

Evaluation of Commonly Used Rapid Test Kits and Serological Serial Testing Algorithm for Diagnosis of HIV-1 and 2 Antibodies in Port Harcourt, Nigeria

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Abstract

Three diagnostic rapid test kits (ChemBio HIV-1/2 Stat-Pak, Alere Determine™ HIV-1/2, and Core HIV-1/2) for human immunodeficiency virus types 1 and 2 (HIV-1/2) antibodies detection were evaluated against a gold standard fourth generation ELISA (Dia-Pro). Hundred and seventy two serum samples were collected from Lulubriggs Health Center, University of Port Harcourt, Rivers State. All samples were tested for HIV-1/2 using the three rapid test (parallel algorithm and serial algorithm), and retested using the 4th generation ELISA. The results obtained were compared with those from the ELISA. The Determine™ rapid strips had sensitivity of 66.7%, specificity of 100%, positive predictive value 100%, negative predictive value 89.4% and total agreement of 91.3%. Stat-pak and Core HIV-1/2 rapid strips both had sensitivity of 71.7%, specificity of 100%, positive predictive value 100%, and negative predictive value 90.7% and total agreement of 92.4%. Determine was mostly used by the participant laboratories 18 of 30 (60%). The performance evaluation of the testing algorithm showed that parallel algorithm is more accurate in comparison to serial algorithm. The result of this study reveals that most rapid test are less sensitive and the accuracy of serial testing algorithm is low, this implies that most acute HIV infection will be missed with rapid assay and false negative results will be reported as actual negative with serial algorithm.

Keywords: HIV; ELISA; Rapid assay; Serial algorithm; Parallel algorithm

Introduction

The diagnosis of human immunodeficiency virus (HIV) can be made by several methods, including rapid HIV tests (immunochromatographic assays), and enzyme-linked immunosorbent assays (ELISAs), p24 antigen assays, and polymerase chain reaction (PCR). Only rapid tests offer point-of-care HIV testing, while all other tests need to be performed in a laboratory. Laboratory-based testing may be associated with logistical problems such as delays in the availability of results, particularly in developing countries where difficulties with transport are often encountered [1].

Several rapid HIV tests are available for point-of-care HIV testing, which may avoid the need for laboratory-based testing [2]. The World Health Organization (WHO) has endorsed the use of rapid HIV tests in order to scale up HIV testing since 2004, particularly in resource-poor settings [3]. Point-of-care assays form an integral part of HIV testing in high prevalence settings such as South Africa, which has the largest population of people infected with HIV in the world, with a prevalence of 16.9% among adults (aged 15 to 49) reported in 2008 [4]. Point of-care assays offer several advantages, for example the opportunity for health care practitioners to provide results to patients immediately, with fewer patients being lost to follow-up. Rapid diagnosis of HIV will also expedite patient referral for care [5]. The disadvantages of using rapid HIV assays include limited sensitivity (especially when compared to laboratory-based HIV tests) and the

problem that acute HIV infection is not detectable with the majority of rapid HIV assays available, as they only detect HIV-1/2 antibodies [2].

Fourth generation laboratory-based HIV assays have the ability to detect antibodies to HIV-1/2 and p24 antigen simultaneously, which reduces the window period to an average of 2 weeks (most rapid HIV assays only detect HIV antibodies, with an average window period of 3 to 4 weeks) [6,7]. Alere (Waltham, USA) has recently launched the Determine™ HIV-1/2 Ag/Ab Combo, which is the first rapid fourth generation HIV assay [8].

In diagnosis of HIV infection different testing approach is adopted to meet a specific need. Each country identifies, adopts and implements an appropriate HIV testing algorithm suitable for use in its environment; this algorithm must be reviewed annually to determine if the test performance is as expected and if any changes need to be made to the algorithm [9].

A number of factors contribute to the selection of a specific algorithm in a country, these factors include; test performance, test availability in the country, ease of use, and cost. Performance of test is one of the factors that determine a country's algorithm. This performance depends on sensitivity and specificity of the test under consideration with respect to the gold standard.

