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NEW INDIRECT IMMUNOFLUORESCENCE ASSAY AS A CONFIRMATORY TEST FOR HUMAN IMMUNODEFICIENCY VIRUS TYPE 1

M. K. KIPTOO, S. S. MPOKE and Z.W. NG'ANG'A

ABSTRACT

Background: 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). However, western blot is expensive and mostly performed in developed countries. A technique more superior or comparable to WB and adaptable to developing countries must be sought. In an effort to identify such a technique, this study determined the efficiency of indirect immunofluorescence assay (IFA) to detect antibodies to HIV-1.

Objective: To determine the accuracy and sensitivity of an in-house immunofluorescence assay (IFA) to detect antibodies to HIV-1 in plasma.

Design: A comparative study to evaluate the performance of indirect immunofluorescence assay (IFA) and western blot (WB) techniques in the detection of antibodies to HIV-1.

Setting: Kenya Medical Research Institute, Centre for Virus Research. The study was conducted between June and December 2001.

Methods: The evaluation of IFA as a technique for detecting antibodies to HIV-1 utilized a total of 400 samples. For these samples, IFA was compared with ELISA and particle agglutination (PA) (manuscript under preparation). Of the 400 samples, there were discrepant results in the three assays in only 36 samples. IFA was compared with Western blot (WB) to confirm the true HIV-1 serostatus in these 36 plasma specimens. The IFA technique used acetone-fixed HIV-1 infected MOLT-4 cells in one spot on a Teflon coated slide and uninfected MOLT-4 cells alone in a second spot to assess non-specific fluorescence. Western blot was performed according to the instructions of the manufacturer.

Results: The sensitivity and specificity of IFA based on 36 plasma specimens tested was 71.4% and 100% respectively. All samples that were HIV seronegative by WB were also HIV seronegative by IFA. However, two (5.6%) samples were HIV seronegative by IFA but seropositive by WB.

Conclusion: The data obtained show that IFA can be used as a primary confirmatory test in Kenya.

INTRODUCTION

Laboratory diagnosis for the evidence of human immunodeficiency virus type 1 (HIV-1) infection is based on the detection of antibodies to HIV-1 in plasma or serum. Antibodies against various viral structure proteins are measured by a number of simple and sensitive screening tests. These assays include enzyme linked immunosorbent assay (ELISA)(1), particle agglutination (PA)(2), and latex agglutination(3). A reliable confirmatory test is used to re-test all ELISA and rapid test positive specimens because specificity is less than 100%. The serological assays that are known to be effective in confirming positive results are WB(4), IFA(5,6) and radioimmunoprecipitation (RIPA)(7). Western blot has been used widely to confirm ELISA and other rapid tests HIV seropositive

samples. However, WB has certain disadvantages, which include high cost, and it yields indeterminate results(8). The high cost limits its use in most developing countries such as Kenya. The World Health Organization (WHO) and joint United Nations Programme on HIV/AIDS (UNAIDS) has recommended that countries consider testing strategies which use a combination of ELISA and simple/rapid assays rather than ELISA/WB for HIV antibody detection(9).

Immunofluorescence assay is a technique that is used to detect the presence of an infection. Immunofluorescence employs antibodies to which fluorochromes are covalently attached. The fluorochrome is attached to the Fc portion of the antibody rather than the antigen-binding end; thus the antibody is still able to bind to its epitome. In indirect immunofluorescence, the fluorochrome is attached to an antibody raised

against, for example, human antibody. This technique has been applied for the detection of antibody to human immunodeficiency virus type 1. Several studies have reported that IFA is sensitive and specific test for screening and/or confirm HIV ELISA reactive sera/plasma(6,10,11). It has also been suggested that the use of IFA can also reduce the cost. Therefore, in an effort to identify a better or comparable assay to WB and adaptable to developing countries, we have evaluated the performance of an in-house indirect IFA.

