Clinical

International Journal of Integrative Biology
A journal for biology beyond borders

IJIB
ISSN 0973-8363

Indirect Immunofluorescence assay, Particle Agglutination and ELISA for the detection of HIV type 1

Michael K Kiptoo [1,2,*], Zipporah W Ng'ang'a [4], Solomon S Mpoke [3], Saida Osman [1], Ann Mwangi [3], Elijah M Songok [1,5]

[1] *Centre for Virus Research, Kenya Medical Research Institute, Nairobi, Kenya*
[2] *Dept. of Zoological Sciences, Kenyatta University, Nairobi, Kenya*
[3] *Centre for Biotechnology, R & D, Kenya Medical Research Institute, Nairobi, Kenya*
[4] *Dept. of Med. Lab. Sci., Jomo Kenyatta University of Agriculture and Technology, Nairobi, Kenya*
[5] *Dept. of Medical Microbiology and Infectious Diseases, University of Manitoba, Canada*

Submitted: 15 Oct. 2008; Revised: 5 Dec. 2008; Accepted: 3 Jan. 2009

Abstract
Screening of blood and blood products for human immunodeficiency virus (HIV) is routinely performed using the enzyme-linked immunosorbent assay (ELISA), and the results confirmed by western blot (WB). In an effort to identify alternative techniques, two locally prepared test kits were evaluated to determine the performance in comparison to commercial kits. These diagnostic kits were indirect immunofluorescence assay (IFA) and particle agglutination (PA). Blood obtained from 400 patients seeking treatment for sexually transmitted infections (STI) at a special treatment clinic in Nairobi were tested for ant-HIV-1 antibody by ELISA, PA and IFA. Out of 160 samples that were HIV antibody positive by PA, 10 (6.3%) were HIV antibody negative by IFA. Out of 240 samples that were HIV antibody negative by PA, 1(0.4%) was HIV antibody positive by IFA. The IFA results agreed with those of PA with a consistency of 97.3% (389 out of 400). The sensitivity and specificity of IFA was 99.3% and 95.9% respectively. Out of 170 samples that were HIV antibody positive by ELISA, 22 (5.8%) were HIV antibody negative by IFA. The IFA results agreed with those of ELISA with a consistency of 93.8% (375 out of 400). The sensitivity and specificity of IFA was 98.0% and 91.2% respectively. This study demonstrates that PA can be used for epidemiological studies and as a screening test in resource poor settings.

Keywords: Particle agglutination assay, Immunofluorescence assay, sensitivity, specificity, diagnosis, HIV.

INTRODUCTION

The classical algorithms for diagnosis of HIV infection are antibody based using ELISA technology for screening and Western Blotting for confirmation. The enzyme-linked immunosorbent assays are the most frequently used method for screening of blood samples for HIV antibody. Other test systems available include passive particle agglutination (Yoshida *et al.*, 1987), immunofluorescence (Gastaldello *et al.*, 1999), and RIPA (Resnick *et al.*, 1987) bioassays. Western blot is regarded as the gold standard and seropositivity is diagnosed when antibodies against both the *env* and the *gag* proteins are detected. Improvement on the performance of the diagnostic kits has been a continuous process since the first kits were developed.

[*] ***Corresponding author:***
Michael K. Kiptoo, Ph.D.
P.O. Box 54628-00200,
Nairobi, Kenya
Email: mkiptoo@kemri.org

Several diagnostic kits are being developed and evaluated such as the dot blot assays and immunoassays (Ravanshad *et al.*, 2006; Francis *et al.*, 2005). The feasibility and capability of nucleic-acid-based tests as screening and confirmation methods for HIV diagnosis has been assessed. The data has shown that the nucleic-acid- based tests if affordable may constitute an effective alternative HIV diagnostic algorithm (Ren *et al.*, 2008).

However, in many developing countries, the diagnostic kits are imported from other countries. The purchase of these kits relies on donor funding which is always not sufficient. Therefore, there is need to develop locally produced kits. The diagnostic kits imported have to be evaluated before they are used. Choosing the appropriate diagnostic tools for the diagnosis and management of HIV disease will lower the costs for laboratory testing. Furthermore, the accurate information obtained on a patient's disease status is vital for effective treatment of the disease. In this study,

we evaluated the sensitivity, specificity and feasibility of using two locally produced diagnostic tests.

