

ACCURACY EVALUATION OF HIV RAPID TESTS RESOLVED BY TIE-BREAKER: A CASE STUDY OF NIGERIAN SERIAL ALGORITHM

*Article Review by Suleiman Mohammed Aminu, Nigeria
(PhD in Public Health, Texila American University)
Email: - aminusule2000@yahoo.com*

ABSTRACT

INTRODUCTION

The World Health Organization strongly recommended test algorithm for diagnosis of Human Immunodeficiency Virus (HIV) infection. In either parallel or serial algorithms, discordance is commonly encountered and the final result has to be determined by the use of a tie breaker.

OBJECTIVE

The objective of this study is to evaluate the accuracy of HIV rapid test results resolved by tie breaker in a serial algorithm using Western Blot as gold standard.

METHODOLOGY

A total of 110 remnant samples reactive as positive by Determine but giving discordant result with Unigold were collected and retested by national (serial) algorithm from January 2013 to July 2013. Samples were collected in either plain bottles or EDTA container by staff conducting HIV rapid test and transported/stored at 2-8 degree Celsius. Samples were tested according to manufactures leaflet insert. The final result obtained with the serial algorithm was verified with Western Blot technique.

RESULT

Out of the 10 discordant results, 9 were confirmed HIV positive by serial algorithm but only 8 were confirmed positive by Western Blot technique. Thus, there was 1(11%) false positive obtained with serial algorithm. Samples that were reactive by Determine were 110 (100%), 94(85.5%) reactive for Unigold and 93(85%) reactive results with Stat Pak.

CONCLUSION

One out of every 9 HIV results resolved by tie breaker as positive is false positive. This accounts for 11%. There is need therefore to introduce further means of verifying positive results resolved

by tie breaker to avoid placing patients on anti retro vial drugs wrongly and subjecting people to psychological trauma.

INTRODUCTION AND BACKGROUND

HIV/AIDS infection has been described as the most dangerously serious pandemic infection ever known in the history of mankind. More than 40 million people are presently living with the virus globally; and more than 70% of the victims live in Sub Saharan Africa. People infected with HIV remain asymptomatic for many years until when their body's immune system is completely weakened and opportunistic infections begin to set in. During the asymptomatic stage, the only way to diagnose the infection is by laboratory test. Unfortunately thousands of people are yet to know their HIV statuses (Heneke, 2009) and many are not yet willing to take the laboratory test because of fear of stigma. There are also allegations that the HIV test is not reliable. The allegations may not be far from the truth especially when the test was conducted with a single rapid test kit which increases the chances of false positives. To improve the quality of HIV diagnosis, the WHO recommended the use of a combination of two or more rapid test kits to diagnose HIV infection. This sequence of tests is called the HIV testing algorithm.

Since 2007, Nigeria has been involved in the development of rapid testing algorithms for diagnosis of Human Immunodeficiency (HIV). The first phase of rapid test kits evaluation called Phase 1, recommended a serial algorithm; which comprised of Determine as screening test, Unigold as confirmatory test as confirmatory, and the use of Stat Pak as tie breaker if discordance exists. However, in 2012 a Phase 2 evaluation was conducted by the Federal Ministry of Health as a result of which the following three algorithms were selected:

Possible Choices for Screening Test 1	Possible Choices for Confirmatory Test 2	Possible Choices for Tie-breaker
Stat-Pak	Uni-Gold	Sure-Check
Sure-Check	Determine	Stat-Pak
Determine	Stat-Pak	Sure-Check

However, only 2 algorithms were evaluated in this study because they are most commonly used in Nigeria. There has not been any study conducted in Nigeria to establish or compare the accuracies of any of the testing algorithms against the gold standard of Western blot with a view to determining the reliability of the results confirmed by tie breaker.

RESEARCH QUESTION

Are the results of HIV rapid tests resolved by tie breaker in serial algorithm as accurate as the results of HIV test obtained Western blot confirmation in diagnosis of HIV infection?

