HIV DIAGNOSTIC ALGORITHMS IN A RESOURSE-LIMITED SETTING: A COMPARATIVE STUDY

ABSTRACT

Background: Adoption of appropriate HIV testing algorithm is essential for quality HIV diagnostic results. Whether rapid enzyme immunoassay (rEIA) or combination of rapid enzyme immunoassay and enzyme-linked immunosorbent assay (rEIA-ELISA) alternative confirmatory algorithm is enough to make accurate HIV diagnosis has been a subject of controversies. Recent evaluation of national algorithm for HIV testing presented some discrepancies in results. Again, current guideline prescribed discontinuity of Western blot supplemental test and its replacement with Geenius HIV-1/ HIV-2 differentiation test followed by nucleic acid testing. The objective of this study is to compare HIV-1 testing algorithms using ELISA-NAT algorithm as the gold standardfor HIV-1 diagnosis, and rEIA-Western blotand ELISA-Western blotas alternative confirmatory algorithms for HIV-2 diagnosis.

Methods: This is a comparative study involving a cohort of173(98.9%) HIV-positive subjects and two (1.1%) HIV-negative subjects initially diagnosed on the basis of rEIA algorithm. Plasma samples separated from ethylene diamine tetra-acetic acidanticoagulated blood were used for further analyses. Fourth generation HIV antigen-antibody kit was used for further testing to make up rEIA-ELISA algorithm. Analyses with Western blot 1 and 2 assays were done as supplemental tests to make up alternative rEIA-Western blot and ELISA-Western blot confirmatory algorithms. Research subjects were further tested for HIV-RNA under PEPFAR plan. The sensitivity, specificity, positive predictive value and negative predictive value of algorithms were compared using the descriptive statistics of the SPSS version 17.

Results: The HIV testing analyses showed 100% sensitivity, specificity, positive predictive value and negative predictive value from algorithm to algorithm especially for HIV-1 diagnosis.Use of combined ELISA-NAT confirmatory algorithm validated outcomes of rEIA-Western blot and ELISA-Western blot alternative confirmatory algorithm for HIV-1 diagnosis. Among HIV-1 positive samples tested, 50.0-60.7% HIV-2 Western blot indeterminate result was obtained and 7.1-10.7% HIV-1/HIV-2 co-infections were observed.

Conclusion: TherEIA or national algorithm andits combination with enzyme-linked immunosorbent assay are reliable alternative confirmatory algorithms for the diagnosis of established HIV-1infection in resource-limited settings but not for HIV-2. Diagnosing acute HIV infection with current algorithm is limited and still require further review.

Key words: Enzyme immunoassay, enzyme-linked immunosorbent assay, Western blot, indeterminate results, algorithm, sensitivity, specificity

1.0 Introduction

Human immunodeficiency virus (HIV), the causative agent of AIDS has constituted a major public health and socio-economic challenge in the last four decades. An epidemic update in 2022 reported that a global estimate of about 39.0 million (33.1 - 45.7 million) persons are living with HIV compared to 26.6 million (22.6 – 31.2 million) in 2000 [1-2]. Despite current reports of continued reductions in the annual incidence of HIV infection and the benefits of a major breakthrough of expanded access to antiretroviral therapy and reduction in AIDS related mortality, there is continued transmission of HIV. Of the global data reported, World Health Organization(WHO) Africa Region constitute two-third of all people living with HIV as it accounts for 26.5 million (67%) of the global data[1]. While countries across the globe (Nigeria inclusive) are striving to meet up with the United NationsProgramme on HIV/AIDS 95-95-95SustainableDevelopmentGoal (SDG) ofsuccessfully testing 95%of population, placement of 95% of people living with HIV on antiretroviral drugs and achieving 95% viral remission, assessment of algorithms for HIV diagnosis is very crucial[2]. There have been debates on sufficiency of rapid enzyme immunoassay-based algorithms for HIV-1 diagnosis [3]. Findings from a household-based study that evaluated the performance of HIV rapid testing algorithm through a Nigeria HIV/AIDS Indicator and Impact Survey (NAIIS) showed a national HIV prevalence of 1.4% compared to HIV prevalence of 4.1% reported in 2010 [4-5],

representing 65.8% reduction in HIV prevalence between 2010 and 2022. One key point of concern was that same study showed high discordant rates (approximated 43.7%) between Determine and Unigold rapid test devices (the two key HIV-1/2 screening kitsadopted by Nigeria for HIV diagnosis),andpositive predictive value (PPV of94.5%) of the national algorithm with a false positive rate of about 5.5%. Another study reported a very good performance of the national HIV serial testing algorithm with only 1.1% discordance rate between Determine and Unigold rapid test devices observed in the retrospective component of the study [6-7]. Discrepancies in reports require further studies on the national HIV rapid serial testing algorithm.