MATERIALS AND METHODS

Clinical samples and processing: Peripheral blood samples from 400 individuals seeking treatment for sexually transmitted infections 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: After the initial screening with IFA, ELISA and PA, thirty-six samples showing discrepant results in the three tests were subjected to IFA and Western blot. IFA was performed as shown in Figure 1. Western blotting was carried out according to instructions of the manufacturer (Lia Tek HIV III, Organon-Teknika).

Cells: The HIV-negative cell line MOLT-4 No. 8 (12) and the HIV-1 infected MOLT-4 cell line (MOLT-4/MN) were obtained from Fujirebio Inc. Ube Plant, Japan. The cells were cultured at 37°C and 5% CO_2 in RPMI 1640 supplemented with heat inactivated foetal bovine serum (FBS), 3% L-glutamine and kanamycin ($60\mu\text{g/ml}$). Cells were seeded at a concentration of 3×10^5 cells/ml twice weekly in fresh medium to maintain cells in logarithmic growth phase. The HIV negative cell line MOLT-4 No. 8 was used as the control.

Immunofluorescence assay: The immunofluorescence assay was performed essentially as described by Gallego *et al.*(13). For the preparation of antigen slides, the MOLT-4/MN cells were sedimented at 800rpm for 5 minutes. The cells were washed three times with 10ml cold phosphate buffered saline (PBS) and resuspended in PBS to obtain a cell concentration of 1.5×10^6 cells/ml. Five microlitre ($5\mu\text{l}$) aliquots of the cell suspension were placed on six wells of a 12-well slide. The above procedure was carried out for MOLT-4 No. 8 cells and then added to the remaining six wells. The slides were air-dried for 1 hour and fixed in cold acetone for 30 minutes. The slides were stored at -80°C for later use.

Immunofluorescence assay testing procedure: The procedure for carrying IFA was as summarised in Figure 1. To detect anti-HIV-1 antibodies; the plasma specimens were diluted 1:20 in PBS. Ten microlitre of diluted plasma were incubated with fixed cells on the slide for 30 minutes in a humidified chamber at 37°C , washed three times with PBS for 5 minutes. The slides were then incubated with $10\mu\text{l}$ of fluorescent isothiocyanate-conjugated anti-human immunoglobulin G (IgG) (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.

RESULTS

The interpretation of IFA results was based on typical cytoplasmic staining patterns of MOLT-4/MN cells (Figure 2). False positive reaction was detected when both MOLT-4 # 8 and MOLT-4/MN cells were stained (non-specific fluorescence). The performance of the IFA was as summarised in Table 1.

Figure 1

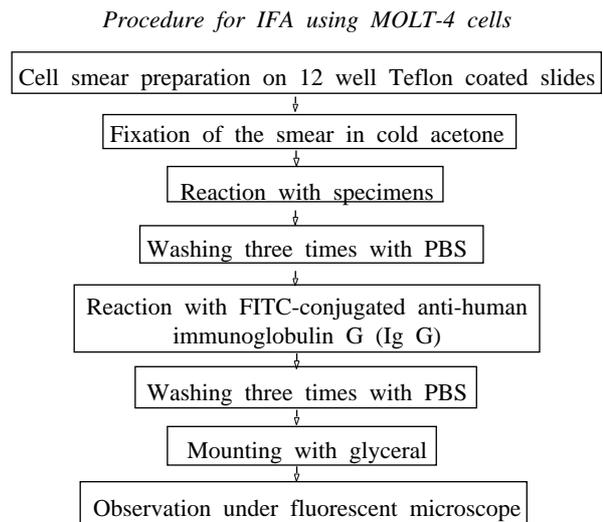


Figure 2

Immunofluorescence assay staining of HIV-1 infected MOLT-4 cells

(a) Control spot reacted with positive plasma (Mg x400)

(b) Control spot reacted with positive plasma
(Mg x1000)

Sensitivity= 71.4%, Specificity= 100%, Efficiency= 94.4%, Where N= HIV antibody negative, P= HIV antibody positive.

