Indeterminate human immunodeficiency virus western blot results in Iranian patients with discordant screening assay results

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ABSTRACT

Objective: The Western blot (WB) assay is the most widely accepted confirmatory assay for the detection and confirmation of antibodies to human immunodeficiency virus type 1 (HIV-1) and 2 (HIV-2). However, indeterminate WB reactivity to HIV-1 and HIV-2 proteins may occur in individuals who do not appear to be infected with HIV.

Methods: In this study, we describe the results of indeterminate WB reactivity in Iranian patients with discordant screening assays. The samples were obtained from the Iranian Blood Transfusion Center, Tehran, Iran and evaluated in the Biotechnology Process Development Center, Pasteur Institute of Iran, Tehran, Iran between 2003 and 2004. A total of 4707 were tested for the presence of HIV-1 antibodies.

Results: Six hundred and four (12.8%) patients tested for HIV were positive for HIV-1 antibody. Nine (1.49%) have discordant results among screening assays and indeterminate WB results as interpreted by Centers for Disease Control and Prevention (CDC) criteria. Most (66.7%) of these indeterminate WB results were due to p24 reactivity. However, 2 (22.2%) display reactivity to both gp41 and gp120 proteins [Positive by World Health Organization (WHO) criteria]. Of 9 WB assays initially indeterminate by the CDC criteria and with follow-up samples, 8 (88.8%) became negative when retested subsequently while one (11.1%) remained indeterminate for more than a year and were thus considered negative. In addition, all the indeterminate samples were negative when assessed by polymerase chain reaction assay.

Conclusion: In general, there was an 88.8% concordance between the CDC and WHO criteria for an indeterminate WB result. The CDC II criteria best met the specified objectives for diagnosis in our setting.

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Although the Western blot (WB) assay is probably the most widely accepted confirmatory assay for the detection and confirmation of antibodies to human immunodeficiency virus type 1 (HIV-1) and type 2 (HIV-2),[1-4] indeterminate WB profiles in HIV-uninfected subjects are frequent and are as high as 23-53% in some populations.[5,6] The type of HIV predominantly circulating in Iran is subtype A and B.[7] The performance of WB assays in identifying samples from individuals infected with HIV-1 subtype A has not been widely investigated. The frequency and profiles of indeterminate WB reactivity to HIV-1 proteins among the Iranian population have not been systematically investigated.[7] In this report, we describe the profiles of indeterminate WB reactivity in blood samples found to be reactive by rapid testing and enzyme-linked immunosorbent assay (ELISA) screening assays.

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Methods. The samples were obtained from the Iranian Blood Transfusion Center, Tehran, Iran and evaluated in the Biotechnology Process Development Center, Pasteur Institute of Iran, Tehran, Iran between 2003 and 2004. Overall, 4103 negative and 604 positive and indeterminate sera were collected. We performed the HIV screening for samples using a rapid test (ACCON HIV1/2/O, USA) and ELISA (Vironostika-HIV Uni-Form II plus O). Specimens with discordant results or those testing reactive by both rapid test and ELISA were confirmed by WB (HIV Blot 2.2; Genelabs Diagnostics). The algorithm for HIV testing is shown in **Figure 1.**[8,9] Specimens testing reactive were then confirmed by another methodologically different ELISA (HIV 1/2 Rec; Pasture Institute of Iran, Iran). We evaluated only discrepant results using WB (HIV Blot 2.2; Genelabs Diagnostics). In ACCON Tri-Line rapid test, the captured HIV proteins are the recombinant protein of HIV-1 and HIV-2, corresponding to a region overlapping the junction between the gp120 and gp41 fragment of the envelope (env) protein, plus a highly purified peptide, which corresponds to a region of the env transmembrane protein of HIV-1. In Vironostika ELISA, the HIV antigens are a mixture of HIV-1 p24, HIV-1 gp160, HIV-1 ANT70 peptide, and HIV-2 env peptide (amino acids 592 to 603). In HIV-1/2 Rec Pasture Institute of Iran ELISA, the HIV antigens comprise the highly purified immunodominant antigens of the core and env proteins of HIV-1 and an immunodominant peptide of the HIV-2 env. In our laboratory, a WB test is interpreted as positive for HIV-1 antibodies according to the Centers for Disease Control and Prevention second revision (CDC II) criteria,[3,8] requiring the presence of at least 3 bands; one from each gene product group of gag, pol, and env.[3] Three observers read all the WB results independently. Also, we compared the performance of the CDC criteria with those of the World Health Organization (WHO) (a specimen is interpreted as positive when there is reactivity to at least 2 env bands).[4] We assess the HIV-1 viral load in plasma using polymerase chain reaction assay [nucleic acid sequence-based amplifications (NASBA); Organon Teknika]. All assays were carried out as specified by the manufacturers. Factors associated with false-positive ACCON Tri-line and ELISA and indeterminate WB test results were examined by univariate analysis (chi-square and t-test where appropriate) and multivariate analysis (logistic regression). The statistical analysis was performed using the SPSS statistical package version 13.0.

