Original article

Evaluation of Virologic Methods for Early Detection of HIV-1 in a Resource-limited Setting: Performance and Cost Analysis.

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Abstract

Introduction
Rapid testing and detection of acute HIV infection are two important arms in the prevention of HIV infection. Virologic testing for HIV remains the mainstay for early diagnosis of the infection. Nucleic acid-based testing for HIV however, requires expensive laboratory infrastructure and well-trained personnel, thereby making it not easily accessible in Low-Middle- Income Countries (LMIC). HIV DNA polymerase chain reaction is currently used by few laboratories in many LMIC to detect HIV in children born of HIV-positive mothers before 18 months. Challenges relating to timely result notification can be reduced if the Early Infant Diagnosis (EID) Programme is decentralized and with easy access to laboratory facilities using other tests with high performance characteristics.
Methods
We evaluated the performance of five assays to identify HIV antibodies, p24 antigen, proviral DNA or viral RNA in 109 infants born to HIV-positive mothers in Yaounde, Cameroon.
Results
The test performance (using plasma) of the HIV p24 antigen ELISA by Perkin Elmer, Roche Amplicor HIV-1 DNA PCR and the Abbott Realtime HIV-1 assay was 100% identifying 12 positive cases. A positive and significant correlation between the HIV-1 RNA viral load and HIV p24 antigen level was found ($p<0.05$).
Conclusion
Therefore, HIV p24 antigen detection by ELISA can be used for early diagnosis of HIV and thus recommended for a decentralized EID Programme in LMIC.

KEY WORDS:
HIV, antigen, detection, immunoassay, sensitive, Cameroon
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Resumé
Évaluation des outils virologiques pour le diagnostic précoce du VIH-1 dans les pays à ressources limitées : Analyse de la performance et du coût
Introduction
Les tests de diagnostic rapide et le dépistage de l'infection aigue du VIH constituent deux approches importantes dans la prévention à l'infection du VIH. Les tests virologiques demeureraient l'approche optimale pour un diagnostic précoce. Toutes fois, les tests détectant le matériel génétique du VIH exigent une plateforme onéreuse et une grande expertise, les rendant ainsi inaccessibles dans les pays à ressources limitées (PRL). La PCR (réaction de polymérisation en chaine) à l'ADN est couramment utilisée dans certains laboratoires des PRL comme outil de diagnostic de l'infection à VIH chez les enfants de <18 mois nés de mères séropositives. Les défis dans le délai du rendu des résultats seraient réduits par une décentralisation du Programme Nationale de Diagnostic Précoce (PNDP), facilitant ainsi l'accès à des tests aussi performants et moins onéreux
Méthodes
Nous avons évalué les performances des cinq tests pour la détection des anticorps du VIH, l'antigène p24, l'ADN proviral ou l'ARN virale chez 109 enfants nés des mères séropositives à Yaoundé au Cameroun.
Résultats
La performance de l'ELISA antigène p24 (Perkin Elmer), de Roche Amplicor HIV-1 DNA-PCR et de Abbott Realtime HIV-1 était de 100% sur les 12 cas positifs. Une corrélation positive et significative ($p<0.05$) a été observée entre la quantification de l'ARN virale et de l'antigène p24 du VIH.
Conclusion
Il en ressort que la détection de l'antigène p24 par ELISA serait une bonne alternative pour le diagnostic précoce du VIH dans les PRL, et pour la décentralisation du PNDP.