RATIONALE AND JUSTIFICATION FOR THE STUDY

Rapid test for HIV is cheap, easy to use and results can be ready within 15 to 30 minutes. With a kit's sensitivity of 99.9%., there is a chance of misdiagnosing one true HIV positive as HIV negative in every 1000 persons testing positive when such kit is used as first line screening test. The aim of this research therefore is to evaluate the accuracy of HIV rapid test which have been resolved by tie breaker. A primary concern about rapid testing is the poor testing sensitivity and the unacceptably of high rate of false positive results. In the Rakai district of Southwestern Uganda, a region heavily plagued by HIV, trials conducted on the three most commonly used rapid HIV tests in Uganda revealed critical inaccuracies in rapid testing results. When the results of the rapid tests were retested using traditional tests that have been proven to be acceptably reliable, such as the western blot test and an enzyme immunoassay, it was discovered that 129 of the 295 people who were diagnosed as HIV-positive were actually HIV-negative (Gray et al, 2007). Thus, nearly 45% of the patients who received positive results received them in error. Western blot technique will therefore be used as gold standard for confirmation of HIV infection in this study.

OBJECTIVE

The primary objectives of the study are to:

- a. To determine the accuracy of final HIV result resolved by tie breaker in Nigerian serial algorithm using western blot as gold standard.

RESEARCH OUTCOMES

The outcome of this research would be useful in selecting the appropriate testing algorithm in Nigeria and whether further confirmatory tests will be necessary after resolution of results by tie breaker for any patient diagnosed as either HIV negative or positive by tie breaker.

LITERATURE SEARCH STRATEGIES

Past literatures and relevant publications were searched on Internet using terms like HIV rapid test, challenges of HIV rapid test, and flaws in HIV test". Search engines like pubmed, ScienceDirect, and HINARI were also searched.

LITERATURE REVIEW

A primary concern about rapid testing is the poor testing sensitivity and the unacceptably of high rate of false positive results. In the Rakai district of Southwestern Uganda, a region heavily plagued by HIV, trials conducted on the three most commonly used rapid HIV tests in Uganda revealed critical inaccuracies in rapid testing results. When the results of the rapid tests were retested using traditional tests that have been proven to be acceptably reliable, such as the western blot test and an enzyme immunoassay, it was discovered that 129 of the 295 people who were diagnosed as HIV-positive were actually HIV-negative (Gray et al, 2007). Thus, nearly 45% of the patients who received positive results received them in error. Similarly, another study analyzed one

of most popular rapid testing algorithms used in Cameroon, an algorithm which first tests with a rapid test called Determine and then confirms results with another rapid test called Immuno-Comb II. The algorithm demonstrated a sensitivity of 100% but a specificity of only 91.5. %. Therefore, although this rapid testing combination produces virtually no false negatives, it regularly leads to false positive result (Aghoken *et al*, 2009). A second commonly used testing algorithm employed by the Cameroonian Ministry of Health results in a specificity of 98.8%, which is significantly higher than that of the first testing combination. Yet despite the improvement in specificity, 2 out of every 100 people who are tested with this algorithm are still receiving false positive results. “2% false HIV positive individuals in a high burden country such as South Africa with more than 5 million HIV infections will correspond to about 100,000 people falsely declared HIV positive (ibid). Nigeria has a population of more than 150 million. However, no studies conducted in Nigeria to evaluate the reliability of result obtained by tie breaker as confirmatory of HIV testing, hence the need for this study.

IMPACT OF HV DIVERSITY ON SEROLOGICAL DIAGNOSIS

It has been reported that HIV diversity has an impact on its serological diagnosis (Lihana et al, 2012). And a very heterogenous distribution and dominance of different sub types (including sub types N, O and P) are found in Africa. Nigeria is not an exception. Significant data have reported the circulation of CRF02_AG, sub type G, su-subtype A3, CRF06_cpx, and other recombinants in significant proportion. Peeters. Et al, 2000; McCutchan et al 1999). Since recombination may introduce genetic and biological consequences that are far greater than those resulting from the steady accumulation of single mutations it is therefore essential to continually study the likely influence of these diversities on serological diagnosis.

