AD_____

INDETERMINATE HIV-1 WESTERN BLOTS: ETIOLOGY, NATURAL HISTORY, AND PSYCHOLOGICAL REACTIONS

FINAL REPORT

CONNIE CELUM

SEPTEMBER 16, 1992

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

Contract No. DAMD17-90-Z-0042

University of Washington
Harborview Medical Center
325 Ninth Avenue
Seattle, Washington 98104

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: University of Washington; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): Harborview Medical Center, 325 Ninth Ave, Seattle, WA 98104	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U.S. Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD17-90-Z-0042
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, Maryland 21702-5012	10 SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 63105A; PROJECT NO. 3M2-63105DH29; TASK NO. AD; WORK UNIT ACCESSION NO. DA33551

11. TITLE (Include Security Classification)

Indeterminate HIV-1 Western Blots: Etiology, Natural History, and Psychological Reaction

12. PERSONAL AUTHOR(S)

Connie Celum, MD, MPH

13a. TYPE OF REPORT	13b. TIME COVERED	14 DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 8/17/90 TO 8/16/92	9/16/92	102

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	RA 1; Western blots; Retroviruses; Indeterminate tests; Natural history
65	08		
06	03		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Previously described causes of indeterminate HIV-1 Western blots (IWB) include the evolving antibody response during HIV-1 seroconversion, antibody cross-reactivity due to HIV-2 infection, autoimmune disease, and alcoholic hepatitis. Most cases of IWB remain unexplained, however. Two hundred forty-four cases were referred to the study because of one or more previous repeatedly reactive HIV-1 enzyme immunoassay (EIA) and IWB and 145 EIA negative controls were frequency-matched by testing site. Risk factors for IWB were

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION	
☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	Unclassified	
22a. NAME OF RESPONSIBLE INDIVIDUAL: Mary Frances Bostian	22b TELEPHONE (Include Area Code): 301-619-7326	22c. OFFICE SYMBOL: SGRD-RMI-S

DD Form 1473, JUN 86 Previous editions are obsolete. SECURITY CLASSIFICATION OF THIS PAGE

evaluated among the 123 cases who were still EIA repeatedly reactive with an IWB at visit one and 112 controls who were both EIA and Western blot negative.

Six (3.4%; 95% CI=0.6%, 6.1%) of the 178 cases who were followed for six months or longer seroconverted. The specificities of HIV-1 culture, PCR, serum p24 antigen, the recombinant Cambridge BioSciences Recombugen (or, CBr3), Syva Microtrak, and the synthetic peptide assay, Genetic Systems GENIE, were 97-100% among the nonseroconverters.

Independent risk factors for IWB among the nonseroconverter cases were: autoantibodies (positive antinuclear antibodies or rheumatoid factor) (O.R. 2.3; 95% CI=1.2,4.5); sexual contact with a prostitute since 1978 (O.R. 5.6; 95% CI=1.5, 21), history of STDs (O.R. 0.5, 95% CI=0.2,0.9), and elective testing for HIV (O.R. 2.3; 95% CI=1.1,4.7). Separate analyses for men and women showed different risk factors for IWB. Among men, these risk factors included sex with a prostitute since 1978 (O.R. 5.8; 95% CI=1.6,20.5) and a tetanus booster in the past two years (O.R. 5.6; 95% CI=1.4,23). Among women, parity (O.R. 1.4; 95% CI=1.1,1.7) and elective testing for HIV (O.R. 3.8; 95% CI=1.4,10.4) were positively associated with IWB , and history of STDs (O.R. 0.2; 95% CI=0.08,0.6) was negatively associated with an IWB. No cross-reactivity was detected when the IWB sera were tested for antibodies to HIV-2, HTLV-1, FIV, FeLV, or BIV.

The cases reported significantly more anxiety and depression than the controls at the first study visit. High-risk behavior was strongly associated with anxiety and depression for both cases and controls. The notification of the IWB accounted for the majority of the difference in anxiety among the cases.

TABLE OF CONTENTS

INTRODUCTION

The sensitivity and specificity of the human immunodeficiency virus type-1 (HIV-1) enzyme-linked immunosorbent assay (EIA) are greater than 99% [1-5]. Specimens that are repeatedly reactive by HIV-1 EIA are confirmed by a supplemental test, usually the Western blot which detects antibodies to denatured HIV-1 proteins. The HIV-1 Western blot has a reported specificity of 98% [5]. However, between 4 and 20% of sera that are repeatedly reactive by HIV-1 EIA are interpreted as indeterminate by Western blot [6-9]. The proportion subsequently classified as indeterminate varies according to the immunoblot and the interpretative criteria used and the prevalence of HIV-1 infection in the population tested [8]. In addition, up to 33% of HIV-1 EIA negative specimens have had indeterminate banding patterns on Western blot in reports of EIA negative volunteers for HIV-1 vaccine trials [10,11].

Indeterminate HIV-1 Western blots (IWB) in HIV-1 infected individuals may result from antibody production against viral core antigens early in HIV-1 infection [12-14] or loss of core antibodies late in HIV-1 infection [15,16]. In HIV-1 negative individuals, IWB have been shown to result from cross-reactive antibody to HIV-2 [17-20] or cross-reactive autoantibodies and alloantibodies [21-26]. In earlier studies of blood donors, the seroconversion rate ranged from 0-5% and was seen exclusively among donors who initially denied HIV risk behaviors and who had p24 bands on the initial Western blot [23-26]. Conversely, 18 of 20 (90%) high-risk individuals from anonymous testing sites in San Francisco who had initial p24 bands only seroconverted to a positive Western blot in two to four weeks [14]. Etiologies of IWB other than HIV seroconversion have otherwise not been well-characterized and have been described primarily in case series and case reports.

Individuals with a reactive HIV-1 EIA and IWB are currently excluded from blood donations and have had difficulty obtaining life and disability insurance, U.S. immigration status, and visas for foreign travel, regardless of their risk history. Persons notified of a reactive HIV-1 EIA and IWB are often concerned not only about their risk of HIV seroconversion but also whether the IWB reflects any underlying medical condition [27]. Our study assessed risk of seroconversion through 6-9 months prospective follow-up of the

cases and risk factors for IWB other than early HIV infection through a case-control study. Cases included individuals with and without risk factors for HIV infection, who were referred to the study because of repeatedly reactive EIAs and IWB. Controls were EIA negative persons recruited from the same testing sites. In addition, we assessed anxiety and depression related to the IWB through comparison of cases and controls and comparison of high- and low-risk cases.

We found that IWB were associated with a low risk of seroconversion of 3.4%. HIV-1 culture, polymerase chain reaction, and the recombinant HIV-1 tests, Cambridge BioSciences Recombigen and Syva Microtrak, and synthetic peptide Genetic Systems GENIE, had specificities of 97-100% and all had negative predictive values of 100%. Sixty-nine percent of cases had an identifiable etiology for IWB unrelated to HIV infection, such as autoantibodies, autoimmune disease, parity, or a recent tetanus booster. Cases were significantly more anxious than controls and the majority of this anxiety was attributed to notification about the indeterminate HIV result.

METHODS

Study Population

This case-control study was initiated at the University of Washington in March 1988 [28]. Cases included men and women 13-85 years of age with a repeatedly reactive EIA and an IWB in the past, who were referred from testing sites in Washington and Oregon states. The HIV-1 Western blot interpretation for Western blots performed on subjects prior to study enrollment was accepted for study entry. Individuals with a prior diagnosis of HIV seropositivity or AIDS and recipients of experimental HIV-1 vaccine were excluded from the study. Cases were recruited by letters sent to providers about the study together with the HIV-1 Western blot result (i.e., the IWB). The rate of response by cases is undefined, given the recruitment from providers and occasional use of anonymous testing.

Controls who had a negative HIV-1 EIA within the past three months were recruited from the same HIV-1 testing sites and were frequency-matched by HIV-1 testing site. After informed consent was obtained, cases and controls were interviewed about HIV risks and general medical history, and were examined. Cases were asked to refer current sexual partner(s) to the study for evaluation and HIV-1 antibody testing.

Study Design

Cases were followed prospectively for 6-9 months to determine the rate of seroconversion. Cases and controls were administered a questionnaire about medical history and risk behaviors for HIV and other sexually-transmitted diseases (STDs). The subset of cases who did not seroconvert and who still had a reactive EIA and indeterminate Western blot at visit one were compared with EIA negative, Western blot negative controls to ascertain etiologies for IWB other than acute HIV-1 seroconversion. Cases and controls were administered a psychological questionnaire to assess anxiety and depression at the first study visit.

Due to the heterogeneity of cases referred from low-risk (i.e., blood banks) as well as high-risk testing sites (e.g., AIDS Prevention Project and Sexually Transmitted Disease clinic),

cases and controls were frequency-matched by HIV testing sites and stratified by testing site in the multivariate analyses. The cases and controls were stratified into three groups (blood donors; high-risk testing sites of the AIDS Prevention Project and the STD clinic; and the women's and prenatal clinics) to reduce potential confounding variables introduced from the heterogenous mix of high- and low-risk cases from different testing sites. Low-risk cases for whom random controls could not be enrolled (i.e., life insurance applicants and patients referred by private physicians) were matched to blood donor controls.

Laboratory Testing

The University of Washington Virology laboratory and the Washington State Public Health laboratory performed EIAs and Western blots for the study. Both laboratories subscribe to the College of American Pathologists Proficiency panel for HIV-1 antibody testing. Cases were followed prospectively with repeat HIV-1 EIAs and Western blots every three months for six to nine months to detect seroconversion.

Dupont (Biotech Research Laboratory Inc., Rockville, MD) and Genetic Systems (Seattle, WA) EIAs and Epitope (Beaverton, OR) Western blots were performed on study subjects. The CDC interpretive criteria were used for Epitope Western blots; a Western blot was considered positive if antibodies were present to two of the following HIV-1 viral proteins: p24, gp41, and gp120 or gp160 [8]. Western blots without any bands were considered negative and blots with bands not meeting the criteria for a positive blot were interpreted as indeterminate. The diagnosis of HIV-1 infection was based upon seroconversion to a positive Western blot with persistently reactive EIA. Positive Western blots were repeated.

The screen for autoantibodies included antinuclear antibodies (ANA) and rheumatoid factor. ANA testing was performed using an initial 1:40 serum dilution on rat liver and 1:100 dilution on HEp-2 cells. Rheumatoid factor testing was performed using latex agglutination at a serum dilution of 1:20. A titer of $\geq$ 1:10 was considered a positive ANA or rheumatoid factor. Lymphocyte subset analyses were performed by flow cytometry for cases and for a subset of the controls. The screen for other infectious diseases included serum VDRL,

antibody to herpes simplex types 1 and 2 by Western blot [29], and serum hepatitis B surface antigen and antibody. EIAs for HTLV-1 and HIV-2 were assessed in a subset of randomly selected 116 and 91 cases, respectively. To ascertain possible cross-reactivity with animal retroviruses, serum from a subset of 27 cases who reported raw milk ingestion or farm animal contact were tested for antibodies to bovine leukemia (BLV) and immunodeficiency virus (BIV) by p24 agar immunodiffusion and Western blot. Sera from 26 cases with a pet cat were tested for reactivity on Western blots for feline leukemia virus (FeLV) and for feline immunodeficiency virus (FIV) [30].

Sera were screened for the presence of antibodies to class I HLA antigens using a panel of T-lymphocytes for the presence of antibodies from 50 donors of known HLA type. Sera were screened for the presence of antibodies to class II HLA antigens using a panel of B-lymphocytes from 25 donors of known HLA type. In both assays a modified microlymphocytotoxicity assay was used to detect complement-fixing antibody [31].

IgG antibodies to tetanus toxoid were measured by EIA, according to the manusfacturer's instructions (The Binding Site, Birmingham, UK) [32]. The EIA was run in duplicate and the mean of the two results was obtained.

Supplemental HIV-1 tests

HIV-1 supplemental tests were performed on sera and peripheral blood mononuclear cells obtained from cases at the first study visit. HIV-1 culture was performed using the cocultivation method with peripheral blood mononuclear cells from seronegative donors and sampling the supernatant for p24 antigen every three days for one month, using the the antigen capture EIA (Abbott Laboratory, Chicago, Illlinois) [33]. Serum p24 antigen assays were performed, using the same antigen capture EIA method with acid neutralization confirmation of positive results [34-36]. Polymerase chain reaction was performed by CETUS Corporation (Emeryville, CA), Roche Biomedical Laboratories (Research Triangle Park, NC), the University of Washington Retroviral Laboratory, and SRA Laboratory, using the SK 38/39 and SK 101/145 primer pairs [37]. SRA performed PCR for HIV-2 for two cases with residence

in West Africa, using primer pairs SK100/104 and the HIV-2 specific primer SK 89/90. Cell lysates were obtained from cryopreserved PBMCs and amplification competency was checked by amplification of a conserved region within the HLA-DQ alpha locus with primer pair GH 25/26. HIV-1 DNA amplification was performed as described previously [37] and each specimen was run in duplicate for both primer sets. HIV-1 proviral sequences were considered present if both primer pairs were positive in duplicate, indeterminate if only one of the duplicate reactions was positive for one or both primer pairs, and not present if neither primer pair resulted in a positive signal.

Two recombinant antigen EIAs, Cambridge Biosciences CBr3 or Recombigen (Cambridge, MA) and Syva Microtrak (Palo Alto, CA), and one synthetic peptide, Genetic Systems GENIE (Seattle, WA) were perfomed. CBr3 and Microtrak are recombinant-based EIAs that have proteins from two conserved gene products--the carboxy-terminal half of gp120, the amino-terminal half of gp41, and all of p24. The GENIE is derived from synthetic peptides of HIV-1 gp41 and HIV-2 gp36 [38].

Psychological instruments

At the first study visit, cases and controls were administered the following questionnaires: the 13-item Beck Depression Inventory (BDI) [39,40], the Symptom Checklist-90 (SCL-90) anxiety subscale [41], and a 16-item scale regarding life events in the past six months, including two questions related to the decision to be tested for HIV and the impact of the result of the HIV test. The 13-item BDI is a self-report measure that is a shortened version of the 21-item BDI and has correlated well with clinical diagnoses of depression in previous studies [40]. The BDI provides a quantification of the intensity of depressive symptoms on a scale of 0-4, no or minimal depression; 5-7 mild; 8-15 moderate; and scores of $\geq$ 16 correlating with severe depression. The SCL-90 anxiety subscale consists of 10 items measured on a 5 point scale. The SCL-90 anxiety is standardized to norms derived from large samples of non-psychiatric outpatients with separate standardizations for men and women [40]. The lowest standardized score is 36 for males and 38 for females. In logistic regression analyses that

included depression and anxiety as independent variables to predict case-control status, a dichotomized variable was created from the continuous BDI and SCL-90 scores; a score of one standard deviation above the mean was used (i.e., a BDI score of ≥5 and a SCL-90 score of ≥ 65=1 and BDI <5 and SCL-90 <65 =0).

The 16-item life event scale is a modification of the 14-item life event scale used in previous studies to assess the impact of stressful life events in the previous six months [42]. Two additional questions were included int he life event scale about HIV testing and the impact of the HIV test result, which were coded into a dichotomous variable of 0 for no stress related to the HIV result and 1 for mild to severe distress. In addition, cases only were administered a 9-item questionnaire related to the impact of the indeterminate Western blot, which was derived from interviews with the first 20 cases referred to the study (Appendix B).

A subset of 31 cases were administered the same psychological questionnaire at a follow-up visit. These responses were compared to their responses at the first study visit to determine the change in anxiety and concerns about the IWB over time.

Statistical methods

Demographic variables and potential HIV risk factors were compared using the Chi-square test and Fisher's exact test for categorical data and Student's t-test for continuous data. The Mann-Whitney test and Kruskal-Wallis tests were used for comparing continuous distributions when the assumption of a normal distribution was not appropriate. Ninety-five percent exact binomial confidence intervals for the seroconversion risk were calculated.

To assess risk factors for IWB other than HIV-1 infection, the nonseroconverter cases and controls were compared in terms of reported autoimmune illness, viral illness in the preceding three months, past history of tuberculosis or a positive skin test for TB (purified protein derivative or PPD), parity, immunization and transfusion history, past STDs, and risk behaviors for HIV since 1978. To reduce potential misclassification bias from inclusion of EIA or Western blot negative cases and EIA negative, Western blot indeterminate controls, risk

factor analyses were restricted to only the cases who were still EIA repeatedly reactive and IWB, and to controls who were both EIA and WB negative, at visit one.