MOTS CLÉS
VIH, antigène, détection, test immuno-enzymatique, sensible, Cameroun

INTRODUCTION

Detection of HIV can be done by direct or indirect testing using different specimens (whole blood, saliva, plasma, serum, dried blood spot, dried plasma spot, etc) depending on the purpose of testing, availability of laboratory facilities and trained personnel. Routine screening for HIV in Cameroon is aimed at detecting specific antibodies to HIV proteins but the limitation of these procedures is the serologic window of three months which allows the risk of getting false negative results (Brauer et al, 2013). However, HIV p24 protein can be detected on average from 10 to 14 days after infection and positive seven days after HIV RNA test (Fiebig et al, 2003, Schupbach et al, 2003; Pilcher et al, 2010) and earlier than HIV antibody during acute infection. Although HIV p24 antigen exhibit a diurnal variation (Schupbach et al, 2003), ELISA developed to detect it are reliable and therefore can be used as an alternative diagnostic tool which is less costly and easier to perform than PCR-based assays. Steps to ensure early detection of HIV are important arms in an effective Prevention and/or Treatment Programme of the health system considering the cost to the individual or the health care system.

Since 2007, HIV-1 DNA PCR technique using dried blood spot specimens (DBS) has been the standard of testing infants born to HIV-positive mothers (Nkenfou et al, 2012) in all ten Regions of Cameroon by two National Reference Laboratories (Figure 1). In addition, serologic testing from age 18 months is recommended. To overcome this barrier, alternate technologies such as nucleic acid testing to detect viral RNA, proviral DNA or assays to detect p24 antigen (HIV core protein) can be used to reduce the diagnostic window to about two weeks (Schupbach et al, 2003).

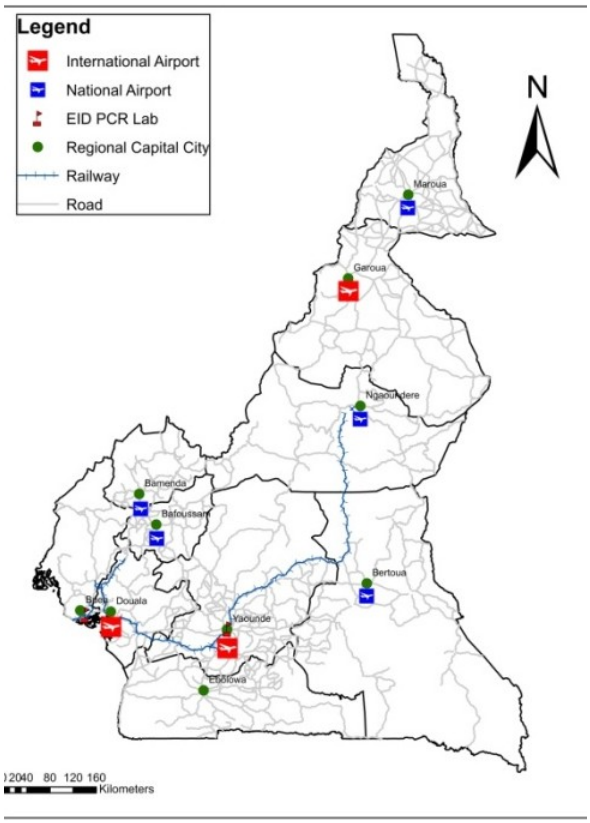

Figure 1: Map of Cameroon showing the ten administrative Regions, the road network, train stations, airports and the National Reference Laboratories for the HIV Early Infant Diagnosis (EID) Programme.

We have used Cameroon as an example of a Low- and Middle- Income Country (LMIC) as defined by the World Health Organization (WHO), to present the challenges encountered in the different EID Programs. In Cameroon, the coverage of the EID Programme was 10.7% and a HIV prevalence 7.1% in 2011 compared to 8.9% in 2010 (National AIDS Control Committee 2011 Report). Similarly, children born of HIV-positive mothers older than 18 months were tested for HIV antibodies and 24.8% was reported positive in 2010 (National AIDS Control Committee 2011 Report, Figure 2).

