Reliability of testing and potential impact on HIV prevention in Nigeria

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Summary

Several factors including variability of human immunodeficiency virus (HIV), laboratory facilities, cost and competence of personnel handling the tests are some of the important factors that affect accuracy and reliability of HIV testing in most parts of Africa. Recently investigators in Africa have observed that antibody detection assays based on antigens derived from HIV-1 subtype B show moderate to significantly lower sensitivity for detection of infection by various non-B subtypes. In this study, we evaluated the reliability of two EIA and 12 rapid HIV-1/2 test kits that are commercially available in Nigeria using the Western immunoblotting technique as reference. A panel of 100 sera from Western blot confirmed symptomatic or asymptomatic HIV-1 infected persons and 90 seronegative patients from those referred for testing in our laboratory were used for this study. Each sample was tested with two HIV-1/2 EIA, and 12 HIV-1/2 rapid test kits commercially available at one time or the other for HIV-1/2 testing in Nigeria. Overall, the sensitivity of the two EIA kits were 100% and 91.0% with specificity of 96.7% and 91.1% respectively. The sensitivity of the rapid test kits ranged from 88% to 98.0% with specificity of 92.2% to 100%. Further analysis showed significant variation in the sensitivity and specificity of the same kit based on whether an individual had asymptomatic or symptomatic infection The results of this study highlight the problem of diagnosis of HIV infections in Africa. It shows that the sensitivity of most of the rapid assays shall not be adequate for detection of early infection. The implications of possible misdiagnosis on the various intervention strategies that rely predominantly on correct HIV status of an individual are enormous. Thus, there is an urgent need for review of the current HIV testing assays or algorithms in Nigeria and other parts of Africa.

Keywords: *HIV, testing, reliability, sensitivity, specificity*

Résumé

Plusieurs facteurs inclus la variabilité du virus immunodéficitaire, les facilités de laboratoire, le coût et la compétence du personnel performant les analyses affectent la précision et la validité des test du VIH dans la plupart des pays Africain. Récemment des investigations ont observées que les tests de détection des anticorps

dérivés des VIH-& sous type B démontraient une sensitivité faible que les infections sous type non-B. Dans cette étude, nous évaluons la validité de deux tests EAI et 12 VIH1 -2 en vente au Nigeria utilisant la technique de western blot comme référence. Les sérums des 100 patients infectés du VIH1 confirmés et 90 individus séronégatifs étaient examinés. Chaque échantillon était testé avec deux types de Kit HIV-EIA et 12HIV1/2 commercialement disponible pour le test du VIH au Nigeria. EN générale, la sensibilité des 2 tests (EIA et 12VIH1/2) étaient 100% et 97% avec une spécificité de 96.7% et 91.1% respectivement. La sensibilité des tests rapides variait de 88-98% avec une spécificité de 92.2-100%. Une analyse approfondie démontrait une variation signifiante en sensitivité et spécificité même si l'individu avait des symptômes ou sans infection. Les résultats de cette étude illuminent le problème de diagnosie des infections du VIH en Afrique. Elle démontrait que la sensitivité de la plupart des tests rapide parait moins adéquate pour la détection des infections ; Les implications de fausse diagnosie par les différentes stratégies d'interventions basé sur le statut propre des VIH sont énorme. Ainsi, il y a un besoin urgent de revoir les tests de diagnosie au Nigeria et d'autres part d'Afrique.

Introduction

Several factors including variability of human immunodeficiency virus, laboratory facilities, cost and competence of personnel handling the tests are some of the important factors known to affect accuracy and reliability of HIV testing in most parts of Africa [1,2]. While some of these factors can be managed to a great extent in middle to high level laboratory settings in some developing countries, the problem of genetic variability is a serious one in most parts of Africa with circulation of multiple HIV-1 and or HIV-2 subtypes [1,2,3,4]. Although most of the HIV-1/2 kit manufacturers claim incorporation of antigens with a broadly, reactive epitopes that can accurately detect all known HIV-1 and HIV-2 subtypes as well as group O viruses, it is well known that genetic variability of the virus can reduce the efficiency of various HIV assays [2,3]. Several groups of investigators in Africa have reported that antibody detection assays that are based on antigens derived from HIV-1 subtype B have

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shown moderate to significantly lower sensitivity for detection of infection by various non-B subtypes [2,3,5,6].