Results. A total of 604 specimens (12.8%) were HIV-1 antibody positive. Of these, 9 (1.49%) show equivocal results, with discordant serological data and indeterminate WB results. Most of the indeterminate WB results were due to antibodies against the HIV-1 core antigen p24.[10,11] In addition, reactivity to pol p51, pol p66, and env gp41 antigens were frequent.[11,12] A total of 2 samples displayed reactivity to both env glycoproteins gp41 and gp120/160. These samples can be considered as positive by the WHO criteria.[4] In general, there was 88.8% concordance between the CDC and WHO criteria for an indeterminate result. Most of the initially indeterminate WB assays, including samples considered positive by the WHO criteria, were negative when retested. One subject with initial indeterminate WB profile was negative throughout 10 months of follow-up, at which time it should be seroconverted.[13] Only 2 samples remained persistently indeterminate (as long as 12 months) without developing any WB reactivity that indicated seroprogression. In addition, all indeterminate WB assays, including the 2 samples that persistently show indeterminate WB profiles, were assessed by NASBA for HIV-1 viremia. Plasma HIV-1 viremia was not detected in any of the above specimens. None of the indeterminate WB test results turned out to be a pre-seroconversion sample.

In multivariate analysis, false-positive ACCON Tri-Line test results were independently associated with lower hemoglobin levels whereas false-positive ELISA results were independently associated with male gender and lower hemoglobin levels.[11,12,14] Samples that are viscous or that contain a precipitate may form a residue and can interfere with the assay. It appears possible, therefore, that alterations of blood viscosity may lead to the false-positive ACCON Tri-Line test results that we observed in this study. Of note, one patient had a transient false positive ACCON Tri-Line test result at a visit when he was diagnosed with hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. We did not find any association between false-positive test results and symptomatic medical conditions. The performance of the screening assays is summarized in **Table 1**.

Discussion. The results of this study indicate that indeterminate WB reactivity to HIV-1 proteins using the WHO criteria may occur among Iranians who do not otherwise appear to be infected with HIV-1. In most cases, the indeterminate WB results were due to reactivity to p24 antigens, although reactivity to other HIV-1 antigens was also present.[6,11] None of the indeterminate WB test results turned out to be a pre-seroconversion sample.[13] In our setting, using negative HIV-1 serology at follow-up visits as a "gold standard" for the subjects being uninfected with HIV-1, the WHO

