Evaluation of HIV ELISA Diagnostic Kit developed at the Institute of Primate Research, Nairobi, Kenya

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SUMMARY

The first diagnostic kits utilizing the enzyme-linked immunosorbent assay (ELISA) technique were developed in mid-eighties, and since then, this technique has become an increasingly important tool for screening multiple samples of blood or serum for presence of antibodies to various infectious pathogens, especially human immunodeficiency virus (HIV) in blood banks. However, most of the commercial diagnostic kits currently available in the market are too expensive, hence not easily affordable in most Diagnostic Laboratories. We designed an ELISA kit for diagnosis of HIV and compared it with some of the commercial kits. We used blood samples from the blood bank at the National Public Health Laboratory Services (NPHLS) in Nairobi and from patients referred to the Kenya Medical Research Institute (KEMRI) for HIV screening. Two commercial kits were used, Wellcozyme HIV Recombinant kit and Recombigen (*env & gag*) HIV-1 EIA kit. Out of 1350 samples tested by Recombigen (*env & gag*) HIV-1 EIA kit, 419 (31.0%) were positive while 421 (31.2%) samples were positive by Wellcozyme HIV Recombinant kit. Our ELISA kit detected a total of 431 positive samples out of 1350 (31.9%), which was almost identical to the results from the other kits. Our kit was nearly identical in terms of sensitivity and specificity to the other two commercial kits used in this study. Thus our ELISA system, which is much cheaper than the commercial kits currently available in the market, offers a more affordable system for routine HIV tests.

[Afr. J. Health Sci. 2002: 9:117-122]

Introduction

Almost 20 years after its first appearance, the HIV /AIDS epidemic continues to pose as a public health threat worldwide [1]. Research efforts have increased the level of knowledge about the mode of HIV transmission and the determinants of AIDS, morbidity and mortality among HIV/AIDS infected individuals. However, given the lack of an effective chemotherapeutic agent HIV prevention remains a priority. Some success in controlling the spread of this disease has been achieved in some countries such as Uganda and Thailand, where HIV prevalence had been very high, but is now on decline or has stabilized [2]. In Sub-Saharan Africa, HIV infection is predominantly spread by heterosexual

transmission [3]. Reports from hospitals in African cities severely affected by the HIV/AIDS epidemic have shown that AIDS is a leading cause of death among young adults between 20 and 40 years of age [4].

In Kenya, the first AIDS case was reported in 1984, and by the end of that year, seven more cases were confirmed by the National AIDS Control Program [5]. Since then, infection rates have steadily increased. For example, in 1990 prevalence of 3.3% was reported and this rose to 5.6% by 1992. By June 1996 about 66,000 cases of AIDS had been reported to the Kenyan Ministry of Health. However, it is suspected that only about one-third of cases are reported to the Ministry of Health or the National AIDS/STDs Control Program. This may be attributed to a number of factors including: (a) Individuals not seeking or able to afford adequate health/hospital care; (b) the stigma attached to a diagnosis of the disease (c) infected individuals may have refused HIV testing, (d) individuals with HIV infection who may have died as a result of other complications or illnesses prior to a diagnosis of HIV; (e) Inaccurate HIV diagnosis; and (f) HIV diagnosis hampered due to cultural beliefs like witchcraft [6,7]. Serological diagnosis of human immunodeficiency virus (HIV) is based on detection of HIV specific antibodies. Anti-HIV screening assays were primarily developed in an effort to protect the blood supply. Increasing testing has since been required in some situations for obtaining life insurance, marriage certificates, immigration documents, and for seroprevalence survey. The first HIV test became available in 1985 [1]. Since then, diagnostic methods that rely on detection of HIV-specific antibodies in serum and using enzyme-linked secondary antibodies to detect immobilized primary antibodies on a solid support (called enzyme-linked immunosorbent assay, ELISA) have been developed. These techniques are very sensitive and specific [8-10], and the implication of this is that most infections can be detected at an early stage of infection [11]. This method is also relatively simple and can be easily automated with high reproducibility. Thus ELISA have become one of the most widely spread method used to detect HIV antibodies [12].

