REPORT OF LABORATORY PERFORMANCE EVALUATION OF INSTI HIV 1/2 AND INSTI MULTIPLEX HIV 1/2-SYPHILIS ANTIBODY RAPID TEST KITS

FOREWORD

HIV and syphilis are major public health concerns affecting pregnant women and their infants, men who have sex with men (MSM), commercial sex workers, and persons who have multiple sex partners worldwide. Both infections are majorly transmitted through sex, from mother to child and blood transfusion. Co-infection with syphilis can increase the transmission of HIV by both increasing viral shedding through open ulcers and by increasing patient viral load. Mother-to-Child-Transmission (MTCT) of HIV and syphilis has been identified as major cause of perinatal mortality worldwide in the last decade.

Human Immunodeficiency Virus (HIV) infection and Acquired Immune Deficiency Syndrome (AIDS) intervention measures such as Prevention of Mother to Child Transmission (PMTCT), Antiretroviral therapy (ART), HIV Testing Services (HTS), Blood safety and HIV Surveillance put in place 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. Ensuring the quality of HIV testing in support of prevention and care efforts has been identified as priority by the Africa Regional Office of the World Health Organization (WHO/AFRO) and the Nigeria Government.

Rapid test kits (RTKs) evaluation is considered a critical aspect of assuring the quality of test result. It is therefore very important to emphasize that before HIV test kits are utilized, Nigeria evaluates each kit to determine the performance characteristics and suitability for use in- country. However, for development of an effective antenatal program, screening for both HIV and syphilis using a single laboratory test is considered more desirable and as such Insti HIV 1 / 2-Syphilis rapid test kit may be very handy for blood screening and better performing HIV antibody detection devices in the control of HIV/AIDS-Syphilis EepidemicsS in Nigeria.

This Laboratory evaluation determine and confirm the claims of the manufacturer of Insti HIV 1 / 2 and Insti Multiplex HIV 1 / 2-Syphilis rapid test kits and their suitability for use in HIV and Syphilis antibody testing activities in Nigeria.

Rapid test kits (RTKs) evaluation is considered a critical aspect of assuring the quality of test results.

I hereby endorse the recommendations of the NALQAT and approve this report for use in Nigeria.

Professor Isaac F. Adewole, FAS, FSPSP,FRCOG, DSC(Hons)
Honourable Minister of Health

HIV and syphilis are major public health concerns affecting pregnant women and their infants, men who have sex with men (MSM), commercial sex workers, and person/persons who have multiple sex partners worldwide. Both infections are majorly transmitted through sex, from mother to child and blood transfusion. Co-infection with syphilis can increase the transmission of HIV by both increasing viral shedding through open ulcers and by increasing patient viral load. Mother-to-Child-Transmission (MTCT) of HIV and syphilis have been significant causes of perinatal mortality worldwide in the last decade.

The diagnosis, prevention, treatment and management of these diseases require availability of accurate and reliable rapid test devices. However, for development of an effective antenatal program, screening for both HIV and syphilis using a single laboratory test is considered more desirable hence; Insti HIV 1 / 2-Syphilis rapid test kit may be very handy for blood screening. Better performing HIV antibody detection devices remain critical for HIV/AIDS

control activities. This evaluation will determine whether the Insti HIV 1 / 2 and Insti HIV 1 / 2-Syphilis rapid test kits will confirm the claim of the manufacturers and their suitability for deployment into the HIV and Syphilis antibody testing activities in Nigeria.

The protocol developed for the evaluation was reviewed and approved by the National Health Research Ethics Committee.

A panel of well characterized sera collected from the different geo-political zones in Nigeria was used to assess kit performance. A total of 1,506 specimens were used as gold standard comprising of 753 positives and 753 negatives. After evaluation both kits had the same performance for HIV antibody testing with sensitivity of 99.7%; 95% CI (98.9 to 100%) and specificity of 99.7%; 95% CI (98.9 to 99.9%). Insti Multiplex HIV 1 / 2-Syphilis rapid test kit for syphilis antibody testing had sensitivity and specificity of 100% each.

Based on the outcome of this evaluation the following recommendations are made:

1. With a sensitivity and specificity rate of 99.7%, both Insti HIV 1/ 2 and Insti Multiplex HIV 1 / 2-Syphilis antibody rapid test kits should be included in the first

line (screening) test of the algorithm. With a sensitivity and specificity of 100%, Multiplex HIV 1 / 2-Syphilis antibody rapid test kit is hereby recommended for use for the detection of Syphilis antibody in Nigeria.
2. The Insti Multiplex HIV 1 / 2-Syphilis antibody rapid test kit is recommended for use among pregnant women and screening of prospective blood donors for dual detection of HIV and Syphilis antibodies.