Early HIV diagnosis and early knowledge of HIV status can result in tremendous public health benefits through decreasing risk behaviours that could transmit HIV to uninfected persons [7-13] and when issues of HIV sero-discordance in relationships are considered [14]. Brookmeyer observed that a person infected with HIV is projected to develop AIDS in about 10 years, without treatment (1991) [15]. However, with early treatment, a 25-years-old adult can survive, on average 39 more years [16]. This makes rapid testing and early access to care essential to combating the HIV/AIDS pandemic. Therefore, rapid testing remains the entry point to HIV prevention, treatment, care and support due to its advantage of relative ease of performance, cost of testing, qualified personnel requirement and faster turnaround time of less than 30 minutes[17]. However, current prescriptions on HIV testing require continued evaluation of alternative algorithms still in use especially in resource-limited settings to assess their diagnostic performances. This study seeks to evaluate four alternative algorithms namely; rapid enzyme immunoassay (or rEIA algorithm)which is in current use as national algorithm (first algorithm),

combination of rapid EIA and enzyme-linked immunosorbent assay for p24 antigen detection and antibodies to HIV-1/2 (rEIA-ELISA- second algorithm), rEIA with Western blot assay as the supplemental test (rEIA-WB- third algorithm) and ELISA test for p24 antigen detection and antibodies to HIV-1/2 with Western blot as the supplemental test (ELISA-WB – fourth algorithm) using combination of ELISA and nucleic acid testing (ELISA-NAT algorithm) as the gold standard for HIV-1 diagnosis; and rEIA-WB or ELISA-WB alternative confirmatory algorithm was used as the gold standard for HIV-2 diagnosis.

2.0 MATERIALS AND METHODS

2.1 Study Site

This study was performed at the Federal Teaching Hospital, Ido-Ekiti, a tertiary health institution and HIV/AIDS referral and treatment centre located at the headquarter of Ido/Osi local government with an estimated population of 106, 792 people, and serving thousands of patients accessing HIV/AIDS care throughout Ekiti State and neighbouring states such as Kogi, Kwara, Osun and Ondo state. Ido-Ekiti is located 25km from Ado Ekiti, the state capital, and nearly 376.5km to Abuja. The institution is currently the serving Teaching Hospital for Afe Babalola University, Ado Ekiti, Nigeria and Federal University, Oye Ekiti, Nigeria for the training of healthcare professionals including Medical, Nursing, Radiography and Medical Laboratory Science students.Ekiti State is in the South-western region of Nigeria and located between longitudes 4° 45° and 5° 45° East of the Greenwich meridian and latitudes 7° 15° and 8° 15° North of the equator.

2.2 Study Design

This is acomparative study of HIV testing algorithms involvinga cohort of 175 research subjects (173 newly diagnosed HIV-positive and 2 HIV-negative patients) based on national algorithm for HIV testing adopted by the Federal Ministry of Health and the UMD-CDC serial algorithm II [6, 7,18,19-21]. Aliquots of plasma separated from 5mls of EDTA anticoagulated blood collected by venipuncture from the patients initially diagnosed according to the r EIA national algorithm were used for further testing to allow for algorithm comparison [7]. The plasma samples were separated within four hours of collection and placed in aliquots of 1.5mls each for ELISA, Western blot and nucleic acid testing.

2.3 Informed Consent/ Ethical Approval

This research was carried out at the haematology department of the Federal Teaching Hospital, Ido Ekiti (FETHI) following informed consent from participating research subjects and the ethical approval from the Human Research and Ethics Committee (HREC) of Federal Teaching Hospital, Ido Ekiti.Due to the fact that all specimens used in this study hadlinkageto personal identifiers, this study was determined by the authors to be mainly for research not involving identifiable human subjects.

2.4 HIV Testing by Rapid Enzyme Immunoassay (rEIA or the National Algorithm)

The Federal Ministry of Health adopted the Centre for Disease Control and Prevention (CDC) serial algorithm II guideline on HIV-1/2 diagnosis for HIV rapid antibody techniques which prescribed HIV 1 / 2 antibodies testing with Determine kit (*Alere Medical Co. Ltd, Chiba, Japan*) as the first line screening test (RT1). Non-reactive result

by Determine ended the testing protocol. Research samples reactive for HIV 1/2 were repeated with Unigold kit (RT2) as the second line of testing (Trinity Biotech, Wicklow, Ireland) which works based on immunochromatographic 'sandwich' principle. Discordant results were first repeated by a superior research scientist and where indeterminate results were obtained, tie breaker kit, Stat-Pak (Chembio Diagnostic Systems Inc, Medford, NY, USA) which also works based on immunochromatographic principle but adopts lateral flow technology for HIV antibody detection was used. Stat-Pak is the only prequalified WHO rapid test device that exceeds WHO performance thresholds and with 100% sensitivity and 100% specificity[22].Analyses were carried out according to manufacturer instructions. The final resultsgave the HIV diagnosis of the patients based on the national algorithmand were compared with those obtained from sandwich ELISA technique and Western blot assays.

2.5 Biorad Genscreen ULTRA HIV Ag-Ab Assay (ELISA ASSAY)

The 175 samples were further tested with fourth generation Genscreen™ ULTRA HIV Ag-Ab ELISA kit which was designed for the simultaneous detection of HIV p24 antigen and antibodies to HIV-1 (groups M and O) and HIV-2 in human serum or plasma. That enabled the evaluation of combined rEIA and ELISA algorithm on HIV testing [20,23-24].

