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EVALUATION OF COMMERCIAL HIV TEST KITS USED IN NIGERIA

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ABSTRACT
Background: Accurate and reliable diagnosis of HIV plays a central role in any effective HIV intervention. We decided to evaluate 4 commercial HIV test kits to determine their reliability for use in developing countries.

Methods: Serum samples obtained from clients accessing tertiary health services at the STI clinic, Jos University Teaching Hospital were used to evaluate Sdbioline, Diaspot, Determine and DIALAB Elisa kits. A Western blot was used as the reference kit.

Results: DETERMINE kit gave 34 positive and 58 negative reactions and the positive sera were all confirmed by Western blot while **DIASPOT** kit gave 27 false negative results, which was at variance with the reference kit result. Other kits were **SDBIOLINE** with 5 false positive and **DIALAB Elisa** kit, which gave one false positive, and one false negative result.

Conclusion: We conclude that Determine, SDbioline and DIALAB Elisa kits are reliable for HIV antibody testing in Nigeria and other developing countries.

Key Words: HIV, Test Kits, Nigeria. *(Accepted 22 May 2008)*

INTRODUCTION

The diagnosis of HIV infection has played very critical roles over the past two decades in detecting and monitoring infection as well as patient management. HIV infection is established by detecting antibodies to the virus, viral antigens, viral RNA/DNA, or by culture[1]. To test for the viral particle or the viral antigen is a difficult procedure. The most common method used is serology for antibody detection by ELISA or agglutination. Positive results are then usually confirmed by western blot (immunoblot) or further specific tests such as competitive ELISA[1].

As technology evolved, screening, confirmatory and HIV monitoring assays have greatly improved[2] in terms of quality and speed. Coupled with this development is the unprecedented increase in the number of test kits that are available for determining HIV status. Test kits that are simple, instrument-free simple/rapid tests are commercially available for measuring antibodies by enzyme-linked immunoassay (EIA). If performed properly, these tests have a sensitivity and specificity exceeding 98 %[3]. Moreover, they are also less expensive and do not require high level of technological expertise to perform or interpret and produce fewer indeterminate results[4].

For small blood-collection centers and hospitals in developing countries, screening tests are needed that have the following specific characteristics; high level of sensitivity and specificity, long shelf life at ambient temperatures, reasonable cost, and ease and rapidity of performance[5].

Accurate and reliable results are essential when testing HIV because the implications of false positive or false negative results are serious and affect the objectives of voluntary counseling and testing.

For the reasons stated above, we evaluated a variety of commercial HIV test kits in order to identify appropriate test kits for use in a developing country.

MATERIALS AND METHODS

Study Area

The evaluation was carried out using a panel of 92 sera obtained from clients who came for Voluntary Testing and Counseling (VCT) between January and March 2006, at the Special Treatment Clinic, (STC) of the Jos University Teaching Hospital (JUTH), a tertiary care center servicing about 19 states in north central Nigeria and the federal capital territory.

Study Design

This is a prospective, anonymous and unlinked study.

Assay Kits

Four commercially available assay kits were evaluated: SDbioline HIV-1/2 3.0 (Biotec laboratories Ltd UK) Diaspot HIV 1/ 2(no manufacturer or place of manufacture indicated) and Determine™ HIV 1/ 2 (Abbot Japan) and one ELISA kit-DIALAB Elisa (Biorad, France). A Western blot (QualiCode™ immunetics Inc, USA) kit was used as the reference. All the kits were used within the expiration dates.

The kits were chosen after a survey of the various

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assay kits used for HIV testing of patients in eight laboratories of public and private health institutions in Jos metropolis. The characteristics of the assay kits are described in Table I.

Specimen Collection and Laboratory Processing
After pre-test counseling, the cubital-fossa was cleansed with 70% isopropyl alcohol and excess alcohol was wiped off using sterile gauze. Five milliliters of venous blood was obtained from the cubital fossa into a sterile Z-10 plastic container without anticoagulant and labeled with a number and no personal identifiers or names were used. The specimen was allowed to clot, and centrifuged at 1,500rpm for 10minutes in a clinical centrifuge (Hettich, Rotanta, centrifuge, Germany), to separate the serum. Aliquots of sera were extracted and stored in cryo-vials at -20^{0}C until tested. The test was performed with each assay kit according to the manufacturer's instructions.
Each S/R assay kit was evaluated with the entire panel of 92 sera and their results recorded before evaluating another assay kit. The evaluation was conducted with the entire panel of 92 sera before any repeat assay was carried out if an invalid result was obtained. The results were read visually. The ELISA evaluation was carried out for the entire panel of 92 sera as well as a positive and a negative control samples. The ELISA result was calculated according to the manufacturer's instructions using the data obtained with the ELISA Micro plate reader (ELx800 universal BIO-TEK Instruments Inc. USA). All positive specimens obtained, were subjected to a Western blot assay. The results were read visually. Band positions were compared to those on the reference card developed using the HIV-1/2 positive control serum.

Data Management
The results of the evaluations were recorded in a Laboratory worksheet. Any test that was repeated for any reason was entered and reasons for the repeat noted.

Ethical Approval
The Ethical committee of Jos University Teaching Hospital approved this research protocol.

