Original Research Article

Laboratory Performance Evaluation of Wantai HIV 1/2 Rapid Test Kit

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; and Accuracy

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.

METHODOLOGY

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.

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.

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.

Sample Size

The sample size was derived using the following formula:

$$n=z^2p(1-p)/d^2$$

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.

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.

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 -20ºC 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.

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.

Specimen Analysis at the Reference Laboratory

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.

Reports of laboratory evaluations are disseminated to stakeholders to guide policy formulation and implementation. The evaluation was a cross-sectional laboratory-based performance characteristic with a specimen sample size of 1357. These were obtained from clients from six selected health facilities in each of the six geopolitical zones of

Nigeria. The role of the study participants in this evaluation was well explained to them to make informed decisions. The risk of the study to the participants was very minimal, and the potential benefits were well defined while assuring them of the confidentiality of the information obtained from them. The specimen collected at the six selected health facilities were tested using the HIV test kits in the National testing algorithm before they were retrieved to the reference Laboratory at the Public Health In-Vitro Diagnostics Control Laboratory, Yaba Lagos. At IVD, the specimen was characterised and used to evaluate the Wantai HIV rapid test kit. Sensitivity, specificity, accuracy, and the mean global rating scores of the Wantai HIV rapid test kit were calculated.

RESULTS

Performance Characteristics of Wantai RTK

The reference laboratory characterised one thousand, three hundred and fifty-three (1353) samples. Of the 1353 samples, 697 were HIV positive and 656 negatives (i.e., tested positive or negative concordantly with the gold standard). These were used as the standard for the evaluation of the Wantai. Wantai RTK accurately detected 694 (99.6%) positive and 649 (98.9%) negative samples. As shown in Table 2, Wantai recorded 99.0% true positive and 99.5% true negative cases ($p<0.001$).

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

Instrument	Total	Positive	Negative
Gold standard	1353	697	656
Wantai RTK	1353	694	649
% Accuracy of Wantai RTK		99.6%	98.9%

Table 2: Predictive value of Wantai screening test for HIV

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

**** significant at p<0.01 level**

Table 3 shows the Wantai sensitivity was 99.57% [95% CI: 98.75% - 99.91%], and the specificity was 98.93% [95% CI: 97.81% - 99.57%]. The positive predictive value was 99.00% [95% CI: 97.81% - 99.57%], and the

negative predictive value was 99.54% [95% CI: 98.59% - 99.85%] with 99.26% accuracy[95% CI: 98.64% - 99.64%].

Table 3: Sensitivity and specificity of Wantai

Statistics (Wantai)	Value	95% CI
Sensitivity	99.57%	98.75% to 99.91%
Specificity	98.93%	97.81% to 99.57%
Positive Predictive Value	99.00%	97.93% to 99.52%
Negative Predictive Value	99.54%	98.59% to 99.85%
Accuracy	99.26%	98.64% to 99.64%

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%.

Table 4. Testers' Rating of the Wantai Test Kit

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

Note: maximum score per category is 5

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%).

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.

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 un-intended 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.

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.

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.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

Ethical Approval:

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

Consent

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

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