MATERIALS AND METHODS

Clinical samples and processing

Four hundred individuals seeking treatment for sexually transmitted infections at a clinic in Nairobi were, with informed consent requested to participate in the study. Five millilitres of peripheral blood samples were collected in EDTA anticoagulant. The plasma was obtained by centrifugation and then stored at -20˚C until the time for detection of antibodies to HIV.

HIV-1 antibody testing

All the samples were screened with IFA, ELISA and PA. Thirty-six samples showing discrepant results in the three tests were subjected to Western blot. These discrepant results have been described in details in a previous report (Kiptoo *et al*., 2004). This current paper focuses on the performance of IFA, PA and ELISA.

Immunofluorescence assay

The immunofluorescence assay was performed essentially as described by Kiptoo *et al.,* (2004). Briefly, the plasma specimens were diluted 1:20 in phosphate buffered saline (PBS). Ten microlitre of diluted plasma were incubated with fixed HIV-infected cells for 30 minutes in a humidified chamber at 37˚C, washed three times with PBS for 5 minutes. The slides were then incubated with 10 ul of fluorescent isothiocyanate-conjugated anti-human immunoglobulin G (Ig G) (Dako, Denmark) diluted 1: 100 in PBS for 30 minutes at 37˚C in a humidified chamber. After washing three times with PBS, the slides were mounted with 90% glycerol buffer on coverslips and examined under a fluorescence microscope.

Particle agglutination assay

This was carried out as described by Yoshida *et al.*, (1987). For the preparation of viral antigen-coated particles, gelatin particles, imported from Fujirebio, Japan, were washed in saline and phosphate buffered saline. These were resuspended in phosphate buffered saline as a 5% (vol/vol) solution. The particles were then activated in 5ppm tannic acid and adjusted to 5% (vol/vol) in phosphate buffer. The HIV-1 antigen obtained from Fujirebio was evaluated at three dilutions of 1:3, 1:3.5, and 1:4 for sensitization of the particles, and the best concentration was used. Excess antigen was washed off in saline. The particles were then suspended in freeze drying medium and lyophilized.

Particle agglutination assay testing procedure

The test plasma sample was serially diluted in volumes of 25 ul per well in a U-shaped microtiter plate. Portions (25ul) each of 1% (vol/vol) antigen-sensitized particles and unsensitized particles were added to the wells containing 1:16 (final dilution, 1:32) and 1:8 (final dilution, 1:16) diluted plasma, respectively. The contents of the wells were mixed with a tray mixer and then allowed to stand at room temperature for 2 hours. The interpretation of PA results was based on agglutination of the gelatin particles. A result was considered positive when unsensitized particles did not agglutinate and sensitized particles gave a definite agglutination pattern (Fig. 1).

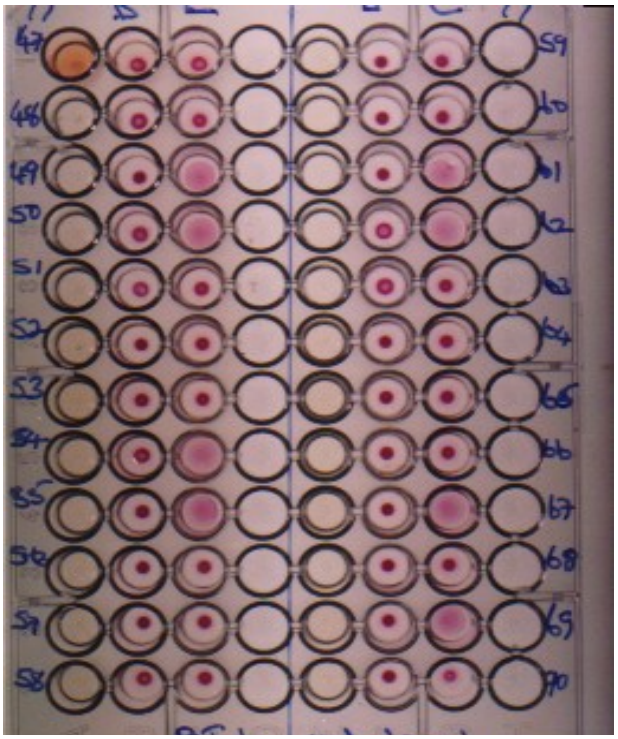

Figure 1: Particle agglutination assay results

In cases where mild visible reaction or both unsensitized and sentitized particles showed agglutination, the specimens were retested after an absorption procedure. Briefly, 350 ul of reconstituted unsensitized particles were mixed with 50 ul of specimen and incubated at room temperature for 20 minutes. The mixture was then centrifuged and 50 ul of supernanat used for retesting.

