

Predictive value of two commercial human immunodeficiency virus serological tests in cases with indeterminate Western blot results

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Background and Purpose: Serodiagnosis of human immunodeficiency virus (HIV) infection typically requires repeatedly reactive positive screening test followed by Western blot (WB) assay. When WB assay result is indeterminate, the results of follow-up WB assay may remain inconclusive. This study evaluated use of enzyme-linked immunosorbent assay (ELISA) and particle agglutination test (PAT) as sequential screening tests with WB assay for the diagnosis of HIV infection.

Methods: From January 1, 2000 to December 31, 2003, a total of 565 serum samples collected from individuals with a previous positive or borderline positive ELISA test for HIV at regular check-up (281 samples) and a second group of individuals with known risk of HIV exposure or suspected infection based on clinical presentation (284 samples) were tested for HIV infection by ELISA, PAT and WB assay.

Results: The result was positive for HIV infection and confirmed by WB assay in 197 samples (22.5%), indeterminate in 127 samples (22.5%) and negative in 241 samples (42.6%). The sensitivity and specificity of ELISA were 100% and 21.6% and of PAT were 99.5% and 95%, respectively. Among the 197 HIV-infected cases, all ELISA and PAT results were concordant with WB assay except 1 (1/197) with negative PAT. Positive ELISA, positive PAT and indeterminate WB assay results were found in 9 of the 284 samples (7 individuals) from at-risk patients. Among these 7 individuals, 5 were later proved to have HIV infection. WB assay in 1 of the 7 individuals remained indeterminate 1 year later, and the remaining case was lost to follow-up.

Conclusion: We suggest initial ELISA followed by PAT as sequential screening for HIV infection. When both screening tests are concordant but subsequent WB assay is indeterminate, further evaluation (such as nucleic acid amplification test) should be arranged as soon as possible.

Key words: Agglutination tests, enzyme-linked immunosorbent assay, HIV, Western blotting

Introduction

Serodiagnosis of human immunodeficiency virus (HIV) infection typically requires initial screening for antibodies by enzyme-linked immunosorbent assay (ELISA) followed by confirmation of repeatedly reactive sera by Western blot (WB) assay. Many of these tests have very good sensitivity and specificity [1,2]. Although

the ELISA is a relatively inexpensive screening test, it is machine-dependent and requires well-trained technicians. On the contrary, particle agglutination test (PAT) overcomes most of these problems. In addition, a previous study suggested that PAT might pick up seroconversion earlier than ELISA [3]. PAT has been shown to be reliable as a screening test, with sensitivity and specificity of more than 99% in blood donors [4-8].

At present, diagnosis of HIV infection depends on the use of a screening test, typically ELISA or PAT combined with WB assay. Previous data showed that the percentage of indeterminate WB assay in repeatedly reactive individuals ranged from 30.4% to 85% [9,10].

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Repeat blood sample collection for retesting WB assay is generally recommended [9]. However, the results of follow-up WB assays may remain inconclusive and the accuracy of identifying acute HIV infection may be lowered and the diagnosis would be delayed.

Recent studies showed that addition of nucleic acid amplification testing to an HIV testing algorithm increased the identification of cases of infection [11, 12]. Nucleic acid amplification testing may help to solve the problems of repeated WB assays which yield indeterminate results. The costs of such tests, however, are considerable and may not be feasible in regions with limited economic resources.

In this study, we evaluated the sensitivity and specificity of 2 different screening tests (ELISA and PAT) in sequential screening tests with WB assay for the diagnosis of HIV infection. The percentage of indeterminate WB assays and subsequent follow-up results were also analyzed.

Methods

Blood sample

From January 1, 2000 to December 31, 2003, a total of 281 serum samples were obtained from a group of foreign laborers who had had a previous positive or borderline positive ELISA test in regular physical check-up. A second group of 284 samples was obtained from patients with clinical presentation leading to suspicion of HIV infection or known risk factors identified at the outpatient department, wards or anonymous screening service in our hospital. Samples were sent for ELISA, PAT and WB assay.

ELISA for anti-HIV antibodies

The Murex HIV-1.2.0 ELISA kit (Murex Biotech Inc., UK) was used according to the manufacturer's instructions. The 96-well plate coated with recombinant antigen of HIV-1 and HIV-2 was incubated with 100 µL serum sample (dilution 1/50) for 30 min at 40°C. After washing, 100 µL of anti-human immunoglobulin coupled to an enzyme (peroxidase) was added to each well and incubated for 30 min at 40°C. 100 µL of substrate (tetramethyl benzidine; TMB) was then added and incubated for 30 min, followed by addition of 50 µL of stop solution. Color-change caused by enzymatic digestion of the substrate (TMB) was detected by spectrometry. The optical density was read at 492/620 nm. The cut-off value of optical density indicating a positive result was 0.8.