2.6 Western Blot Supplemental Assay and Quality Control Measures (Confirmatory Test)

The NEW LAV BLOT 1 and 2 kits are designed to detect human anti-HIV-1 and anti-HIV-2 antibodies in serum or plasma by immunoblotting in order to confirm a positive

anti-HIV-1 or anti-HIV-2 response and specify its antigenic specificity within the scope of AIDS diagnosis[25]. The assay was performed in this study as a supplemental test for HIV confirmation in order to evaluate the diagnostic performance of the rEIA and rEIA-ELISA alternative algorithms using both rEIA-WB and ELISA-WB as alternative confirmatory and gold standard for HIV-2 diagnosis. Its performance was evaluated for HIV-1 diagnosis using ELISA-NAT as the gold standard for HIV-1 diagnosis. The Western blot assays were performed for HIV-1 and HIV-2 according to the manufacturer instructions. The prescribed precautions were followed as quality controls to prevent cross-reactivity, subjective or technical errors that might be associated with assay procedures. Besides, known positive and negative controls supplied with the kit were performed in parallel with the research samples for each test batch run in order to validate test results and correctly interpret the bands.

NEW LAV BLOT I and II Interpretation

Biorad NEW LAV BLOT I and II kits for HIV-1 and HIV-2 respectively typically contain the specific proteins shown in Table 1below.

Table 1: Specific Protein Bands on Biorad Western Blot I and II Confirmatory Kits

NEW LAV BLOT I Strips Protein Bands:	gp160, gp110/120, p66, p55, p51, gp41, p40, p31, p24 and p18
NEW LAV BLOT II Protein Bands:	gp140, gp105/125, p68, p56, gp36, p34, p26 and p16

Key: gp= Glycoproteins; p= Protein

The interpretations of the results were based on the World Health Organization (WHO) and Consortium for Retrovirus Serology Standardization (CRSS). While WHO criterion relied on two of the envelope proteins (gp160, gp110/120, gp41) with or without one of the core proteins (p55, p40, p24 and p18) or enzyme proteins (p66, p51 and p31) as the minimum requirement for a HIV-1 positive result, CRSS like most other regulatory bodies relies on one of the envelope proteins with one of core proteins or enzyme proteins as the minimum criteria for a positive result [26-27].Incomplete banding profiles of tested strips showing specific reactivity to any of the viral proteins not compatible with the approved criteria for a positive interpretation are thus considered indeterminate. Absence of all bands except the control band of the test strip has been universally accepted to imply a negative result. Some regulatory bodies including WHO has advocated that band showing weak reaction to p17 be also interpreted as negative result. Same WHO and CRSS criteria were used in interpreting results of NEW LAV BLOT II for plasma samples analysed for HIV-2. The WHO and CRSS criteria for interpreting WB results were compared with the FDA-approved guideline for Du Pont WB licensed kit for HIV-1, CDC, American Red Cross (ARC) and the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) criteria.Thecriteria of different regulatory bodies for interpreting Western Blot HIV-1 and HIV-2 positive, negative and indeterminateresults are shown in Tables 2 and 3.

Table 2. Criteria for Interpreting Western Blot Results for HIV-1

Regulatory Body	Minimum Criteria for HIV-1 Positive WB Result	Criteria for HIV-1 Negative WB Result	Criteria for HIV-1 Indeterminate WB Result
CDC	At least two of p24, gp41, gp120/160	No band at all	Incomplete banding profiles that do not meet the prescribed

			criteria for positive result
WHO	Two env Bands	No HIV-specific band	Incomplete banding profiles that do not meet the prescribed criteria for positive result
CRSS	1 Env + 1 Pol or 1 Gag band	No band at all	Incomplete banding profiles that do not meet the prescribed criteria for positive result
ARC	1 Env + 1 Pol +1 Gag band	No band at all	Incomplete banding profiles that do not meet the prescribed criteria for positive result
ASTPHLD	Any two of p24, gp41, gp120/160	No band at all	Incomplete banding profiles that do not meet the prescribed criteria for positive result
FDA	p24, p31 and gp41 or gp120/160	No band at all	Incomplete banding profiles that do not meet the prescribed criteria for positive result

Key: CDC: Centre for Disease Control and Prevention; WHO: World Health Organization; CRSS: Consortium for Retrovirus Serology Standardization; ARC: American Red Cross; ASTPHLD: Association of State and Territorial Public Health Laboratory Directors; Env: Envelope; Pol: Reverse transcriptase; Gag: Group antigens; gp: Glycoprotein; p: protein

Table 3. Criteria for Interpreting Western Blot Results for HIV-2

Regulatory Body*	Minimum Criteria for HIV-2 Positive WB Result	Criteria for HIV-2 Negative WB Result	Criteria For HIV-2 Indeterminate WB Result
CDC	At least two of p26, gp36, gp105	No band at all	Incomplete banding profiles that do not meet the prescribed criteria for positive result
WHO	Two Env Bands	No HIV-specific band	Incomplete banding profiles

prescribed			that do not meet the criteria for positive result
CRSS prescribed	1 Env + 1 Pol or 1 Gag	No band at all	Incomplete banding profiles that do not meet the criteria for positive result
ARC prescribed	1 Env + 1 Pol +1 Gag band	No band at all	Incomplete banding profiles that do not meet the criteria for positive result
ASTPHLD prescribed	Any two of p26, gp36, gp105	No band at all	Incomplete banding profiles that do not meet the criteria for positive result

Key: CDC: Centre for Disease Control and Prevention; WHO: World Health Organization; CRSS: Consortium for Retrovirus Serology Standardization; ARC: American Red Cross; ASTPHLD: Association of State and Territorial Public Health Laboratory Directors; Env: Envelope; Pol: Reverse transcriptase; Gag: Group antigens; gp: Glycoprotein; p: protein

*****No FDA-criteria for interpreting HIV-2 results by Western blot was discovered during the course of this study

Nucleic acid testing (NAT)

Further analysis was done for 105(of the samples randomly selected for HIV-RNA detection for HIV-1 confirmation using COBAS AmpliPrep/COBAS Taqman polymerase chain reaction (real-time PCR) through PEPFAR plan. Combined results of ELISA and NAT served as the gold standard for HIV diagnosis.