Conditional logistic regression was used to compare the cases and controls for risk factors for indeterminate Western blots, stratified by three groups of testing sites--1) blood donors and low-risk cases from disability and life insurance screening, 2) high risk testing sites (AIDS Prevention Project and Sexually Transmitted Disease clinic), and 3) women's and prenatal clinics. Analyses were also performed for males and females combined and separately.

The psychological data were analyzed in three ways. The cases and controls were compared by univariate analyses in terms of demographics, self-reported history of depression and psychotropic medication use, and scores on the Beck Depression Inventory and the SCL-90 anxiety subscale. Multivariate analyses were also performed for the cases only in which the SCL-90 anxiety score was the dependent variable and independent variables were entered, i.e., demographic, history of depression and psychotropic medication use, the BDI score, and the dichotomous variable drived from indicating distress due to the results of the HIV test (item 16 on the life event scale). Analyses restricted to cases only were performed to analyze differences in expectations and impacts of the IWB among high- and low-risk cases. Finally, the subset of 31 cases with psychological data from follow-up visits were analyzed in terms of changes in anxiety and depression scores and concern about the IWB over time.

RESULTS

Of 244 persons with indeterminate HIV-1 Western blots referred and enrolled in the study, 178 were followed for six months or longer and were included in the calculation of seroconversion rate and specificities of supplemental tests. Sixty-three percent of cases were referred from blood banks and 31% from the Seattle-King County Department of Public Health clinics, primarily the AIDS Prevention Project and the Sexually Transmitted Diseases clinic, and 5% from women's and prenatal clinics. One hundred four (44%) cases electively sought HIV-1 testing due to concern over possible exposure to HIV-1; the remaining 132 (56%) were routinely screened for HIV-1 as blood donors, military recruits, life insurance or immigration applicants.

One hundred forty-five EIA negative controls were recruited from the same HIV-1 testing sites as the cases. Ninety-one (63%) controls were recruited from blood banks, 47 (33%) from the AIDS Prevention Project and Sexually Transmitted Diseases clinic, and 5 (4%) from prenatal clinics.

Eighty-three current sexual partners of 83 cases were enrolled in the study. Fifty-seven (72%) were partners of index cases who were blood donors, 18 (23%) had partners from the AIDS Prevention Project and the Sexually Transmitted Disease clinic, and 4 (5%) from women's and prenatal clinics.

Characteristics of cases, sexual partners of cases, and controls are shown in Appendix A, Table 1. Cases and controls were similar demographically, but the cases' sexual partners were significantly more likely to be married, have a family income greater than $20,000, have fewer sexual partners in the past three months, and fewer of the partners reported a history of STDs than cases or controls.

HIV serologies among cases, controls, and cases' sexual partners

Results of HIV-1 EIA and Western blots from the first study visit are shown in Appendix A, Table 2. Of 244 cases referred to the study because of previous reactive EIA and IWB, 139 still had repeatedly reactive EIA and of these, 124 also had IWB at the first study

visit and did not seroconvert to a positive Western blot. These 124 cases are further analyzed for risk factors for IWB other than HIV infection. Of the 145 EIA negative controls, 38 (26%) had indeterminate Western blots and the remaining 112 with negative Western blots were included as controls for the risk factor analyses. It is of interest that banding patterns included p17 and p24 (core) bands in 87% of the 155 cases who had indeterminate Western blots at study visit one compared to 50% of 38 controls and 14% of the 21 cases' sexual partners with IWB ($P < 0.001$).

Five partners of the cases were EIA repeatedly reactive; one was negative by Western blot and one was indeterminate with a p55 band. Three (4%) of the cases' sexual partners were confirmed seropositive by Western blot, two of whom had HIV risk behaviors (a bisexual man and a hemophiliac). The third sexual partner was married to a recent immigrant from West Africa. Her husband, the case, had a stable p17 band on Western blot and negative HIV culture and PCR; the source of her infection is uncertain.

Risk of seroconversion among cases

The risk of seroconversion was determined for cases with $\geq$ six months follow-up beyond the initial IWB. Of the 178 cases with six months or greater follow-up, 72% remained indeterminate by Western blot at follow-up visits and 28% developed negative blots. We have previously reported on 89 of these individuals, among whom four (4.5%) seroconverted [23]. In the larger sample of 178 individuals, a total of six seroconverted (3.4%; 95% confidence interval, 0.6% - 6.1%). All six seroconverters had a p24 band on the initial Western blot prior to seroconversion and had recent HIV risk behavior. Five of the six seroconverters progressed from a p24 band only to a positive Western blot within one month. The sixth seroconverted after 10 months, with ongoing unprotected homosexual activity during the study period. He was likely infected after his six month visit, given his stable indeterminate Western blot (p24 only) and a negative recombinant HIV-1 p24 and gp41 EIA at his six month visit. Two low-risk cases and one high-risk case had false positive Western blots during

follow-up with the presence of p24 and envelope bands, but were determined to not be HIV-infected through HIV-1 culture, PCR, and recombinant HIV-1 EIAs.

Supplemental HIV-1 tests

To aid in a more rapid determination of the HIV serostatus of persons referred with IWB, we analyzed the specificity and negative predictive value of several supplemental HIV-1 tests. HIV-1 culture was negative in 122 of 122 nonseroconverters, polymerase chain reaction was negative in 177 of 178, and serum p24 antigen was negative in 192 of 192 nonseroconverter cases, resulting in specificities of 99.4%-100%. However, given the low sensitivity of serum p24 antigen [34-36] and the time and expertise needed to perform HIV-1 culture and polymerase chain reaction, we sought more rapid assays for use in the algorithm we proposed to evaluate persons with IWB [28]. The two recombinant assays, Cambridge BioSciences Recombugen (or, CBr3) and the Syva Microtrak, and the synthetic peptide assay, Genetic Systems GENIE, had specificities of 99%, 97%, and 100% respectively, and all had negative predictive values of 100% (Appendix A, Table 3). Given the EIA format of the Recombugen and Microtrak and the dipstick methodology of the GENIE, these supplemental tests appear promising as rapid and accurate methods for ruling out HIV-1 infection, especially in persons with p24 bands.

The difficulty in evaluating supplemental tests is determining their sensitivity in early HIV infection due to the few number of seroconverters identified in our study. Using a panel of commercially available seroconverter sera, we compared the sensitivity of the Recombugen, Microtrak, and GENIE (Appendix A, Table 4) and found that they were positive from 12 to 14 days before the conventional Genetic Systems HIV-1 EIA, indicating a high sensitivity relative to the conventional EIA and Western blot.

The results of supplemental tests in one of the seroconverters from our study are shown in Table 5, Appendix A. Within two days, the EIA went from a high negative R-value of 0.9 with weak p24 and gp41 bands on Western blot and a strongly positive p24 antigen of >1,000 pg/ml to a positive EIA (R-value of 1.8) and positive Western blot with p24, gp41, and

gp120/160 bands. Peripheral blood mononuclear cells were not available from the initial visit. His PCR and PBMC culture were positive a month later at the time his EIA was positive with a R-value of 4.0 and the HIV-1 Western blot had all viral bands, but the serum p24 antigen was negative. These data demonstrate how more than one supplemental tests may need to be performed in a person with an IWB during the seroconversion window due to the dynamic and variable amount of cell-free and proviral HIV present early in HIV seroconversion.

Risk factors for IWB

The demographics of the 124 nonseroconverter cases who had repeatedly reactive EIA and IWB at visit one and the 112 EIA and Western blot negative controls were similar, except that the median number of years of education was lower for cases than controls (Appendix A, Table 6).

Univariate Analyses

The 124 cases who did not seroconvert and were EIA repeatedly reactive and indeterminate by Western blot at visit one were compared with the 112 EIA and Western blot negative controls in terms of general medical history, autoantibodies, hepatitis B antibodies, lymphocyte subsets, and HIV risk factors (Appendix A, Table 7). In univariate analyses, cases had significantly higher prevalences of autoantibodies (rheumatoid factor and/or antinuclear antibodies), history of sexual contact with a prostitute, tetanus booster within the past two years, history of autoimmune disease (systemic lupus erythematosus, rheumatoid arthritis, juvenile onset diabetes, thyroiditis, and Crohn's disease), were less likely to be tested electively for HIV, and a lower frequency of reported prior STDs. The 67 (31%) cases with autoantibodies in our study usually had low titer ANAs (median titer of 40, range 10-640) or rheumatoid factor (median titer of 320, range 20-10,240).

Among men, cases significantly more often reported sexual contact with a prostitute since 1978, any high risk behavior since 1978, and a tetanus booster in the past two years, had

a significantly higher prevalence of autoantibodies, and a higher proportion of cases than controls had shared needles during injection drug use.

In comparison with female controls, female cases were significantly more often parous, and more often sought testing for HIV infection, more often reported a viral-like illness in the preceeding three months, had a lower prevalence of antibody to hepatitis B surface antigen, and more often gave a history of autoimmune disease.

Multivariate analyses

The results of the conditional logistic regression analyses are also shown in Table 7. The independent variables associated with IWB among males and females combined included presence of autoantibodies, history of sex with a prostitute, a less frequent history of STDs, and more frequent elective testing for HIV. The variables that were independently and significantly correlated with IWB for males were sex with a prostitute and a tetanus booster in the past two years. For females these variables were parity, less frequent history of STDs, elective testing for HIV, and viral illness in the past three months. Fifteen female cases (13%) and four (6%) female controls were pregnant at study enrollment ($P=0.07$). Seven of the pregnant cases had risk factors for HIV, but none seroconverted.

HLA antibodies and lymphocyte subsets

HLA antibodies were tested for 78 cases; anti-class I HLA reactivity was found in 13 (17%) and definite anti-class II HLA reactivity could not be identified in any of the samples. All but one of the cases who demonstrated anti-class I HLA reactivity were multiparous females. There was no correlation between HLA antibodies, a positive ANA or rheumatoid factor, and presence of p17 or p24 banding patterns on Western blot.

Cases and controls did not differ in median absolute CD4 count (1018 versus 1124) or median percentage CD4 lymphocytes (48% versus 50%; $P>0.2$ for both comparisons).

Tetanus IgG among cases and controls

Given the association between a tetanus booster in the past two years and IWB, anti-tetanus IgG titers were compared between cases and controls. The median titer of IgG was 254 in the 16 male cases tested (range 46-4056) and 310 in the 5 male controls tested (range 245-

4056) (P=0.06 by Mann-Whitney test). Higher titers of tetanus IgG were associated with more recent vaccination.

Herpes simplex type-2 antibodies among cases and controls

To pursue the association between high-risk behavior and IWB among the male cases, antibodies to herpes simplex type-2 were measured among male cases and controls. The prevalence of HSV-1 and HSV-2 specific antibodies among male cases was 5 (19%) and 20 (74%) of 27 male cases, respectively, and 11 (55%) and 3 (15%) of 20 male controls, respectively (P=0.02 for HSV-1 and P<0.001 for HSV-2). In comparison among females, the prevalence of HSV-1 and HSV-2 antibodies was 17 (59%) and 9 (31%) of 29 female cases, respectively, and 9 (37%) and 5 (21%) of 24 female controls, respectively (P=0.21 and P=0.60).

Cross-reactivity among other human and animal retroviruses among cases

Of 116 cases tested, only one case had a weakly reactive HTLV-1 EIA and an indeterminate HTLV-1 Western blot. Ninety-one cases tested for HIV-2 by the synthetic peptide GENIE for HIV-2 gp36 were negative. Two cases with residence in West Africa or with West African sexual partners were tested for HIV-2, one by HIV-2 EIA and RIPA and one by HIV-2 PCR; both cases were not infected with HIV-2.

Among 27 cases who reported raw milk ingestion or farm animal contact, none had antibodies to bovine immunodeficiency virus. Among the cases who reported a cat as a pet and were tested by Western blot for antibodies to FeLV and to FIV viruses, 8 of 26 (31%) had antibodies apparently reactive against p15e of FeLV, the transmembrane protein, compared to 4 of 14 (29%) controls (P > 0.2). No cases or controls had antibodies against FIV.

Pyschological impact of the IWB

One hundred eighty-nine cases and 116 controls who had completed the psychological questionnaire at visit one were compared. The cases and controls were similar in age, gender, marital status, income, high-risk behavior since 1978, history of anxiety, depression, and previous psychotropic medication use, all of which could be associated with current anxiety and depression (Appendix A, Table 8). The cases reported significantly higher amounts of

anxiety, as demonstrated by their median standardized SCL-90 anxiety subscale of 62 (range 36, 82) compared to 49 among the controls (range 36, 82; P<0.0001 by Kruskal-Wallis test of significance). The score on the 13-item Beck Depression Inventory was higher among the cases (median 3; range 0, 21) than controls (median 2; range 0, 18), but the difference was not statistically significant (P=0.19). High-risk behavior was strongly associated with higher anxiety and depression scores among both cases and controls (Appendix A, Table 9). High-risk cases had higher anxiety scores than high-risk controls who had higher scores than low-risk cases (P<0.001).

Anxiety and concerns about the IWB among cases

In multiple regression models, the independent predictors of anxiety among the cases at study visit one were frequency of thinking about the IWB, younger age, the Beck Depression Inventory score, distress related to notification of the IWB (as indicated on the life event scale), and male gender. The model was able to explain 65% of the variance in anxiety scores among the cases. In multiple regression models with the continuous Beck Depression Inventory score as the dependent variable, the independent predictors were the SCL-90 anxiety score and a history of anxiety and depression. The regression model explained 31% of the variance in BDI scores among the cases.

The psychological questionnaire that was piloted and derived from interviews with the first 20 cases referred to the study was administered to the cases only to ascertain the impact of notification of the IWB. The 62 high-risk cases had significantly higher anxiety and depression scores than the 113 low-risk cases (P<0.001). In addition, the high-risk cases were more likely to indicate that they thought the IWB was related to HIV infection, that the Western blot might turn positive in the subsequent six months of serologic follow-up, and were less likely to feel reassured if they did not seroconvert. The six seroconverter cases had similar anxiety and depression scores relative to the nonseroconverter cases (P=0.36 and 0.29, respectively for differences in the SCL-90 and BDI scores). The small number of seroconverters, however, limits the power to detect a difference between the two groups.

A subsample of 31 cases completed psychological questionnaires at follow-up study visits. A Beck Depression Inventory score of $\geq$ 5 at the first study visit was highly correlated with a score of $\geq$ 5 at follow-up visits ($\underline{P}$=0.01). Similarly, a standardized SCL-90 score of > 65 at the first study visit was correlated with a score of $\geq$ 65 at follow-up visits ($\underline{P}$=0.02) and the frequency of thinking about the IWB at the first study visit was correlated with the frequency of thinking about the IWB at follow-up visits ($\underline{P}$=0.02). Specific anxiety related to the IWB at the first study visit was significantly lower at the last follow-up visit ($\underline{P}$=0.002).

DISCUSSION AND CONCLUSIONS

With increasing pressure to screen more of the population for antibodies to HIV-1, more indeterminate HIV-1 serologic results will be generated [21,43]. This is the first case-control study to examine the etiology and psychological impact of indeterminate HIV-1 Western blots in persons at either high- or low-risk for HIV infection as well as to evaluate the cases' sexual partners and to follow the cases prospectively. Previous reports comprised case series of either high-risk persons, such as the San Francisco cohort, or low-risk persons, such as blood donors [14,23-26]. We compared both high- and low-risk cases with controls for medical conditions and exposures that might result in autoantibodies or alloimmunization and prospectively followed the cases for six months or longer to determine whether they seroconverted to a positive Western blot. The risk of seroconversion was 3.4% and was observed only among persons with recent high-risk behavior. The low risk of seroconversion in our sample population was comparable with that of earlier reports of seroconversion in 0% to 5% in blood donor cohorts [23-26]. Seroconverters in the earlier blood donor cohorts had p24 antibodies on initial Western blot and retrospectively admitted to HIV risk behaviors.

We found the specificity and negative predictive value of the recombinant assays, the Cambridge BioSciences Recombugen and Syva Microtrak, and the synthetic peptide, Genetic Systems GENIE, to be very high (97%-100%) and would recommend their use in the evaluation of persons with IWB, especially those with p24 bands. A negative supplemental assay can provide additional reassurance and potentially shorten the follow-up of persons with IWB [28]. In our study two blood donors without identifiable risk of HIV infection and one male with multiple heterosexual partners had false positive immunoblots with the presence of envelope bands. Supplemental tests confirmed the lack of HIV-1 infection in these three cases.