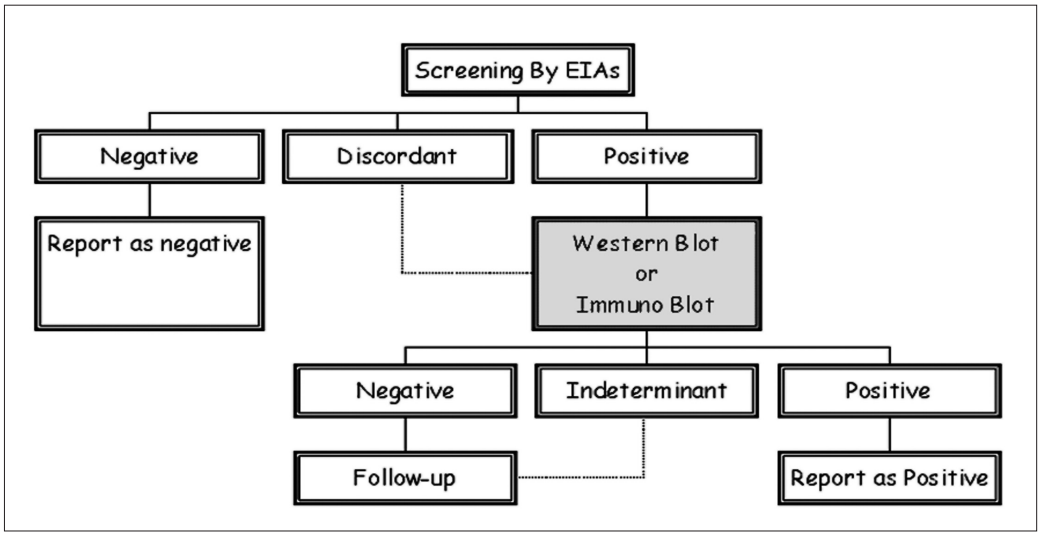

Figure 1 - Proposed human immunodeficiency virus testing algorithm for screening and confirmation of infection.

Table 1 - Performance of screening assays compared with the gold standard, Western blot.

Assay type	ACCON tri-line	ELISA (HIV-1 and 2 Rec, Pasture Inst.)	ELISA (Vironostika-HIV Uni-Form II)
Sensitivity (%)	97.4	100	100
Specificity (%)	99.5	99	98.6
PPV	95.5	90.9	89.9
NPV	99.7	100	100

PPV - positive predictive value, NPV - negative predictive value, HIV - human immunodeficiency virus, ELISA - enzyme-linked immunosorbent assay.

criteria would have misclassified 2 samples. The most strict interpretation criteria set by CDC proved to be the most accurate in the Iranian context.[3,5,8] Thus, due to the reported relatively high frequency of the indeterminate WB profile in apparently healthy HIV-seronegative,[5,8] it could be concluded that, in general, stricter criteria may be required for interpretation of WB test results on Iranian samples. Many initially indeterminate results that subsequently become negative or remain indeterminate are probably the result of nonspecific reactions between antibodies to residual cell components on the WB strips or are due to the presence of hyper gammaglobulinemia or of cross-reacting antibodies to some infections or are due to infection with an unknown but related retrovirus.[11-14] Human immunodeficiency virus-1 vaccine trials are under way in many developing countries. One of the major practical obstacles for HIV-1 vaccine trials is the development of tests that discriminate between vaccinated and those who are truly infected. This is particularly relevant for those candidate who receive vaccines that elicit antibodies to a limited number of HIV-1 proteins. The WB profiles of such vaccinated will have to be studied and put in the context of the indeterminate WB profiles, as described in this paper. These findings indicate that it is important to assess the background of WB profile of HIV-uninfected individuals in a given community before embarking on vaccine trials. However, in the present study we did not find any association between false-positive test results and other viral infections. However, one patient had a transient false-positive ACCON Tri-Line test result at a visit when he was diagnosed with HBV and HCV infections. The interpretation of indeterminate WB test results, particularly those with discordant results in 2 screening assays, such as rapid tests and ELISA, are a challenge to laboratories in the developing or least developed countries. An important implication of this is how to address these issues on counseling. Although such problems may not be frequent (<1%), counseling should be tailored differently for individuals with equivocal HIV-1 test

results. Follow-up of such cases, in combination with an offer to obtain a repeat blood sample for re-testing, is one of the most important measures. This would be a more practical approach than to use alternative laboratory tests, such as determination of serum viral load, to help clarify such cases.[13,15] Viral load determinations are still largely not available in many developing countries where HIV-1 infection is also highly prevalent. New HIV-1 subtypes and circulating recombinant forms of HIV-1 are being discovered regularly.[15] Thus, the performance of WB assays in correctly identifying samples from individuals infected with these viruses needs constant attention.

In conclusion, each laboratory must adopt the criteria that provide specified objectives for diagnosis. In the Iranian setting, it is recommended to opt for the most stringent criteria of interpretation of the WB, namely, CDC revision II. It may well be that these criteria prove optimal for other Middle Eastern contexts too.

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