The world health organization standard recommended algorithm for Nigeria is serial algorithm in which the result of the first test determines whether an additional testing is required. Serial algorithm is 2.5 fold less costly than the parallel algorithm [10].

Many HIV diagnostics kits have flooded Nigeria market, at the individual level, inaccurate testing can lead to a false positive or negative result, both of which have impact on the health and mental well-being of the patient and entire communities. Since HIV-1 and 2 sero-diagnoses is a backbone of VCT (volunteer counselling and testing), HIV diagnosis and an essential part in the fight against HIV/AIDS epidemic, it is therefore essential to identify and select a suitable diagnostics kit for the country and design suitable algorithm for HIV testing in the country. The aim of this study was to ascertain the performance of commonly used diagnostics kits in Port Harcourt and validate the HIV testing algorithm being used. The objectives are: To evaluate the performance of rapid test used in Port Harcourt if it meets the WHO recommended standard of specificity and sensitivity, to review the serial testing algorithm adopted in Nigeria and to evaluate parallel testing algorithm for HIV1/2 screening.

Kit type	HIV infected	HIV uninfected	HIV prevalence (%)
Determine			
Positive	30 (true+)	0 (false+)	17.4
Negative	15 (false-)	127 (true-)	-
Stat-Pak			
Positive	32 (true+)	0 (false+)	18.6
Negative	13 (false-)	127 (true-)	-
Core			
Positive	32 (true+)	0 (false+)	18.6
Negative	13 (false-)	127 (true-)	-
Dia-Pro	45	127	26.1

Table 1: Result obtained with the various assay. +: Positive, -: Negative; Number test=172.

Materials and Method

Study area and population

The study was carried out in LulluBriggs health center in University of Port Harcourt, laboratories were randomly visited in Port Harcourt. Port Harcourt is in Rivers State which is in South-South region of Nigeria. A total of one hundred and seventy two (172) patients attending LuluBriggs Health Center University of Port Harcourt from April to June 2013 consented to participate. Thirty laboratories were visited in Port Harcourt.

Sampling technique

Randomly selected laboratories were visited in Port Harcourt and information about the type of rapid assay used was obtained. Blood sample was collected randomly from patients visiting Lulubriggs Health Center. Demographic information about each screened person was obtained (age and sex). All information about screened persons were kept confidential.

Sample collection, preparation and storage

Five millilitres of peripheral blood samples were collected by venous puncture using sterile syringes and needles. The blood samples were dispensed into sterile universal bottles. Serum was extracted by centrifugation of blood specimens obtained at 3000 rpm for 10 min and stored frozen at -20°C until analysed in the laboratory.

Assay	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Accuracy (%)	PPV (%)	NPV (%)
Determine	66.7 (58.7-74.7)	100 (-)	91.3	100	89.4
Stat-Pak	77.1 (71.1-83.1)	100 (-)	92.4	100	90.7
Core	77.1 (71.1-83.1)	100 (-)	92.4	100	90.7

Table 2: Performance of three rapid HIV test compared to the gold standard (Dia-Pro).

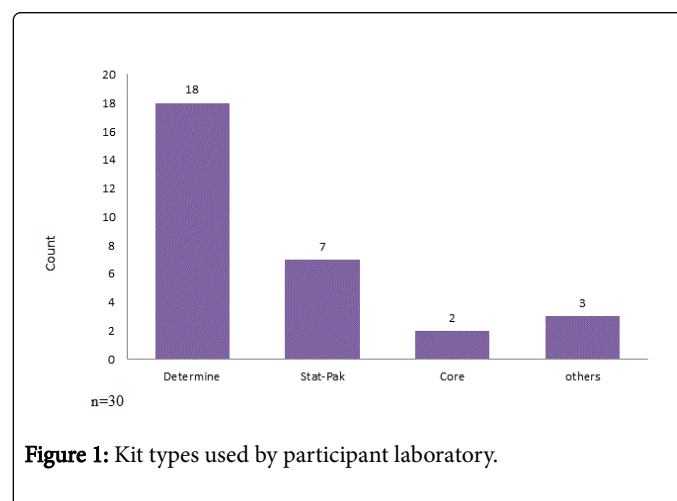


Figure 1: Kit types used by participant laboratory.