The cases had immunoblot banding patterns that differed from patterns of the current sexual partners of the cases and the controls; the cases had anti-core (p17 or p24) antibodies whereas the majority of the controls and cases' sexual partners who were EIA nonreactive but

had IWB had polymerase bands. Previous studies have shown that up to 33% of EIA nonreactive individuals will be indeterminate on Western blot, often with p24 or polymerase reactivity, depending on which immunoblot is used [10,11].

In previous case reports and case series, individuals with IWB have had T cell lymphoma, multiple sclerosis, and dermatologic disorders [44], passive transfer of antibody by hepatitis B immune globulin [45], injection drug use [46], alcoholic liver disease [47], class II HLA antibodies [48], cross-reactivity with HIV-2 [17-20,49], and autoantibodies such as those found in patients with systemic lupus erythematosus or Sjogren's disease [50]. A recent study by Jackson and coworkers of 99 Minnesota blood donors with indeterminate HIV-1 blots found no evidence for HIV-1 or HIV-2 infection [26]. One case series from the New York Blood Center in Syracuse suggested an association between indeterminate HIV-1 immunoblots and bovine immunodeficiency virus, but subsequent confirmatory assays did not confirm BIV reactivity [24]. We found no association between HIV-1 IWB and serologic reactivity to other known human and animal retroviruses (i.e., HTLV-1, HIV-2, BIV, or feline leukemia or immunodeficiency viruses).

A potential non-HIV basis for IWB was identified in a majority of the nonseroconverter cases in the present study. For example, autoantibodies (either a positive antinuclear antibody or rheumatoid factor), recent tetanus vaccination, and among women, parity, were risk factors for IWB. Autoantibodies could perhaps cause an IWB through cross-reactivity with epitopes of human cellular or HIV core proteins, or this could be a secondary association if both the IWB and autoantibodies were caused by another factor. Parity could reflect alloimmunization during pregnancy, producing antibody to cellular proteins that comigrate with HIV proteins on the Western blot. False-positive EIA in multiparous females have been associated with presence of HLA-DR4 antibodies [51]. Although 12 of 13 cases in our study with anti-class I HLA reactivity were multiparous females, we found no significant relationship of HLA antibodies to IWB and anti-class II reactivity was not observed. Golding and colleagues have demonstrated homology between HIV-1 gp41 and

human class β_1 domains [52], however, most of our cases had cross-reactivity to core proteins, either p17 or p24.

Autoantibodies are found in 5-10% of the population, and these autoantibodies might cross-react with normal cellular determinants of the H-9 or CEM cells in which HIV-1 is cultured prior to Western blot production. Of 22 cases with a reported autoimmune history, 13 (59%) reported a history of autoimmune thyroid disease. However, we did not specifically measure antithyroglobulins. There was no correlation between a reported history of autoimmune disease and the presence of autoantibodies (either a positive ANA or rheumatoid factor).

Although a link between human retroviruses and certain autoimmune illnesses has been suggested [50,53-57], only limited data exist to support this hypothesis. Of note, p24 reactivity has been reported among one-third of persons with systemic lupus erythematosus [54,55].

A recent tetanus booster could result in immunogenic polyclonal stimulation of B cells and production of antibody with cross-reactivity to epitopes of HIV-1 or cellular proteins. A similar effect of influenza vaccine has been reported [58], but we did not observe an association with recent influenza vaccination in our study (data not shown).

Stratified analyses of men and women found different risk factors to be associated with IWB. The strongest risk factors for IWB among males were sexual contact with a prostitute and sharing of needles during injection drug use. Previous studies have indicated that antibodies to HSV-2 is a surrogate marker for sexual activity and STDs [59,60]. The prevalence of HSV-2 antibodies was higher among both male cases than controls corroborating their risk histories; however, HSV seropositivity was not correlated with high-risk behavior or past number of sexual partners among the male cases. These data, in conjunction with the similar rate of indeterminate HIV-1 Western blots among the cases' sexual partners as controls, argue against either a sexually-transmissible or blood-borne agent responsible for IWB [61].

The most significant risk factor for IWB among women was parity. Although there was a trend towards current pregnancy as a risk factor for IWB among female cases with an odds ratio of 3.2 (95% CI of 0.8, 12.8), the sample size was small and the difference was not statistically significant. For pregnant women with indeterminate HIV-1 serologies due to alloimmunization, rapid evaluation protocols and counseling will be necessary not only to avoid unnecessary anxiety related to an indeterminate test result but also to determine the HIV status of the mother and fetus [28].

The cases reported significantly more anxiety than the controls at the first study visit, but had similar rates of previous psychiatric morbidity. Interestingly, high-risk behavior was strongly correlated with higher anxiety and depression scores for both cases and controls. This finding could lead to possible interventions in post-test counseling if providers can help individuals to see the link between their high-risk behavior and anxiety and ways to modify their risk-taking behavior.

The higher amount of anxiety among cases, but not depression, is consistent with other studies which have found anxiety to be an early response to a stressful event, such as notification of an indeterminate HIV result or premature coronary heart disease [42]. Consistent with this hypothesis was the ability of our multivariate model to explain a larger proportion of the variance in anxiety among cases than depression. The multivariate analyses that examined predictors of anxiety among the cases indicated that previous psychologic morbidity and current concerns about the IWB, as indicated in the 16-item life event score and the 9-item questionnaire administered to cases only, were the strongest predictors of anxiety.

The anxiety among a subset of 31 cases persisted at follow-up visits, but specific anxiety related to the IWB decreased over the follow-up period. If providers are able to address the specific concerns of individuals notified of an IWB about etiologies (such as autoantibodies and cross-reacting antibodies), some of that anxiety can be reduced. The six months follow-up that is currently recommended by CDC can accentuate the anxiety and uncertainty about the

IWB. Shorter follow-up with the use of supplemental tests with high negative predictive value can reduce the anxiety and uncertainty that often accompany notification of an IWB.

There are several limitations to our study. We attempted to recruit a population-based sample of high- and low-risk individuals with IWB referred from the community. We attempted to recruit EIA negative controls from the same distribution of testing sites to match for the heterogeneous mix of high- and low-risk cases. However, we were not able to recruit EIA negative controls from private providers and life insurance companies, so we used blood donor controls to match these low-risk cases and may have introduced bias into the case-control matching. The source of potential bias could explain why some variables, such as fewer STDs were reported by female blood donor and low-risk life insurance cases than the female blood donor controls. We attempted to control for the heterogeneity of our study population by frequency-matching cases and controls by testing site and by performing conditional logisitc regression using three strata of testing sites (blood banks, high-risk testing sites such as the AIDS Prevention Project and the Sexually Transmitted diseases clinic, and women's clinics). Sample size limited our power to perform subset analyses by specific testing site (eg., blood donors).

Since blood donors referred to our study had been deferred from donating blood for up to three years following repeated EIA reactivity and IWB, we were concerned that time to study enrollment could be a confounder. Analyses limited to cases whose first IWB was within six months of study enrollment, however, demonstrated the same risk factors for IWB (data not shown). Recall bias may have accounted for cases having a higher likelihood of remembering recent exposures, such as tetanus vaccination. The problem of multiple comparisons could result in some associations appearing significant by chance.

Case-control studies usually involve examination of potential exposure risk factors for a relatively rare disease outcome. Case-control methodology to examine risk factors for a reactive laboratory test that is not always reproducible over time or with different manufacturer's tests presented methodologic issues. Many cases referred to us because of prior repeatedly reactive EIA and IWB were EIA nonreactive at the first study visit, and a

subset of these EIA nonreactive cases were also negative by Western blot. Conversely, 26% of the EIA nonreactive controls had an IWB at the first study visit. To reduce potential misclassification, we performed the analyses including only those cases who were still EIA RR and IWB at visit one and the controls who were both EIA nonreactive and Western blot negative. Power was not sacrificed; the variables implicated as independently and significantly associated with an IWB in the analyses that included all 238 nonseroconverter cases and 145 controls were the same as those shown in Table 7.

Our data have five main implications for facilitating the management of indeterminate HIV Western blots in the clinical setting. First, approximately one third of persons who present with IWB will not be repeatedly reactive by follow-up EIA and retesting within one month to identify this group is warranted [28]. Second, less than five percent of persons with IWB in this study were infected with HIV-1, and the most important factor for seroconversion was high-risk behavior, which could be determined from the risk history. As previously reported [28], selective supplemental testing for HIV should decrease the period of uncertainty directly associated with repeat testing at three and six months as currently recommended by the CDC [9]. The use of rapid supplemental HIV-1 tests with a high negative predictive value, such as recombinant p24 and gp41 EIAs, can be a useful aid in the evaluation of persons with IWB. Supplemental testing was also useful in ruling out HIV infection in three cases with false positive Western blots.

Our third major finding was that no cross-reactivity with known human retroviruses, HTLV-1 or HIV-2, or animal retroviruses was demonstrated in our study population. As additional retroviruses are identified, serologic and virologic studies should be performed among persons with indeterminate HIV-1 serologies. Fourth, 69% of persons with IWB had an identifiable factor as a potential etiology for the IWB reactivity: parity among the female cases, a history of autoimmune disease, prostitute contact, presence of autoantibodies, or a recent tetanus booster. Medical history will identify subjects with a potential immune-related basis for IWB, such as parity, autoimmune diseases, autoantibodies, recent viral illness, and recent vaccination.

Fifth, even with intensive counseling at the first study visit, a high proportion of both low- and high-risk cases were still anxious about the IWB. Discussion of the overall low-risk of seroconversion unless the person has recently engaged in high-risk behavior, other non-HIV etiologies for IWB, such as autoantibodies, and the use of supplemental tests to rule out infection may help to reduce patient's anxiety about the meaning of the IWB.

As HIV-1 testing becomes more widespread, laboratories and clinicians must be prepared to evaluate and counsel persons who have indeterminate serologies [43]. This presents a challenge to clinicians given the relatively infrequency of indeterminate HIV-1 Western blots. Both individuals notified of indeterminate HIV-1 serologies and their providers can benefit from educational materials that accompany the test result. We include in Appendix C examples of the brochures we have designed and distributed through the University of Washington Virology laboratory. Evaluation of persons with IWB should include an assessment of HIV risk behavior as well as potential immune-related etiologies by history, but persons with a low-risk of seroconversion should be reassured and an extensive medical evaluation is not usually indicated.

Acknowledgments

We thank the following individuals for their assistance and collaboration: Todd Damrow, PhD, MPH and Delores Villareal, MT from the Washington State Public Health Laboratories; Paul Swenson, PhD and June Nakata from the Seattle-King County Department of Public Health laboratory; and, Joan Dragavon, MS of the University of Washington Virology laboratory for performing HIV-1 EIAs and Western blots; Kim Chaloupka and the staff of the University of Washington Retrovirus laboratory for performing the HIV-1 cultures; Chester Roberts, PhD of Walter Reed Army Institute of Research, Retrovirology Division, for performing CBre3, HTLV-1, and HIV-1 and HIV-2 PCR assays; Danny Youngs and Karen Nelson, PhD of the Puget Sound Blood Center for testing for HLA antibodies; and Diane Whetstone, PhD from the University of Iowa for performing bovine immunodeficiency virus serologies.

We thank the following clinics and blood banks for referrals to the study: the Seattle-King County Department of Public Health AIDS Prevention Project and Sexually Transmitted Diseases clinic at Harborview Medical Center, University of Washington and Harborview Medical Centers prenatal and women's clinics, Portland American Red Cross, and Puget Sound and Pierce County Blood Centers.

We appreciate Noel Weiss, PhD, for his review of our manuscript and his valuable suggestions.

REFERENCES

1. Reesink HW, Huisman JG, Gonsalves M, et al. Evaluation of six enzyme immunoassays for antibody against human immunodeficiency virus. Lancet 1986; ii:483-6.
2. Burke DS, Brundage JF, Redfield RR, et al. Measurement of the false positive rate in a screening program for human immunodeficiency virus infections. N Engl J Med 1988;319:961-4.
3. Weiss SH, Goedert JJ, Sarngadharam MG, Bodne AJ, Gallo RC, Blattner WA. Screening tests for HTLV-III (AIDS agent) antibodies. JAMA 1985;253: 221-5.
4. Ward JW, Grindon AJ, Feorino PM, Schable C, Parvin M, Allen JR. Laboratory and epidemiologic evaluation of an enzyme immunoassay for antibodies to HTLV-III. JAMA 1986;256:357-61.
5. Update: Serologic testing for HIV-1 antibody--United States, 1988 and 1989.MMWR 1990;39:380-2.
6. Carlson JR, Bryant ML, Hinrich SH, et al. AIDS serology testing in low- and high-risk groups. JAMA 1988;253:3405-8.
7. Schwartz JS, Dans PE, Kinosian BP. Human immunodeficiency virus test evaluation, performance, and use. JAMA 1988;259:2574-9.
8. CDC, Interpretation and use of the Western blot assay for serodiagnosis of Human Immunodeficiency Virus Type 1 infections. MMWR 1989;38(No.S-7):1-7.
9. McDonald KL, Jackson JB, Bowman RJ, et al. Performance characteristics of serologic tests of human immunodeficiency virus type 1 (HIV-1) antibody among Minnesota blood donors. Ann Intern Med 1989;110: 617-21.
10. Midthun K, Garrison L, Clements ML, et al. Frequency of indeterminate Western blot tests in healthy adults at low risk for human immunodeficiency virus infection. J Infect Dis 1990; 162: 1379-82.
11. Westblom TU, Belshe RB, Gorse GJ, et al. Characteristics of a population volunteering for human immunodeficiency virus immunization. Intl J STD AIDs 1990; 1: 126-128.

12. Gaines H, Sonnerborg A, Czajkowski J, et al. Antibody response in primary human immunodeficiency virus infection. Lancet 1987;i:1249-53.

13. Ranki A, Krohn M, Allain J-P, Franchini G, Valle SL, Antonen J. Long latency precedes overt seroconversion in sexually-transmitted human immunodeficiency virus infection. Lancet 1987;ii:590-3.

14.Wilbur JC, Louie B, Soncuya E, Barnes J, Myers R, Back A. Evaluation of indeterminate results in HIV serology: Distinguishing seroconversions from false positives [Abstract 829]. In: Program and abstracts of the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, CA: American Society for Microbiology, 1988.

15. Biggar RJ, Melbye M, Ebbesen P, et al. Variation in human T lymphotrophic virus III (HTLV-III) antibodies in homosexual men: decline before onset of illness related to acquired immune deficiency syndrome (AIDS). BMJ 1985;291:997-8.

16. Farzadegan H, Polis MA, Wolinsky SM, et al. Loss of human immunodeficiency virus type 1 (HIV-1) antibodies with evidence of viral infection in asymptomatic homosexual men. Ann Intern Med 1988; 108:785-90.

17. O'Brien TR, George JR, Holberg SD. Human immunodeficiency virus type 2 infection in the United States: Epidemiology, diagnosis, and public health implications. JAMA 1992; 267: 2775-9.

18. DeCock KM, Porter A, Kouadio J, et al. Cross-reactivity on Western blots in HIV-1 and HIV-2 infections. AIDS 1991; 5: 859-60.

19. Myers RA, Patel JD, Joseph JM. Identifying HIV-2 seropositive individuals by reevaluating HIV-1 indeterminate sera. J Acquir Immune Defic Syndr 1992; 5: 417-23.

20. George JR, Rayfield MA, Phillips S, et al. Efficacies of U.S. Food and Drug Administration-licensed HIV-1 screening enzyme immunoassays for detecting antibodies to HIV-2. AIDS 1990, 4: 321-6.

21. Rhame FS, Maki DG. The case for wider use of testing for HIV infection. N Engl J Med 1989; 320: 1248-51.

22. Ranki A, Johansson E, Krohn K. Interpretation of antibodies reacting solely with human retroviral core proteins. N Engl J Med 1988; 318: 448-9.

23. Kleinman S, Fitzpatrick L, Secord K, Wilke D. Follow-up testing and notification of anti-HIV Western blot atypical (indeterminant) donors. Transfusion 1988; 28: 280-2.

24. Dock NL, Lamberson HV, O'Brien TA, Tribe DE, Alexander SS, Poiesz BJ. Evaluation of atypical human immunodeficiency virus immunoblot reactivity in blood donors. Transfusion 1988;28:412-8.

25. Dock NL, Kleinman SH, Rayfield MA, et al. Human immunodeficiency virus infection and indeterminate Western blot patterns. Arch Intern Med 1991; 151: 525-30.

26. Jackson JB, MacDonald KL, Cadwell J, et al. Absence of human immunodeficiency virus infection in blood donors with indeterminate Western blot tests for antibody to HIV-1. N Engl J Med 1990; 312: 217-22.