Statistical Analysis

Data generated from the study were analysed with the descriptive statistics of the SPSS (Statistical Package for Social Sciences Inc. Chicago, USA) and the sensitivity and positive predictive value of the of the algorithms were derived.

3.0 Results and Discussion

3.1.Rapid Enzyme Immunoassay Alternative Confirmatory Algorithm for HIV-1/2 Diagnosis (National Algorithm)

Based on the rEIA or the national algorithm, 173 gave concordant positive results with Determine (RT1) and Unigold kits implying final positive test results. One showed concordant negative results with both Determine and Unigold, implying a final negative result. One showed discordant results with RT1 and RT2 and was further repeated with Stat-Pak (RT3), negative result was obtained. That implied that based on the use of rapid kits as sole enzyme immunoassays (EIA) for this alternative algorithm without any supplemental test, 173 (98.9%) of tested samples were positive for HIV 1/2 as shown in Table 4.

Table 4: Rapid Enzyme Immunoassay Alternative Confirmatory Algorithm for HIV-1/2 Diagnosis

Total no. of samples tested	Determine positive	Determine Negative	Unigold Positive	Unigold Negative	Discordant result	Stat-Pak positive	Stat-Pak Negative	**Final Result:** Pos. (%) Neg. (%)
175	174	1	173	2	1	0	1	**173(98.9) 2(1.1)**

Key: % = Percentage

3.2 Combined Rapid Enzyme Immunoassay-ELISA Alternative Confirmatory Algorithm (rEIA-ELISA) for HIV-1/2 Diagnosis

Similarly,the combined rEIA-ELISA algorithmresulted in 173 (98.9%)HIV-1/2positiveand 2(1.1%) negative test results as shown in Table 5.

Table 5: Rapid Enzyme Immunoassay-ELISA Alternative Confirmatory Algorithm for HIV-1/2

Diagnosis

Total no. of samples: N (%)	ELISA positive: N (%)	ELISA negative: N (%)	Final Result of rEIA: Pos.: N (%) Neg.: N (%)	rEIA-ELISA Alternative Confirmatory AlgorithmResults: Positive: N (%) Negative.: N (%)
175 (100)	173 (98.9)	2 (1.1)	173 (98.9) 2 (1.1)	173(98.9) 2(1.1)

Key: N = Absolute number; % = Percent; ELISA = Enzyme-linked immunosorbent assay;
rEIA= Rapid enzyme immunoassay;
rEIA-ELISA= Rapid enzyme immunoassay-Enzyme-linked immunosorbent assay

3.3 Western Blot Assay (Biorad NEW LAV BLOT-1) Results and rEIA-WB Alternative Confirmatory Algorithm for HIV-1 Diagnosis

Table6showed the results of Western blot confirmatory test with NEW LAV Blot I for HIV-1 confirmation. Out of the 175 samples tested, 173 (98.9%) were HIV-1 positive irrespective of the criteria used. Therefore,the results of the rEIA and ELISA could be attributed to HIV-1 seropositivity. More than 95% (165/173) of samples that tested positive showed reaction and expressed nearly all the protein bands on the nitrocellulose membrane strips. Nearly 3.0% (5/173) of HIV-1 positive samples tested did not express gp41. Interestingly, no HIV-1 Western blot indeterminate (WBi) results were obtained in this study. Whether rEIA-WB or ELISA-WB algorithm was adopted, the results for HIV-1 were consistent, showing the recommended WHO alternative confirmatory algorithm to be of similar sensitivity and specificity.

Table 6: Western Blot Assay (BIORAD NEW LAV BLOT-1) Results and Rapid EIA-WB / ELISA-WB Alternative Confirmatory Algorithm for HIV-1 Diagnosis

Total no. of samples tested: N (%)	HIV-1 positive: N (%)	HIV-1-negative N (%)	Final Result of Western Blot 1 Pos.: N (%)	Neg.: N (%)	rEIA-WB or ELISA-Western Blot Alternative Confirmatory Algorithm Pos.: N (%)	Neg.: N (%)
175 (100)	173 (98.9)	2 (1.1)	173 (98.9)	2 (1.1)	173(98.9)	2(1.1)

Key: N = Number of samples tested; % = Percent; Pos.: Positive; Neg. Negative

3.4 Assessment of Alternative rEIA and rEIA-ELISA Algorithm Based on Serial rEIA-WB/ ELISA-WB Alternative Confirmatory Algorithm for HIV-1

Assessment of the sensitivity, specificity, positive predictive value and negative predictive value of the alternative algorithms based on the use of either the combination of rEIA and Western blot assay or ELISA and Western blot assay confirmatory algorithm showed 100.0% each for sensitivity, specificity, positive predictive value and negative predictive value. Table 7 showed the results. Irrespective of algorithm used for interpretation, 173 (98.9%) was HIV-1 positive and 2 (1.1%) was negative for HIV-1.