Reference assay

Alere Determine™ HIV-1/2, Chembio Stat- pak HIV-1/2, and CORE HIV-1/2, were rapid assays used. Determine™ HIV-1/2 and Chembio Stat-pak HIV-1/2 has HIV1/2 antigen conjugated at one end and two reaction window labelled as test and control window. Core assay is a dual recognition rapid enzyme immunoassay and is based on the specific detection of anti-HIV-1 and anti-HIV-2 by antigens that bind to both antibody binding sites. Dia-pro an automated 4th generation Enzyme Immunoassay for determination of HIV 1/2/0 and p24 antigen in human serum and plasma used as gold standard.

Criteria	Determine	Stat-Pak	Core	Dia-pro
Speed	15-30 min	15-30 min	15-30 min	>3 hours
Test accuracy				
Sensitivity	66.70%	71.10%	71.10%	highly sensitive
Specificity	100%	100%	100%	highly specific
Ease of test	very easy	very easy	very easy	not easy

Reagent stability	2-30°C	2-30°C	2-30°C	2-8°C
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Table 3: Characteristics of the HIV kits.

Rapid test screening of the serum samples using serial and parallel testing algorithm

Determine™ and Stat-Pak was selected for use in serial testing algorithm. Determine™ was used to first test all the samples, the reactive samples were retested using Stat-Pak to check for discordance. All samples were tested with three rapid test (Determine™ Combo assay HIV1/2, Core HIV1/2, Stat-Pak HIV1/2) using a parallel algorithm. All the rapid kits are immune-chromatographic assay designed for the qualitative detection of HIV-1/2 antibodies. Testing was performed and results were interpreted following manufacturer’s instructions.

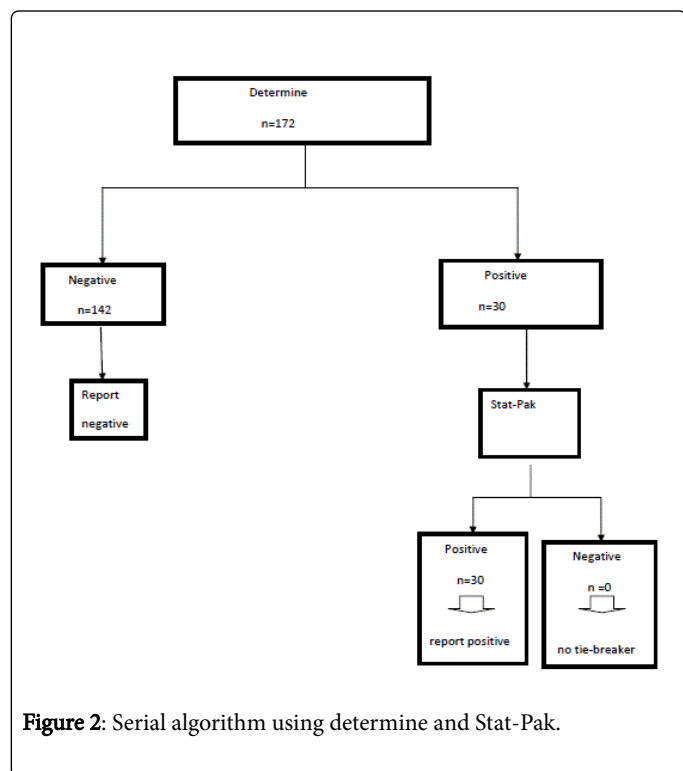


Figure 2: Serial algorithm using determine and Stat-Pak.

ELISA screening of the serum samples

All specimens were retested using Dia-Pro a 4th generation enzyme immunoassay for determination of antibodies to all subtypes of HIV1/2 and HIV-1 antigen (p24) in human serum or plasma. Testing was performed and results were interpreted following manufacturer’s instructions. Test validity and evaluation were measured using the result of the automated ELISA as the gold standard. Sensitivity, specificity, positive predictive value and negative predictive values were calculated.