27. Courouce AM. Following up indeterminate HIV-1 Western blots. Lancet 1989; 2: 1330-1.

28. Celum CL, Coombs RW, Lafferty W, et al. Indeterminate human immunodeficiency virus type 1 Western blots: Seroconversion risk, specificity of supplemental tests, and an algorithm for evaluation. J Infect Dis 1991; 164: 656-64.

29. Ashley RL, Militoni J, Lee F, et al. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. J Clin Microbiol 1988; 26: 662-7.

30. Shelton GH, Grant CK, Cotter SM, et al. Feline immunodeficiency virus and feline leukemia virus infections and their relationships to lymphoid malignancies in cats: A retrospective study (1968-1988). JAIDS 1990; 3: 623-630.

31. ASHI Laboratory Manual, Second Edition, ASHI, Lenexa, KS. 1990.

32. Rubin RL, Fu-Lin T, Lucas AH, et al. IgG subclasses of anti-tetanus toxoid antibodies in adult and newborn normal subjects and in patients with systemic lupus erythematous, Sjogren's syndrome, and drug-induced autoimmunity. J Immunol 1986; 137: 25222-7.

33. Jackson JB, Coombs RW, Sannerud K, et al. Rapid and sensitive viral culture method for human immunodeficiency virus type 1. J Clin Microbiol 1988; 26: 1416-8.

34. Kenny C, Parkin J, Underhill G, et al. Human immunodeficiency virus antigen testing. Lancet 1987; 1: 565-6.

35. Kellogg DE, Kwok S. Detection of human immunodeficiency virus. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ. eds. PCR protocols. San Deigo: Academic Press, 1990: 337-47.

36. Kenny C, Parkin J, Underhill G, et al. Human immunodeficiency virus antigen testing. Lancet 1987; 1: 565-6.

37. Busch MP, Taylor PE, Lenes BA, et al. Screening of selected male blood donors for p24 antigen of Human immunodefiency virus type 1. N Engl J Med 1990; 323: 1308-12.

38. Murphy VM, Celum CL, Roberts CR, et al. Recombinant antigen and synthetic peptide assays as supplemental tests for indeterminate HIV-1 Western blots. VIII International Conference on AIDS, PoC 4442, Amsterdam, NL, July 19-24, 1992.

39. Beck AT, Steer RA, Garbin MG. Psychometric properties of the Beck Depression Inventory: Twenty-five years of evaluation. Clin Psych Review 1988; 8 (1): 77-100.

40. Derogtais LR. The SCL-90 Manual I: Scoring, administration, and procedures for the SCL-90. John Hopkins University School of Medicine, Clinical Psychometrics Unit, Baltimore, MD, 1977.

41. Beck AT, Beck R. Screening depressed patients in family practice. Postgrad Med 1972; 52 (6): 81-5.

42. Horowitz MJ, Simon N, Holden M, et al. The stressful impact of news of premature heart disease. Psychosom Med 1983; 45: 31-40.

43. Celum CL, Coombs RW. Indeterminate HIV-1 Western blots: Implications and considerations for widespread HIV testing. J Gen Int Med; in press.

44. Ranki A, Johansson E, Krohn K. Interpretation of antibodies reacting solely with human retroviral core proteins. N Engl J Med 1988; 318: 448-9 (letter).

45. Schlech WF, LeeSH, Cook J, et al. Passive transfer of HIV antibody by Hepatitis B immune globulin. JAMA 1989; 261: 411-3.

46. Moore JD, Cone EJ, Alexander SS. HTLV-III seropositivity in 1971-1972 parenteral drug abusers--A case of false positives or evidence of viral exposure? N Eng J Med 1986; 314: 1387-8 (letter).

47. Mendenhall CL, Roelle GA, Grossman CJ, et al. False positive tests for HTLV-III antibodies in alcoholic patients with hepatitis. N Engl J Med 1986; 314: 921-2 (letter).

48. Sayers MH, Beatty PG, Hansen JA. HLA antibodies as a cause of false-positive reactions in screening enzyme immunoassays for antibodies to human T-lymphotropic virus Type III. Transfusion 1986; 26: 113-5.

49. Centers for Disease Control. Update: HIV-2 infection--United States. MMWR 1989; 33: 572-80.

50. Talal N, Dauphinee MJ, Dang H, et al. Detection of serum antibodies to retroviral proteins in patients with primary Sjogren's syndrome. Arthritis and Rheumatism 1990; 33: 774-781.

51. Kunhl P, Seidl S, Holzberger G. HLA DR4 antibodies cause positive HTLV-III antibody ELISA results. Lancet 1985; 1: 1222-23.

52. Golding H, Robdy FA, Gates FT, et al. Identification of homologous regions in human immunodeficiency virus type 1 gp41 and human class II ß1 domain. J Exp Med 1988; 167: 914-23.

53. Garr RF, Fermin CD, Hart DJ, et al. Detection of a human intracisternal A-type retroviral particle antigenically related to HIV. Science 1990; 250: 1127-9.

54. Talal N, Garry RF, Schur PH, et al. A conserved idiotype and antibodies to retroviral proteins in systemic lupus erythematosis. J Clin Invest 1990; 85: 1866-71.

55. Talal N, Dauphinee MJ, Dang H, et al. Evidence suggesting a retroviral etiology for human autoimmune disease, in Progress in Immunology, Vol. VII, e. F. Melchers, et al. pp. 837-41.

56. Ciampolillo A, Mirakian R, Schulz T, et al. Retrovirus-like sequences in Grave's disease: Implications for human autoimmunity. Lancet 1989; i: 1096-1100.

57. Humphrey M, Baker JR, Carr FE, et al. Absence of retroviral sequences in Grave's disease. Lancet 1991; 337: 17-18.

58. Press release from American Association of Blood Banks (FAXNET), December 19, 1991.

59. Johnson RE, Nahmias AJ, Madger LS, et al. A seroepidemiologic survey of the prevalence of herpes simplex virus type 2 infection in the United States. N Engl J Med 1989; 321: 17-21.

60. Koutsky LA, Stevens CE, Holmes KK, et al. Underdiagnosis of genital herpes by current clinical and viral-isolation procedures. N Engl J Med 1992; 326: 1533-9.

61. van der Poel CL, Lelie PN, Reesink HW, et al. Blood donors with indeterminate anti-p24 reactivity in HIV-1 Western blots: absence of infectivity to transfused patients and in virus culture. Vox Sang 1989; 56: 162-7.

APPENDIX A

TABLES

TABLE 1

DEMOGRAPHIC AND HIV RISK FACTORS OF CASES , THEIR SEXUAL PARTNERS, AND EIA NEGATIVE CONTROLS:

	CASES	CONTROLS	CASES' SEX PARTNERS	
NUMBER OF CASES	238	145	80	p-value
AGE				
Median (range)	37 (13,85)	35 (17,69)	38 (19,62)	NS
SEX				
Male (%)	113 (48%)	68 (47%)	42 (53%)	NS
RACE				
Caucasian (%)	211 (89%)	125 (86%)	73 (91%)	NS
MARITAL STATUS				
Never married (%)	78 (33%)	61 (42%)	13 (16%)	<0.001
Married (%)	108 (46%)	58 (40%)	57 (71%)	
Divorced/widow (%)	51 (22%)	26 (18%)	10 (13%)	
EDUCATION				
≥ 12 yrs (%)	212 (90%)	136 (94%)	74 (94%)	NS
ANNUAL FAMILY INCOME				
Greater than $20,000	137 (61%)	75 (54%)	53 (73%)	0.03
SEXUAL PREFERENCE				
Heterosexual (%)	210 (90%)	121 (85%)	76 (95%)	NS
Bisexual (%)	9 (4%)	7 (5%)	2 (3%)	
Homosexual (%)	13 (6%)	12 (8%)	2 (3%)	
Never sexually active (%)	2 (1%)	3 (2%)	0 (0%)	
NUMBER OF SEXUAL PARTNERS				
Median, past 3 months	1 (0,90)	1 (0,6)	1 (0,2)	.002
Median, past year	1 (0,300)	1 (0,40)	1 (0,6)	NS
PAST STDs*	82 (36%)	66 (46%)	14 (18%)	<.001
HIGH-RISK SEXUAL PARTNER				
Since 1978 (%)	71 (30%)	42 (29%)	16 (20%)	.16
PAST PROSTITUTION	11 (5%)	3 (2%)	1 (1%)	NS
TRANSFUSION 1978-85	11 (5%)	4 (3%)	4 (5%)	NS
PAST IV DRUG USE	21 (9%)	8 (6%)	5 (6%)	NS
HEMOPHILIA	1 (0.4%)	1 (.7%)	1 (1%)	NS
ANY RISK FOR HIV	80 (34%)	45 (31%)	23 (29%)	NS

LEGEND FOR TABLE 1:

Cases were referred to the study because of one or more previously repeatedly reactive human immunodeficiency virus type-1 (HIV-1) enzyme-linked immunosorbent assay (EIA) and an indeterminate Western blot (IWB). Cases in Table 1 are limited to the 238 cases who did not seroconvert, excluding the 6 seroconverters. Cases were encouraged to refer their current sexual partner(s) to the study. Controls were EIA negative and frequency-matched with the cases by HIV-1 testing site. P-values refer to the differences between cases, controls, and current sexual partners of the cases.

*STDs include gonorrhea, chlamydial infection, and genital herpes

TABLE 2

RESULTS OF HIV-1 EIA AND WESTERN BLOTS IN CASES, CONTROLS, AND CASES' SEXUAL PARTNERS AT FIRST STUDY VISIT

	CASES	CONTROLS	CASES' SEXUAL PARTNERS
NUMBER OF CASES	244	145	83
EIA			
Repeatedly reactive (%)	139 (57%)	0 (0%)	5 (6%)
EPITOPE WESTERN BLOT			
Negative	83 (35%)	107(74%)	58 (71%)
Indeterminate	155 (55%)	38 (26%)	21 (26%)
p17 only	60	6	2
p24 only	66	13	1
p17 and p24 bands	9	1	0
env only	2	1	1
other bands	19	17	17
Positive*	5 (2%)	0 (0%)	3 (4%)

LEGEND FOR TABLE 2:

Genetic Systems or Dupont enzyme immunoassays (EIA) and Epitope Western blots were performed for the study subjects on sera obtained at the first study visit. Western blots were interpreted as positive using CDC interpretative criteria (if at least two of the following anti-HIV antibodies were present: p24, gp41, and/or gp120/160).

* Five of the six seroconverters had a positive Western blot at the first study visit; all six seroconverters had been referred with a p24 band on initial blot. The sixth case seroconverted 10 months after his initial Western blot showed a p24 band only with ongoing risk behavior during the study period.

TABLE 3

UW IWB STUDY RESULTS

HIV-1 WESTERN BLOT	SYVA MICROTRAK +	SYVA MICROTRAK -
seroconverters	5	0
nonseroconverters	1*	104

Sensitivity	=	100%
Specificity	=	99%
Positive predictive value	=	83%
Negative predictive value	=	100%

HIV-1 WESTERN BLOT	CBr3 +	CBr3 -
seroconverters	4	0
nonseroconverters	4*	111

Sensitivity	=	100%
Specificity	=	97%
Positive predictive value	=	50%
Negative predictive value	=	100%

HIV-1 WESTERN BLOT	GENIE +	GENIE -
seroconverters	5	0
nonseroconverters	0	92

Sensitivity	=	100%
Specificity	=	100%
Positive predictive value	=	100%
Negative predictive value	=	100%

TABLE 4

RESULTS

ANTI-HIV 1 SEROCONVERSION PANEL C

Boston Biomedica, Inc.

			Epitope Western Blot												
Sample	Day	GSC HIV-1 EIA R-val	p17	p24	p31	p41	p51	p55	p66	gp120	gp160	WB result	Syva	CBr3	GENIE
1	0	0.13										NEG	-	-	-
2	7	0.08										NEG	+	+	-
3	9	0.13		+							+/-	INDET	+	+	+/-
4	14	0.37		+							+	POS	+	+	+
5	16	0.58		+							+	POS	+	+	+
6	21	1.0		+						+	+	POS	+	+	+
7	23	1.3		+						+	+	POS	+	+	+
8	28	2.0		+					+	+	+	POS	+	+	+
9	30	1.6		+	+/-	+/-	+/-		+	+	+	POS	+	+	+
10	35	3.2		+	+	+	+		+	+	+	POS	+	+	+
11	42	4.8		+	+	+	+	+/-	+	+	+	POS	+	+	+
12	44	4.4		+	+	+	+	+/-	+	+	+	POS	+	+	+
13	55	3.8	+/-	+	+	+	+	+	+	+	+	POS	+	+	+
14	57	5.0	+/-	+	+	+	+	+	+	+	+	POS	+	+	+
15	62	4.9	+/-	+	+	+	+	+	+	+	+	POS	+	+	+
16	64	3.9	+	+	+	+	+	+	+	+	+	POS	+	+	+
17	69	4.2	+	+	+	+	+	+	+	+	+	POS	+	+	+
18	71	5.9	+	+	+	+	+	+	+	+	+	POS	+	+	+

Bold type denotes positive result

TABLE 5

Laboratory Results in Seroconverters Case #5

Time	EIA & WB results	CD4 Count (%)	HIV Culture	PCR	p24 Ag	ANA
0	EIA 0.9, p24 (w), gp160	N/A	N/A	N/A	N/A	N/A
2 days	EIA 1.8 p24, 41, 120/160				+ (>1000)	
1 mo	EIA 4.0 all bands	479 (20%)	+ Cells + Plasma	+	–	1:40 (sp)
1.5 mos	EIA 4.4 all bands	686 (23%)	N/A	+	–	

TABLE 6

DEMOGRAPHIC CHARACTERISTICS AND HIV RISK FACTORS OF CASES AND CONTROLS

	CASES*	CONTROLS*	
NUMBER OF CASES	124	112	p-value
AGE			
Median (range)	35 (13,85)	35 (17,69)	NS
SEX			
Male (%)	53 (43%)	50 (42%)	NS
RACE			
Caucasian (%)	104 (84%)	98 (88%)	NS
MARITAL STATUS			
Never married (%)	49 (39%)	50 (45%)	NS
Married (%)	50 (41%)	43 (38%)	
Divorced/widow (%)	25 (20%)	19 (17%)	
EDUCATION			
Median (range)	14 (0,22)	15 (5,25)	0.03
ANNUAL FAMILY INCOME			
Greater than $20,000	93 (75%)	87 (75%)	NS
SEXUAL PREFERENCE			
Heterosexual (%)	109 (91%)	95 (86%)	NS
Bisexual (%)	6 (5%)	6 (6%)	
Homosexual (%)	5 (4%)	8 (7%)	
NUMBER OF SEXUAL PARTNERS			
Median, past 3 months (range)	1 (0,4)	1 (0,6)	NS
Median, past year (range)	1 (0,50)	1 (0,40)	NS
PAST STDs**	48 (40%)	53 (48%)	NS
HIGH-RISK SEXUAL PARTNER			
Since 1978 (%)	50 (40%)	35 (31%)	NS
PAST PROSTITUTION	7 (6%)	2 (2%)	NS
TRANSFUSION 1978-85	6 (5%)	3 (3%)	NS
PAST IV DRUG USE	11 (9%)	7 (6%)	NS
ANY RISK FOR HIV	55 (45%)	39 (35%)	NS

LEGEND FOR TABLE 6:

* Cases were persons referred to the study because of one or more previous repeatedly reactive human immunodeficiency virus type-1 (HIV-1) enzyme-linked immunosorbent assay (EIA) and an indeterminate Western blot (IWB). Cases in Table 2 are limited to those who were still EIA RR and IWB at visit one and does not include the six seroconverters. Controls were EIA negative and frequency-matched with the cases by HIV-1 testing site. Controls in Table 2 were those who were both EIA and Western blot negative at the first study visit. Cases were encouraged to refer their current sexual partner(s) to the study.