PAT for anti-HIV

The Serodia-HIV PAT (Fujirebio Inc., Japan) was used according to the manufacturer's instructions. The gelatin particles were coated with inactivated HIV antigens. Twenty µL of specimen was mixed with gelatin, and then diluted 2-fold. The reactive samples were left at room temperature for 2 h before reading. If the specimen contained HIV antibodies, cross-linking between gelatin and antibodies occurred. A specimen which showed agglutination (final dilution 1:32 for HIV-1) was regarded as positive.

WB analysis

The New LAV blot I WB assay (Bio-Rad Company, USA) was used according to the manufacturer's instructions. Briefly, 20 µL of specimen was incubated with 2 mL of buffer for 2 h at room temperature. The contents of each cell were drained completely. After washing 3 times, 2 mL of conjugate solution was added to each cell, and the sample was incubated for 1 h under slow shaking. Two mL of color development solution was added and the color of the mixture was monitored. A specimen was interpreted as positive when there was reactivity to at least 2 *env* bands according to World Health Organization criteria [13]. Medical records were retrospectively reviewed to determine HIV status if samples in the at-risk group showed indeterminate WB assay and positive PAT. HIV infection was confirmed when positive result was obtained for WB assay or plasma HIV-1 RNA viremia by reverse transcriptase-polymerase chain reaction (RT-PCR) [Amplicor HIV-1 Monitor; Roche Diagnostic Systems, Branchburg, NJ, USA].

Results

Prevalence and age distribution of HIV serological survey

The gender and age distributions of all cases are shown in Fig. 1. Among the 281 samples in the ELISA-positive group, HIV infection was demonstrated by WB assay in 10. The HIV seroprevalence rate in the ELISA-positive group was 3.6%. All of the HIV-infected patients in this group were males. The average age of this group was 38.9 years. 187 cases in the at-risk group were proved to have HIV infection by WB assay (Table 1). The HIV-1 seroprevalence in the at-risk group was 65.8%, with the male-to-female ratio 10.7. The average age of male patients was 38.9 years and that of female patients was 41.7 years. Among the 197 HIV-infected cases, ELISA

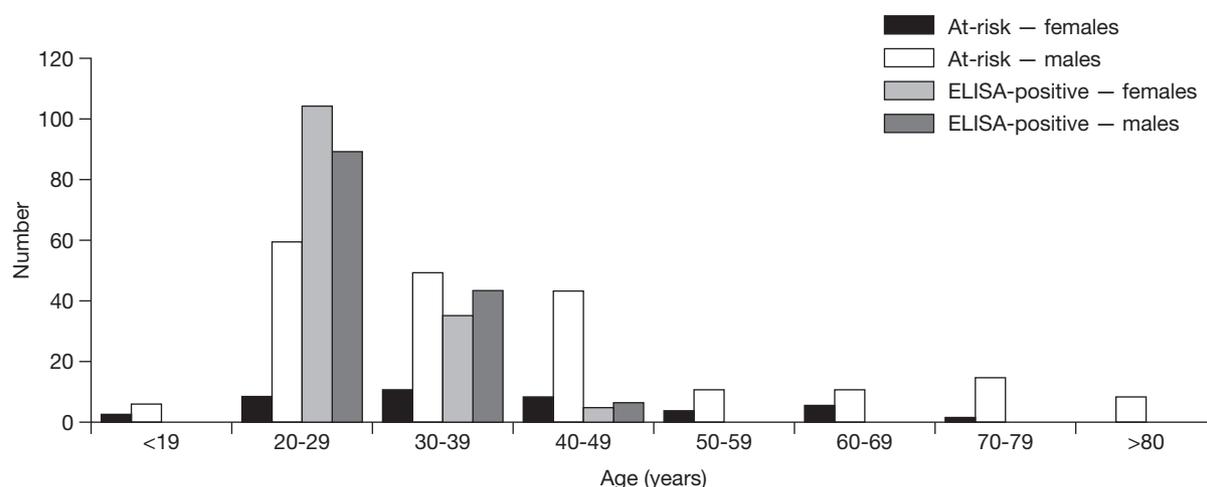


Fig. 1. Gender and age distribution of the enzyme-linked immunosorbent assay (ELISA)-positive and at-risk groups.

and PAT results were all concordant with WB assay except for 1 case with negative PAT and positive ELISA (data not shown).