Enzyme-linked immunosorbent assay

This was carried out according to instructions of the manufacturer (Vironostika HIV Uni-Form II, Organon-Teknika BV, Boxtel, Holland). A mixture of HIV antigens coupled to horseradish peroxidase (HRP)

served as the conjugate with tetramethylbenzidine (TMB) and peroxide as the substrate. The development of color suggested the presence of antibody to HIV-1, while no or low color suggested absence of antibody to HIV-1.

Western blot

Western blotting was carried out according to instructions of the manufacturer (Lia Tek HIV III, Organon-Teknika BV, Boxtel, The Netherlands). Briefly, strips were incubated with test specimen, washed, incubated with the enzyme conjugate, washed again and incubated with a chromogen. Brown bands were visualized wherever alkaline phosphatase-labeled antibody was bound, and the color development was proportional to the amount of specific antibody present in the sample.

RESULTS

Comparison of immunofluorescence assay and particle agglutination

Out of 160 samples that were HIV antibody positive by PA, 10 (6.3%) were HIV antibody by IFA. Out of 240 samples that were HIV antibody negative by PA, 1 (0.4%) was antibody positive (Table 1). The IFA results agreed with those of PA with a consistency of 97.3% (389 out of 400). The sensitivity and specificity of IFA was 99.3% and 95.9% respectively, while positive and negative predictive values were 99.3% and 95.9% using PA as the reference test.

Table 1: Comparison of immunofluorescence assay and particle agglutination

	PA result		
IFA result	Positive	Negative	Total
Positive	150	1	151
Negative	10	239	249
Total	160	240	400

Table 2: Comparison of immunofluorescence assay and enzyme-linked immunosorbent assay

	ELISA result		
IFA result	Positive	Negative	Total
Positive	148	3	151
Negative	22	227	249
Total	170	230	400

Comparison of immunofluorescence assay and enzyme-linked immunosorbent assay

Out of 170 samples that were HIV antibody positive by ELISA, 22 (5.8%) were HIV antibody negative by IFA (Table 2). The IFA results agreed with those of ELISA with a consistency of 93.8% (375 out of 400). The sensitivity and specificity of IFA was 98.0% and 91.2% respectively, while the positive and negative predictive values were 98.0% and 91.2% respectively using ELISA as the reference test.

DISCUSSION

In this study, we have demonstrated that there was an overall concordance of 97.3% between IFA and PA. However, some discrepancies were found between IFA and PA. There were 10 (6.3%) samples that were HIV antibody positive by PA but negative by IFA. These findings are similar to a previous report (Poljak *et al.*, 1997) where it was found that 5 samples that were positive by PA were negative by IFA. The biological explanation for these discrepancies is not clear but may be due to different sensitivities of the tests. Whereas the PA test can detect both IgM and IgG, the IFA test detected only IgG. Interestingly, when the 10 discordant samples were tested by western blot, 9 were negative and one positive. This suggests that results by PA must be confirmed by another test.

There was an overall concordance of 94.4% between IFA and WB. The results between IFA and Western blot have been discussed previously by Kiptoo *et al*., (2004). A few discrepant cases were nevertheless noted between ELISA, WB, PCR and IFA. There were four samples that were ELISA positive but negative for WB, IFA and PCR (Kiptoo *et al*., 2004). These samples were clearly false positives by ELISA. False positive ELISA results have been reported previously (Doran *et al.*, 2000; Auwant *et al.*, 1990). Misdiagnosis of HIV can lead to psychosocial difficulties and psychiatric morbidity, public health and epidemiological implications and can lead to medico-legal conflict. It is important to be mindful of the reality of erroneous and false positive HIV test results.

