (one of the kits -Bundi HIV 1/2, gave inconsistent results in the field and was not considered for inclusion in the Phase II evaluation). The FMoH also implemented additional phase I evaluations, resulting in the emergency of more kits approved for HIV testing services in Nigeria [1]. These kits, be they imported or locally manufactured, were
always evaluated at the request of the manufacturers or their vendors in Nigeria. This kit is also to undergo phase one evaluation at the request of the manufacturer's representative in Nigeria. The study's main objective is to evaluate the laboratory performance characteristics of the Wantai HIV rapid test kit. Also, to determine the sensitivity, specificity, and accuracy of the Wantai HIV rapid test kit and establish the ease of use of the Wantai HIV rapid test kit.

2. METHODOLOGY

2.1 Study Design

This study was a cross-sectional laboratory-based performance characteristics study with specimens obtained from Nigeria's six geopolitical zones. These specimens were subsequently characterised in the reference laboratory and are used to evaluate the test kit. Analysts were asked through a structured questionnaire to rate the ease of performing the test, clarity of reading and interpretation of the test result, ergonomics. These factors can affect the outcome of test results with test devices. This process is called global rating. They are mathematically weighted, and it contributes to the rating of whether a device can be used for testing and its sensitivity and specificity. When sensitivity and specificity (accuracy) are combined with the global rating, the score is composite. Apart from meeting specified sensitivity and specificity requirements, it has been determined that a kit must also have a minimum of 90% composite score deployed for HTS in Nigeria.

2.2 Study Site

Specimen for the evaluation were obtained from the six geopolitical zones where the positive and negative were confirmed using the National HIV testing algorithm. The specimen was transported to the Reference Laboratory for further testing to obtain the gold standard for the evaluation.

2.3 Study Population

Blood samples for this evaluation were collected from clients between 18 and 60 years old, attending HTS, blood bank and STI clinics, and PLHIV on ART less than 12 months.

2.4 Sample Size

The sample size was derived using the following formula:

\[ n = z^2 \frac{p(1-p)}{d^2} \]

Where,

- \( n \) = sample size
- \( Z \) = Standard normal deviate corresponding with specified Confidence Level
- \( p \) = expected sensitivity and specificity of the test, respectively
- \( q = 1 - p \)
- \( d \) = tolerable error margin (a measure of precision).

In this evaluation, the expected sensitivity and specificity were 99%, with a confidence level of 95% and the error margin fixed at 0.9%. With the above specification, the sample size determined for the study is 469 positives and 469 negatives (a total of 938). To compensate for invalid samples due to loss of quality in storage and transportation and discordance, the size is adjusted by 5%, thus bringing the final size to 985. A total of one thousand three hundred and fifty-seven (1,357) samples were obtained from the six (6) study sites.

2.5 Sample Collection Sites

Specimens were collected from the six geopolitical zones of Nigeria (Southeast, southwest, south-south, northwest, northeast, and north-central). In the southeast, the specimen was collected from the University of Nigeria Teaching Hospital, Enugu, Enugu state. Lagos State University Teaching Hospital (LASUTH) was the site in the southwest, and University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers in the south. The specimen was collected from the Infectious Disease Hospital, Kano, Kano in the northwest, Federal Teaching Hospital Gombe, Gombe in the northeast, and PLASVERIC, Jos, Plateau in the north-central zone. The specimen was collected and transferred to the reference testing laboratory, Public Health IVD Laboratory Lagos.

2.6 Specimen Collection, Processing, and Testing at the Sites

Ten millilitres (10mls) of whole blood were collected in EDTA vacutainer tubes through venous puncture from participants. These specimens were coded, centrifuged and plasma harvested into cryovials in three aliquots of 2mls each. The aliquots were stored at -20°C before transportation to the reference testing laboratory. No information was required from the clients as
the focus was the test performance for sensitivity
and specificity. Specimen were coded based on
the abbreviation of the state where the samples
were obtained and the serial number (EN for
UNTH Enugu, LA for LASUTH Lagos, RV for
UPTH Port Harcourt, KN for AKTHKano, GM for
FTH Gombe, PL for Plasveric). Individuals
accessing services at the service points indicated
above in the selected facilities, upon knowledge
of HIV status, were asked for consent. The focal
Medical Laboratory Scientist in each facility was
responsible for collecting, processing, and
storing all specimens at their sites. The cold
chain was maintained during transportation to the
reference laboratory.

2.7 Specimen Transportation

The specimen was packaged using the triple
packaging system with frozen icepacks in
cryovial boxes to maintain the cold chain. An
aliquot of the sample was maintained at the
collection sites as a backup. All packaged
specimens were taken to the reference
laboratory by the Laboratory Staff of NASCP
within twelve hours of the packaging via air and
road transportation. Specimen transported were
accompanied by sample manifest, duplicated
with one of the copies retained at the collection
site.