Initially assays were made of virus lysate antigens from infected cell lines and were used to coat the solid phase, but unfortunately antibodies directed against lymphocyte antigens were found to cause unspecific reactions [11]. Subsequently, the use of recombinant HIV proteins and synthetic HIV peptides provided a considerable improvement in the specificity of ELISA [1]. The use of recombinant antigens enabled specific HIV epitopes to be more densely 'packed 'on the solid phase and thus yielded not only improved specificity but also enhanced sensitivity [1].

A variety of different ELISA kits are commercially available, and they mainly consist of HIV recombinant antigens immobilized on a solid support, and utilize a secondary antibody conjugated to an enzyme, and substrate detection system. The solid supports can be either "wells" of microtitre plate, plastic beads, or a type of paper, usually nitrocellulose. In the most common type of ELISA, addition of sample to the antigen-coated bead or wells enables binding of specific antibody (anti-HIV) if present, to the antigen. A secondary antibody conjugated to an enzyme or fluorochrome is added and incubated to allow binding to the immobilized primary antibody (if present). The presence of bound antibody is then visualized by addition of enzyme substrate with a chromogen, resulting in a coloured solution whose optical density can be measured. The commercial kits currently available are very expensive since they are imported, and are not affordable in most hospitals in developing countries. Thus we designed a kit at the Institute of Primate Research, Karen, Nairobi, which is cheap to produce and has a similar sensitivity and specificity as the currently available commercial kits. In this report, we evaluated the usefulness of this kit in routine screening of serum for HIV infection.

Materials and Methods

Human serum samples were obtained from the NPHL, Nairobi, and the KEMRI, Nairobi, Kenya. Two commercial ELISA kits were used to test the serum for HIV infection; the Wellcozyme HIV Recombinant kit (Wellcome Diagnostics, Dartford, England) and Recombigen (*env & gag*) HIV-1 EIA kit (Cambridge Biotech Corporation, USA). Both kits consist of 96-well ELISA plates coated with recombinant HIV *env*elope (gp120, gp41) and matrix (p24) proteins, and utilize peroxidase-conjugated secondary antibodies, 0.05% hydrogen peroxide and tetramethylbenzidine for detection of primary antibody.

The IPR ELISA kit was developed as follows. Briefly, we used a 20 amino acid synthetic peptide derived from a conserved, immunodominant region of HIV-1 transmembrane glycoprotein, gp41 (GWGGCSGKICTTAVPWNAS; Research Genetics, Huntsville, AL). The peptide was used to coat the wells of an ELISA plate (Falcon, Becton Dickinson, Oxnard, CA) at a concentration of 40µg/ml in bicarbonate buffer, pH 9.6 (100µl per well) and incubated overnight at 4°C. The plates were washed 3 times with Tris-buffered saline, containing 1% Tween-20 (TBS-T). Non-specific binding sites were blocked using 5% fat-free milk in TBS for one hour at room temperature. The samples were diluted 1:100 in TBS-T and 100µl dispensed into each well and incubated for 3 hours at 37°C. The plates were washed 3 times, then 100µl of peroxidase-conjugated goat anti-human IgG (Nordic Labs, Capistrano Beach, CA) added to each well and incubated for 2 hours at 37°C. Following a further wash, positive reactivity was detected by incubating the plates for 20 minutes with 1 mg/ml o-phenylenediamine tetrahydrochloride (Sigma Immunochemical Co., St. Louis, MO) in urea hydrogen chloride buffer, pH 5.0. The reaction was then stopped by addition of 50ul of 4M sulphuric acid, and the optical density of each well read spectrophotometrically at 50nm using a DYNEX MR ELISA plate reader (Dynex Technologies, Germany). The samples were assayed in duplicate and considered positive if the mean optical density (OD) for the sample wells were greater than the mean OD of the negative control serum plus 3 standard deviations of the mean OD negative control sample.