Out of seven samples that were HIV antibody positive by WB, two (28.6%) were HIV antibody by IFA. All 29 samples that were HIV antibody negative by WB were also HIV antibody negative by IFA. The IFA results agreed with those of WB with a consistency of 94.4% (34 out of 36).

DISCUSSION

In this study, IFA and Western blot were compared as tests for detecting antibodies to human immunodeficiency virus type 1 under conditions where descriptant results have been obtained from IFA and two other serological tests, ELISA and PA. We have demonstrated that there was an overall concordance of 94.4% between IFA and WB. All samples that were negative by WB were also negative by IFA. However, two samples were negative by IFA but positive by WB. These findings are significant in that they demonstrate that the IFA technique is potentially more specific compared to WB.

At the moment there is no one test, which can be referred as a gold standard for HIV testing. Currently, the "ultimate" test for the detection of HIV-1 infection is the Polymerase Chain Reaction (PCR)(14). The PCR technique is not ideal for comparison with other serological assays as it is nucleic acid based rather than antibody based, and as with WB is also relatively expensive. The recommendation for HIV screening, therefore, is the combinations of ELISA and rapid tests. However, even with these combinations, sometimes a conclusive result is not determined, necessitating the use of other tests such as WB, PCR and now from our study, IFA.

The sensitivity and specificity of IFA was 71.4% and 100% respectively using western blot as the reference test. These findings are similar to another report(15) where the sensitivity of IFA ranged between 76.7% and 97.6% when the six major western blot interpretive criteria was used(16). In a previous study(17) sensitivity and specificity of 100% for IFA as a confirmatory test was reported. Similarly, Abraham and co-workers(15) reported a sensitivity and specificity of 97.6% and 97.8% respectively when IFA was compared with western blot.

In this study, IFA had a PPV of 100% using WB as the reference test. This is very important for HIV diagnosis since PPV measures the probability that a positive result indicates the presence of the disease. Specificities and sensitivities reported here for IFA show that it could be used for preliminary confirmation.

When compared with WB, IFA has the following advantages. The IFA is superior to WB with respect to the ease of use and rapidity(18). The IFA takes less

(c) Test spot reacted with positive plasma
(Mg x400)

(d) Test spot reacted with positive plasma (Mg x1000)

Table 1

Sensitivity and specificity of IFA using WB as the reference test

IFA	WB		Total
	P	N	
P	5	0	5
N	2	29	31
Total	7	29	36

than two hours to perform making it significantly faster than WB test, which is usually an overnight test. The IFA can also be used in quantitative analysis of serum antibody. The in-house IFA reagents are relatively easy to prepare with a shelf life of several months (16). Furthermore, interpretation of fluorescence patterns in IFA is easy when a control cell is used to assess non-specific fluorescence. In this study, three people were allowed to read the results independently and later compared to the results. In all the cases that were examined, no discrepancies were observed. The simplicity, quick turnaround time and good reproducibility makes the IFA an attractive or supplemental to WB as a confirmatory test for ELISA/rapid tests-positive sera/plasma.

When compared to the current methods for determining HIV status (combinations of ELISA and rapid tests) in Kenya, IFA has certain operational advantages. First, the technical capacity to carry out IFA has now been installed in our institute, which may therefore serve as a reference laboratory. However, it is expected that immunofluorescence microscopy is routinely performed in many laboratories or health facilities across the country, and so this technique can easily be adapted to such local settings. Second, IFA is a reliable and sustainable test. Most of the kits in the Kenyan market (ELISA and other rapid tests) are currently obtained as donations. When such donations stop, the kits must be purchased, but they are relatively expensive and so long-term sustainability of this strategy is not guaranteed. Therefore, IFA can easily be the test of choice for confirming those results where other methods have given inconclusive results and hence has the properties of a primary confirmatory test for HIV-1 diagnosis in Kenya. We propose a testing algorithm consisting of initial testing with rapid/simple and ELISA assays then the positive samples will be tested with IFA.

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