Results

Actual HIV result

A total of 172 samples were screened using three HIV rapid test (Determine, Core and Stat-pak), and retested using a 4th generation ELISA (DIA-pro) as the gold standard. Table 1 shows the HIV result obtained with the assay, 30 serum samples were reactive with Determine™, 32 serums were reactive with Core and Stat -Pak and 45 reactive serums with Dia-Pro which is the actual HIV positive result.

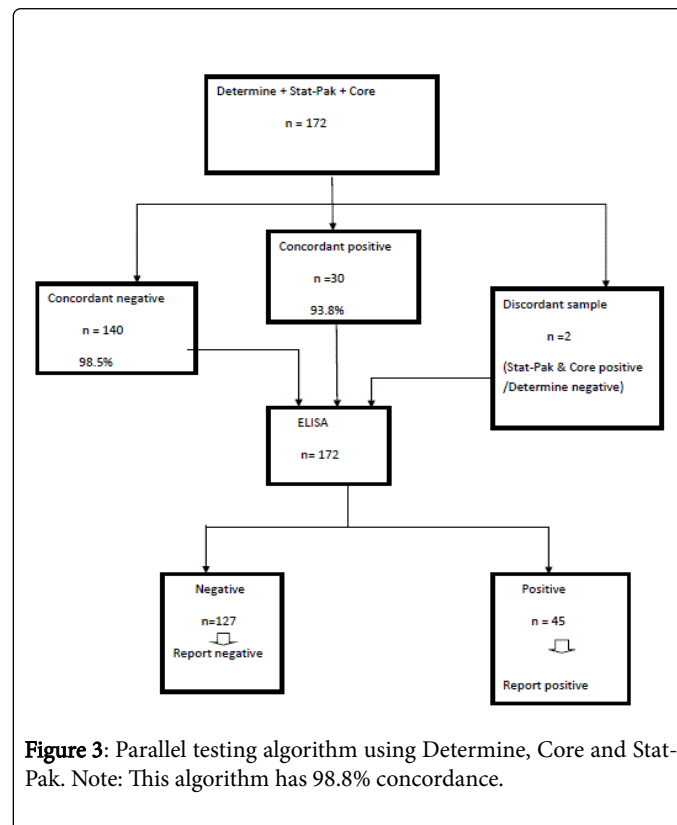


Figure 3: Parallel testing algorithm using Determine, Core and Stat-Pak. Note: This algorithm has 98.8% concordance.

Performance of the rapid assays

In Table 2 the performance of three the rapid HIV test compared with the gold standard was presented. Core and Stat-Pak had higher sensitivity of 71.7% than Determine™ with sensitivity of 66.7%. The specificity of all the assays was 100% showing that there is 100% probability that all reactive serum were actually positive. Accuracy of Core and Stat-Pak performance was 92.4% while that of Determine was 91.3%.

Frequency of the use of rapid kits by participant laboratories

Determine was the most used kit by the participant laboratories 18 (60%) with Core being less used 2 (6.6%) as shown in Figure 1.

Characteristics of the rapid kits

Table 3 describes the characteristics of the various rapid kits, the various kits met the criteria of ease of use, stability, speed, but came short on sensitivity, which the WHO recommendation is 99%. The speed of Dia-pro, not easy to use, not stable at room temperature, although highly sensitive makes it impractical in point-of-care service.

Serial testing algorithm

Figure 2 shows serial algorithm in which Determine was first used, the non-reactive samples with Determine were reported negative. The reactive samples were further confirmed with Stat-Pak, there was no discordant sample thus no tie-breaker (third test).

Parallel testing algorithm

With parallel testing algorithm there were two (2) discordant samples and thirty (30) concordant samples and Dia-Pro the gold standard was used as a tiebreaker thereby giving the actual result as shown in Figure 3.