** STDs include gonorrhea and chlamydial infection and genital herpes.

TABLE 7

RISK FACTORS FOR INDETERMINATE HIV-1 WESTERN BLOTS

--Univariate and multivariate comparisons between nonseroconverter cases (restricted to EIA RR and IWB) and EIA and WB negative controls at visit one--

	CASES N=124	CONTROLS N=112	UNIVAR O. R.	MULTIVAR O.R.	p value
Autoantibodies*	39 (34%)	19 (18%)	2.3 (1.2, 4.4)	2.3 (1.2, 4.5)	0.01
Sex with prostitute	16 (13%)	5 (5%)	3.2 (1.1,8.7)	5.6 (1.5,21)	0.01
History of STDs**	35 (29%)	39 (35%)	0.6 (0.3,1.1)	0.5 (0.2, 0.9)	0.03
Elective testing for HIV	49 (40%)	61 (55%)	1.9 (1.0, 3.5)	2.3 (1.1, 4.7)	0.03
Tetanus booster past 2 yrs	22 (18%)	10 (9%)	2.2 (1.0,4.8)	–	
Autoimmune history	13 (11%)	4 (4%)	3.2 (1.0,10.1)	–	

	MALE CASES N=53	CONTROLS N=50	UNIVAR O. R.	MULTIVAR O.R.	p value
Sex with prostitute	15 (29%)	4 (8%)	4.6 (1.4, 15.1)	5.8 (1.6,20.5)	0.007
Tetanus booster past 2 yrs	12 (24%)	3 (6%)	4.8 (1.3, 18)	5.6 (1.4, 23)	0.02
Shared needles in IDU***	9 (18%)	3 (6%)	3.3 (0.8,13.2)	-	
Autoantibodies*	19 (39%)	8 (18%)	3.0 (1.2, 7.8)	–	
Any high risk since 1978	29 (57%)	18 (36%)	3.0 (1.1 7.4)	–	

	FEMALE CASES N=71	CONTROLS N=62	UNIVAR O. R.	MULTIVAR O.R.	p value
Parity	50 (69%)	30 (49%)	1.2 (1.0,1.5)	1.4 (1.1,1.7)	0.004
History of STDs**	18 (25%)	23 (37%)	0.3 (0.1, 0.8)	0.2 (0.08, 0.6)	0.006
Elective testing for HIV	26 (36%)	35 (57%)	2.2 (1.0, 5.1)	3.8 (1.4, 10.4)	0.009
Viral illness past 3 mos	40 (56%)	26 (42%)	1.8 (0.9, 3.6)	-	
Hepatitis B surface Ab	6 (10%)	14 (25%)	0.3 (0.1, 0.9)	–	
Autoimmune history****	12 (17%)	4 (7%)	2.8 (0.9,9.3)	–	

LEGEND FOR TABLE 7:

To reduce potential misclassification from inclusion of cases who were EIA and/or Western blot negative and controls who were EIA nonreactive but had IWB, the risk factors for IWB were compared between the nonseroconverter casss who were EIA RR and IWB and the EIA and Western blot negative controls, using serologic results from visit one. Conditional logistic regression was performed with three strata of cases and controls--blood donors, high-risk cases, and women's and prenatal clinics. Low-risk cases for whom random controls could not be enrolled (i.e., life insurance applicants and patients referred by private MDs) were matched with blood donor controls.

*Autoantibodies = antinuclear antibodies (ANA) or rheumatoid factor

** STDs include gonorrhea, chlamydia, and genital herpes.

***IDU=injection drug use

****autoimmune history includes systemic lupus erythematosus, rheumatoid arthritis, juvenile onset diabetes mellitus, Sjogren's disease, thyroiditis, and Crohn's disease

TABLE 8

ANXIETY AND DEPRESSION AMONG CASES AND CONTROLS

	CASES N=181	CONTROLS N=116	p-value
Age			
Mean (± SD)	38 (± 12)	37 (±12)	0.84
Sex			
Female (%)	93 (51%)	54 (47%)	0.49
Marital status			
Married	77 (43%)	52 (45%)	0.35
Never married	62 (34%)	45 (39%)	
Divorced/widowed	42 (23%)	19 (16%)	
Race			
Caucasian	166 (92%)	99 (85%)	0.21
Black	7 (4%)	7 (6%)	
Family income			
≥ $ 20,000/yr	105 (61%)	59 (53%)	0.22
Any HIV risk behavior since 1978*	65 (36%)	36 (31%)	0.49
History of depression or anxiety	65 (36%)	34 (29%)	0.29
Prior psychotropic medications	43 (25%)	28 (28%)	0.80
SCL-90 standardized anxiety subscale**			
Median (± range)	62 (36, 82)	49 (36, 82)	<0.0001
Beck Depression Inventory (13-item)***			
Median (± range)	3 (0,21)	2 (0, 18)	0.19

Legend for Table 8:

*Risk behaviors for HIV included male-to-male sexual contact; sexual contact with an injection drug user, prostitute, bisexual man, or known HIV seropositive; injection drug use; recipient of blood products between 1978 and 1985.

**The Symptom Checklist (SCL-90) anxiety subscale is a 10-item self-administered questionnaire about symptoms of anxiety, which is standardized to nonpsychiatric outpatient norms. The lowest standardized score possible for males is 36 and for females is 38.

***The BDI is the 13-item Beck Depression Inventory. Scores of 0-4 are considered no or minimal depression; 5-8 mild; 8-15 moderate; and $\geq$16 severe depression.

TABLE 9

ANXIETY AND DEPRESSION STRATIFIED BY RISK HISTORY

SCL-90 ANXIETY SUBSCALE*: Median (&range) of scores by case-control status and risk**

	CASE	CONTROL	
HIGH-RISK	N=65	N=36	
	67.5	64	
	(36,82)	(36,82)	
			P=0.0001
LOW-RISK	N=116	N=79	
	57	44	
	(36,82)	(36,72)	

BECK DEPRESSION INVENTORY***: Median (& range) of scores by case-control status and risk**

	CASE	CONTROL	
HIGH-RISK	N=65	N=36	
	4.5	5.0	
	(0,19)	(0,18)	
			P=0.0001
LOW-RISK	N=116	N=79	
	2	2	
	(0,21)	(0,15)	

LEGEND FOR TABLE 9:

*The Symptom Checklist (SCL-90) anxiety subscale is a 10-item self-administered questionnaire about symptoms of anxiety, which is standardized to nonpsychiatric outpatient norms. The lowest standardized score possible for males is 36 and for females is 38.

**Risk behaviors for HIV were ascertained for the period from 1978 until the first study visit and included male-to-male sexual contact; sexual contact with an injection drug user, prostitute, bisexual man, or known HIV seropositive; injection drug use; recipient of blood products between 1978 and 1985.

***The BDI is the 13-item Beck Depression Inventory. Scores of 0-4 are considered no or minimal depression; 5-8 mild; 8-15 moderate; and $\geq$16 severe depression.

APPENDIX B

MEDICAL HISTORY INSTRUMENT

INDETERMINATE WESTERN BLOT STUDY

ID RECORD (REV891030)

ID.0	Subject ID	_ _ _ _
ID.0A	Subject Status 0=case; 1=control; 2-9=case's SP	_
ID.0B	Referral Site 01=APP;02=PSBC;03=TacBC;04=Health Dept; 05=STD;06=PP;07=UW/HMC Women's Clinic; 08=Methadone Clinic;09=TB Clinic; 10=Immigration;11=PMD;12=Portland Red Cross; 13=Military;14=Insurance;	_ _
ID.0C	Enrollment Date	_ _ _ _ _ _
ID.1	DEMOGRAPHICS	
ID.1A	Sex 1=M;2=F	_
ID.1B	Marital Status 1=Never married;2=Married;3=Divorced 4=Widowed;5=Separated	_
ID.1C	Race 1=Cauc;2=Blk;3=NAI;4=Hisp;5=Asian;6=Other	_
ID.1D	Years of education	_ _
ID.1E	Occupation 1=Health Care;2=Agric;3=Prof; 4=Clerical;5=Manuf;6=Admin 7=Other:specify_____	_
ID.1F	Gross family income Code 0-9 corresponding to 1st digit of ten thousand ex: <10,000=0;50,000=5;>90,000=9	_
ID.1G	Number of people supported	_
ID.1H	Census tract	_
	Write in address & zipcode_____	

ID.1I Birthdate _ _ _ _ _ _

ID.1J Birthplace _
0=Foreign born;1=AK,HI,WA,OR,CA;
2=MT,ID,WY,NV,UT,CO,AZ,NM;3=ND,SD,NE,KS,MN,IA,MO;
4=OK,TX,AR,LA;5=WI,IL,MI,IN,OH;6=KY,TN,MS,AL;
7=NY,NJ,PA;8=ME,VT,NH,MA,RI,CT;
9=WV,MD,DE,DC,VA,NC,SC,AL,GA,FL

ID.1K FAMILY HX

ID.1Ka Autoimmune disease _

ID.1Kb Hemophilia _

ID.1L LIVED IN HIGH RISK AREA IN p10y

ID.1La San Francisco _

ID.1Lb New York/New Jersey _

ID.1Lc Miami _

ID.1M TRAVELED TO FOREIGN HIGH RISK AREA p5y

ID.1Ma Central Africa _

ID.1Mb Haiti _

ID.1N EVER SERVED IN ANY MILITARY _
0=no, 1=yes

ID.1Na Date of service _ _ _ _

ID.2 HIV RISK FACTORS

ID.2A Reason for HIV screen _
0=blood donor;1=considering pregnancy;
2=new SP;3=concern over p sexual exposure;
4=past IVDU;5=is pregnant;6=life insurance;
7=military/immigration screen;8=HC worker c exp;
9=other_____

ID.2B Secondary reason for HIV screen _
Use ID.2A codes

ID.2C Sexual preference _
1=homosexual;2=bisexual;3=heterosexual

ID.2D SP evaluated _
0=none;1=SP refuses;
2=SP will participate

MALES:

ID.2E Had vasectomy _

ID.2F Been circumcized _

INDETERMINATE WESTERN BLOT STUDY

BEHAVIORAL HISTORY (REV891030)

		1	3	6	9
BE.0	Subject Id	____			
BE.0A	Visit Number				
BE.0B	Visit Date	_____	_____	_____	_____
BE.1	MISC HISTORY				
	Have you ever: 0=no;1=yes;9=unk;.=ND				
BE.1A	Had tatoo/acu	_	_	_	_
BE.1Aa	# mo 1st tat/acu	___	___	___	___
BE.1B	Raw dairy prod p5y	_	_	_	_
BE.2	ANIMAL EXPOSURE				
BE.2A	Farm animals	_	_	_	_
BE.2Aa	# months	___	___	___	___
BE.2B	Owned a cat 0=no; 1=yes, healthy 2=yes, sick..... 3=yes, hx of Felv shots	_	_	_	_
BE.2Ba	Cat wound p1y 0=no; 1=scratched only; 2=bitten only; 3=both	_	_	_	_
BE.2Bb	Owned other pets 0=no;1=dog; 2=rodent;3=other	_	_	_	_

BE.3	SMOKING HISTORY				
BE.3A	Smoked >100 cig LT	_	_	_	_
BE.3Aa	Currently smoke	_	_	_	_
BE.3Ab	Average # cig/day	_ _	_ _	_ _	_ _
BE.3Ac	# month's smoked	_ _ _	_ _ _	_ _ _	_ _ _
BE.3Ad	# month's quit	_ _ _	_ _ _	_ _ _	_ _ _
BE.4	DRINKING HISTORY				
BE.4A	Hx of ETOH prob 0=no; 1=yes,practicing 2=yes, recovering	_	_	_	_
BE.4Aa	Ave # drinks/wk	_ _ _	_ _ _	_ _ _	_ _ _
BE.5	DRUG USE HISTORY				
BE.5A	Marijuana use p5y	_	_	_	_
BE.5Aa	Ave #x's/wk	_ _	_ _	_ _	_ _
BE.5B	Crack use p5y	_	_	_	_
BE.5Ba	Ave #x's/wk	_ _	_ _	_ _	_ _
BE.6	SEXUAL HISTORY				
BE.6A	Age 1st IC c F	_ _	_ _	_ _	_ _
BE.6Aa	# F SP L/T	_ _	_ _	_ _	_ _
BE.6Ab	# F SP p1y	_ _	_ _	_ _	_ _
BE.6Ac	# female SP p3m	_ _	_ _	_ _	_ _
BE.6Ad	# new F-SP p3m	_ _	_ _	_ _	_ _
BE.6B	Age 1st act c male	_ _	_ _	_ _	_ _
BE.6Ba	# M SP L/T	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
BE.6Bb	# M SP p1y	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc	# male SP p3m	_ _	_ _	_ _	_ _
BE.6Bd	# new M-SP p3m	_ _	_ _	_ _	_ _

BE.6Bc	Specific activities p3m				
BE.6Bc1	#x's PV s condom	_ _ _	_ _ _	_ _ _	_ _ .
BE.6Bc2	#x's PV c condom	_ _ _	_ _ _	_ _ _	_ _ .
BE.6Bc3	Ever had anal IC	_	_	_	.
BE.6Bc4	#x's PR s condom	_ _ _	_ _ _	_ _ _	_ _ .
BE.6Bc5	#x's PR c condom	_ _ _	_ _ _	_ _ _	_ _ .
BE.6Bc6	#x's OV s dam	_ _ _	_ _ _	_ _ _	_ _ .
BE.6Bc7	#x's OV c dam	_ _ _	_ _ _	_ _ _	_ _ .
BE.6Bc8	#x's OP s condom	_ _ _	_ _ _	_ _ _	_ _ .
BE.6Bc9	#x's OP c condom	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc10	#x's IC c menses	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc11	#x's spermicidals	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc12	#x's diaphragm	_ _ _	_ _ _	_ _ _	_ _ _
BE.6Bc13	#x's cervical cap	_ _ _	_ _ _	_ _ _	_ _ _
BE.7A	Sex with:				
BE.7Aa	Homo/Bisexual male	_	_	_	_
BE.7Ab	IVDU	_	_	_	_
BE.7Ac	Male prostitute	_	_	_	_
BE.YAd	Female prostitute	_	_	_	_
BE.7Ae	Person HIV area	_	_	_	_
BE.7Af	Person with ARC	_	_	_	_
BE.7Ag	Person with AIDS	_	_	_	_
BE.7Ah	HIV + person	_	_	_	_
BE.8	HX OF:				
BE.8A	Hemophilia 0=no;1=Hemophilia A 2=Hemophilia B; 3=Von Wildebrand's Date of dx.....	_	_	_	_
BE.8Aa	Uses FF 0=no;1=currently; 2=prior to 1985	_	_	_	_
BE.8Aa1	#x's/yr	_ _	_ _	_ _	_ _

BE.8Ab	Uses cryo 0=no;1=currently; 2=prior to 1985	_	_	_	_
BE.8Ab1	#x's/yr	_ _	_ _	_ _	_ _
BE.8B	Transfusion 1978 0=no;1=yes	_	_	_	_
BE.8Ba	# units	_	_	_	_
BE.8Bb	Date of transfusion (MMYY)	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _
BE.8C	Worked as a prostitute When_____	_	_	_	_
BE.8D	IVDU	_	_	_	_
BE.8Da	Date last used (MMDDYY)	_ _ _ _ _ _	_ _ _ _ _ _	_ _ _ _ _ _	_ _ _ _
BE.8Db	Shared needles	_	_	_	_
BE.8Dc	Specific drug used 1=cocaine;2=heroin;3=other	_	_	_	_
BE.8Dd	Most common IVDU 1=cocaine; 2=heroin; 3=other	_	_	_	_
BE.8A	Any high risk	_	_	_	_

INDETERMINATE WESTERN BLOT STUDY

MEDICAL HISTORY (REV891024)

ME.0	Subject ID		____		
ME.0A	Visit Number	1	3	6	9
ME.0B	Visit Date	______	______	______	______
ME.1	PAST MEDICAL HISTORY 0=no;1=yes;9=unk;.=ND				
ME.1A	Allergies 0=no;1=yes, not desens 2=yes, desens	_	_	_	_
ME.1B	Surgery 0=no;1=yes	_	_	_	_
ME.1C	Hospitalizations 0=no,1=yes Specify	_	_	_	_ .....
ME.1D	Current meds 0=no;1=yes Specify	_	_	_	_ .....
ME.1E	DC'd meds p3m 0=no;1=yes Specify	_	_	_	_ .....
ME.1F	Viral illnesses p3m 0=no;1=URI;2=LRI; 3=gastroenteritis; 4=other..... Date of illness	_	_	_	_ .....
ME.1G	Depression ≥ 2 wk 0=no;1=yes	_	_	_	_
ME.1Ga	Date dx (MMYY)	____	____	____	____
ME.1Gb	Rx antidepressants 0=no;1=yes	_	_	_	_
ME.1Gc	Rx psychotherapy 0=no;1=yes	_	_	_	_