Diagnostic value of 2 commercial HIV serological kits

The diagnostic results of the screening assays are summarized in Table 2. The sensitivity of ELISA in both groups was 100%. The sensitivity of PAT in the ELISA-positive group was 100% and in the at-risk group was 99.4%. The specificity of follow-up ELISA in the ELISA-positive group was 20.6% and that in the at-risk group was 32.5%. The specificity of PAT in the ELISA-positive group was 95.6% and that in the at-risk group was 92.5%.

Diagnostic flow chart of 2 different sequential screening tests

Flow charts of 2 different sequential screening protocols (positive ELISA test followed by repeated ELISA or PAT) in the ELISA-positive group are shown in Fig. 2. Fig. 2A shows that after repeated ELISA test, 241 specimens were subjected to WB assay and HIV infection was identified in 10 of these patients. Fig. 2B shows the diagnostic flow chart for the ELISA positive

group with PAT; 20 specimens positive on ELISA and PAT were then subjected to WB assay. HIV infection was identified in 10 of these patients.

Follow-up of patients with indeterminate WB result

Indeterminate WB results were found in 91 patients in the ELISA-positive group and in 2 of 36 patients in the at-risk group. No further follow-up data were available for patients in the ELISA-positive group. The final HIV status in the at-risk group with indeterminate WB test results was evaluated using medical charts. In at-risk patients with positive result of ELISA and PAT, HIV infection was demonstrated by nucleic acid amplification test in 5 of 7 cases, and 3 patients were lost to follow-up. Among patients with positive ELISA and negative PAT result (total 13 cases), HIV infection was demonstrated in 1 patient in another hospital about 1 year later. WB results of 3 other patients remained indeterminate, and 9 patients were lost to follow-up (Table 3).

Discussion

In this study, samples of patients in the ELISA-positive group were collected from foreign laborers screened at

Table 1. Human immunodeficiency virus (HIV)-1 seroprevalence determined by Western blot assay

Category	Negative	Positive	Indeterminate	Seroprevalence (%)	Total
ELISA-positive group ^a	180	10	91	3.6	281
At-risk group ^b	61	187	36	65.8	284

Abbreviation: ELISA = enzyme-linked immunosorbent assay

^aSamples were obtained from patients with positive ELISA reports at routine check-up.

^bSamples were obtained from patients with known risk of HIV exposure or via clinical suspicion of infection (designated "at-risk").

Table 2. Performance of screening assays compared to Western blot assay

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
ELISA	100	21.6	51.0	100
ELISA-positive (previous)	100	20.6	6.5	100
At-risk	100	32.5	86.4	100
PAT	99.5	95.0	94.2	99.6
ELISA-positive (previous)	100	95.6	55.6	100
At-risk	99.4	92.5	98.3	97.4

Abbreviations: PPV= positive predictive value; NPV= negative predictive value; ELISA= enzyme-linked immunosorbent assay; PAT = particle agglutination test

referral clinics and those of at-risk patients were collected from citizens of Taiwan who visited hospital (outpatient and inpatient departments and anonymous screening services). These patients either had risks for HIV exposure or were suspected of HIV infection according to clinical presentation. Thus, these groups represent a highly specific subpopulation.

The specificity of ELISA test was 20.6% and 32.5% in the ELISA-positive and at-risk group, respectively, which is much lower than that of in previous studies [5,10]. The difference, however, is

probably attributable to the use of specimens from blood donors or healthy subjects in these previous studies. The specificity would be expected to decrease when both numerator (truly negative samples) and denominator (test negative samples) decrease.

The positive predictive value of ELISA was 6.5% in the ELISA-positive and 86.4% in the at-risk group. The positive predictive value of PAT was 55.6% in the ELISA-positive and 98.3% in the at-risk group. As the positive predictive value of HIV tests may vary with the seroprevalence of the tested population, when the