Discussion

A wide range of HIV tests are available in Nigeria. The challenge today is to identify the most suitable assays for a given set of circumstances without compromising the reliability of test results. This research work evaluated the performance of three rapid test kits (Determine™, Core and Stat-pak) against a 4th generation ELISA (DIA-pro) and serial testing algorithm for HIV diagnosis used in Port Harcourt.

The Determine kit did not show superiority for HIV antibody detection when compared to other rapid assays despite its wide use in Port Harcourt. The result of this evaluation shows 66.7% with 15 false negative, for Determine in comparison with 71.1% sensitivity with 13 false negative for Stat-Pak and Core. In concordance with Rosenberge et al. [11] and Pavie et al. [12] evaluation studies Determine was not more sensitive than other kits used in point-of-care.

Also an evaluation by Dessie et al. [13] showed that Determine™ HIV-1/2 sensitivity, specificity, positive and negative predictive values were 60.5%, 98.9%, 88.5% and 94.9% respectively, which also agree with the result obtained from this study. However, the result showed performance that was less than that claimed by the manufacturer's insert.

In this study, the rapid assay detected HIV-1/2 antibodies with a low sensitivity. Rapid HIV assays are generally not as sensitive as HIV automated ELISA for the detection of HIV antibodies, in particular during HIV seroconversion [12], assays that detect p24 antigen reduce the diagnostic window period of HIV testing. Most rapid assays have poor sensitivity to diagnose acute HIV infection as they only detect antibodies against HIV-1/2, therefore it is not surprising that the rapid assay did not detect HIV reliably in specimens with relatively low signal to cut-off values on automated HIV ELISAs. Also the first available rapid assays were initially developed for detection of B subtypes antibodies. However in Africa of HIV-1 non B subtypes and CRF (circulating recombinant factor) poses a diagnostics problem. HIV antigenic diversity has been implicated in poor antibody detection [14].

Furthermore the low sensitivity of the rapid test in comparison with the Dia-Pro ELISA suggest that the detection of p24 antigen in a sample gives a better result, and the process of incubation and washing could contribute to validity, as heat denaturation may aid in dissociation of non-specific antigen-antibody complexes [15]. The rapid assay, does not require heat denaturation. Previously published data suggested that the presence of immune complexes in a specimen due to antibodies that bind p24 antigen may interfere with the detection of p24 antigen in an assay [16].

It has been suggested that patients on combination of antiretroviral therapy, have decreased anti-HIV antibody titer, causing false negative test result. However none were falsely negative with rapid tests although all were on antiretroviral therapy.

The fact that the Determine test was used more than the other kit 18 of 30(60%) visited laboratories, could the result of WHO recommendation of Determine as a standard kit for HIV antibody detection [17,18].

According to the WHO, an ideal test for the rapid diagnosis of HIV infection should be rapid, inexpensive, highly sensitive and specific, and easy to perform and interpret [3]. In addition to these characteristics, ideal rapid tests should possible to be stored at room temperature, should have long shelf lives, and should require no additional equipment or auxiliary supplies in order to be performed [19]. The rapid assays used in this study fulfil these criteria and, as such, serves as a powerful tool for point-of-care HIV diagnosis. So in absence of more sensitive assays these tests may still be used to detect HIV-infected persons, since the specificity is high [20,21].

The use of rapid test serial algorithm for the screening of HIV infection was not valid according to this study, many reactive serums were assumed to be negative. Thus parallel algorithm in which more than one rapid test is used in point of care should be adopted, since for now it is not possible to get ELISA machine in all remote areas.

The use of single rapid kit provide an alternative in situations in which parallel algorithm is impractical. However, more than 10% of HIV-positive cases would be missed due to the poor sensitivity of the rapid test kit. Notification of false-negative results could have serious personal and public health consequences.

Conclusions

Ensuring the quality of HIV testing in support of prevention and care efforts has been identified as a priority by Center for Disease Control and Prevention. The result obtained from this study shows that the performance of the commonly used rapid kits in Port Harcourt is low and the serial testing algorithm is not valid due to the low sensitivity of the kits.

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