DEVELOPMENT OF HIV TESTING ALGORITHM IN NIGERIA

The Government of Nigeria (GON), in collaboration with several development partners, in 2005, established a multi-agency working group charged with the responsibility of evaluating HIV rapid test kits and recommending their appropriate use at points of services in Nigeria.

The working group, using an internationally standardized method, created a set of criteria which guided the selection of nine HIV rapid test kits for evaluation. One major criterion for kit selection was non-reliance on refrigeration. Test performance was assessed in a one week laboratory exercise to determine sensitivity, specificity and operational characteristics of individual tests. A panel of well characterized sera collected from the different geo-political zones in Nigeria was used to assess kit performance.

Based on their characteristics and performance, six of the tests were selected for further evaluation, three test kits were dropped from further consideration due to poor performance, cost or complexity. The remaining six kits (Bundi, Determine, Double Check Gold, StatPak, SureCheck and UniGold) all had 100% sensitivity and high specificity, ranging from 97.9 to 100%.

In practice individual tests are not used for diagnosis of HIV; tests are used in combinations (algorithms) to increase diagnostic accuracy. Since a single specimen panel was used in this

evaluation various algorithms could be proposed and the sensitivity/specificity of these algorithms calculated. Using the six test kits listed above there are 120 possible serial test algorithms. All of these were 100% sensitive, specificity ranged from 99.1 to 100%.

Parallel algorithms were also proposed and when compared to serial algorithm there was no difference in accuracy. There was however, a substantial difference in cost. Serial algorithms, in general, are half the cost.

Based on the outcome of this evaluation the following recommendations were proposed:

1. Serial testing should be adopted for use in Nigeria. In this evaluation it was as accurate as and more cost effective than parallel testing.
2. Three possible serial test algorithms are proposed.
3. These algorithms are built around four tests which performed well in this evaluation, have a proven record of use in Nigeria, satisfy concerns for purchasing locally produced products and make use of kits soon to be available in large supply in Nigeria.
4. All HIV diagnostic testing, especially that using rapid tests, should be linked to a well developed training program and a comprehensive quality assurance system.
5. A formal evaluation of HIV rapid test performance is an ongoing process that begins prior to implementation of testing and continues after testing programs have been scaled- up in the field. The following algorithms have been widely in used based on this work

SERIAL ALGORITHMS

<u>Screening Test</u>	<u>Confirmation of Positives</u>	<u>Tie-breaker</u>
Determine	StatPak	
UniGold	StatPak	Bundi
Determine	UniGold	StatPak

However, Bundi and operational challenges and limitations long the line and had to be withdrawn from the market. Therefore this study will evaluate the performance of the only optional algorithm of Determine – Unigold- Stat Pak.

Nigeria is a highly-populated country of 140 million people with an HIV prevalence rate around 5% (Antenatal Clinic [ANC] Survey, 2005). It is a multi-ethnic society with a large proportion of the population living in rural settings (64%).

Traditionally, HIV testing has been the gateway to HIV/AIDS prevention, treatment, care and support. To date, many African countries have evaluated simple, rapid HIV testing as a tool for fighting the HIV epidemic. All of these studies have demonstrated that the use of rapid testing

strategies can be an important part of overall HIV testing in resource-poor settings, where cold storage capacity, reliable power, efficient transportation and sufficient numbers of skilled laboratorians may not be readily available.

The use of HIV rapid testing has also dramatically increased the proportion of tested individuals who receive their results. Prior to the availability of rapid testing, same-day results were not available, and an estimated one-third of those tested did not return to learn their HIV status. The Government of Nigeria (GON) is currently working to expand quality HIV counseling and testing (HCT) services as a prevention intervention, and as an entry to care and treatment. Therefore, the need for well-evaluated, reliable testing products whose performance and use is quality-assured is essential and urgent.