The standard serological algorithm recommended by the WHO is initial serial testing with two different enzyme immunoassays (EIA) followed by a confirmatory testing. In most situations, a readily available and most convenient confirmatory test is the Western immunoblotting technique. However, various alternative methods such as double EIA, double rapid or single rapid tests have also been recommended for resource-limited countries [7,8]. Due largely to high cost of the standard method, coupled with severe dearth of well trained laboratory personnel to cope with the demand for HIV testing in Nigeria, most of the hospitals in the country now rely solely on the use of a single ELISA or a single rapid test for diagnosis of HIV-1/2 infection.

In this study, we evaluated the reliability of two EIA and 12 rapid test kits that are commercially available for HIV-1/2 testing in Nigeria using the Western immunoblotting technique as reference.

Materials and methods

Subjects and samples collection

Aliquots of a panel of 100 sera from Western blot confirmed symptomatic (60) or asymptomatic (40) HIV-1 infected persons and 90 seronegative patients referred for testing in our laboratory in the department of virology, University College Hospital were used for this study. The samples were derived from groups of patients, spouse of infected persons, persons from some high and low risk groups who volunteered for HIV counseling and testing.

HIV-1/2 Assay kits

Two commonly used commercial HIV-1/2 EIA kits in Nigeria, Genscreen® and Human® were evaluated. In addition, 12 HIV-1/2 rapid test kits available at one time or the other for HIV-1/2 testing in Nigeria were included for the evaluation. These are Capillus®, Determine®, Genie II®, Immunocomb®, Hexagon®, HEMA®, SD®, Unigold®, Global Device®, Bispot®, Triline® and Efosa®.

Aliquots of each serum sample were tested with the HIV-1/2 test kits according to each manufacturer's instructions. Interpretation of the results was also based on the specific guidelines for each kit. The sensitivity, specificity as well as the positive and negative predictive values of each of the assays were calculated using the result of Western immunoblotting of each serum sample as the reference.

Results

The performance of each of the HIV-1/2 EIA and rapid test kits commonly used in Nigeria evaluated in this study is shown in the table 1. Overall, the sensitivity of the two

EIA kits were 100% and 91.0% with specificity of 96.7% and 91.1% for Genscreen® and Humane® respectively. For the 12 rapid test kits evaluated, the sensitivity ranged from 88% to 98.9% with specificity of 90.9% to 100%. (Table I).

Further analysis of the results by clinical condition of the HIV positive persons whose samples were tested showed significant variation in the sensitivity and specificity of the same kit based on whether an individual had asymptomatic or symptomatic infection. The rates varied by up to 20% with some of the rapid test kits, based on whether the subjects were asymptomatic or symptomatic HIV positive in the same geographical area (Table II). Similarly, the positive predictive values varied from 80.5%-100% and 87.9%-100% among asymptomatic and asymptomatic respectively.

Discussion

The effectiveness of control of the spread of HIV and treatment of infected persons in any country depends largely on provision of accurate and reliable diagnosis of the infection. Reliability of the assay techniques is imperative for unambiguous identification of infected individuals among other conditions. The results of this study shows the contrary in Nigeria and therefore, a need for urgent review of the current HIV testing assays and or algorithms in the country.

The results of the study indicate that the performance of most of the assays is less than optimal in the country even under a tertiary hospital laboratory setting where this study was carried out. Overall, only EIA test kit designed to detect both HIV-1/2 antibodies and antigens of multiple viral subtypes could be reliably recommended for HIV screening under the prevailing circulating viral strains and stage of HIV epidemic in the country. On the other hand, such assays with very high sensitivity will have lower specificity as observed in this study. This is also of concern in the phase of massive HIV treatment programmes in the country. False positive results may lead to wrong enrolment of persons for antiretroviral drugs.

Another interesting observation from the results of the study is the wide variation in the sensitivity of the same assay kit based on the clinical conditions of HIV infected persons in the same geographical locations. It shows that the sensitivity of most of the assays is lower for detection of early infection with most of the circulating HIV strains in the country. Although most commercial HIV tests manufacturers claim the use of antigens that can detect antibodies to all known subtypes, the sensitivity of these assays for CRFs or other highly divergent strains of the virus may not be as high as presented [2,6]. Hence, such strains of HIV may continue to escape detection even under the best laboratory conditions.