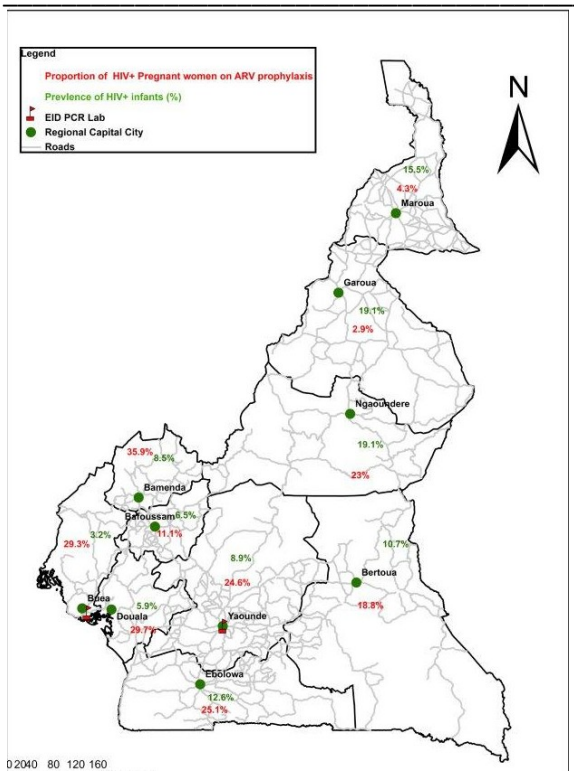

Figure 2: Map of Cameroon showing PMTCT Coverage by administrative Region and HIV prevalence in infants born to HIV-positive mothers in 2011 (NACC 2011 Report).

Challenges relating to collection and handling of the dried blood spot (DBS) specimens, transportation of specimens to the National Reference Laboratories (NRLs) and dispatch of results to the care-providers in the PMTCT sites are common in the ongoing EID Programme. To surmount such challenges an easy-to perform virologic testing alternative, was evaluated in a population where a broad genetic variation of HIV-1 is reported (Torimiro et al, 2009). We hereby report the performance of two PCR protocols using RNA and DNA respectively, two HIV-1 p24 antigen assays (rapid and ELISA) and one HIV-1 antibody test for clinical use and a cost-effectiveness comparative analysis.

METHODS

Study design:

Specimen collection: collection of 3mL of blood by venepuncture from 109 infants born of HIV-positive mothers and aged between 6 to 72 weeks, and 32 born of HIV-negative mothers was done at the Mother-Child Hospital of the Chantal Biya Foundation. Blood The plasma and dried blood spots (DBS) collected on filter paper (Whatman BFC 180) were prepared and stored at 4^{o}C before analysis and at -80^{o}C and -20^{o}C after analysis, respectively. The technicians who carried out the PCR assays were blinded from the results of the HIV-1 p24 antigen testing to avoid bias in the interpretation of all the results.

HIV TESTING: Each specimen was tested for specific HIV-1 & HIV-2 envelope antibodies, p24 antigen, HIV-1 RNA or HIV-1 proviral DNA following the manufacturers' instructions and using the following assays. The gold standard used in this study was the Roche Amplicor HIV-1 DNA assay version 1.5.

Roche Amplicor HIV-1 DNA assay Version 1.5: The Amplicor HIV-1 DNA Test is a qualitative *in vitro* test for the detection of HIV-1 proviral DNA in human whole blood from dried blood spot specimens. The test utilizes amplification of target DNA by PCR and nucleic acid hybridization for the detection of HIV-1 DNA. The test permits multiplexed PCR amplification of HIV-1 target and HIV-1 Internal Control DNA (http://www.roche.com).

Abbott RealTime HIV-1 m2000^{RT} assay: The Abbott RealTi*me* HIV-1 assay is an *in vitro* reverse transcription-polymerase chain reaction (RT-PCR) assay for the quantization of HIV-1 on the automated *m*2000 System using human plasma from HIV-1 infected individuals over the range of 40 to 10,000,000 copies/mL of RNA viral load. The Abbott RealTi*me* HIV-1 assay is intended for use in conjunction with clinical presentation and other laboratory markers for disease prognosis and for use as an aid in assessing viral response to antiretroviral treatment as measured by changes in plasma HIV-1 RNA levels. Although this assay is not intended to be used as a donor screening test for HIV-1 or as a diagnostic test, it was used in this case to confirm the presence of HIV-1 infection (http://www.abbottmolecular.com/products/infectious-diseases/realtime-pcr/hiv-1-assay.html).