ME.1I	Autoimmune dis 0=no;1=RA;2=Lupus; 3=thyroiditis;4=Addison's 5=Cushing's;6=other	_	_	_	_
ME.1J	Diabetes 0=no;1=IDDM;2=NIDDM Date of dx.....	_	_	_	_
ME.1K	Ever tested TB 0=no;1=yes	_	_	_	_
ME.1Ka	Reason initial test 0=routine; 1=health related; 2=work related	_	_	_	_
ME.1Kb	Reason mult test Same as ME.1Ka	_	_	_	_
ME.1Kc	Result initial test 0=neg; 1=borderline; 2=pos	_	_	_	_
ME.1Kd	Result mult test Same ME.1Kc	_	_	_	_
ME.1L	Liver disease 0=no;1=hepatitis 2=cirrhosis;3=alcohol; 4=other..... Date of dx.....	_	_	_	_
ME.1M	Cancer 0=no;1=yes..... Date of dx.....	_	_	_	_
ME.1N	Zoster 0=no;1=single dermatome 2=mult dermatomes Date of dx.....	_	_	_	_
ME.1O	Skin diseases 0=no;1=seborrhea; 2=recurrent staph; 3=psoriasis; 4=vitiligo 5=other..... 6=multiple	_	_	_	_
ME.1P	Mononucleosis 0=no;1=presumptive; 2=+monospot	_	_	_	_

ME.1Q	Immunizations p2y 0=no;1=tetanus; 2=pneumococcal 3=Hepatitis B; 4=other	_	_	_	_
ME.1Qa	# mo	_ _	_ _	_ _	_ _
ME.1R	Gamma glob p2y 0=no;1=yes	_	_	_	_
ME.1Ra	# mo g globulin	_ _	_ _	_ _	_ _
FEMALES:					
ME.2A	# of pregnancies	_	_	_	_
ME.2Aa	# of deliveries	_	_	_	_
ME.2Ab	# of SAB	_	_	_	_
ME.2Ac	# of TOP	_	_	_	_
ME.2B	Currently pregnant	_	_	_	_
ME.2Ba	# months gestation	_	_	_	_
ME.2C	Rhogam injection 0=no;1=yes	_	_	_	_
ME.2Ca	# mo since Rhogam	_ _	_ _	_ _	_ _
ME.2D	Abnl PAP 0=no;1=ABNL	_	_	_	_
ME.2Da	Date last abnl (MMYY)	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.2E	SP had vasectomy	_	_	_	_
ME.2Ea	SP circumcized	_	_	_	_
ME.4	STD's				
ME.4Aa	Genital herpes	_	_	_	_
ME.4Ab	Date primary	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4Ac	# recurrences/yr	_ _	_ _	_ _	_ _
ME.4Ad	Uses acyclovir	_	_	_	_
ME.4Ae	Sexual IC c recur	_	_	_	_

ME.4B	#x's Gonorrhea	_	_	_	_
ME.4Ba	Date last x	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4C	#x's NGU/CERV	_	_	_	_
ME.4Ca	Date last x	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4D	#x's syphilis	_	_	_	_
ME.4Da	Date last x	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4E	#x's Genital warts	_	_	_	_
ME.4Ea	Date of dx	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4F	#x's genital ulcers	_	_	_	_
ME.4Fa	Date of dx	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4G	#x's vaginitis	_	_	_	_
ME.4Ga	Date last x	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4H	Hepatitis	_	_	_	_
ME.4Ha	Date of dx	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
ME.4I	Any STD	_	_	_	_
ME.5	CURRENT MEDICAL HISTORY				
	In past month, have you had:				
ME.5A	Lymphadenopathy	_	_	_	_
ME.5B	Fever >101	_	_	_	_
ME.5C	Night sweats	_	_	_	_
ME.5D	Fatigue	_	_	_	_
ME.5E	Weight loss >10 lbs	_	_	_	_
ME.5F	Anorexia	_	_	_	_
ME.5G	Cough	_	_	_	_
ME.5H	Dyspnea	_	_	_	_
ME.5I	Diarrhea >3 x/d	_	_	_	_
ME.5J	Skin problems	_	_	_	_
ME.5K	Mouth sores	_	_	_	_
ME.5L	Oral thrush	_	_	_	_

ME.5M	Easy bruising	–	–	–	–
ME.5N	Easy bleeding	–	–	–	–
ME.5O	Other	–	–	–	–

INDETERMINATE WESTERN BLOT STUDY

PHYSICAL EXAM (REV092589)

PE.0	Subject Id	____			
PE.0A	Visit Number	1	3	6	9
PE.0B	Visit Date	_____	_____	_____	_____
PE.1	EXAM 0=nl;1=abnl;.=ND;*=unable to do				
PE.1A	Mental status	_	_	_	_
PE.1B	Fundi	_	_	_	_
PE.1C	Oral exam	_	_	_	_
PE.1Ca	Thrush	_	_	_	_
PE.1Cb	Hairy leukoplakia	_	_	_	_
PE.1Cc	Kaposi's	_	_	_	_
PE.1Cd	Other	_	_	_	_
PE.1D	Skin exam	_	_	_	_
PE.1Da	Lesions	_	_	_	_
PE.1E	Abdominal exam	_	_	_	_
PE.1Ea	Liver 0=nl;1=enlarged_____	_	_	_	_
PE.1Eb	Spleen 0=nl;1=enlarged_____	_	_	_	_
PE.1F	Pulmonary exam	_	_	_	_
PE.2	NODES				
PE.2A	Ant cerv	_	_	_	_
PE.2B	Post cerv	_	_	_	_
PE.2C	Pre/post avricular	_	_	_	_
PE.2D	Axillary	_	_	_	_
PE.2E	Inguinal	_	_	_	_

INDETERMINATE WESTERN BLOT STUDY

LABORATORY RESULTS (REV891030)

LR.0	Subject Id					
LR.0A	Visit #					
LR.0B	Visit Date					
LR.1	ELISA 0=NR 1=borderline 2=Repeatedly reactive					
LR.1A	OD ratio to cut-off					
LR.1B	OD value					
LR.1C	ELISA Brand 1=Dupont 2=Epitope 3=other 9=unk					
LR.2A	WESTERN BLOT 1 0=negative; 1=indeterminate 3=positive					
LR.2Aa	WB Brand 1=Dupont 2=Epitope					
LR.2Ab	Bands present:					
LR.2Ab1	p17/18					
LR.2Ab2	p24					
LR.2Ab3	p31					
LR.2Ab4	gp41					
LR.2Ab5	p51					
LR.2Ab6	p55					
LR.2Ab7	p66					
LR.2Ab8	gp120					
LR.2Ab9	gp160					
LR.2Ab10	other/HLA					
	specify	.....	.....	.....	.....	.....

LR.2B	WESTERN BLOT 0=negative 1=indeterminate 2=positive	–	–	–	–	–
LR.2Ba	WB Brand 1=Dupont 2=Epitope	–	–	–	–	–
LR.2Bb	Bands present:					
LR.2Bb1	p17/18	–	–	–	–	–
LR.2Bb2	p24	–	–	–	–	–
LR.2Bb3	p31	–	–	–	–	–
LR.2Bb4	gp41	–	–	–	–	–
LR.2Bb5	p51	–	–	–	–	–
LR.2Bb6	p55	–	–	–	–	–
LR.2Bb7	p66	–	–	–	–	–
LR.2Bb8	gp120	–	–	–	–	–
LR.2Bb9	gp160	–	–	–	–	–
LR.2Bb10	other	–	–	–	–	–
	specify	.....	.....	.....	.....	.....
LR.3	RIPA 0=neg;1=borderline 2=positive		–	–	–	–
LR.3A	Bands present					
LR.3Aa	p24		–	–	–	–
LR.3Ab	p55		–	–	–	–
LR.3Ac	gp120/160		–	–	–	–
LR.4	PCR-CETUS 0=neg;1=borderline; 2=positive		–	–	–	–
LR.5	PCR-UofW 0=neg;1=borderline; 2=positive		–	–	–	–
LR.5	Serum p24 Ag		–	–	–	–

LR.6	T-cell subsets				
LR.6A	%T4	_ _	_ _	_ _	_ _
LR.6Aa	AbsT4	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
LR.6B	%T8	_ _	_ _	_ _	_ _
LR.6Ba	AbsT8	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
LR.6C	T4:T8	_._ _	_._ _	_._ _	_._ _
LR.7	Rheumatoid Fx	_	_	_	_
LR.7A	Titer 1:	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
LR.8	ANA	_	_	_	_
LR.8A	Titer 1:	_ _ _ _	_ _ _ _	_ _ _ _	_ _ _ _
LR.8B	Pattern 1=speckled 2=diffuse 3=homo 4=nucleoler	_	_	_	_
LR.9	RPR	_	_	_	_
LR.10	Other antibodies	_	_	_	_
LR.11	HTLV-1 ELISA 0=neg;1=WR;2=pos	_	_	_	_
LR.12	HTLV-1 western blot 0=nl;1=inde;2=pos	_	_	_	_
LR.12A	Bands present				
LR.12Aa	p15	_	_	_	_
LR.12Ab	p19	_	_	_	_
LR.12Ac	p24	_	_	_	_
LR.12Ad	gp21	_	_	_	_
LR.12Ae	gp46	_	_	_	_
LR.12Af	p40x	_	_	_	_
LR.12Ag	p96	_	_	_	_
LR.12Ah	other	_	_	_	_
	specify	.....	.....	.....	.....

LR.13	FeLV WB 0=neg;1=bordeline; 2=pos	–	–	–	–
LR.14	FIV WB 0=neg;1=borderline; 2=pos	–	–	–	–
LR.15	BLV p24 Agar	–	–	–	–
LR.16	Dupont ENV 9	–	–	–	–
LR.17	HIV cultures				
LR.17A	Plasma	–	–	–	–
LR.17Aa	Day positive	– –	– –	– –	– –
LR.17B	Cells	–	–	–	–
LR.17Ba	Day positive	– –	– –	– –	– –
LR.18	HIVAGEN	–	–	–	–
LR.18A	Bands present				
LR.18Aa	Ip24	–	–	–	–
LR.18Ab	Kp55	–	–	–	–
LR.18Ac	Kp41	–	–	–	–
LR.18Ad	Kp120N	–	–	–	–
LR.18Ae	Kp120CC	–	–	–	–
LR.18Af	Kp66/31	–	–	–	–
LR.18Ag	Ip120	–	–	–	–
LR.19	Hepatitis panel				
LR.19A	HBsAg	–	–	–	–
LR.19B	HBsAb	–	–	–	–
LR.19C	HBcAB	–	–	–	–
LR.19D	PCR-HB rev tran pri	–	–	–	–

APPENDIX B

PSYCHOLOGICAL INSTRUMENT

INDETERMINATE WESTERN BLOT PSYCHOLOGICAL QUESTIONNAIRE

Variable

Study No. ____ ____ ____ ____

Study Visits: ____
0=prior to 1st visit; 1=initial; 3=3 mos; 6=6mos; 9=9mos

Subject Status: ____
0=case; 1=control

Site: ____ ____
1 = AIDS Prevention Project
2 = Puget Sound Blood Center
3 = Tacoma/Pierce County Blood Center
4 = Dept of Public Health
5 = HMC STD Clinic
6 = Planned Parenthood
7 = UW/HMC Women's Clinic
8 = Methadone Clinic
9 = TB Clinic
10=immigration
11=private medical doctor
12=Portland Red Cross
13=military
14=insurance
15=other_____

Today's date ____/____/____
M D Y

Date notified of HIV test result ____/____
M Y

The following questions pertain to your experiences and feelings about your indete Western blot result. There are no right answers to these questions. We are inter your thoughts and reactions at the present time.

1. What do you think an indeterminate Western Blot means?

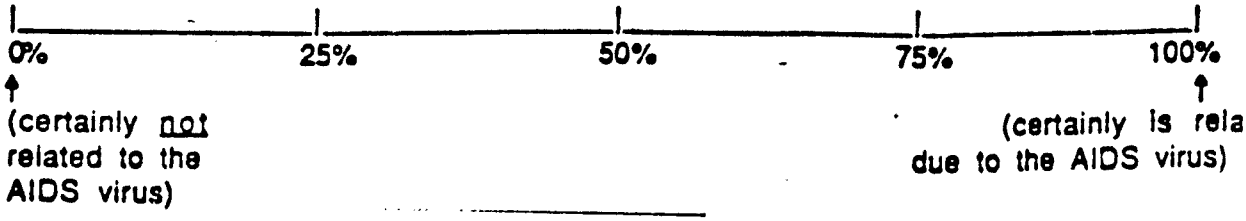
a) The likelihood that my Western Blot is indeterminate due to a limitation of the laboratory or the test is:
[Place an "X" on the scale]

0% 25% 50% 75% 100%

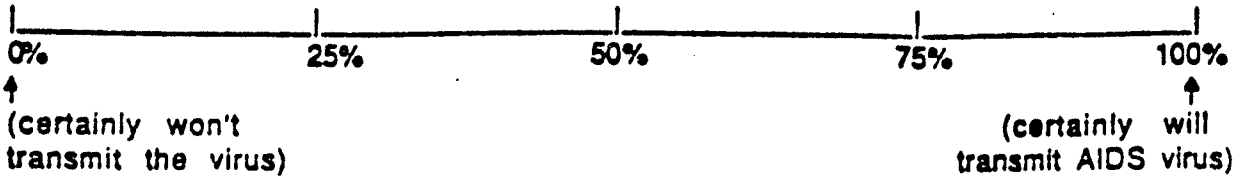
(my indeterminate is not due to a limitation of the lab or test)

(my indeterminate is certainly a limit of the lab or test)

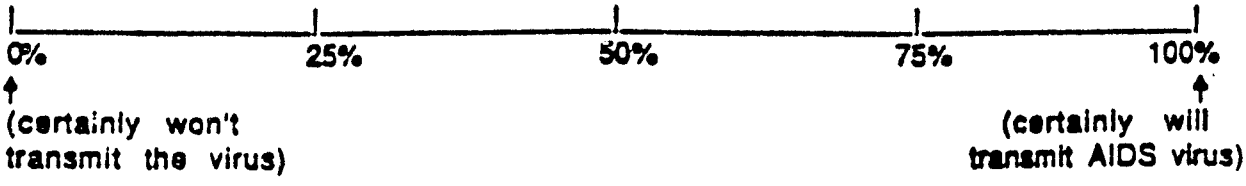
b) The likelihood that my Western Blot is indeterminate because of some abnormality in my blood related to the AIDS virus is:



2. Please indicate what you think the likelihood is that you will transmit the AIDS v your sexual partner.



3. Please indicate the likelihood of your transmitting the AIDS virus through non-sexu contact to household members or work associates.



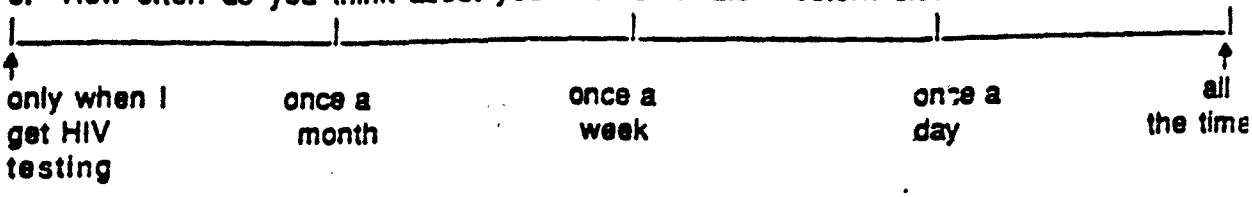
4. Please indicate which of the following you think is most likely to happen to your indeterminate Western blot.
[check one response only]

My Western blot is most likely to:
turn negative_____
remain indeterminate_____
turn positive_____

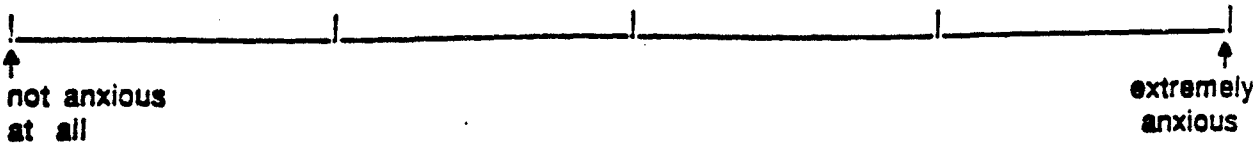
5. If your Western blot does not turn positive over the next six months, will you feel completely reassured that you have not been exposed to the AIDS virus?
[check one response only]

Yes_____
No_____
Uncertain____

6. How often do you think about your indeterminate Western blot?



7. How anxious are you now about your indeterminate Western blot?



SYMPTOM CHECKLIST 90 ANXIETY SUBSCALE

Below is a list of complaints that you may have had since being notified your AIDS test result. Read each item carefully and select one of the descriptions that best describes HOW MUCH DISCOMFORT THAT PROBLEM HAS CAUSED YOU SINCE YOU RECEIVED YOUR AIDS TEST RESULT.