Table 7: Assessment of Alternative rEIA and rEIA-ELISA Algorithm Based on Serial rEIA-WB/ ELISA-WB Alternative Confirmatory Algorithm for HIV-1

Algorithm Variables	Total Number of Samples tested	HIV-1 Pos. Cases	HIV-1 Neg. Cases	Sensitivity %	Specificity %	PPV %	NPV %
	Frequency (%)	Frequency (%)	Frequency (%)				

rEIA (National Algorithm)	175 (100.0)	173 (98.9)	2 (1.1)	100.0	100.0	100.0	100.0
rEIA-ELISA Algorithm	175 (100.0)	173 (98.9)	2 (1.1)	100.0	100.0	100.0	100.0
rEIA-Western Blot Alternative - Confirmatory Algorithm	175 (100.0)	173 (98.9)	2 (1.1)	-	-	-	-
ELISA-Western Blot Alternative Confirmatory Algorithm	175 (100.0)	173 (98.9)	2 (1.1)	-	-	-	-

Key: Positive predictive value; NPV= Negative predictive value; % = Percentage; WB = Western blot; rEIA = Rapid Enzyme immunoassay; ELISA = Enzyme-linked immunosorbent assay; Pos. = Positive; Neg. = Negative

3.5 Protein Band Patterns of Western Blot Assay Indeterminate results

Based on WHO criteria,17/26 (60.7%) showed indeterminate results and9/26 (32.2%) were confirmed negative for HIV-2. NEW LAV BLOT 2 band patterns (not shown in Table 6)showed 3/17 (10.7%) WBiwere due to the expression of p68, p26 and p16 protein bands; 1/17 (3.6%) expressed p68 and p26 bands; 4/17 (14.3%) expressed p26 and p16; 6/17 (21.4%) expressed p26 protein band; 1/17 (3.6%) expressed the gp105 and p26 bands, 1/17 (3.6%)expressed p68, p56, gp36, p26 and p16 bands and another 1/17 (3.6%)expressed gp105, p68, p26 and p16. No HIV-2 positive case based on WHO criterion.

Based on CRSS criteria, 3/26 (10.7%) was positive for HIV-2. The Western blot assay showed p68, p56, gp36, p26 and p16; gp105, p68, p26 and p16; and gp105 and p26 protein bands respectively. Exactly 14/26 (50.0%)gave indeterminate results and the rest 9/26 (32.2%) were confirmed HIV-2 negative. Of the 50% WBi results, 3/14 (10.7%)

were due to the expression of p68, p26 and p16 protein bands; 1/14 (3.6%) expressed p68 and p26 bands; 4/14 (14.3%) expressed p26 and p16; and 6/14 (21.4%) expressed p26 protein band. Using ARC criteria for interpretation, 2/28(7.1%)were positive; 15/26 (53.6%) were indeterminate and the remaining 9/26 (32.2%) were confirmed negative for HIV-2.Table 8showed theHIV-2 Western blot indeterminate results.

Table 8: Patterns of Western Blot Supplemental Assay Protein Bands for HIV II Indeterminate Results

Protein Bands Patterns	Western Blot Criteria Interpretations				
	WHO	CRSS†	ARC	WHO	CRSS†
ARC	Pos. (%)	Pos. (%)	Pos. (%)	WBi (%)‡	WBi (%)
WBi (%)					
p68, p26 and p16	0	0	0	10.7	10.7
p68 and p26	0	0	0	3.6	3.6
p26 and p16	0	0	0	14.3	14.3
p26	0	0	0	21.4	21.4
gp105 and p26	0	3.6	0	3.6	0
p68, p56, gp36, p26 and p16	0	3.6	3.6	3.6	0
gp105, p68, p26 and p260	3.6	3.6	3.6	0	0
Overall Protein Bands Expressed	0	10.8‡	7.2‡	60.8‡	50.0 53.6

Key: WHO: World Health Organization; CRSS†:Consortium for Retrovirus Serology Standardization and other criteria for WB interpretations such as CDC: Centre for Disease Control and Prevention; ASTPHLD:Association of State and Territorial Public Health Laboratory Directors; ARC: American Red Cross; Pos.: Positive; ‡ = Approximated figures are higher than real figures by 0.1

3.6 Western Blot HIV-2 Indeterminate and HIV-1/HIV-2 Co-infection Patterns among Randomly Selected HIV-1 Research Subjects

Twenty-eight samples (100.0%), consisting of 26 (92.9%) HIV-1 randomly selected samples and 2 (7.1%) plasma samples already established HIV-1 negative, were tested for HIV 2 in parallel with the manufacturer supplied positive and negative controls. Overall, out of the 28 (100.0%) HIV-1 randomly selected samples tested, HIV-2 WBi results ranged from 14 – 17 (50.0 – 60.7%); 11 (39.3%) were HIV-2 negative while HIV-1/HIV-2 coinfection ranged from 2 – 3 (7.1-10.7%). The HIV-2 positivity was on the basis of interpretive criteria other than WHO criteria. The WHO criteria showed no HIV-1/HIV-2 co-infection of 0%. HIV-1/HIV-2 coinfection rate of 10.7% were obtained based on CRSS, CDC, ASTPHLD interpretive criteria; and 7.1% on the basis of American Red Cross criterion.