Results

The Wellcozyme HIV Recombinant kit was used as the gold standard to analyse the sensitivity of the IPR kit because it's sensitivity and specificity, calculated by the manufacturer were both given as 100%. Of the 1,350 serum samples tested with this kit, 421 samples tested positive (31.2%), and this was taken to reflect the true positive samples since the sensitivity and the specificity of the kit was given to be 100%. The same samples were tested using the Recombigen kit, and 419 samples turned out positive with this kit. The sensitivity of this kit was given as 100% and the specificity was 99.97%, thus the results obtained were as expected.

We then tested the same samples using the IPR ELISA kit as described in materials and methods. Four hundred and seven samples out of 1,350 samples (29.8%) were positive (Table 1). The sensitivity of the kit, calculated as a percentage of the true positives (using the results from the Wellcozyme HIV Recombinant kit as the standard reference) from total number of positive samples detected by the kit was 99.3% while the specificity was 90% (Table 1). The positive predictive value for this kit was 75%. Statistical evaluations were performed using normal approximation [13] to test the hypothesis, and there was no statistical difference in the population testing positive irrespective of the kit used ($X^2 = 0.759, p > 0.05$).

Table 1. **Comparison of the reactivity of the IPR kit with the commercial kits used in the study**

Status	IPR kit	Recombigen	Wellcozyme
Percentage positive	29.7%	31.0%	31.2%
True positive	410	419	421
False positive	5	0	0
True negative	9	10	10
False negative	7	2	0
Sensitivity	99.3%	99.5%	100%
Specificity	90.0%	100%	100%
Negative predictive value	98.3%	100%	100%
Positive predictive value	75.0%	83.3%	100%

Discussion

The analytical laboratory goals for the development of a new anti HIV kit are aimed at obtaining a high sensitivity and specificity, detection of antibodies as early as possible during seroconversion, easy processing and high robustness, suitability for automatic apparatus, high precision and unspecific reactions by known potential interfering factors. In addition, the kit should be affordable. The latter requirement is especially applicable in developing countries, where AIDS is spreading very fast, and one of the problems is diagnosis in early stages because the test kits available are beyond reach of many people. Thus the affordability should be one of the considerations when designing new diagnostic kits, in addition to performing at least as well as existing test kits but preferably exceeding in all essential aspects [14].

In this study, we used a commercially available kit (Wellcozyme HIV Recombinant kit) to determine the HIV positive serum samples because its sensitivity and specificity was determined to be 100% (manufacturer's quality control analysis). As a further control, we tested the same samples using another commercial kit (Recombigen). The results obtained with these two kits were almost identical, the Wellcozyme kit showed 421 positive samples while the Recombigen kit recognized the same samples as positive except for two samples that were just below the cut-off point.

The results from assaying the same samples using our IPR kit showed almost identical results. The kit detected 407 of the 421 positive samples (determined by the Wellcozyme kit), showing that this kit was able to diagnose HIV infected serum. The sensitivity and specificity of this kit was comparable to those of the commercial kits. The small differences observed in the results with these three kits may have emanated from the fact that different domains were used as the HIV capture antigen. The Wellcozyme kit utilizes HIV-1 recombinant core and *env* antigens purified from *Escherichia coli* transfected with the relevant genes from HIV-1, the Recombigen kit utilizes recombinant HIV-1 *env* and *gag* antigen also from *E. coli*, while in the IPR kit, we used a 20 amino acid peptide from a conserved portion of the *env* gene. Thus each of these kits has different specificities for different antibodies because they present different epitopes. It is possible that our kit detected less samples than the other kits because of the limitations inherent in the use of short peptides as capture antigens in ELISAs. The short peptides will only bind to a small population of the antibodies in serum; they would not bind to antibodies to conformational epitopes, while the use of the whole protein would have the advantage of binding most of the available antibodies in serum, thus increasing the sensitivity.