0=Not at all; 1=A little bit; 2=Moderately; 3=Quite a bit; 4=Extremely

A. There has been a negative effect on my self-esteem. A._____

B. Nervousness and shakiness inside. B._____

C. Trembling. C._____

D. Suddenly scared for no reason. D._____

E. Feeling fearful. E._____

F. Heart pounding or racing. F._____

G. Feeling tense or keyed up. G._____

H. Spells of terror or panic. H._____

I. Feeling so restless you couldn't sit still. I._____

J. The feeling that something bad is going to happen to you. J._____

K. Thoughts and images of a frightening nature. K._____

BECK DEPRESSION INVENTORY

Below are 13 sets of statements. Please circle the statement in each set which you believe to be most descriptive of yourself. Be sure to read all statements in each group before making your choice.

A. 0. I do not feel sad.
1. I feel sad or blue.
2. I am blue or sad all the time and I can't snap out of it.
3. I am so sad or unhappy that I can't stand it.

B. 0. I am not particularly pessimistic or discouraged about the future.
1. I feel discouraged about the future.
2. I feel I have nothing to look forward to.
3. I feel that the future is hopeless and that things cannot improve.

C. 0. I do not feel like a failure.
1. I feel I have failed more than the average person.
2. As I look back on my life, all I can see is a lot of failures.
3. I feel I am a complete failure as a person (parent, husband, wife).

D. 0. I am not particularly dissatisfied.
1. I don't enjoy things the way I used to.
2. I don't get satisfaction out of anything anymore.
3. I am dissatisfied with everything.

E. 0. I don't feel particularly guilty.
1. I feel bad or unworthy some of the time.
2. I feel quite guilty.
3. I feel as though I am very bad or worthless.

F. 0. I don't feel disappointed in myself.
1. I am disappointed in myself.
2. I am disgusted with myself.
3. I hate myself.

G. 0. I don't have any thoughts of harming myself.
1. I feel I would be better off dead.
2. I have definite plans about committing suicide.
3. I would kill myself if I had the chance.

H. 0. I have not lost interest in other people.
1. I am less interested in other people than I used to be.
2. I have lost most of my interest in other people and have little feelings for them.
3. I have lost all of my interest in other people and don't care about them at all.

I. 0. I make decisions about as well as ever.
1. I try to put off making decisions.
2. I have great difficulty in making decisions.
3. I can't make any decisions at all any more.

J. 0. I don't feel I look any worse than I used to.
1. I am worried that I am looking old or unattractive.
2. I feel that there are permanent changes in my appearance and they make me look unattractive.
3. I feel that I am ugly or repulsive looking.

K. 0. I can work about as well as before.
1. It takes extra effort to get started at doing something.
2. I have to push myself very hard to do anything.
3. I can't do any work at all.

L. 0. I don't get any more tired than usual.
1. I get tired more easily than I used to.
2. I get tired from doing anything.
3. I get too tired from doing anything.

M. 0. My appetite is no worse than usual.
1. My appetite is not as good as it used to be.
2. My appetite is much worse now.
3. I have no appetite at all any more.

Mark the appropriate column to the degree which you believe the event has caused you distress. If the event did not occur, please check that column ("did not occur").

LIFE EVENT	did not occur	no distress	mild distress	moderate distress	severe distress
A. Change in school situation					
B. Change in work situation					
C. Engagement or marriage					
D. Change in marital status (divorce or separation)					
E. Change in relationship with spouse/ significant other					
F. Pregnancy or birth of a child					
G. Serious family arguments (not including spouse)					
H. Death of family member or friend					
I. Change in residance to a different city/town					
J. Change in financial situation/status					
K. Financial debt					
L. Change in physical health (or injury)					
M. Serious illness or injury of close family member					
N. Divorce or separation of parents					
O. Decision to be tested for HIV (AIDS virus)					
P. Results of HIV test					

Indicate which of the above experiences (A-P) has been the major stressor (problem) in your life: _____
If there has been another experience that is not listed above, please indicate it _____

APPENDIX C

PUBLICATIONS, ABSTRACTS, AND BROCHURES

Indeterminate Human Immunodeficiency Virus Type 1 Western Blots: Seroconversion Risk, Specificity of Supplemental Tests, and an Algorithm for Evaluation

Connie L. Celum, Robert W. Coombs, William Lafferty, Thomas S. Inui, Pamela H. Louie, Carol A. Gates, Bruce J. McCreedy, Richard Egan,* Thomas Grove, Steve Alexander, Thomas Koepsell, Noel Weiss, Lloyd Fisher, Lawrence Corey, and King K. Holmes

Departments of Medicine, Laboratory Medicine, Epidemiology, and Biostatistics, University of Washington, and HIV/AIDS Epidemiology Office, Washington Department of Health and Social Services, Seattle; Roche Diagnostic Research Laboratories and Chiron Corporation, Emeryville, and SmithKline Beecham Clinical Laboratories, Van Nuys, California; Center for Molecular Biology, Roche Biomedical Laboratories, Research Triangle Park, North Carolina; Dupont Glasgow Research Laboratory, Glasgow, Delaware; Cambridge Biotech, Rockville, Maryland

The human immunodeficiency virus type 1 (HIV-1) Western blot is indeterminate in 10%–20% of sera reactive by EIA. Eighty-nine individuals with prior repeatedly reactive EIA and indeterminate Western blots were followed prospectively to study the risk of seroconversion and specificity of supplemental tests. Four high-risk cases seroconverted within 10 months after enrollment (seroconversion risk, 4.5%, 95% confidence interval, 1.2%–11.1%). Among cases with p24 bands initially, 4 (18.2%) of 22 high-risk individuals seroconverted compared with 0 of 33 low-risk cases ($P = .03$). Specificities of HIV-1 culture, serum p24 antigen, polymerase chain reaction, and recombinant ENV 9 EIA were 100%, 100%, 98.6%, and 94.4%, respectively. An expedited evaluation protocol is proposed. Low-risk individuals with nonreactive EIAs upon repeat testing do not need further follow-up; high-risk individuals should be followed serologically for at least 6 months, especially those with p24 bands on Western blot.

The first laboratory step in human immunodeficiency virus type 1 (HIV-1) antibody detection is the EIA which has a reported sensitivity and specificity of >99% [1–5]. Specimens that are repeatedly reactive by HIV-1 EIA are confirmed by a more specific supplemental test, which is usually the Western blot. The Western blot detects antibodies to specific denatured HIV-1 proteins, such as core (p17, p24, and p55), polymerase (p31, p51, p66), and envelope (gp41, gp120, gp160) proteins [6–8]. The Western blot has a reported specificity of 97.8% [5]. About 10%–20% of sera that are repeatedly reactive by HIV-1 EIA are interpreted as indeterminate by Western blot [8–11].

Indeterminate HIV-1 Western blots (IWBs) may be due to antibody production against viral core antigens early in HIV-1 infection [12–14], loss of core antibodies late in HIV-1 infection [15, 16], cross-reactive antibody to HIV-2 [17], or cross-reactive antibody due to autoantibodies or alloimmunization [18–21]. Because an IWB may represent recent HIV-1 infection and incomplete antibody production, the Centers for Disease Control (CDC) recommends that all individuals with IWBs be retested over 6 months. The CDC recommends that a low-risk individual be considered HIV-negative if the Western blot is still indeterminate or becomes negative after 6 months. Longer follow-up, HIV-1 testing of sex and drug-using partners, and additional immunologic and virologic evaluation are recommended for high-risk individuals with IWBs [8].

Individuals with IWBs are currently excluded from blood donations and have had difficulty obtaining life and disability insurance, US immigration status, and visas for foreign travel. Concern about possible HIV-1 infection among those with IWBs has resulted in uncertainty about appropriate procedures for notification, counseling, and evaluation. A clearer estimation of the risk of seroconversion among individuals with IWBs is needed. Accurate identification of HIV-1 infection among individuals with IWBs may be possible by use of supplemental HIV-1 tests, including HIV-1 culture, serum p24 antigen assay, polymerase chain reaction,

Received 24 January 1991; revised 22 May 1991.

Presented in part: VI International Conference on AIDS, San Francisco, June 1990 (abstract SC-220); VII International Conference on AIDS, Florence, June 1991 (abstract 4449).

Informed consent was obtained from patients or their guardians, and the study protocol was approved by the University of Washington institutional review board.

The opinions, conclusions, and proposals contained herein are those of the authors and do not necessarily represent the views of the Robert Wood Johnson Foundation or other supporting agencies.

Grant support: Centers for Disease Control Family of Seroprevalence Studies, Robert Wood Johnson Foundation Clinical Scholars Program, University of Washington Center for AIDS and STD Research, and National Institutes of Health (AI-27757, AI-27664).

Reprints or correspondence: Dr. Connie Celum, Division of Infectious Diseases, Harborview Medical Center ZA-09, 325 9th Ave., Seattle, WA 98104.

* Present address: Syva Corporation, Palo Alto, California.

The Journal of Infectious Diseases 1991;164:656–64

0022-1899/91/6404-0004$01.00

radioimmunoprecipitation assay, and recombinant HIV-1 antigen assays, but the sensitivity and specificity of these supplemental tests in individuals with IWBs is not known.

This study was designed to assess the risk of seroconversion and the specificity of supplemental HIV-1 tests in a prospective cohort of both low- and high-risk individuals referred because of repeatedly reactive EIAs and IWBs.

Methods

Study population and design. A prospective cohort study with 6–9 months of follow-up was initiated at the University of Washington in March 1988. The cohort included men and women 16–70 years of age with one or more repeatedly reactive EIAs and an IWB, who were referred from testing sites in Washington and Oregon. We accepted the HIV-1 Western blot interpretive criteria of the referral laboratory for Western blots done on subjects before study enrollment. Individuals with a prior diagnosis of HIV seropositivity or AIDS were excluded from the study. Subjects were interviewed about HIV risks [22] and general medical history and were examined.

Laboratory studies. The three reference laboratories doing EIAs and Western blots for the study subscribed to the College of American Pathologists proficiency panel for HIV-1 antibody testing. Dupont (Biotech Research Laboratory, Rockville, MD) EIA and Epitope (Beaverton, OR) and Dupont Western blots were used. The CDC interpretive criteria were used for both Epitope and Dupont Western blots; a Western blot was considered positive if antibodies were present to two of the following HIV-1 viral proteins: p24, gp41, and gp120/gp160 [8]. Western blots without any bands were considered negative, and blots with bands not meeting the criteria for a positive blot were interpreted as indeterminate.

Cases were followed prospectively with repeat HIV-1 EIAs and Western blots every 3 months. The diagnosis of HIV-1 infection was based on seroconversion (a positive EIA and Western blot) or on isolation of HIV-1 in culture from peripheral blood mononuclear cells (PBMCs). Positive Western blots were repeated to rule out laboratory error.

Specificity of supplemental HIV-1 tests. Determination of the specificity of supplemental tests was based on test results from the individuals who did not develop a positive HIV-1 culture or positive Western blot during ≥6 months of follow-up. Supplemental tests were done on sera and cells obtained from subjects at the first study visit and on samples from 81 HIV-1 EIA–negative controls recruited from the same HTV testing sites.

HIV-1 cultures and serum p24 antigen. Cell-free plasma and PBMCs were cultured for HIV-1 as previously described [23, 24]. Culture supernatants were sampled for HIV-1 p24 antigen with the antigen-capture EIA following the manufacturer's protocol (Abbott Laboratory, Chicago, IL) every 3 days for 1 month. Serum p24 antigen assays were done by the same antigen-capture EIA method [25–27]. Positive serum samples were tested in a confirmatory antibody-neutralization assay.

Polymerase chain reaction (PCR). PCR was done by Cetus (Emeryville, CA) and Roche Biomedical Laboratories (Research Triangle Park, NC) using the SK38/39 and SK101/145 primer pairs for the HIV-1 *gag* gene [28, 29]. Cell lysates were obtained from cryopreserved PBMCs, and amplification competency of specimens was checked by amplification of a conserved region within the histocompatibility locus antigen–DQα locus with primer pair GH26/27 [30]. HIV-1 DNA amplification was done as described by Kellogg and Kwok [28]. Each specimen was run in duplicate for both primer sets. HIV-1 proviral sequences were considered present if both primer pairs were positive in duplicate, indeterminate if only one of the duplicate reactions was positive for one or both primer pairs, and not present if neither primer pair resulted in a positive signal.

Serologic assays. Two serologic EIAs of recombinant HIV-1 antigens were done by their manufacturers: HIVAGEN (SmithKline Beecham Clinical Laboratories, Van Nuys, CA) and ENV 9 (Dupont Glasgow Research laboratory, Glasgow, DE). The HIVAGEN panel comprised five recombinant HIV-1 antigens produced in *Escherichia coli:* Ip24 represents the entire sequence of p24, Kp55 the complete sequence of p55, Kp66/31 the complete reverse transcriptase genome and 40% of endonuclease, Kp41 40% of the amino-terminus of gp41, and Igp120 98% of gp120 [31]. A HIVAGEN result that showed Ip24, Kp55, or Kp66/31 and either Kp41 or Igp120 was considered positive, and any other pattern of reactivity was considered indeterminate. ENV 9 used a single HIV-1 envelope peptide (the carboxy-terminus of gp120 and half of the gp41 sequence) produced in *E. coli* [32].

Radioimmunoprecipitation assays (RIPAs) were done by Biotech using HIV-1–infected H-9 cells labeled for 8 h with [^{35}S]methionine [33]. Samples reactive with the envelope glycoproteins gp120 and gp160 were considered positive.

Statistical methods. Demographic and HIV risk factors were compared using χ^2 analysis and Fisher's exact test for categorical data and Student's *t* test for continuous data. Logistic regression was used to compare the proportion of cases with reactive versus nonreactive HIV-1 EIA at visit one with respect to the proportion with past high-risk sex partners while controlling for time between initial HIV tests and study enrollment and for the number of HIV tests before study enrollment. The Mann-Whitney test was used for comparing continuous distributions when the assumption of a normal distribution was not appropriate. Specificity of the supplemental HIV-1 tests was analyzed by comparing results of the supplemental tests with the 6-month Western blot result and isolation of HIV-1 by culture as the reference standards. Ninety-five percent confidence intervals (CI) for the seroconversion risk were calculated using exact binomial methods.

Results

Of 147 individuals referred and enrolled in the study as of May 1990, 89 were followed for ≥6 months and were included in this analysis. Five subjects moved or were lost to follow-up and were not included in the analysis, and the remaining 53 have been followed for <6 months. Subjects were referred primarily from blood banks (49%) and from Department of Public Health clinics (29%). The reasons cited for HIV-1 testing were routine HIV-1 screening for

blood donors, military recruits, or life insurance or immigration applicants (58%), concern over past sexual exposures (27%), current or past intravenous drug use (2%), pregnancy (5%), needlesticks in health care workers (2%), and other reasons such as prior blood-product transfusion or hemophilia (6%).

The subjects were divided into three groups for analysis based on the Dupont HIV-1 EIA and Western blot results on samples obtained at the first study visit. Those in group 1 were four individuals who seroconverted, three of whom seroconverted by the first visit and one who seroconverted 10 months after study enrollment. Group 2 comprised 50 who were still repeatedly reactive by Dupont HIV-1 EIA, with an R-value (ratio of sample to cutoff) ≥0.8 at the first study visit. Group 3 comprised 35 who were no longer reactive by Dupont HIV-1 EIA, with an R-value <0.8 at the first study visit. The seroconversion risk was 4 of 89 (4.5%; 95% CI, 1.2%–11.1%).

The demographics of the three groups were similar except for marital status, with group 3 containing the highest proportion of married subjects (table 1). The proportion of subjects reporting a high-risk sex partner since 1978 was significantly higher for groups 1 and 2 (100% and 34%, respectively) than for group 3 (14%) ($P < .001$, χ^2 analysis). The proportion of cases reporting past bisexual or homosexual male partners was significantly higher for group 1 (75%) than for group 2 (8%) or group 3 (9%) ($P < .001$ for both comparisons). The median time from initial IWB to study enrollment was shortest for group 1 (1 month; range, 0.5–3), intermediate for group 2 (2.5 months; range, 0.5–36), and longest for group 3 (13 months; range, 0.5–51) ($P < .01$ for group 1 vs. group 3; $P < .05$ for group 1 vs. group 2; and $P = .003$ for group 2 vs. group 3). When the proportion of cases with a high-risk sex partner since 1978 was compared between groups 2 and 3 while adjusting for the time between initial IWB and study enrollment and for the number of HIV-1 EIAs before study enrollment, the difference in high-risk sex partners approached statistical significance ($P = .06$).