1.0 BACKGROUND

Globally, the pandemic of HIV/AIDS has continued to constitute serious health and socio-economic challenges.

The first HIV/AIDS cases in Nigeria were reported in 1986 and since then the HIV/AIDS epidemics have continued to spread and attract due attention.

HIV/AIDS interventions such as PMTCT, ART, HTS, Blood safety and HIV Surveillance put in place 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. Detection of specific antibodies in the blood or other body fluids is the main method of testing for HIV and the standard procedures for diagnosis of HIV infection. 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.

Also, HIV and syphilis are major public health concerns affecting pregnant women and their infants, men who have sex with men (MSM), commercial sex workers, and person who have multiple sex partners worldwide. Both infections are majorly transmitted through sex, from mother to child and blood transfusion. MSM have particularly high rates of HIV and syphilis co-infection, documented to be 47 to 72%

in some areas of the United States. Co-infection with syphilis can increase the transmission of HIV by both increasing viral shedding through open ulcers and by increasing patient viral load. Mother-to-Child-Transmission (MTCT) of HIV and syphilis have been significant causes of perinatal mortality worldwide in the last decade. While the PMTCT of HIV is well-resourced, syphilis often remains undiagnosed and untreated, resulting in more than 520,000 adverse pregnancy outcomes in low-resource countries. The diagnosis, prevention, treatment and management of these diseases require availability of accurate and reliable rapid test devices. The laboratory diagnosis of these infections is based on the demonstration of antibody in serum or plasma and the causative organisms in blood. However, for development of an effective antenatal program, screening for both HIV and syphilis using a single laboratory test is considered more desirable hence; Insti HIV 1 / 2-Syphilis rapid test kit may be very handy for blood screening. Better performing HIV antibody detection devices remain critical for HIV/AIDS control activities. This

evaluation will determine whether the Insti HIV 1 / 2 and Insti Multiplex HIV 1 / 2-Syphilis rapid test kits will confirm the claim of the manufacturers and their suitability for deployment into the HIV and Syphilis antibody testing activities in Nigeria.

2.0 METHODS

2.1 Study Design

This is a prospective cross-sectional study in which the sensitivities and specificities of INSTI HIV1 / 2 and INSTI Multiplex HIV 1/2-Syphilis antibody rapid test kits were evaluated. Blood specimen were collected from pregnant women attending antenatal clinic, clients attending, Sexually Transmitted Infections (STI) clinics and apparently healthy blood donors presenting at blood banks. Plasma were harvested, characterised and used to evaluate the test kits.

2.2 Study Sites

The blood samples were collected from the populations indicated above at the following sites:

Geopolitical Zone	State	Location of Site
South East	Enugu	University of Nigeria Teaching Hospital
South West	Lagos	Lagos University Teaching Hospital
South South	Cross-River	University of Calabar Teaching Hospital
North West	Kano	Amino Kano University Teaching Hospital
North East	Gombe	Federal Teaching Hospital Gombe

North Central	Platea u	Jos University Teaching Hospital

2.3 Sample Size

For the evaluation of HIV antibody testing capabilities of the kits under evaluation ,the sample size were 1,352 and 600 for Syphilis. These sample sizes were derived using the following formula:

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

Where

n = sample size (rounded up to 660 and 1,500 respectively)

Z score= 1.96 at 95% Confidence Interval

p = 16% (the arithmetic mean of the prevalence of HIV in the general population) and less than 1.5% for Syphilis.

q = 1- pc

d = 2% (measure of precision).

i. For Syphilis:

In order to make room for a 95% confidence level and possible attritions, 660 (330 positive and 330 negative) specimen were collected from all the zones and transferred to the reference testing laboratory. This sample size was achieved with each zone providing 110 plasma specimen consisting of 55 positive and 55 negative specimens.

ii. For HIV:

For the purpose of this evaluation, the expected sensitivity is 99% and the error margin fixed at 0.0075. With above specification, a sample size determined for the study was 1,352 rounded up to 1,500 (750 positive and 750 negative) to guide against possibility of invalid specimen in the process of transportation, storage, testing and discordance. The sample size was achieved with each zone providing 250 specimen consisting of 125 each of positive and negative.

2.4 Sample Collection

10mls of whole blood were collected in EDTA vacutainer tubes through veno-puncture from all participants. The samples were centrifuged and the plasma harvested into cryovials in three aliquots of 2mls each and stored at minimal temperature of -20ºC before transportation to the reference testing laboratory. No information were required from the participants as the focus was the test performance. Samples were coded based on the abbreviation of the state where the samples were collected, (EN for UNTH Enugu, LA for LUTH Lagos, CR for UCTH Calabar, KN for AKTH Kano, GM for FTH Gombe, PL for JUTH) and serial number was assigned to each sample.