Based on manufacturer's prescribed WHO and CRSS criteria, and when compared with other criteria for interpreting WB negative result, the exact two samples, 2/28 (7.1%) that tested negative for HIV 1/2 antibodies when rEIA, ELISA and NEW LAV BLOT I assay kits were used also tested negative with NEW LAV BLOT II kit.

The summary ofHIV-1/HIV-2 co-infection and indeterminate results among the randomly selected research subjects based on different criteria of interpretationwere shown in Table 9.

Table 9: HIV-2 Western Blot Indeterminate and Coinfection Patterns among Randomly Selected HIV-1Positive Subjects Using Different Interpretive Criteria

Regulatory Body*	HIV-1Negative samples tested	HIV-1 Positive samples tested	HIV-2 Indeterminate	HIV-2 Positive	HIV-2 Negative	HIV-1/HIV-2 Coinfection

	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)	Frequency (%)
ALL	2 (7.1)	26 (92.9)	14 (50.0) - 17 (60.7)	2 (7.1) - 3 (10.7)	11 (39.3)	2 (7.1) - 3 (10.7)
WHO	-	26 (92.9)	17 (60.7)	0 (0)	9 (32.2)	0 (0)
CRSS	-	26 (92.9)	14 (50.0)	3 (10.7)	9 (32.2)	3 (10.7)
CDC	-	26 (92.9)	14 (50.0)	3 (10.7)	9 (32.2)	3 (10.7)
ASTPHLD	-	26 (92.9)	14 (50.0)	3 (10.7)	9 (32.2)	3 (10.7)
ARC	-	26 (92.9)	15 (53.6)	2 (7.1)	9 (32.2)	2 (7.1)

Key: *No FDA interpretation criteria for interpreting Western blot HIV-2 results was discovered in thecourse of this study. WHO: World Health Organization; CRSS: Consortium for Retrovirus Serology Standardization; CDC: Centre for Disease Control and Prevention; ASTPHLD: Association of State and Territorial Public Health Laboratory Directors; ARC: American Red Cross.

Discussion

Based on the guideline of UNAIDS/WHO (WHO, 2004), three alternative testing algorithms were recommended in dealing with different scenarios for HIV testing. These algorithms, designated strategies I, II, and III, were designed to eliminate the use of confirmatory tests, such as Western blot and nucleic acid testing, and hence reduce the testing costs for resource-limited countries. In particular, strategy I (requiring only one test) is intended for use in diagnostic testing in populations with an HIV prevalence of >30% among persons with clinical signs and symptoms of HIV infection and in blood screening and surveillance testing in populations with an HIV prevalence of >10%. Strategy II (requiring two tests) is a moderate approach for use in surveillance testing in populations with an HIV prevalence of ≤10% and in diagnostic testing in populations with a prevalence of ≤30% among persons with clinical signs and symptoms of HIV infection or >10% among asymptomatic persons. The most stringent approach is strategy III (requiring three tests utilizing different antigens and/or different test

principles) for diagnostic testing in populations with an HIV prevalence of ≤10% among asymptomatic persons [26]. National guideline for HIV testing in Nigeria adopted II that use two kits for HIV diagnosis. Third kit came in as tie-breaker in sero-discordant cases.

This UMD-CDC algorithm II was recently evaluated by Patel et al [4] and Iriemenam et al [7] in 2022 with differing outcomes.Study findings corroborated the outcomes of the latter as sero-discordant rate was 0% in this study. Critical evaluations of the former and latter studies revealed the possible causes of the differences. While the former prevalence study on general population capturing six geopolitical zones in Nigeria reported high discordant rate of 43.7% between RT1 and RT2 and false positive rate of 5.5% and it was based on field study with first 50 tests by field testers and quality control checks but with accompanied challenges relating to failed testers'complete monitoring due to security challenges, the latter compared the outcomes of both field and reference laboratory based (controlled) testing[4, 7]. This study was similarly a laboratory-based study. Thus, issues relating to right sampling techniques, training and retraining of field testers, involvement of quality control officers, inclusion of known positive and negative control samples for each group of testers, avoidance of use and reuse of testing materials to prevent cross contamination, experience in careful interpretation of results based on assessment of risk factors, and batching of the numbers of samples or patients to be tested within a given time frame to eliminate subjective errors are all areas to look into to reduce false positives, enhance national algorithm performance while scaling up enrolment of HIV positive patients for antiretroviral therapy [7, 27-28]. From a different viewpoint, differences instudy design might contribute to differences NAIIS reports and outcomes of this study. While NAIIS

report was a cross-sectional prevalence and national algorithm assessment study using general population including asymptomatic subjects with evident declining HIV prevalence in recent years, current study focussed on algorithm assessment using cohorts of research subjects initially diagnosed as HIV-1/2 positive based on national algorithm [4]. Hence, the adoption of UMD-CDC algorithm strategy III as advocated by Patel et al in the face of dwindling trends in HIV incidence in Nigeria may not be out of place.