However, the sensitivity of our kit was reasonably high, suggesting it can be used for mass screening of serum samples.

The other advantage of our kit is that it is relatively cheap. Although the use of already-assembled kits has its advantages in that all the conditions have already been predetermined and the reagents prepacked and ready-to-use, the high cost generally means that the use of these kits is unavailable to the majority of people in the developing countries. Since our kit was designed and locally made and tested, we are able to keep down the costs of manufacturer and thus the cost of using the kit. The peptide can be synthesized locally, and the ELISA plates and other reagents purchased in bulk. The cost of testing one sample using our kit is Kshs. 350 compared to Kshs. 550 using the Wellcozyme kit and Kshs. 600 using the Recombigen kit. Thus our kit is cheap to use and affordable to many patients who would otherwise have not undergone an HIV test.

In summary, we have developed a kit that is easy to use and affordable in developing countries, especially in Kenya. The kit is comparable to the expensive ELISA kits currently in use, in sensitivity, specificity and in the predictive value (Table 1). We are currently working on improvements of this kit by addition of peptides from conserved portions of the *pol* and *env* region of HIV to increase the sensitivity.

Acknowledgments

References

1. Constantine NT, Callatan JD and Watts DT. Retroviral testing: Essentials for Quality Control and Laboratory Diagnosis. *Journal of Virological Methods* 1994; 25: 35-43.
2. Giovanni R and Nancy P. Development of an assay for simultaneous detection antibodies to HIV-1, HIV-2 and HIV-1 subtype O (HIV-O). *Infection* 2000; 23: 322-333
3. Piot P, Maitha GM and Hillier SL. Association of cervical ectopy with heterosexual transmission of HIV: results of a study of couples in Nairobi, Kenya. *Journal of Infectious Disease* 1990; 164: 588-591
4. De Cock KM, Barrier B and Diary L. AIDS-the leading cause of adult death in the West African city of Abidjan, Ivory Coast. *Science* 1990; 249:793-796
5. National AIDS and STDs Control Program. Background, Projections, Impact and Interventions. Nairobi, Kenya, 1996.
6. Termmerman M, Ndinya-Acholla JO and Ambani J, Piot P. The right not to know HIV test results. *Lancet* 1995; 345: 964-970.
7. Gatei M, Okeyo TM, Karithi M and Ndolo M. Kenya national AIDS/STD control Program: Home based care for people living with Aids: *Leaflet Ministry of Health, Kenya.* 1998; Pp. 87
8. Lelie PN and Reesink HW. Evaluation of three second-generation and three confirmatory assays for antibodies to human immunodeficiency virus. *Vox Sang.* 1988; 54: 84-91
9. Lelie PN, Vander-Poel CL, Reesink HW, Huisman HG and Buocher CA. Efficacy in the latest generation of antibody assays for (early) detection of HIV-1 and HIV-2 infection. *Vox Sang* 1989; 569: 59-61.
10. Reesink HG, Lelie PN and Huisman A. Evaluation of six enzyme immunoassays for antibody against human immunodeficiency virus. *Lancet* 1986; 11:483-486.

11. Sload EM, Pitt E and Chiarello RJ. HIV testing: State of the art. *Journal of the American Medical Association* 1991; 266: 2861- 2866.

12. Ward JW, Grindon AJ, Feorino PM, Schable C, Parvin M and Allen JR. Laboratory and epidemiology evaluation of an enzyme immunoassay for antibodies to HTLV-III. *Journal of the American Medical Association* 1986; 256: 357-361.

13. Jerrold HZ. Biostatistical analysis. 2nd Ed., 1984; pp. 400-401

14. Bachmann P, Beyer J, Brust S, Engelhardt W, Gurtler LG, Habbermehl KO, Thormehl R. Multicentre study for diagnostic evaluation of an assay for simultaneous detection of antibodies to HIV-1, HIV-2 and HIV-1subtype 0 (HIV-0). *Infection* 1995; 23: 322-333