2.8 Specimen Analysis at the Reference
Laboratory

2.8.1 Characterisation of positive and
negative panels

All specimens were retested using Determine
and Unigold rapid test kits. Specimens that
tested positive or negative concordantly with
these kits were used as a standard for evaluating
the Wantai HIV test kit. Specimen with discordant
test results after testing with Determine and
Unigold were not used as standard. The HIV
antibody characterised samples were tested with
the Wantai HIV RTK according to the
manufacturer’s instructions by the NALQAT. Kit
controls and in-house positive and negative controls were included in all
procedures.

Table 1. Comparison of Wantai test results with the Gold Standard

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Total</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold standard</td>
<td>1353</td>
<td>697</td>
<td>656</td>
</tr>
<tr>
<td>Wantai RTK</td>
<td>1353</td>
<td>694</td>
<td>649</td>
</tr>
<tr>
<td>% Accuracy of Wantai RTK</td>
<td>99.6%</td>
<td>98.9%</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Predictive value of Wantai screening test for HIV

<table>
<thead>
<tr>
<th>HIV outcomes</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
<th>$X^2$ (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>694 (99.0%)</td>
<td>7 (1.0%)</td>
<td>701 (51.8%)</td>
<td>1313.30 (≤0.001)**</td>
</tr>
<tr>
<td>(True positive)</td>
<td>(False positive)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>3 (0.5%)</td>
<td>649 (99.5%)</td>
<td>652 (48.2%)</td>
<td></td>
</tr>
<tr>
<td>(False negative)</td>
<td>(True negative)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>697 (51.5%)</td>
<td>656 (48.5%)</td>
<td>1353 (100.0%)</td>
<td></td>
</tr>
</tbody>
</table>

** significant at p<0.01 level

Table 3. Sensitivity and specificity of Wantai

<table>
<thead>
<tr>
<th>Statistics (Wantai)</th>
<th>Value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>99.57%</td>
<td>98.75% to 99.91%</td>
</tr>
<tr>
<td>Specificity</td>
<td>98.93%</td>
<td>97.81% to 99.57%</td>
</tr>
<tr>
<td>Positive Predictive Value</td>
<td>99.00%</td>
<td>97.93% to 99.52%</td>
</tr>
<tr>
<td>Negative Predictive Value</td>
<td>99.54%</td>
<td>98.59% to 99.85%</td>
</tr>
<tr>
<td>Accuracy</td>
<td>99.26%</td>
<td>98.64% to 99.64%</td>
</tr>
</tbody>
</table>

Table 4. Testers’ rating of the Wantai test kit

<table>
<thead>
<tr>
<th>Domains for Assessing the Ease of Use</th>
<th>Median Score*</th>
<th>Percentage score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collecting correct volume of plasma/sera</td>
<td>4.5</td>
<td>90.0%</td>
</tr>
<tr>
<td>Reading the test Result within the time limit</td>
<td>5</td>
<td>100.0%</td>
</tr>
<tr>
<td>Line visibility</td>
<td>5</td>
<td>100.0%</td>
</tr>
<tr>
<td>Interpreting the result</td>
<td>5</td>
<td>100.0%</td>
</tr>
<tr>
<td>Learning the procedure</td>
<td>5</td>
<td>100.0%</td>
</tr>
<tr>
<td>Design of the Kit</td>
<td>5</td>
<td>100.0%</td>
</tr>
<tr>
<td>Packaging size</td>
<td>4</td>
<td>80.0%</td>
</tr>
<tr>
<td>Packaging integrity</td>
<td>3</td>
<td>60.0%</td>
</tr>
<tr>
<td>Waste Generated</td>
<td>5</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

Note: maximum score per category is 5

Table 4 shows the tester’s rating of the Wantai test kit. Collecting the correct volume of plasma/serum was at a 90.0% rate, reading the test result within the time limit, line visibility, interpreting the result, learning the procedure, design of the kit and waste generated were all at the rate of 100.0%. The packaging size was 80%, and packaging integrity was 65%.

3.2 Testers’ Rating

The maximum score attainable was 45. The median global score from all the testers for this test kit was 40.4 (89.8%).

3.3 Composite Score

The accuracy of the test kit was assigned a weight of 70%, while the global score (based on testers’ ratings) was given a weight of 30%. The composite score was determined as the weighted mean of accuracy and global score. The combined score for Wantai RTK was 96.4%. A comparison of the composite score of this kit with those evaluated earlier shows that Wantai ranks among the top five test kits when considering the performance characteristics and the ease of use.