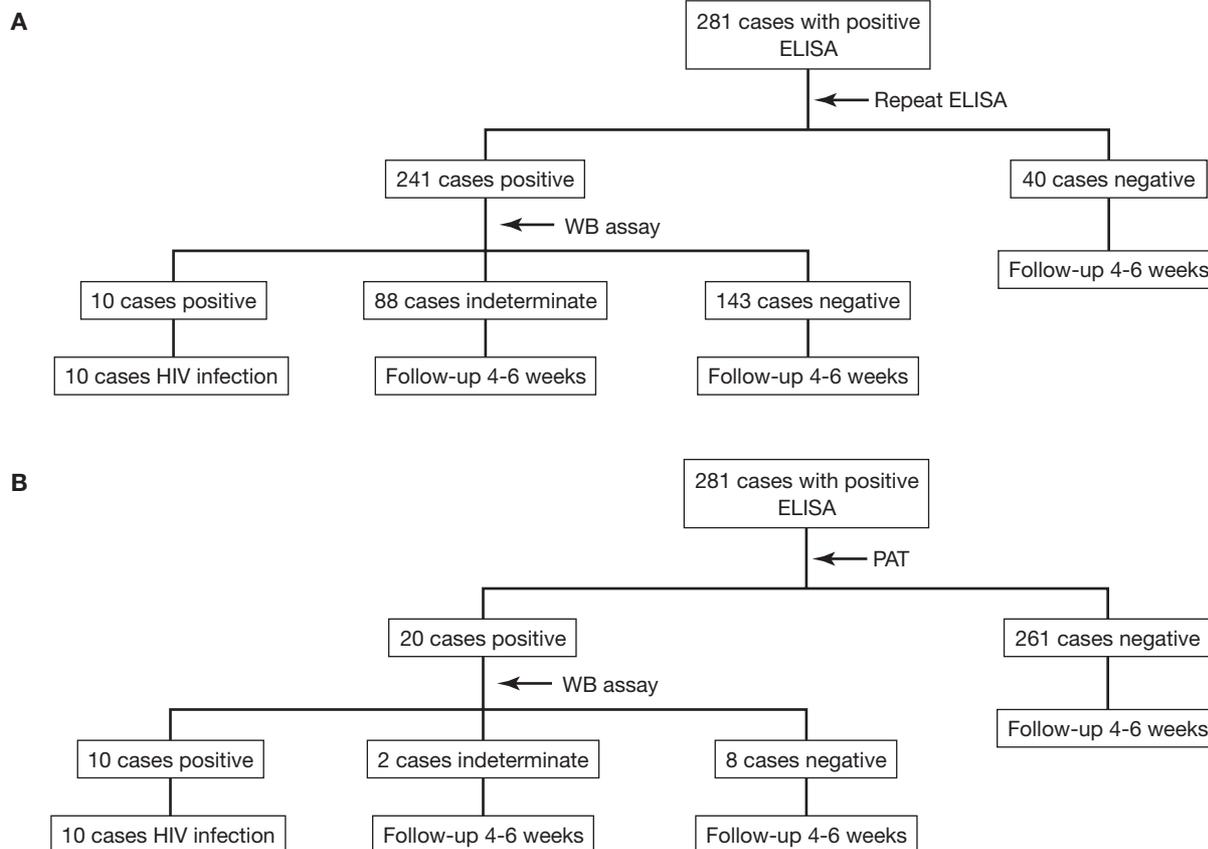


Fig. 2. Flow charts of sequential screening tests for the diagnosis of human immunodeficiency virus (HIV) infection. A) Repeat enzyme-linked immunosorbent assay (ELISA). B) ELISA test followed by particle agglutination test (PAT). WB = Western blot.

Table 3. Follow-up of human immunodeficiency virus (HIV) status in at-risk group patients with positive enzyme-linked immunosorbent assay (ELISA) test and indeterminate Western blot (WB) test^a

	HIV infection demonstrated	HIV infection not demonstrated	Lost to follow-up
ELISA-positive, PAT-positive (n = 7)	5 ^b	1	1
ELISA-positive, PAT-negative (n = 13)	1	3	9

Abbreviation: PAT = particle agglutination test

^aIn total, 20 cases were followed for up to 2 years.

^bHIV infection was diagnosed by WB assay in 4 patients within 2 months; HIV infection was diagnosed by HIV viral load test in 1 patient.

prevalence increases, the false-positive rate will decrease and the positive predictive value will increase. The seroprevalance of the at-risk group was higher than that of the ELISA-positive group, indicating that the positive predictive value of ELISA and PAT in the at-risk group should be higher.

Previous studies reported that PAT and ELISA were reliable screening tests for HIV infection [5,7,14]. In this study, the sensitivity of PAT in ELISA-positive patients was 100%. In the at-risk group, 1 patient had positive ELISA and positive WB assay, but negative PAT, resulting in sensitivity of 99.4%. ELISA may be more suitable for use as an initial screening test, as it is comparatively inexpensive and can test a batch of samples at once. In addition, 1 HIV-infected case was missed when PAT was the initial screening test in this study. Furthermore, a follow-up PAT is better than repeated ELISA if the initial ELISA is positive, because PAT is rapid, infrastructure-free and can be performed regardless of the number of samples, with no need to wait for a batch of samples for economic reasons. PAT also had higher specificity and positive predictive value in this study.