Comparison of these results with those obtained from the fourth generation ELISA assay and the immunoblotting assays showed that all assays including rEIA scored 100% sensitivity for all the positive sera. Previous studies on the use rEIA have shown that Determine and Unigold have 100% sensitivity in consonance with the manufacturers' findings [29-30]. While the use of sandwich ELISA kits such as HIV ULTRA Ag-Ab detection kit cannot be underestimated and the value of supplemental tests for HIV diagnosis cannot be over-emphasized [20, 31-34], study results corroborate the findings of many researchers on the effectiveness of the national algorithm and rEIA-ELISA algorithm in resource-limited settings [9]. Although outcomes of this study on specificity and negative predictive value is grossly limited and require further substantiation due to inadequate HIV-negative samples involved, it at least, gave a clue on the specificity and negative predictive value (NPV) of kits used and algorithm tested. For instance, on the specificity of Determine kit used in the study, one false positive HIV 1/2 obtained from this study pictured possible slightly lower specificityof the kit compared to those of Unigold and Stat-Pak EIA kits which were evaluated to be 100% based on the results of this study (data not shown) and from different panels of

samples tested by the manufacturers. The positive predictive values (PPV) of the rapid test kits were 99.43%, 100% and 100% for Determine, Unigold and Stat-Pak respectively. This finding poses some cautions to testers and clinicians when interpreting positive results from Determine kit only based on the recommendation of the World Health Organization on serial algorithm I. Its sole use in HIV testing must be limited to alternative algorithm strategy I [19]. The use of the three kits in arriving at the final test results as prescribed by the national algorithm have greatly enhanced the diagnostic performance of rEIA or national algorithm [7].

Although ELISA-based Genscreen ULTRA HIV Ag-Ab. fourth generation kits detectp24 antigen and antibodies to HIV-1/2 by enzyme-linked immunosorbent assay and do not differentiate HIV-1 and HIV-2, they still play significant roles in HIV screening and diagnosis in resource-limited settings [20].In fact, studies showed that repeatedly reactive samples by EIAsin established infectiondo not require supplemental test such as Western blot, and NAT [3, 35]. Our findings were consistent with Yuksel et al's with respect to HIV-1 confirmation but limited by non-further testing for HIV-2 RNA by NAT or for HIV-1 and HIV-2 differentiation test by Geenius HIV-1/HIV-2 immunochromatographic supplemental analysis.In contrast to findings by Nasrullah et al, there were no HIV-1 WBi findings among the repeatedly reactive HIV-1 positive cases by the national algorithm or rEIA-ELISA algorithm and that showed 100% sensitivity of these alternative algorithms in HIV-1 diagnosis. Again, in contrast to their findings where some repeatedly reactive samples were confirmed HIV-1 negative by Western blot and NAT, findings in this study confirmed positivity of repeatedly reactive samples by Western blot and NAT. For instance, out of the 105 (60.0%) of samples

repeated for HIV-RNA detection by nucleic acid testing, 103 (98.1%) repeatedly reactive cases by rEIA and rEIA-ELISA algorithms were HIV-1 positive and the repeatedly reactive HIV-1 negative cases (1.9%) were similarly confirmed negative by NAT. Differences in results might not be unconnected with the differences in the category of subjects tested in the two studies or false repeatedly reactive findings by rEIA/ELISA due to the presence of immune complexes in the plasma of research subjects that have been infected with other pathogens as at the time of sample collection, recent vaccination, patients with strong immune stimulation or autoimmune disorders, allergies or pregnancy [35-36]. Those might have been confirmed negative by the Western blot assay or NAT.

Traditionally, a Western blot (WB) or less frequently, an immunofluorescence assay (IFA) was used to confirm antibodies to HIV-1 or HIV-2 after it has been identified by a rEIA for HIV antibody screening test [4, 31, 35]. Current guideline regards Western blot assay as the historic gold standard for HIV testing due to its inability to detect acute HIV infection, possible misidentification of HIV-1 as HIV-2 or delay in diagnosis resulting from its cumbersome procedure and advocate for its discontinuity as a supplemental test [(CDC, 2016, 2014). Same guideline made provision for laboratories that still use WB as a supplemental test. Up till date, Western blot assay continues to be used as a supplemental test for HIV-1 and HIV-2 in settings where NAT is not in place [31]. In Nigeria, Western blot assay, more often than not is used for research purposes [4]. Although many developed countries still use WB to confirm HIV testing results that are positive either by point-of-care (POC) rapid tests or laboratory-based procedure [4, 7, 31], others like the United Kingdom set an algorithm that use fourth generation ELISA

as initial diagnostic test and Nucleic acid testing (NAT) by polymerase chain reaction as the second line test for HIV diagnosis. It is believed that such settings adopted CDC 2023guidelineand by the guideline prescription, detection ofp24 antigen only without the antibodies to HIV-1 or HIV-2 followed by HIV viral particle detection by NAT can diagnose acute HIV-1 or HIV-2 infectionelse, the approach has sets of limitations [37]. Resource poor countries where cold storage capacity, reliable power supply, efficient transportation, good laboratory infrastructure constitute major challenges and sufficient trained personnel may not be readily available, alternative rapid confirmatory approaches recommended by the World Health Organization (WHO) have been adopted [4, 7, 27]. Again, to further strengthen the national algorithm, fourth generation ELISA assay kits can be used. It has been recommended that repeatedly reactive samples by rEIA-ELISA algorithm may not warrant further testing with supplemental test or nucleic acid testing but the category of samples have to be defined [3,35]. Besides, the adoption of these alternative confirmatory, cost-effective and comparable algorithms have increased HIV test uptake in resource-limited settings like ours and reduced risks of degenerated status and high HIV transmission rates to infected partners [5, 39] contrary to some settings where more complex algorithms with associated high costs of tests, non-availability of equipment and trained personnel as well as difficulties in interpreting Western blot results have constituted major hindrances to HIV test uptake in endemic population.