2.5 Specimen Storage

All specimens from the sites were stored at -200C for short term storage and -800C for long term storage until analysis.

2.6 Specimen Transportation

The specimen were packaged using the triple packaging system with frozen icepacks in cryovial boxes to maintain

cold chain. One aliquot of the sample from each participant was maintained at the collection site as backup. All packaged samples were sent to the reference laboratory by the Laboratory Staff of NASCP within twelve hours of the packaging via air and/or road transportation. All samples transported were accompanied by a sample manifest with a duplicate copy maintained at each collection site.

2.7. Plasma Specimen Characterization and analysis

All the plasma specimen from the six sites were transported frozen to NEQAL, Saye, Zaria for characterization.

2.7.1.HIV testing:

All specimen were tested in parallel using the screening and confirmation test kits in the national testing algorithm (Determine and UniGold). All samples testing concurrently positive and negative were used as gold standard for the evaluation.

2.7.2. Syphilis testing:

All Syphilis specimens were tested using Treponema Pallidum Haem Agglutination (TPHA). All samples testing positive and negative for TPHA were used as gold standard for the evaluation of the INSTI MULTIPLEX syphilis antibody testing.

2.8. Testing of the Kits under Evaluation

i. Trained and proficient Medical Laboratory Scientists performed all the testing activities.

ii. All testing were done strictly according to manufacturers' instructions.

iii. Each Medical Laboratory Scientist rated each kit evaluated using a structured questionnaire. (Reference)

2.9 Data Presentation

Data obtained after testing was presented in the table below and subsequently analysed for sensitivity, specificity, Positive and Negative Predictive Values of the kits under evaluation.

Table 1:Data Collation Table of the Test Results Of Kits Under Evaluation

Test Result of kit under evaluation	Gold Standard (Positive)	Gold Standard (Negative)	Total
Positive test	A (True Positive)	B (False Positive)	A + B
Negative Test	C (False Negative)	D (True Negative)	C + D
Total	A +C	B +D	

2.9 Data Analysis

Sensitivity was calculated by dividing true positives of the kits under evaluation by total gold standard positives (A/(A+C)).

Specificity was calculated by dividing true negatives of the kit under evaluation by total gold standard negatives (D/(B+D)).

Positive Predictive Value (PPV) was calculated by dividing true positives by total positives tested by the kits under evaluation (A/(A+B)).

Negative Predictive Value (NPV) was calculated by dividing true negatives by total negative tested by the kit under evaluation (D/(C+D)).

2.10. Specimen Archiving

All plasma brought to the reference laboratory were stored at -800C and shall remain so stored for a minimum of five years after completion of the evaluation exercise.

3.0 RESULTS AND ANALYSIS

A total of 1,510 plasma specimen was received from the six study sites. During characterization at the reference lab (NEQAL, Saye Zaria), four specimen had discordant test results with the two test kits used to determine the gold standard. They were therefore excluded. We therefore ended up with 1,506 specimen as gold standard. After characterization, we ended up with 753 as positive standard and 753 also as negative standard. These were used to evaluate the two Insti test kits. The Insti HIV 1/2 test kit during testing had one that tested invalid.

Table 2: HIV Antibody Test Result with Insti HIV 1 / 2 Rapid Test Kit

INSTI HIV 1/2	Gold Standard (Positive)	Gold Standard (Negative)	Total
Positive test	751	2	753
Negative Test	2	751	753
Total	753	753	1506

Performance of Insti HIV 1 / 2 Rapid Test Kit

Sensitivity = 99.7%; 95% CI (98.9 to 100%)

Specificity = 99.7%; 95% CI (98.9 to 99.9%)

Positive Predictive Value = 99.7%

Negative Predictive Value = 99.7%

Note: one test device had invalid test.

Table 3: HIV Antibody Test Result with Insti Multiplex HIV 1 / 2-Syphilis Rapid Test Kit

INSTI MULTIPLEX HIV 1 / 2-Syphilis	Gold Standard (Positive)	Gold Standard (Negative)	Total
Positive test	751	2	753
Negative Test	2	751	753
Total	753	753	1506

Performance of HIV Antibody Test Result with Insti Multiplex HIV 1 / 2-Syphilis Rapid Test Kit

Sensitivity = 99.7%; 95% CI (98.9 to 100%)

Specificity = 99.7%; 95% CI (98.9 to 99.9%)

Positive Predictive Value = 99.7%

Negative Predictive Value = 99.7%

Table 4: Syphilis Antibody Test Result with Insti Multiplex HIV 1 / 2- Syphilis Rapid Test Kit

INSTI MULTIPLEX HIV 1 / 2-Syphilis	Gold Standard (Positive)	Gold Standard (Negative)	Total
Positive test	18	0	18
Negative Test	0	428	428

Total	18	428	446

Performance of Syphilis Antibody Test Result with Insti Multiplex HIV 1 / 2- Syphilis Rapid Test Kit

Sensitivity = 100%

Specificity = 100%

Positive Predictive Value = 100%

Negative Predictive Value = 100%

Note: After random sampling from sites, only two syphilis antibody positive specimen were confirmed. This number was too small to be used for the analysis. We therefore obtained known sero-positive plasma, which were further characterised and confirmed ones formed part of the positive gold standard.