The implications of possible misdiagnosis on the various intervention strategies that rely predominantly

Table 1: Performance of some HIV test kits evaluated in Nigeria

Name of test kit®	Sensitivity (95 % CI)	Specificity (95 % CI)	Positive Predictive Value (NPP) (95 % CI)	Negative Predictive Value (NPP) (95 % CI)
Genscreen	100%	96.7%[95.8-97.7]	97.1%(96.2-98.1)	100%
Human	91%(90.1-92.0)	91.1%[90.2-92.1]	91.9%(91.0-92.9)	90.1%(89.3-91.1)
SDS	89%(88.1-90.0)	92.2%[91.3-93.2]	92.7%(91.8-93.7)	88.3%(87.5-89.2)
Hema	88%(87.2-88.9)	100%	100%	88.2%(87.4-89.1)
Hexagon	92%(91.1-93.0)	93.3%[92.4-94.3]	93.9%(93.0-94.9)	91.3%(90.4-92.3)
Capillus	99%(98.0-100)	97.8%[96.9-98.9]	98.0%(97.0-99.0)	98.9%(97.9-99.9)
Immunocomb	98%(97.0-99.0)	100%	100%	97.8%(96.9-98.9)
Genie II	90%(89.2-91.0)	100%	100%	90.0%(89.2-91.0)
Determine	97%(96.1-98.0)	100%	100%	96.8%(95.9-97.8)
Unigold	96%(95.1-97.0)	98.9%[97.9-99.9]	99.0%(98.0-100)	95.7%(94.8-96.7)
Global device	93%(92.1-94.0)	98.9%[97.9-99.9]	98.9%(97.9-99.9)	92.7%(91.8-93.7)
Bispot	94%(93.1-95.0)	98.9%[97.9-99.9]	98.9%(97.9-99.9)	93.7%(92.8-94.7)
Triline	94%(93.1-95.0)	100%	100%	95.7%(94.8-96.7)
Efosa	94%(93.1-95.0)	100%	100%	95.7%(94.8-96.7)

Table 2: Performance HIV test kits for HIV infected symptomatic and symptomatic persons in Nigeria

Name of Test kit®	Asymptomatic (n=40)			Symptomatic (n=60)		
	Sensitivity (95 % CI)	PPV (95 % CI)	NPV (95 % CI)	Sensitivity (95 % CI)	PPV (95 % CI)	NPV (95 % CI)
Genscreen	100%	93.0 (92.1-94.0)	100	100%	95.2 (94.3-96.2)	100
Human	82.5% [81.8-83.4]	80.5 (79.8-81.4)	92.1 (91.3-93.1)	96.7% [95.8-97.7]	87.9 (87.1-88.9)	97.6 (96.7-98.6)
SDS	77.5% [76.9-78.4]	81.6 (80.9-82.5)	90.2 (89.4-91.2)	96.7% [95.8-97.7]	89.2 (88.4-90.2)	97.6 (96.7-98.6)
Hema	77.5% [76.9-78.4]	100	90.9 (90.1-91.9)	95.0% (95.9-97.8)	100	96.8 [94.1-96.0]
Hexagon	80.0% [79.3-80.9]	84.2 (83.5-85.2)	91.3 (96.9-98.8)	100%	90.9 (90.1-91.9)	100
Capillus	97.5% [96.6-98.5]	95.1 (94.2-96.1)	98.0 (97.0-99.0)	100%	96.8 (95.9-97.8)	100
Immunocomb	95.0% [94.1-96.0]	100	97.8 (90.5-92.3)	100%	100	100
Genie II	87.5% [86.7-88.5]	100	94.7 (93.8-95.7)	91.7% (93.8-95.7)	100	94.7 [90.9-92.7]
Determine	95.0% [94.1-96.0]	100	97.8 (96.9-98.8)	98.3% [97.3-99.3]	100	98.9 (97.9-99.9)
Unigold	92.5% [91.7-93.5]	97.4 (96.5-98.4)	96.7 (95.8-97.7)	98.3% [97.3-99.3]	98.3 (97.4-99.3)	98.9 (97.9-99.9)
Global device	87.5% [86.7-88.5]	97.2 (96.3-98.2)	94.7 (93.8-95.7)	96.7% [95.8-97.7]	98.3 (97.4-99.3)	98.9 (97.9-99.9)
Bispot	87.5% [86.7-88.5]	97.2 (96.3-98.2)	94.7 (93.8-95.7)	98.3% [97.3-99.3]	98.3 (97.4-99.3)	98.9 (97.9-99.9)
Triline	92.5% [91.7-93.5]	100	96.8 (95.9-97.8)	98.3% [97.3-99.3]	100	98.9 (97.9-99.9)
Efosa	92.5% [91.7-93.5]	100	96.8 (95.9-97.8)	98.3% [97.3-99.3]	100	98.9 (97.9-99.9)