Immunocomb HIV-1 & HIV-2 ag-ab TriSpot Rapid test: The ImmunoComb II HIV 1 & 2 Tri-spot Ag-Ab test (Orgenics) is a rapid EIA test intended for the qualitative and differential detection of antibodies to HIV-1 and HIV-2, and HIV-1 p24 antigen in human plasma or serum (www.productosweens.com/eng/immuno1.swf).

Alliance HIV-1 p24 Antigen ELISA : The Alliance HIV-1 p24 Antigen ELISA (Perkin Elmer) was used for the detection of HIV-1 Group M and HIV-1 Group 0 Subtypes in plasma. This kit provides reagents for immune complex disruption (ICD) of antigen/antibody complexes in serum and plasma samples and proprietary antibodies increasing the sensitivity of the assay. The quantity of free HIV-1 antigen in a specimen was determined by comparing its absorbance with that of known HIV-1 p24 antigen standard curve (http://www.perkinelmer.com/Catalog/Family/ID/AllianceR%20HIV1%20P24%20ELISA%20Kit).

Data Analysis

Sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) were calculated as percentages. Linear regression analysis and analysis of variance were used to determine correlation between the different testing methods.

RESULTS

The study population consisted of 109 infants with 75 who were born of HIV-1 infected mothers and of whom 21 (28%) were breastfed and 48 (44%) had no antiretroviral (ARV) prophylaxis. Thirty four children born of HIV-uninfected mothers served as the Control group.

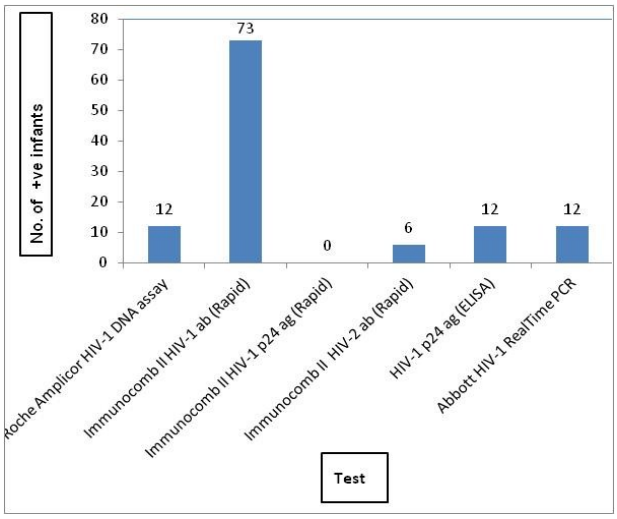

Figure 3 : Results of HIV detection using 4 different assays of different principles and detection markers

The above Figure shows that the virologic assays detected the presence of HIV while the rapid HIV p24 antigen test (ImmunoComb II HIV 1 & 2 Tri-spot Ag-Ab test) gave 100% of false negative results. This test which was the direct assay used, could not differentiate between infant and maternal HIV antibodies supporting the fact that these infants (97%) habour maternal antibodies circulating even at age 18 months. HIV-2 was not detected.

The two virologic assays to detect either HIV-1 p24 antigen or RNA showed acceptable performance whilst comparing with the gold standard, Roche Amplicor HIV-1 DNA PCR (kappa concordance = 1.00). Considering the cost and performance of these assays, laboratory facility and competencies of the laboratory personnel, the ELISA HIV p24 antigen (Perkin Elmer) proved to have high performance characteristics, more cost-effective, and easy-to-use. The ELISA p24 antigen can therefore be brought closer to the infant than any other of the tests that was evaluated and thus suitable for a decentralized National EID Program.