Interestingly, no indeterminate result was obtained for HIV-1 in this study contrary to findings by other researchers [3]. Moreover, the findings of 60.7%, 53.6% and 50.0% WBi results based on WHO, American Red Cross and CRSS (or related) criteria

respectively for HIV-2 assay showed that Western blot cannot be reckoned as confirmatory test for HIV-2 where the prevalence of the virus is low because of possible increase in the risks of Western blot indeterminate results due to cross-reactivities with HIV-1 and other factors which can affect decision making on HIV treatment initiation [17, 35]. Further testing with NAT could have resolved the WBi findings as demonstrated by other studies. Evaluating the 60.7%, 53.6% and 50.0% HIV-2 WBi results based on WHO, ARC and CRSS (or related) criteria, most of the cross-reactivities demonstrated in this study were due to p26 core protein either singly (21.4%) or in combination with others 25.41% and 14.70% respectively based on WHO and CRSS criteria). This is in consonance with theConstantineet al's findings which affirmed that most cross reactions represent antibody induced by the core (p26) and/or Pol antigens (p68, p34), because these are highly conserved between the two different viruses [32-33].

Finally, this study showedHIV 1 - HIV-2 co-infection was 0% based on the WHO interpretive criteria but 10.7% among research subjects based on CRSS, CDC and ASTPHLD criteria, and 7.1% based on ARC criteria. The Cambridge Western blot assay used in NAIIS study was similarly based on CRSS/CDC/ASTPHLD criteria, however the HIV-1/HIV-2 coinfection prevalence was much lower compared with the findings in this study [4]. Outcomes of this study is in agreement with the established facts that HIV-2 infection is prevalent in in West African nations including Guinea-Bissau, The Gambia, Senegal, Cape Verde, Cote d'Ivoire, Mali, Sierra Leone, and Nigeria [40]. If same interpretive criteria used by Landman et al (2009) who reported HIV-1/2 co-infection of 0.3-1% in West Africa [41], were used, these findings among HIV-1 infected subjects in Nigeria can be considered quite alarming. It however, require further confirmation as

WHO interpretive criteria showed 0% HIV-1/HIV-2 coinfection rate. They cited a particular example of a rural area of north-western Guinea-Bissau where HIV-2 prevalence dropped from 8.3% in 1990 to 4.7% in 2000. In a swift attempt to promote HIV testing uptake without compromising quality of results and at the same time ensure HIV-1 and HIV-2 differentiation and early diagnosis of acute HIV infection, different countries are careful in the choice of supplemental tests. Food and Drug Administration approved Geenius HIV 1/2 confirmatory assay in 2013 as a supplemental test and useful tool in HIV-1 and HIV-2 differentiation testing and have since been evaluated in studies[4, 7, 31].According to the US Centre for Disease Control and Prevention and the Association of Public Health Laboratories, the FDA approved Geenius HIV-1/2 antibody differentiation supplemental test requires careful interpretations to forestall misdiagnosis or missed diagnosis and promote early treatment; the FDA similarly gave recommendations on alternative approach to HIV testing [17].The picture of high HIV-1/HIV-2 co-infection in this study without further assessments with either Geenius HIV-1/HIV-2 supplemental test or HIV-2 RNA detection by NATunderscores the insufficiency of rEIA-WB or ELISA-WB algorithm for HIV-2 diagnosis, the need for further evaluation of the study findings, upgrading to CDC current guidelinesaccording to CDC/UNAIDS/APHL joint body on HIV/AIDS and reaching national consensus on HIV-2 diagnosis testing in Nigeria.[17].

Limitation of the Study

Non-performance of HIV-1 and HIV-2 differentiating test in this study at the national HIV serial algorithm levellimited classifying a positive result as HIV-1 or HIV-2 for routine diagnosis but interestingly, antiretroviral therapies that can treat both HIV-1 and HIV-2

are now available. However, optimal comparison between rEIA or rEIA-ELISA alternative algorithm and the gold standard rEIA-WB and ELISA-WB algorithm used in this studycan be made by the inclusion of rapid Geenius HIV-1/HIV-2 differentiating supplemental test which do not compromise the gains interms of quality of results,

Conclusion

The results of this study showed that rEIA or rEIA-ELISA alternative confirmatory algorithm is a sufficient and effective diagnostic algorithm for HIV-1 diagnosis especially in an established infection as it compares in sensitivity and specificity with the more complex testing protocol that adopts rEIA-WB, rELISA-WB and rELISA-NAT approach. However, it is insufficient for HIV-2 diagnosis. Introduction of Geenius HIV-1/HIV-2 immunochromatographic supplemental test rather than Western blot assay will improve the adopted national algorithm, capture acute HIV infection in asymptomatic individualswithout compromising quality or the gains in turnaround time and early initiation of treatment.

Declarations

Ethical Approval and Consent for Participation

This study was performed according to the ethical guidelines of the Human Research and Ethical Committee of the Federal Teaching Hospital, Ido-Ekiti.

Consent for Publication

Not applicable.

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