on correct HIV status of an individual are enormous. Some of such programmes or plans include scaling up of the PMTCT, and VCT in the country. As far as we can ascertain, most of these programmes currently utilize single rapid HIV-1/2 test with little or no internal or external monitoring or necessary quality assurance measures. In a place where the rate of new HIV infections is likely to be high such as in Nigeria, it is important to ensure accurate diagnosis in order not to compromise the objectives of these highly beneficial and promising control measures.

While cost may be an important factor in the implementation of programmes in resource-limited countries, it should be weighed carefully against the benefits or implications of misdiagnosis in a programme like PMTCT or VCT. Misdiagnosed cases of positive pregnant women who in most cases are asymptomatic may result into transmission of HIV from mother to the baby or rapid progression of the disease due to pregnancy related complications or even maternal plus child death. Similarly, misdiagnosed HIV positive cases at a VCT centre have serious implications for the individual and the public at large. It is completely a different situation when an individual did not have the benefit of testing than to be misdiagnosed.

Also very important is the fact that HIV testing of blood units for transfusion in the country is largely based on the use of single HIV-1/2 rapid test kits. Some of such commonly used rapid kits were found to be less than satisfactory with sensitivity of less than 80% for asymptomatic HIV infected persons in this study. Under normal conditions, blood donors will be in this category, expectedly asymptomatic to be eligible to donate blood and whose diagnosis may be missed leading to iatrogenic transfusion associated transmission [9].

Low sensitivity of many of the rapid HIV test kits observed in this study may partly explain several cases of unexplained source of HIV infection in the country mostly among persons with history of transfusion of blood units claimed to have been screened for HIV-1/2 antibodies. Also worthy of note is the fact that majority of blood banks in most parts of the country are based in private laboratories. Previous reports from other parts of the world have shown detection of HIV-1 in seronegative blood donors [9,10]. However, with little or no supervision by the relevant health authorities either at the state or federal level.

The findings presented in this report show that cases of false negative HIV test results due to low sensitivity of assay kits may be high in Nigeria. On the other hand, there may also be problem of specificity with the commonly used assay kit in the country. While the margin of error in HIV testing should be zero, the implications of low sensitivity of assay kits are enormous for the various intervention strategies in the country [11]. The situation becomes more frightening when it is evident that the chances of transfusion associated HIV

transmission remain very high in the country. It may also be reasonable to speculate that the findings reported herein may partly explain the low rate of HIV infection observed in the recent past national HIV sentinel surveys in the country with an algorithm of serial double rapid tests and sometimes a third rapid [12].

Some immediate remedies to the situation will include upgrading of HIV screening laboratories in the major blood banks to test blood units for transfusion by EIAs that can accurately detect HIV-1/2 antibodies and antigens. In places where this may be difficult or impossible for manpower or technical reasons, HIV testing can be done using double parallel rapid test strategy with one kit having at least 99% sensitivity and the other not less than 99% specificity. Such HIV laboratories or centres should be linked with state or regional HIV testing reference centres for monitoring and quality assurance. Accordingly, the current algorithm of single rapid test for PMTCT and VCT should be reviewed to adopt a double parallel rapid or EIA plus Western blot strategy in secondary or tertiary hospital settings. Also very important is the need for rigorous and continuous evaluation and monitoring of HIV test kits in use in the country. On the medium to long term is undoubtedly the requirement for basic research on the virology, immunology and epidemiology of HIV for planning of scientifically sound intervention measures to abate the spread of the virus in the country.

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