In cases where antibody testing may be insufficient to determine whether a patient is infected, it is necessary to perform DNA PCR, a nucleic acid amplification method that allows for the detection of viral DNA integrated into the host cell's genomic DNA (Feron, 2005). PCR is particularly useful in testing infants of HIV-positive mothers; these infants may carry maternal antibody to 15 months of age (Zhang *et al.*, 2008). It should be noted, however, that the HIV-1 DNA PCR assay is currently more costly than the serological tests.

The KEMRI HIV-1 PA kit does not need expensive equipments such as a spectrophotometer (ELISA reader) and plate washer required when using the ELISA system. The test is simple to perform since it is a one-step antigen-antibody reaction, whereas more steps are required in the ELISA. The results can be read easily. Therefore, the KEMRI HIV-1 PA test should be useful

for epidemiological studies and patient screening in resource limited areas.

However, with the availability of commercial rapid tests, it has become possible to cost-effectively screen smaller numbers of samples without a huge initial input for the laboratory (Plate, 2007). Therefore, there is need to develop locally produced rapid single test kits.

Acknowledgement

The authors would like to thank the staff of special treatment clinic for their immense contributions, and the Director, KEMRI for approving this manuscript for publication. This work was funded by Japan International Cooperation Agency through the KEMRI/JICA project.

References

Auwanit W, Ayuthaya PI *et al.* (1990) Immunofluorescence, enzyme-linked immunosorbent assay, particle agglutination and western blot for the detection of antibody to human immunodeficiency virus type 1. *Southeast Asian J. Trop. Med. Public Health* **21**(1):53-59.

Doran IT, Parra E *et al.* (2000) False-positive results and indeterminate human immunodeficiency virus test in pregnant women. *Arch. Fam. Med*. **9**:924-929.

Francis B, Laurence M, *et al.*, (2005) Development and validation of an immunoassay for identification of recent human immunodeficiency virus type 1 infections and its use on dried serum spots. *J. Clin. Microbio.* **43**(9):4441-4447.

Fearon M (2005) The laboratory diagnosis of HIV infections. *Can. J. Infect. Dis. Med. Microbiol.* **16**(1):26-30.

Gastaldello R, Gallego S *et al.* (1999) Efficiency of indirect immunofluorescence assay as a confirmatory test for the diagnosis of human retrovirus infection (HIV-1 and HTLV-I/II) in different risk populations. *Rev. Inst. Med. Trop. S. Paulo*. **41**: 159-164.

Kiptoo MK, Mpoke SS *et al.* (2004) New indirect immunoflorescence assay as a confirmatory test for human immunodeficiency virus type 1. *East Afr. Med. J*. **81**: 222-225.

Plate DK (2007) Evaluation and implementation of rapid HIV tests: the experience in 11 African countries. *AIDS Res. Hum. Retroviruses*. **23**(12):1491-1498.

Poljak M, Zener N, *et al*., (1997) .Particle agglutination test "Serodia HIV-1/2" as a novel anti-HIV-1/2 screening test: comparative study on 3311 serum samples. *Folia Biol (Praha)* ;**43**(4):171-3.

Ravanshad M, Sabahi F *et al.*, (2006) Evaluation of a new dot blot assay for confirmation of human immunodeficiency virus type 1 and 2 infections using recombinant p24, gp41, gp120 and gp 36 antigens. *Saudi Med J.* **27**(1): 31-36.

Ren A, Louie B, *et al*., (2008) Screening and confirmation of human immunodeficiency virus type 1 infection solely by detection of RNA. J. Med. Micribio. **57** (Pt 10):1228-1233.

Resnick L, Shapshak P (1987) Serologic characterization of human immunodeficiency virus infection by Western blot and radioimmunoprecipitation assays. *Arch. Pathol. Lab. Med*. **111**(11):1040-1044.

Yoshida T, Matsui T *et al.* (1987) Evaluation of passive particle agglutination test for antibody to human immunodeficiency virus. *J. Clin. Microbiol.* **25**: 1433-1437.

Zhang Q, Wang L *et al.* (2008) Early infant human immunodeficiency virus type 1 detection suitable for resource-limited settings with multiple circulating subtypes by use of nested three-monoplex DNA PCR and dried blood spots. *J. Clin. Microbiol*. **46**(2):721-726.