GON health care facilities and non-government organizations (NGOs) in Nigeria are currently providing HIV rapid testing for HCT, prevention of mother-to-child transmission (PMTCT), emergency blood transfusions, and clinical diagnosis. When rapid testing is provided in settings where people learn their status, a multiple test algorithm is used. The U.S. President's Emergency Plan for AIDS Relief (PEPFAR) program supports HIV diagnosis through rapid testing, over the past six months more than 350,000 clients attending HCT sites and nearly 100,000 women attending PMTCT programs across Nigeria. In addition, test kits were provide for emergency HIV screening of 20,000 units of blood in the past year.

Enzyme immunoassays (EIA) and Western Blot (WB) technologies have been available in Nigeria; however cost and the required infrastructure have limited their availability. HIV rapid tests offer a cheaper, simpler and faster alternative. HIV Rapid Test Kits (RTKs) have been used in Nigeria for at least ten years. However, they have not always been used in a systematic or standardized fashion, such as a three-test algorithm as recommended by the World Health Organization (WHO). As of 2003, there was no national HIV rapid testing algorithm in Nigeria (though recommendations did exist in the initial VCT manual). Therefore, PEPFAR adopted the testing algorithm used successfully in past HIV ANC surveys. This was a serial algorithm, using Capillus (for screening) followed by Genie II (for all Capillus positive specimens), and Determine as a tie-breaker (in cases where discordant results were seen between the initial two tests).

The first two tests in this algorithm require refrigeration, and it was apparent that this would hinder expansion of HCT beyond tertiary and secondary healthcare facilities. In early 2006 a temporary move was made toward a non-cold chain dependent testing algorithm. Parallel testing was suggested using any two of the following tests: Determine, StatPak, Bundi, Double Check Gold or Inocheck. None of these tests require refrigeration and have eased the burden of cold chain during transport to and storage at testing sites. These tests are also far easier to run and have allowed Nigeria to move toward the use of trained, non-laboratory staff for HIV diagnostic testing at HCT sites. To resolve discordant results Genie II or Capillus was suggested or clients could be referred to a higher level facility for EIA or WB testing. However, both tie-breaker tests require refrigeration, there were concerns over loss of clients and availability of EIA / WB testing. There still existed a need for a completely non-cold chain dependent algorithm.

No formal evaluation of HIV rapid tests for the development of an algorithm has been conducted in Nigeria. HIV rapid tests have been evaluated individually, but multiple test products have not been evaluated with a single, well-characterized specimen set representative of the entire Country.

AVAILABILITY OF HIV RAPID TEST KITS IN NIGERIA

Currently there are dozens of HIV RTKs available commercially, but not all of these are appropriate for use in Nigeria. The following criteria were developed by the evaluation working group established by GON to decide which HIV RTKs should be evaluated. Criteria have been ranked by order of importance. The first five criteria were deemed by the working group to be the most important (presented below in bold text).

- 1) Stability within the climate in Nigeria, and not dependent on cold chain
- 2) Ability to test whole blood
- 3) Easy to perform and interpret
- 4) Low test price
- 5) Ability of manufacturers to produce and provide adequate numbers of testing kits to meet the needs of testing programs in Nigeria
- 6) Prior experience and validation - documented performance in Nigeria and other African countries
- 7) Ability to detect HIV-1, HIV-2 and HIV- type O subtypes
- 8) Ability to detect IgG and IgM antibodies to reduce the window period
- 9) Do not require additional equipment to run tests or read results
- 10) Packaging of test kits not excessively bulky
- 11) Long shelf life (at least one year) and robust
- 12) Test results provided in at least 30 minutes