Using the optical densities of the ELISA HIV p24 antigen and the Roche Amplicor HIV-1 DNA PCR, and the HIV-1 RNA load in logarithmic scale of the twelve positive cases, we found a positive and significant correlation (Figure 4).

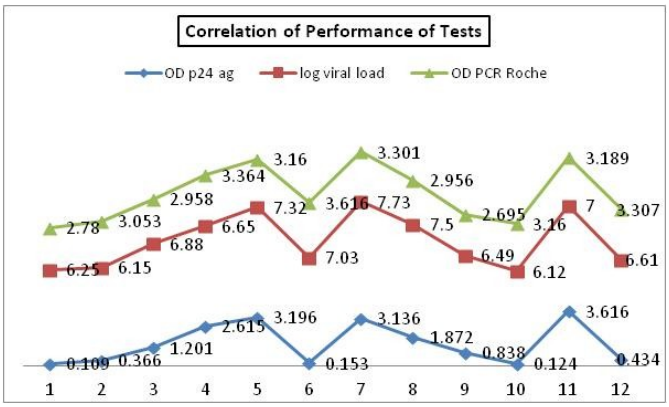

Figure 4: Comparison of load of HIV particles using three virologic assays (ELISA HIV p24 antigen (Perkin Elmer), Abbott HIV-1 RealTime PCR, Roche Amplicor HIV-1 DNA PCR (gold standard)

Linear regression analysis was used to explore the relation between ELISA HIV-1 p24Ag and HIV-1 RNA viral load (log, copies) which was positive and significant.

There is a positive and significant correlation (p=0.03) between HIV-1 RNA load (Log copies) and p24 antigen titre (optical density, Figure 5a).

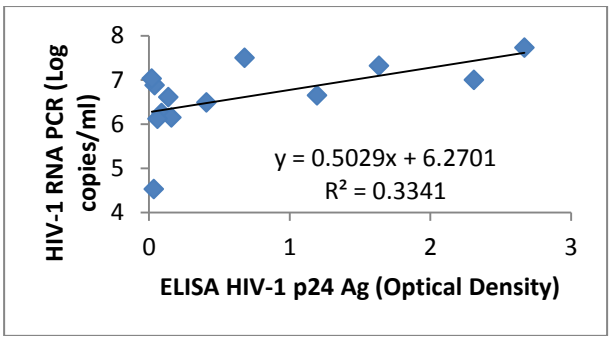

Figure 5a: Correlation between HIV-1 RNA Viral Load (log copies) and quantity of HIV-1 p24 antigen (optical density)

TABLE 1: PERFORMANCE CHARATCERISTICS AND COST OF TESTS

Assay	Performance characteristics of assays				Cost (US$)
	Sensitivity	**Specificity**	**Positive Predictive Value (PPV)**	**Negative Predictive Value (NPV)**	
Immunocomb II HIV-1 antibody	100	37	16	100	5
Immunocomb II HIV-1 p24 antigen	50	100	100	94	
HIV-1 p24 ag ELISA	100	100	100	100	10
Abbott HIV-1 RealTime PCR	100	100	100	100	40
Note: Roche Amplcor HIV-1 DNA PCR (used as gold standard)					**40**

There is a positive and significant correlation (P=0.008) between HIV-1 RNA load (copies) and HIV-1 p24 antigen titre (optical density, Figure 5b).

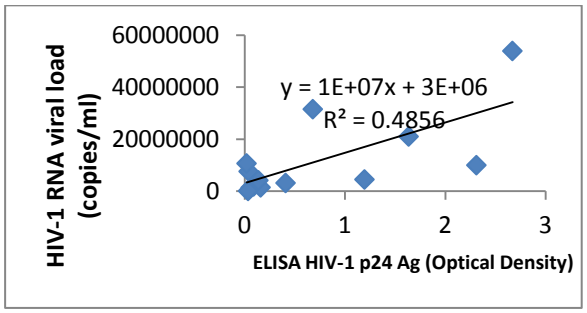

Figure 5b: Correlation between HIV-1 RNA Viral load (copies/mL) and quantity of p24 antigen

Comparing the results of HIV-1 RNA load (in copies or in logarithmic scale) and p24 antigen optical density, the positive and significant correlation (P=0.008) suggests that p24 antigen optical density can be used as a biologic marker for monitoring disease progression and response to antiretroviral treatment. Although the small samples size of our study, similar findings have been reported by other investigators.