Table 5: Summary of Testers' Rating of Insti HIV 1/2 and Insti Multiplex HIV 1/2-Syphilis Antibody Rapid Test Kits

Question	1 Spec. Add. to device	2 Dilu. Add.	3 Reading result	4 Result interpre	5 Learning the test	6 Ease of use	7 Labelling space	8 Kit packaging	9 Waste Gen.

Average Score	1.9	1.6	1.1	1.3	1.3	2.1	2.1	1.3	2,0

The maximum score attainable was 55. The median global score from all the testers for this test kit was 46.0 (84%)

Composite Score

The accuracy of the test kit was assigned a weight of 70% while the global score (based on testers' ratings) was assigned the weight of 30%. A composite score was determined as weighted mean of accuracy and global score. The composite score for Insti HIV 1/2 and Insti Multiplex HIV 1/2-Syphilis for HIV antibody testing is 95% and for Syphilis antibody testing is 95.2%.

4.0 Discussion

It is important to determine the laboratory performance characteristics of test devices so it can be appropriately deployed for routine testing. Key performances to be monitored closely are the ability of the device to detect analyte (sensitivity) without any interference from 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.

This evaluation showed that Insi HIV 1 / 2 and Insti Multiplex HIV 1 / 2-Syphilis antibody rapid test kits were

able to detect antibody to HIV with equal performance characteristics. The addition of ability to detect Syphilis antibody in Insti Multiplex HIV 1 / 2-Syphilis antibody rapid test kit did not affect its performance for the detection of HIV antibody. Insti Multiplex HIV 1 / 2-Syphilis antibody rapid test kit performed very well in its ability to detect Syphilis antibody Insti Multiplex HIV 1 / 2-Syphilis antibody rapid test kit can be comfortably deployed to where concurrent detection of these two antibodies may be necessary.
It seems also, that the ability to detect HIV antibody did not affect its capacity for Syphilis antibody detection.
The performance of these test kits has met the WHO requirement for use in HTS. For the Insti Multiplex HIV 1 / 2-Syphilis antibody rapid test kit, the performance characteristics obviously shows it as a very good test device for Syphilis antibody test detection. However, because of the low prevalence of Syphilis, it was difficult to have enough positive specimen for this evaluation. The small number of positive specimen may have, possibly, not presented an accurate sensitivity for the detection of Syphilis antibody.

Analysts were asked through a structured questionnaire to rate ease of performing the test, clarity of reading and interpretation of the test result, ergonomics, etc. These factors can affect outcome of test result with test devices. This process is called global rating. They are mathematically weighted and it contributes to rating if a device can be used for testing irrespective of its sensitivity

and specificity. When sensitivity and specificity (accuracy) is combined with the global rating, the score is referred to as composite score. Apart from meeting specified requirement for sensitivity and specificity, a kit must also have a minimum of 90% composite score to be deployed for Laboratory and Point of Care testing.

5.0 Recommendations and Conclusion

The performance characteristics of Insti HIV 1/ 2 and Insti Multiplex HIV 1 / 2-Syphilis antibody rapid test kits for detection of antibodies to HIV makes the two devices suitable for inclusion in our HIV testing algorithm. With a sensitivity and specificity rate of 99.7%, both Insti HIV 1/ 2 and Insti Multiplex HIV 1 / 2-Syphilis antibody rapid test kits should be included in the first line (screening) test of the algorithm. With a sensitivity and specificity of 100%, Multiplex HIV 1 / 2-Syphilis antibody rapid test kit is hereby recommended for use for the detection of Syphilis antibody in Nigeria.

The use of Insti Multiplex HIV 1 / 2-Syphilis antibody rapid test kit is recommended for use among pregnant women and screening of prospective blood donors for dual detection of HIV and Syphilis antibodies.

6.0 References

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7.0 Appendices

Please rate the RTK using the following scoring system:

1 (Excellent); 2 (Good); 3 (Average); 4 (Poor); 5 (Very Poor)

1. Specimen addition onto the device.
2. Addition of diluents / wash/ buffer correctly onto the device.
3. Reading the test result.

4. Interpretation of the result.
5. Ease of learning how to perform the test.
6. Overall ease of use.
7. Design of the test device for adequate labelling.
8. Kit packaging.
9. Waste generated.