There were seven HIV rapid tests which met these criteria. Two additional tests were included in this evaluation for the following reasons. OraQuick meets most of the inclusion criteria but is more costly than other tests (\$4.00) and has a shorter shelf life (6 months). However, members of the working group expressed interest in evaluating this test since it has the capacity to test oral fluid and could be used in settings where oral testing is the only viable option. Bundi was also included; this is a new product and therefore does not have a documented performance record (criteria 6). The capacity of the manufacturer to produce adequate numbers of tests to meet the needs of programs in Nigeria is not known (criteria 5). Bundi was included in this evaluation since it is a locally assembled test product, an important issue for the GON. All evaluated kits are listed in

Table 1 along with test characteristics. NAFDAC registration information is also provided for each test.

PRINCIPLES OF HIV RAPID TEST KITS

All the tests studied in this evaluation are qualitative tests for the detection of antibodies to HIV-1 and HIV-2. All of these tests, with the exception of InstantChek, use immunochromatographic technology (also described as lateral flow). Recombinant and/or synthetic proteins representing the immunodominant regions of the envelop proteins of HIV-1 and -2 (such as glycoproteins gp41, gp120 and gp36) are immobilized in the test regions of a reaction strip (nitrocellulose). A small volume of sample (whole blood, plasma or serum) is added to the sample pad at one end of this strip. This pad acts as a filter to remove red blood cells or other blood solids (such as fibrin clots) and provides a substrate for reconstitution and mixing of the sample with the colloid- antigen conjugate (including either selenium or gold as a colorimetric agent).

Some kits include a buffer which is added just after the specimen, this facilitates the flow of liquid through the strip. As the specimen and conjugate migrate through the strip to the immobilized recombinant antigens at the detection window, a red/purple line is formed if HIV specific Ab is present. If antibodies to HIV are absent, the antigen-selenium colloid flows past the patient window.

To ensure assay validity, a procedural control bar is incorporated in the assay strip, which provides an indication that a specimen has been added to the strip and that fluid is flowing adequately through the device. Typically, test results are interpreted within 15 – 20 minutes.

Many lateral flow strips (Bundi, DoubleCheck Gold, First Response, Stat-Pak and UniGold) are incorporated into a plastic cassette. This is then sealed in foil package to preserve the test from humidity. These cassettes allow for easier labeling and handling of the reaction strip. The manufacturers of SureCheck HIV have gone one step further and encased the strip into a clear plastic tube with a small capillary at one end for specimen loading. This tube completely encloses the strip, preventing contamination of the strip or exposure of the specimen to the tester, while at the same time allowing for easy viewing of the test results.

While OraQuick can test whole blood, plasma and serum, it was primarily designed to test oral fluid. To facilitate this, a collection pad is attached at one end of the device.

The makers of Determine do not use a cassette; instead test strips are attached to a flexible foil backing sealed with a foil cover. Ten tests are attached to create a card. One hundred tests are packed into a single envelope measuring 15 by 25 centimeters. This type of packaging greatly reduces the size and weight of test packages, which in turn reduces transport/shipping costs.

InstantChek uses a different test principle: it is a 'flow through' device. HIV antigens are immobilized on a membrane through which specimens are allowed to flow (on to an absorbent pad). If HIV-specific antibodies are present, they bind to the antigen and the addition of colloidal gold particles results in a red spot. A control spot is also incorporated into the membrane.

Efforts were made to ensure that specimens were contributed from sites in all six of the geo-political zones in Nigeria.

METHOD AND STUDY DESIGN

This is a cross sectional study to determine the accuracy of HIV test results resolved by tie breaker rapid test kits. In Nigeria, Determine rapid test strip is used as first screening for HIV. If Determine reacts negative; the result is declared as negative. If Determine test strip indicates positive, the results has to be confirmed by a second line rapid test kit called Unigold. If the Unigold indicates positive, the test results is concluded as positive and is given to the patients. When the two test results disagree (discordance), then a tie breaker HIV rapid test kit (Stat Pak) is used to resolve the discordance.