RESULTS
The results presented in Table 2 were obtained from the evaluation of the three-simple/rapid assay kits, one ELISA assay kit and a Western blot used to confirm all the samples that reacted positive with all the other test kits.
DETERMINE HIV 1/ 2: Of the 92 panel of sera tested for HIV with this assay kit, 34 (37%) gave positive reactions, while 58(63%) gave negative Reactions. These results were confirmed by Western blot.
DIASPOT HIV1/ 2: Out of the 92 HIV tests carried out with this kit, 7(7.6%) gave positive reactions, while 85(92.4%) gave negative reactions. This result does not agree with the reference kit result, which gave 34 positive reactions.
SDBIOLINE HIV1/2 3.0: HIV tests were also carried out with this kit. The kit gave 39(42%) positive reactions, while 53(58%) gave negative reactions out of the 92 evaluations. This result again does not agree with the reference kit value of 34 positive reactions. Five of the HIV antibody sero-positive sera were not confirmed by the Western blot.
DIALAB Elisa HIV ½: Of the 92 panel of sera tested for HIV antibodies using this assay kit, 34(37%) gave positive reactions, while 58(63%) gave negative reactions. This kit gave one positive result and one false negative result as indicated by the confirmation test with Western blot. Also the false negative sera by this assay kit tested positive with Determine and SDBioline assay kits.

Table 1: **Characteristics of the Commercial HIV Antibody Assay Kits.**

Name	DIALAB Elisa	SDbioline	DiaSpot	Determine™	QualiCode™
Test type	Sandwich Elisa	Simple/Rapid	Simple/Rapid	Simple/Rapid	Immunoblot
Antigen type	Recombinant Antigen /synth -etic peptide	Recombinant antigen	Recombinant antigen	Recombinant antigen/synth -etic peptide	HIV-1 viral lystate protein /Recombinant HIV-2 protein
Coating on	flat wells	Membrane	Membrane	Sample pad membrane	Immunoblot
Number of tests per kit	96	40	30	100	24
Volume of serum required (µl)	100	10	5	50	10
Total time Required to Perform the Assay (mins)	100	10	10	15	220
Detection	Microplate Reader	Visual	Visual	Visual	Visual

Table 2: **Performance of the Commercial HIV Antibody Assay Kits.**

Assay kit	No. Negative	No. Positive	No. false Positive	No. false Negative
Determine	56	34	0	0
Diaspot	85	7	0	27
SDBioline	53	39	5	0
Dialab Elisa	56	34	1	1

N=92

DISCUSSION

An evaluation of commercial assay kits used for detecting HIV 1 and 2 antibodies in Jos, Nigeria was undertaken to determine their performance and recommend assay kits that may be used for HIV testing in Nigeria and other developing countries. This study is important because it has been established that early detection of HIV infection can yield public health benefits by decreasing risk behaviours that transmit HIV to uninfected persons. Uninfected persons may also benefit from reliable HIV testing since knowing their HIV status can assist them in reducing or eliminating their risk-taking behaviour. In addition, knowledge of one's own HIV status and that of his or her partner may be an important factor for preventing acquisition and spread of HIV. The recently demonstrated benefits of antiretroviral therapy have underscored the influence of expanding HIV testing services to facilitate early diagnosis and treatment of HIV infected persons. The implication of accurate and reliable results cannot be overemphasized in HIV testing. But HIV testing kits of different technologies and attractive claims by the manufacturers are commercially available in spite of restrictions by regulatory agencies such as Food and Drug Administration (FDA), the National Agency for Food and Drugs Administration and Control (NAFDAC) and National AIDS/STDs Control Programme (NASCP)[6].

The conventional ELISA method for detecting HIV a number of shortcomings including: the assay requires costly instruments and specialized reagents; it is not rapid, it is not suitable for use in small or rural health facilities; and it requires electricity[7, 8, 9] which is often unavailable or unreliable in most developing countries.

The simple/rapid assays are more expensive to use, but more suitable than the ELISAs in small clinics, rural settings, emergency situations, blood banks and where electricity is unreliable; they are easier to perform, read visually, easy to interpret and yield results within minutes.

Out of the four assay kits evaluated in this study, determine a simple/rapid assay kit compared very well with the reference kit. This finding is similar to that reported for a rapid test by Constantine and Ketema[10]. The Dialab Elisa is a sandwich ELISA that also showed excellent performance compared to the reference test of HIV 1/2 antibody test. The high performance by these assay kits may be due to the fact that these two test kits utilize recombinant antigen/synthetic peptide as the antigen type in contrast to the other test kits (SDBioline and Diaspot) that utilize only the recombinant antigen as their antigen type. Interestingly also, Diaspot that showed the widest discrepancy with the reference kit had no manufacturer or place of manufacture indicated anywhere on the kit.

In addition, adherence to the manufacturer's specifications for transport and storage, which is usually difficult to observe in resource-poor settings such as obtained in Nigeria and many other underdeveloped parts of the world, could contribute to the poor performance of some of the kits such as the Diaspot.

The Determine, SDbioline and DIALAB Elisa assay kits performed the best in this evaluation and could be confidently recommended for use in Nigeria. However, by selecting combinations of these rapid tests and ELISA, it should also be possible to use one simple/rapid assay as screening test, and a subsequent one as a confirmatory test. The two-simple/rapid test could be used for screening in emergency settings and resource- constrained areas[8]. The first (screening) test needs to be highly sensitive in order to provide for reliable detection of antibodies in the serum specimen[5].

It is to be noted that in areas of low disease prevalence, it may be possible to use these assays as screening tests to identify patients for presumptive treatment[6].

We conclude that in this study; Determine, SDbioline and DIALAB Elisa kits performed creditably well and are therefore reliable for HIV antibody testing in Nigeria and other developing countries.

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