DISCUSSION

Rapid and early diagnosis of HIV in infants exposed through their mothers has great benefits in the paediatric care and treatment continuum. Although technological advances should bring the laboratory closer to the patient, access to these technologies in resource-limited and geographically remote regions in sub Saharan is poor. The poor road network and limited public and affordable transportation services, poor laboratory infrastructures, geographic isolation and lack of well-trained staff, contribute to the low coverage of the EID Program and delay in the implementation of point-of-care tools for early infant diagnosis of HIV. However, testing with PCR-based methods has achieved successes in the PMTCT Programme in several African countries although the coverage is about 7.9% in Cameroon. In this study, a cost-effectiveness analysis and assay performance evaluation were used to compare another virologic test to a "gold standard" (the RNA PCR-based methods) before implementation in a decentralized Program for laboratories with an ELISA chain of equipments.

Our results are similar with studies carried out in some African countries (Sherman GG et al, 2004, Susan Fiscus et al, 2007). Specimens used in our study covered the entire clinically significant range following infection as well as performance limits with the PCR-based assays. On the other hand, our study included direct detection approaches based on quantification of HIV p24 antigen and DNA, measurement of HIV RNA viral load and indirect or antibody EIA formulated into point-of-care rapid diagnostic test. HIV p24 antigen ELISA gave a sensitivity and specificity of 100% when compared to the gold standard (HIV-1 DNA PCR) as well as the Realtime PCR HIV-1 viral load commercial assays.

To promote universal access to testing of HIV to a broad patient population and in order to achieve the goals of "Test and Treat" Programme, the laboratory technology for screening should be decentralized and use Point-of-Care (POC) assays that have proven to be easy to transport and manipulate, and are cost-effective

The potential benefit of including HIV p24 antigen assays in screening Programs in general could be to detect recent infections prior to emergence of HIV antibodies for example in the blood bank and after known exposure risk (FDA Consumer Report, 2011).

Another important finding from this study is the positive correlation between HIV-1 RNA Viral load and HIV-1 p24 antigen titre. Although it is not clear which appears first or become detectable during acute infection, HIV-1 p24 antigen levels showed a

positive correlation with RNA viral load in our study. In the chronic phase of HIV infection however and advanced disease, RNA load increases while no concomitant increase in p24 antigen levels is observed. Not withstanding, HIV p24 antigen levels correlated significantly with immune parameters in HIV disease and this can be considered a good predictor of disease progression as is viral RNA.

"The HIV Early Diagnosis – Early Treatment" Initiative in resource-limited settings requires that the laboratory be brought closer to the population in terms of diagnosis and monitoring of treatment response. With the PMTCT B+ option for the prevention of vertical HIV transmission, an effective early diagnosis program for both mother and infant would reduce the burden of paediatric HIV infection in the country. However, there is need to evaluate HIV-1 p24 antigen titres compared to viral RNA load with non-B subtypes of HIV-1 circulating predominantly in Cameroon and other African countries, for sensitivity to disease progression, or death as well as for monitoring antiretroviral therapy as has been reported by others (Richard AR et al, 2005, Repress R.A et al, 2005, Tehe A et al, 2006).

CONCLUSION

We conclude that HIV p24 antigen ELISA is sensitive and affordable and can be used for early diagnosis as well as prediction of disease progression in early infection in resource-limited settings such as Cameroon. However, more data is needed to confirm the profile of HIV p24 antigen in disease progression in this population.

CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare in the evaluation of these methods.

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