All remnant samples of samples whose results were resolved by tie breaker from patients attending 3 hospital laboratories (Defense Headquarter Medical Centre, Abuja, 44 Nigeria Army Reference Hospital Laboratory, and 45 Nigeria Air Force Hospital Laboratory) were collected. A total of 110 remnant samples reacting as positive by Determine but giving a discordance with Unigold were retested collected for this study from January 2013 to July 2013. All the 110 samples were re-tested following serial algorithm and final results confirmed with Western Blot technique. Samples were collected in either plain bottles or EDTA container by staff conducting HIV rapid test and transported/stored at 2-8 degree Celsius. The research was conducted at Defense Reference Laboratory (DRL) using a serial algorithm below:

Screening Test 1	Confirmatory Test 2	Tie-breaker
Determine	Unigold	Stat Pak

The results were tabulated and all the samples tested by Western Blot (New Lav Blot1) to confirm the presence or absence of antibodies against HIV in the sample.

SAMPLE COLLECTION

Blood samples were collected from patients from ante cubital fossa vein. Plasma or serum samples were used for the tests.

TESTING PROCEDURE

After all specimens were initially tested in the selected laboratories and presumptive results were released to patients, the discards specimens were collected and assigned new ID numbers between 1 and 110. Standard Operating Procedures and manufacturers/kit's insert/ instructions for each test were used for each test procedure – Western blot and rapid tests.

QUALITY ASSURANCE

To ensure quality is maintained throughout the testing process, a daily Quality Control panel (HIV Sera Care) of positive and negative vial is used before commencing the testing. In

addition, care was taken to ensure that all validation specimens were of high quality. For this reason, all specimens included in this evaluation met the following criteria:

- Properly collected specimens in line with DRL sample collection SOP,
- Properly processed, no obvious signs of hemolysis, fungal or bacterial contamination/growth
- Properly stored, at -20°C
- Freshly collected specimen, not stored for longer than two months at the collection sites
- Clear HIV Enzyme Immunoassay (EIA) sero-status, positive or negative
- Adequate specimen volume, at least 3 ml

DATA COLLECTION AND ANALYSIS

All test results were collected on paper and entered into a spreadsheet database (MS Excel) for analysis. The sensitivity and specificity of each rapid test were calculated by comparing rapid test results with reference results derived from WB testing.

CALCULATION OF SENSITIVITY AND SPECIFICITY

	WB Gold Standard Positive	WB Gold Standard Negative	
Test Positive	A	B	A
Test Negative	C	D	C
	A + C	B + D	

$$\text{Sensitivity} = A/A+C$$

Defined as the ability of an assay being evaluated to correctly detect specimens containing Ab to HIV. In other words, sensitivity is the percentage of true positive HIV specimens identified by the assay under evaluation as positive (A), divided by the number of specimens identified by the reference assays as positive (A+C).

$$\text{Specificity} = D/B+D$$

Defined as the ability of an assay being evaluated to correctly detect specimens that do not contain Ab to HIV. In other words, specificity is the percentage of true negative specimens identified by the assay being evaluated as negative (D), divided by the number of specimens identified by the reference assays as negative (B+D).

In practice, individual tests are not used for the diagnosis of HIV in patients. International recommendations (WHO, Guidelines for Appropriate Evaluations of HIV Testing Technologies in Africa, page 4) support the use of multiple tests as part of a testing algorithm to improve the overall accuracy of diagnosis.