4. DISCUSSION

It is essential to determine test devices’ laboratory performance and operational characteristics appropriately for routine testing. Key performances to be monitored closely are the ability of the device to detect the analyte (sensitivity) without any interference from the unintended analyte (specificity). Other factors that may affect these variables include ease of performing the test, clarity of reading, and interpretation of the test result. The Western Blot (WB) assay is regarded as the gold standard for confirming HIV infection. However, it is highly time-consuming and requires technical competence, even though the results were similar to Wantai RTK in this study [6]. The WHO recommends blood-based fast diagnosis to achieve the same-day testing and treatment [7].
However, due to a lack of data demonstrating the efficacy of RTs as a confirmatory HIV test for the Gold standard, the RT technique has not been widely recommended. Nevertheless, several studies have reported high specificity and sensitivity, even though single studies could not give sufficient statistical power [8–10].

This evaluation showed that Wantai HIV RTK could detect antibodies to HIV with a sensitivity of 99.6% and specificity of 98.9%. The National AIDS/STI Control Program (NASCP) also approved the use of the Wantai HIV 1&2 RTK for HIV/AIDS algorithm testing after relevant bodies validated its sensitivity, specificity, and accuracy [11]. As stated, the Public Health In-vitro Diagnostic Control Laboratory (PHIVDCL) specimen was characterised and utilised to evaluate the Wantai HIV RTK. The evaluation of the cross-sectional study also determined the sensitivity, specificity, accuracy, and mean global rating ratings of the Wantai HIV RTK. Additionally, the report noted that the test's sensitivity, specificity, and accuracy were 99.6%, 98.9%, and 99.3%, respectively, closely correlating with the result of this present study, which further prompted the suggestion that the Wantai HIV RTK is included in the algorithm's first line (screening) tests[11].

Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, “Rapid Test for Antibody to Human Immunodeficiency Virus (HIV) (Colloidal Gold Device) was also evaluated by WHO in the third quarter of 2012 using serum/plasma specimens [12]. This evaluation reported that the anti-HIV antibody diagnostic kit (colloidal gold) is an immunochromatographic assay for detecting HIV-1/2 antibodies in whole human blood serum and plasma specimens. The authors reported the result in the limited evaluation on a panel of 1079 clinically-derived specimens, which found an initial sensitivity (95% CI) of 99.76% (98.7% - 100%) and an initial specificity (95% CI) of 98.33% (97.0% - 99.2%) compared to the reference assays. The final sensitivity (95% CI) was 100% (99.1% - 100%) and the final specificity (95% CI) was 98.48% (97.2% - 99.3%) compared to the reference assays. A lot-to-lot variation was acceptable except for one dilution series for which there was a 2-fold difference between lots [12]. For eight seroconversion panels, an Anti-HIV antibody diagnostic kit (colloidal gold) was detected on average 0.5 specimens later than the benchmark assay; Enzygnost Anti-HIV 1/2 Plus (Siemens Healthcare Diagnostics).

Huang et al.[13] reported that although the sensitivity and specificity of RT reagents are both more than 99.5%, they may compromise in non-laboratory environments due to unstandardised processes. They further explained that without a quality assurance and assessment system, it could diminish the sensitivity of RT. Unstandardised procedures can result in a false negative rate of up to 5.4 percent for RT [14]. Walensky et al.[15] also reported some difficulties RT test encounters, such as rechecking the same sample and relatively low sensitivity for early HIV infection.

5. CONCLUSION

Several significant advancements in HIV testing have occurred over the last two decades. Serologic techniques based on recombinant antigens have been developed to provide advantages in various testing contexts. These advancements are fast tests performed on a fingertip blood sample with minimal procedural steps. This test kit's performance meets the WHO’s criteria for HTS use. Overall, our study demonstrated that Wantai HIV RTK is equally effective as the Gold standard. It can be widely utilised for HIV early therapy on the same day of identification when performed according to established management guidelines. Quality assurance and quality control of RTs are critical and time-consuming tasks in a devolved setting where such practices are not ingrained in the culture. Therefore, it is essential to acquire the knowledge and skills necessary to undertake quality control at an RT site. The time has come for rapid tests to take a more central role in raising the rate of HIV infection detection. The Wantai HIV RTK’s performance characteristics for detecting antibodies to HIV makes the device suitable for inclusion in our HIV testing algorithm.

6. RECOMMENDATION

The test’s sensitivity, specificity, and accuracy were 99.6%, 98.9%, and 99.3%, respectively. Based on the above results, it is recommended that Wantai HIV rapid test kit should be included among the first line (screening) tests of the HIV testing algorithm.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is no conflict of interest between the authors and
producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

CONSENT
As per international standard or university standard, patients’ written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL
Ethical approval was obtained from the National Health Research Ethics Committee of Nigeria before the commencement of the study.

COMPETING INTERESTS
Authors have declared that no competing interests exist.

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1. FMOH. Laboratory-Based HIV Rapid Test validation in Nigeria Phase 1; 2007.

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