Follow-up data were not available for the ELISA-positive group in this study. In the at-risk group, 7 cases with positive ELISA and positive PAT had indeterminate results. HIV infection was diagnosed by WB assay in 4 of these patients during the following 2 months. HIV infection was diagnosed by RT-PCR in another of these patients, while another's WB assay remained indeterminate 1 month later, and 1 was lost to follow-up (Table 3). In contrast, among patients with positive ELISA and negative PAT, HIV infection was diagnosed in 1 patient at another hospital 1 year later, 1 patient's WB assay remained indeterminate 1 year later, 1 patient's WB assay was negative 1 year later, 1 patient's ELISA was negative 3 years later, and 9 patients were lost to follow-up. As a considerable proportion of patients were lost to follow-up in this study, we were not able to determine the serologic outcome of HIV

infection. Previous studies [11,12] demonstrated that further assay such as nucleic acid amplification helps to identify the presence of acute infection and should be arranged as soon as possible.

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References

1. Reesink HW, Lelie PN, Huisman JG, Schaasberg W, Gonsalves M, Aaij C, et al. Evaluation of six enzyme immunoassays for antibody against human immunodeficiency virus. *Lancet* 1986; 2:483-6.
2. Behets F, Disasi A, Ryder RW, Bishagara K, Piot P, Kashamuka M, et al. Comparison of five commercial enzyme-linked immunosorbent assays and Western immunoblotting for human immunodeficiency virus antibody detection in serum samples from Central Africa. *J Clin Microbiol* 1991;29:2280-4.
3. Chairri U, Sirivichayakul S, Phanuphak P, Panmoung W, Ubolyam S. Comparison of the sensitivity of various anti-HIV tests in early seroconversion sera. *Asian Pac J Allergy Immunol* 1991;9:95-100.
4. Yoshida T, Matsui T, Kobayashi S, Yamamoto N. Evaluation of passive particle agglutination test for antibody to human immunodeficiency virus. *J Clin Microbiol* 1987;25:1433-7.
5. Elavia AJ, Thomas A, Nandi J, Coyaji GD, Bhavalkar-Potdar V. Performance evaluation of a particle agglutination test for antibody to human immunodeficiency virus 1: comparison with enzyme immunoassay. *Vox Sang* 1995;69:23-6.
6. Behets F, Bishagara K, Disasi A, Likin S, Ryder RW, Brown C, et al. Diagnosis of HIV infection with instrument-free assays as an alternative to the ELISA and western blot testing strategy: an evaluation in Central Africa. *J Acquir Immune Defic Syndr* 1992;5:878-82.
7. Poljak M, Zener N, Seme K, Kristancic L. 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)* 1997;43:171-3.

8. Ohya K, Morishima Y, Funato E, Hasegawa I, Kamiya T. Screening of blood donors for antibody to human immunodeficiency virus type I by sensitive particle agglutination assay. *Vox Sang* 1988;55:148-51.
9. Lee JH. Follow-up investigation of indeterminate western blot results for antibody to human immunodeficiency virus type. *J Formos Med Assoc* 1994;93:283-8.
10. Meles H, Wolday D, Fontanet A, Tsegaye A, Tilahun T, Aklilu M, et al. Indeterminate human immunodeficiency virus Western blot profiles in ethiopians with discordant screening-assay results. *Clin Diagn Lab Immunol* 2002;9:160-3.
11. Pilcher CD, Fiscus SA, Nguyen TQ, Foust E, Wolf L, Williams D, et al. Detection of acute infections during HIV testing in North Carolina. *N Engl J Med* 2005;352:1873-83.
12. Pilcher CD, McPherson JT, Leone PA, Smurzynski M, Owen-O'Dowd J, Peace-Brewer AL, et al. Real-time, universal screening for acute HIV infection in a routine HIV counseling and testing population. *JAMA* 2002;288:216-21.
13. Acquired immunodeficiency syndrome (AIDS). Proposed WHO criteria for interpreting results from western blot assays for HIV-1, HIV-2, and HTLV-I/HTLV-II. *Wkly Epidemiol Rec* 1990;65:281-3.
14. Ramalingam S, Kannangai R, Raj AA, Jesudason MV, Sridharan G: Rapid particle agglutination test for human immunodeficiency virus: hospital-based evaluation. *J Clin Microbiol* 2002;40:1553-4.