DATA ANALYSIS

Sensitivities, specificities, positive predictive values and negative predictive values were calculated as described in the HIV Testing Guidelines (5, 6); see Table 3

Table 3: 2 × 2 Table for data analysis

		Results of Gold Standard Test		
		Pos	Neg	
Results of assay	pos	A True Positives	B False Positives	A + B
		C False Negatives	D True Negatives	C + D
Being evaluated	neg	A + C	B + D	A + B + C + D

$$\text{Sensitivity (SENS)} = A / A + C$$

$$\text{Specificity (SPEC)} = D / B + D$$

$$\text{Positive Predictive Value (PPV)} = A / A + B$$

$$\text{Negative Predictive Value (NPV)} = D / C + D$$

In addition, Efficiency (EFF) of the assay, which is the percent of all results that are true results, was calculated as, $EFF = A + D / A + B + C + D$

Sensitivity, specificity, PPV, NPV and efficiencies for serial test algorithms (combination tests) were calculated using the individual sensitivity and specificity values of the RTK assays applied in sequence at several HIV prevalence rates.

In order to calculate positive and negative predictive values for the RTKs at a range of HIV infection prevalence, we use the sensitivity and specificity values calculated from the study data and adjust for the prevalence of disease. The rarer HIV infection may be in a given population, the surer we are that a negative test result indicates no infection, and the less sure we are that a positive

test result indicates infection. Showing the effect of prevalence on the different RTK predictive values is useful to guide clinical as well as policy decisions as the prevalence of HIV infection in persons being tested often varies from the prevalence in the groups used in a study.

The formulas for calculating predictive values are based on Bayes' theorem of conditional probability² and are shown here.

$$\frac{\text{Prev} \times \text{Se}}{\text{Prev} \times \text{Se} + (1-\text{Prev}) (1-\text{Sp})}$$

$$\frac{(1-\text{Prev}) \times \text{Sp}}{(1-\text{Prev}) \times \text{Sp} + \text{Prev} \times (1-\text{Se})}$$

NPV_{Pr} = where PV_{Pr} = predictive value at a known or set prevalence; Prev = true prevalence or the pre-test probability of disease; Se = sensitivity; Sp = specificity

Using these data, a 3 x 3 table was constructed for each RTK at each calculated prevalence level. An example using a prevalence of 0.1% and an RTK with a stated

100% sensitivity and 99% specificity is shown here.

RESULTS

Determine detected HIV antibodies in all the 110 samples collected, while Unigold detected antibodies in only 94. There was discordance in 10 samples. Out of the 10 discordant results the tie breaker (Stat Pak) did not detect antibodies in one of the 10 specimens; thereby agreeing with the second line test kit in 9 specimens. However, when these 10 specimens were subjected to Western Blot confirmation, only 8 samples were found to have antibodies; thus 2 samples were actually confirmed HIV negative by Western Blot. Thus, there were 2 false negative results by the algorithm. Samples that were reactive by Determine were 104, and only 93 results of Stat Pak were concordant with that of Western Blot. On the other hand only 94 results from Unigold were actually agreeing with the results of Western Blot. The result is tabulated as follows:

	Determine	Unigold	Stat Pak	Western Blot
Positive	110	94	93	108
Negative	6	16	17	2
No. False Negative	2	14	15	N/A
Total	110	110	110	110

Using the formulae given above, the following performance parameters were calculated for each rapid test kit.

	Determine	Unigold	Stat Pak	Western Blot
Sensitivity	98.11%	87%	86.11%	
Specificity	96	94	99	
Positive Predictive Value (PPV)	97%	98%	99%	
Negative Predictive Value (NPV)	96%	86%	85%	
Concordance with Western Blot				

Therefore by national algorithm, only 10 samples require the use of a tie breaker, which is Stat pak. The stat pak reacted with only 9 specimens and resolved as reactive. However, when the 10 discordant samples were tested with western blot, 8 samples were confirmed HIV positive while 2 were negative.

DISCUSSION

Human Immunodeficiency Virus (HIV) can lead to Acquired Immune Deficiency Syndrome (AIDS); a condition which renders the immune status of individual severely compromised. It has been estimated that more than 20 million people have died from the disease and more than 40 million people are infected with the virus. The discovery of rapid test kits for HIV have facilitated rapid ways of identifying people infected with the virus faster; cheaper and more accessible especially in low income countries; without the necessarily using sophisticated equipment (Gray, 2007). May publications have revealed the fallacy of using rapid test kits for diagnosis of HIV (Aghokeng, 2009). Thus, the WHO strongly recommended the use of test algorithm to increase test accuracy, where serial or parallel algorithm is used. In Nigeria, serial algorithm is used, where Determine rapid test strip is used as first screening test. If Determine reacts negative; the result is declared as negative. If Determine test strip indicates positive, the results has to be confirmed by a second line rapid test kit called Unigold. If the Unigold indicates positive, the test results is concluded as positive and is given to the patients. When the two test results disagree (discordance), then a tie breaker HIV rapid test kit (Stat Pak) is used to resolve the discordance.

This study discovered that the testing algorithm may still have some short comings compared to Western Blot method of confirming HIV infection. Out of the 10 discordant results subjected to confirmation by tie breaker, 9 were confirmed positive by the tie breaker. However, Western Blot confirmation revealed that only 8 were actually positive. By implication 1 out of every 9 cases resolved by tie breaker will emerge to be a false positive. This accounts for 11% of all HIV test results resolved by tie breaker.

IMPLICATION OF HIV FALSE POSITIVE RESULTS

There are many implications of false positive HIV results in public health context. A woman in Democratic Republic of Congo revealed that her husband divorced her after she was told she was HIV positive; but later she was re-tested and confirmed to be HIV negative. She was in the process

of remarrying a HIV positive from peer group when she was re tested. Other implications include loss of jobs, psychological trauma, loss in self dignity and possible placement on anti retro viral drugs.

LIMITATIONS OF THE STUDY

Limitation of this study is lack of large number of samples yielding discordant results in a testing algorithm to compare its performance with Western Blot.

It is important therefore to verify the HIV seropositivity of every sample resolved by tie breaker by Western Blot.

Advanced research in this area is also very important.

REFERENCES

1. Aghokeng, A., et. al. (2009). "Inaccurate Diagnosis of HIV-1 Groupe M and O is a Key Challenge for Ongoing Universal Access to Antiretroviral Treatment and HIV Prevention in Cameroon." *PLUS One*. 4.11 (2009). Accessed on 2 November 2010. <http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0007702>
2. Gray, R., et al (2007). "Limitations of rapid HIV-1 tests during screening for trials in Uganda: diagnostic test accuracy study." *BMJ*.335.7612 (2007): 335. Accessed on 2 November 2010. <http://www.bmj.com/content/335/7612/188.full>
3. Heneke, M. "An analysis of HIV-related law in South Africa: progressive in text, unproductive in practice." *Transnational Law & Contemporary Problems* 18.3 (2009): 751. Accessed on 9 November 2010. <http://find.galegroup.com.libdb.fairfield.edu/gtx/infomark.do?&contentSet=IAC- Documents&type=retrieve&tabID=T002&prodId=AONE&docId=A211453847&source=gale &srcprod=AONE&userGroupName=a04fu&version=1.0>
4. Lihana R.W., Ssemwanga D., Abimiku A., Ndembi N., (2012). Update on HIV 1 Diversity in Africa: A Decade in Review. *AIDS Rev* 14:83-10. Available [online] at http://www.aidsreviews.com/files/2012_14_2_083-100.pdf
5. McCutchan FE, Carr JK, Bajani M, Sanders-Buell E, Harry TO, Stoeckli TC, Robbins KE, Gashau W, Nasidi A, Janssens W, Kalish ML. (1999). Subtype G and multiple forms of A/G intersubtype recombinant human immunodeficiency virus type 1 in Nigeria. *Virology*., **254**:226-234
6. Peeters M, Esu-Williams E, Vergne L, Montavon C, Mulanga-Kabeya C, Harry T, Ibrionke A, Lesage D, Patrel D, Delaporte E. (2000). Predominance of subtype A and G HIV type 1 in Nigeria, with geographical differences in their distribution. *AIDS Res Hum Retroviruses*. **16**(4):315-25.