Characteristics of the four individuals who seroconverted. Case 1 was a bisexual man with a history of prostitution and intravenous drug use before HIV-1 testing in 1988. He reported symptoms of an acute viral-like syndrome in the month between the IWB (p24 antibody only) and the positive Western blot. Case 2 was a woman with a history of autoimmune disease who had unprotected sexual exposure with an HIV-seropositive bisexual partner. After her initial IWB with a p24 band only, she seroconverted by Dupont blot 2 weeks later and by Epitope blot 4 weeks later. Case 3 was a homosexual man with a viral-like syndrome who had p24 and weak gp160 bands (interpreted by the referring laboratory as indeterminate by the FDA/Dupont criteria) and 3 months later had antibodies against all viral proteins on Western blot. Case 4 was a homosexual man with a persistent p24 band and intermittent p66 band until he seroconverted in the tenth month of follow-up. He reported ongoing high-risk sexual behavior during the study period.

Seroconversion was seen only among individuals with p24 bands on their initial Western blots. The risk of seroconversion among individuals with p24 bands was 4 of 55 (7.3%; 95% CI, 2.0%–17.6%). The risk of seroconversion was 4 (18.2%) of 22 among high-risk individuals with p24 bands and 0 of 33 among low-risk persons with p24 bands ($P = .03$ by Fisher's exact test). The median R-value for the seroconverters was 3.6 (range, 3.3–8.5) at the first study visit.

Estimation of the sensitivity of the supplemental HIV-1 tests is not reliable, given the low number of seroconverters. Case 1 had a positive ENV 9 assay and RIPA at the time the initial Western blot showed a p24 band only. Lymphocytes were not available from that visit. When the Western blot became positive 1 month later at his first study visit, his HIV-1 PBMC culture and HIVAGEN assay were positive, but plasma culture, PCR, and serum p24 antigen assay were negative; repeat PCR was positive 3 months after seroconversion. Case 2 had negative serum p24 antigen assay and HIV-1 PBMC and plasma cultures and indeterminate HIVAGEN EIA but a positive PCR, ENV 9 EIA, and RIPA at the initial study visit when the Dupont Western blot detected p17, p24, gp41, and gp120/160 antibodies (2 weeks after the initial Western blot had p24 antibody only). Case 3 was positive on all supplemental tests (HIV-1 PBMC and plasma culture, serum p24 antigen assay, PCR, and ENV 9 and HIVAGEN EIAs) at the first study visit when the Western blot had antibodies against all viral proteins. Specimens were not available from his initial testing 3 months earlier when the Western blot detected antibodies to p24 and gp160. Case 4 had negative HIV-1 PBMC and plasma cultures, PCR, ENV 9 EIA, and RIPA and indeterminate HIVAGEN EIA (Ip24, Kp55) at his initial study visit when the Western blot showed a p24 band only. He seroconverted after 10 months of follow-up, with a history of high-risk behavior intermittently during the 10 months, and refused repeat supplemental testing.

Western blot and supplemental test results in individuals who did not seroconvert. The median R-value of the Dupont HIV-1 EIA was 2.2 (range, 0.9–4.7) among group 2 subjects at the first study visit compared with 0.2 (range, 0.06–0.7) among group 3 subjects. Forty-two group 2 subjects (84%) had repeatedly reactive EIAs at all study visits and 8 (16%) had one or more nonreactive EIAs at follow-up visits. Conversely, 29 group 3 subjects (82.9%) were nonreactive by EIA at all study visits and 6 (17.1%) were again repeatedly reactive at one or more study visits.

There was 70% agreement between Epitope and Dupont blots among the nonseroconverters; 53 cases were indeterminate by both Epitope and Dupont, 24 were indeterminate by Dupont alone, 1 was indeterminate by Epitope alone, and 7 were negative by both Epitope and Dupont (table 2). Among

Table 1. Characteristics of subjects with indeterminate human immunodeficiency virus type 1 (HIV-1) Western blots (IWBs): comparisons by HIV-1 EIA reactivity at first study visit.

	Group 1	Group 2	Group 3	*P*
No.	4	50	35	
Age, years, median (range)	45.5 (22–58)	35 (16–68)	42 (18–70)	NS
No. male (%)	3 (75)	18 (36)	18 (51.4)	NS
No. white (%)	4 (100)	42 (84)	33 (94.3)	NS
Marital status, no. (%)				
Never married	0	20 (40)	8 (22.9)	.002
Married	0	20 (40)	19 (54.3)	
Divorced/separated	4 (100)	7 (14)	8 (22.9)	
Education, years, median (range)	14 (13–22)	14 (4–20)	14.5 (9–20)	NS
Annual family income >$20,000, no. (%)	2 (50)	22 (46.8)	24 (70.6)	NS
Past sexually transmitted diseases*, no. (%)	3 (75)	15 (30)	11 (31.4)	NS
High-risk sex partner since 1978, no. (%)	4 (100)	17 (34)	5 (14.3)	<.001
Median (range) no. of sex partners past year	4.5 (0–300)	1 (0–50)	1 (0–3)	NS
Sexual preference, no. (%)				
Heterosexual	1 (25)	45 (91.8)	31 (88.6)	<.001
Bisexual	1 (25)	3 (6.1)	2 (5.7)	
Homosexual	2 (50)	1 (2)	1 (2.9)	
Never sexually active	0	0	1 (2.9)	
History of prostitution, no. (%)	2 (50)	3 (6)	0	<.001
Intravenous drug use, no. (%)	1 (25)	4 (8)	2 (5.7)	NS
Blood product transfusion 1978–1985, no. (%)	0	3 (6)	3 (8.6)	NS
Time between initial IWB and first study visit, months, median (range)	1.0 (0.5–3)	2.5 (0.5–36)	13 (0.5–51)	<.05
No. of EIAs before study enrollment, median (range)	1.0 (1–1)	1 (1–5)	1 (1–5)	NS

NOTE. Subjects were referred because of past repeatedly reactive HIV-1 EIA and one or more IWBs before first study visit. Group 1, individuals who seroconverted during study period (1–10 months); group 2 and 3, repeatedly reactive and nonreactive, respectively, by HIV-1 EIA (Dupont) at first study visit. *P* values for categorical data were derived from the summary $3 \times k$ χ^2 statistic for groups 1–3. Those for continuous data were obtained from Mann-Whitney tests and represent comparisons between groups 1 and 2, groups 1 and 3, and groups 2 and 3. NS (nonsignificant), $P > .05$.

* Genital herpes, gonorrhea, chlamydial infection, genital warts, genital ulcerations, and hepatitis B.

the 50 group 2 subjects, antibody to p24 was detected by both Dupont and Epitope blots in 11 (22%), by Dupont blot only in 18 (36%), and by Epitope blot only in 1 (2%) at the first study visit ($P < .01$ for comparison between Dupont and Epitope by McNemar's test). Of the 35 group 3 subjects, 12 (34%) had p24 antibody detected by both Dupont and Epitope blots, 10 (29%) had p24 antibody detected only by Dupont blot, and none had p24 antibody detected by Epitope blot only ($P < .01$).

The specificity of supplemental tests done at the initial study visit was estimated in the 85 nonseroconverters who had negative or IWBs after ≥6 months of follow-up (table 3). All 84 HIV-1 cultures were negative in the nonseroconverters. The PCR assay was negative in all 20 EIA-negative controls (data not shown) and 68 (98.6%) of 69 group 2 and 3 subjects who did not seroconvert. One high-risk individual was initially positive by PCR but negative on repeat PCR testing of the same specimen by two different laboratories. During an additional 9 months of follow-up he remained negative for HIV-1 by Western blot, culture, and four serial PCR assays.

ENV 9 EIA was done for 72 nonseroconverters, 4 of whom had borderline reactivity (R-values of 1.1–1.4). Specificity of ENV 9 was 94.4% in the subjects and 100% in 39 EIA-negative controls (data not shown).

Serum p24 antigen testing was done for 64 nonseroconverters; one was borderline reactive but not neutralizable with anti-p24 antibody, resulting in a specificity of 100%.

HIV-1 RIPA was done for 63 nonseroconverters, of whom 50 were negative (79.4%) and 13 were indeterminate

Table 2. Results of human immunodeficiency virus type 1 Western blots at first study visit after enrollment.

	Group 1	Group 2	Group 3
No.	4	50	35
No. negative (%)	0	0	7 (20)*
No. indeterminate (%)	1 (25)†	50 (100)	28 (80)
Epitope only	0	0	1
Dupont only	0	14	10
Epitope and Dupont	1	36	17
No. positive‡ (%)	3 (75)	0	0

NOTE. Subjects were referred because of past repeatedly reactive human immunodeficiency virus type-1 (HIV-1) EIA and one or more indeterminate Western blots before first study visit. Group 1, individuals who seroconverted during study period (1–10 months); groups 2 and 3, repeatedly reactive and nonreactive, respectively, by HIV-1 EIA (Dupont) at first study visit.

* Negative by both Epitope and Dupont blot. Difference in proportion of groups 2 and 3 who had negative versus indeterminate Western blots was significant ($P < .01$).

† Three of four seroconverters had positive Dupont Western blot at first study visit; all three had had p24 band on initial blot. The fourth seroconverted 10 months after initial Western blot showed p24 band only with ongoing risk behavior during study period.

‡ Western blots were interpreted as positive using Centers for Disease Control interpretative criteria if at least two of the following anti-HIV antibodies were present: p24, gp41, gp120/160.

(20.6%). The specificity of RIPA was 79.4% if the indeterminate RIPAs were considered false-positives or 100% if the indeterminate results were excluded.

HIVAGEN EIA was done for 81 nonseroconverters and 63 EIA-negative controls. Sixty-one (75%) of the 81 nonseroconverters were indeterminate and 1 (1%) was positive, and 13 (21%) of the EIA-negative controls were indeterminate by HIVAGEN ($P < .001$). Of the 61 subjects with indeterminate HIVAGEN results, 36% and 72% had reactivity against the Ip24 and Kp55 antigens, respectively, confirming the *gag* reactivity on Western blot. The specificity of the envelope antigens was 100% for Igp120 and 98.8% for Kp41 among the cases.

In summary, excluding indeterminate RIPA and HIVAGEN EIA results, false-positive PCR ($n = 1$) or ENV 9 ($n = 4$) or HIVAGEN ($n = 1$) EIA results were obtained from six subjects, none of whom was positive on more than one supplemental test.

Discussion

The long-term outcome of persons identified as being repeatedly reactive by screening EIA and indeterminate by Western blot for HIV-1 is not well characterized. A more rapid determination of HIV-1 infection among such persons through delineation of epidemiologic and serologic characteristics would benefit both patients and clinicians. In this cohort study of 89 adults referred because of prior reactive HIV-1 EIAs and IWBs, we found HIV-1 infection in only 4 (12.5%) of 32 high-risk cases and 0 of 57 low-risk cases. Of

Table 3. Specificity of supplemental human immunodeficiency virus type 1 (HIV-1) tests in 85 subjects followed ≥6 months who did not develop positive Western blots.

	Group 2 ($n = 50$)		Group 3 ($n = 35$)	
	High risk ($n = 20$)	Low risk ($n = 30$)	High risk ($n = 8$)	Low risk ($n = 27$)
HIV-1 culture negative	20/20	30/30	8/8	26/26
Polymerase chain reaction				
Negative	17/17	26/26	5/6	20/20
Indeterminate	—	—	1/6*	—
Serum p24 antigen negative	17/17	23/23	7/7	17/17
ENV 9 EIA				
Negative	17/19	27/27	4/5	20/21
Low positive†	2/19	—	1/5	1/21
HIVAGEN EIA				
Negative	2/17	1/30	2/8	14/26
Indeterminate	15/17	28/30	6/8	12/26
Positive	—	1/30	—	—
Radioimmunoprecipitation				
Negative	15/18	19/24	5/7	11/14
Indeterminate	3/18	5/24	2/7	3/14

NOTE. Data are no. tests with specified result/no. done. Subjects were referred because of past repeatedly reactive HIV-1 EIA and one or more indeterminate Western blots before first study visit. Group 1, individuals who seroconverted during study period (1–10 months); groups 2 and 3, repeatedly reactive and nonreactive, respectively, by HIV-1 EIA (Dupont) at first study visit.

* Subsequent testing did not confirm initial positive result.

† The four with low-positive results had borderline specimen-to-cutoff OD values of 1.1 and 1.4.

the 32 high-risk cases, 22 had a p24 band initially, of whom four seroconverted (18%; 95% CI, 5.2%–40.3%); none of the 10 high-risk cases with other bands seroconverted (95% CI, 0–30.9%).

The low risk of seroconversion (4.5%) in our sample population was comparable to that of earlier published studies of blood donors with repeatedly reactive EIAs and IWBs, which reported seroconversion rates of 3%–5% [19–21]. As in our study, the seroconverters in the earlier blood donor cohorts had p24 antibodies on initial Western blot and admitted to HIV risk behaviors. A recent study by Jackson et al. [34] of 99 Minnesota blood donors with indeterminate HIV-1 blots found no evidence of HIV-1 or HIV-2 infection.

During the interval between the first repeatedly reactive EIA and IWB result until enrollment into our study, 39% of cases became nonreactive by EIA, 7 (8%) of whom also were nonreactive by both Epitope and Dupont Western blot. The loss of reactivity on EIA was related to the duration of time between initial testing and the first study visit and the number of prior EIAs done before study enrollment. One explanation for this finding is that these were blood donors who had been tested with earlier generations of less-specific HIV-1 EIAs and Western blots.

An IWB was more persistent than a positive EIA among the nonseroconverters; 79% of the group 3 subjects (no longer EIA-reactive at visit one) still had IWBs. We noted considerable discordance between the proportion of group 2 and group 3 cases who were indeterminate by Dupont and Epitope blots, with the Dupont assay frequently giving indeterminate results on specimens that were negative by Epitope blot. This variability between manufacturers of commercial kits as well as different lots of antigen by the same manufacturer has also been noted by other investigators [35].

Based on our study and the findings of Courouce [36], a nonreactive EIA on a follow-up sample in a low-risk individual with an IWB has a high predictive value for lack of HIV-1 infection, and those individuals do not need further follow-up. Of the 35 group 3 cases, 27 had no risk factors for HIV-1 infection, representing 30% of the total study population who would require no further follow-up by this approach. The remaining 62 subjects (70%) still required additional evaluation based on their risk history or persistent EIA reactivity. Supplemental assays that might more quickly identify or exclude HIV infection would be desirable in this large group.

The low number of seroconverters in our study precluded estimation of the sensitivity of supplemental tests and, therefore, the predictive value of a negative test. Nevertheless, the specificities of HIV-1 culture, PCR, ENV 9 EIA, and serum p24 antigen assay were 100%, 98.6%, 94.4%, and 100%, respectively among the 85 nonseroconverters. We found that HIV-1 culture, PCR, and a recombinant envelope assay were the three most useful supplemental assays. Although HIV-1 culture and PCR have excellent specificity and sensitivity in many laboratories and are reported to be useful in diagnosing the presence or absence of HIV-1 infection [37–40], they are not widely available, currently are technically difficult, and have not been extensively evaluated for sensitivity in this specific context of recently infected individuals with IWBs who have not yet seroconverted.

ENV 9 EIA had a specificity of 94.4% overall, and it or other recombinant envelope assays might be useful as a supplemental test for IWB sera. Prior studies of other recombinant assays, such as CBre3 (Cambridge Biosciences, Boston), have shown high sensitivity in seroconverter panels [41] and excellent negative predictive value in IWBs [42]. The high prevalence of indeterminate recombinant HIVAGEN EIA results in our study population reflected reactivity to one or more *gag* epitopes. The specificity of HIVAGEN recombinant envelope proteins was comparable to that of ENV 9, but the additional core and polymerase proteins did not help to resolve the IWB patterns.

Although the US Army and other investigators have found RIPA to be a sensitive assay compared with Western blot for detecting antibody to HIV-1 envelope glycoproteins during the course of seroconversion [42], we found weak reactivity to p55 or gp120 in 13 (29%) of the 65 nonseroconverters tested. In addition, RIPA is a labor-intensive test that requires radiolabeled lysate and is not practical for routine clinical use.

The p24 antigen assay was 100% specific but detected only one of four seroconverters in our series and was negative in 24 seroconverters before a diagnostic Western blot in another study [38]. A study of p24 antigen screening among male blood donors in the United States found the specificity of p24 antigen to be 100% but the sensitivity only 11.4% [43].

Because the interval from first IWB until study enrollment varied in our cohort, the duration of this interval represents a possible confounder, which we attempted to control for in our analyses. After adjusting for the time and number of prior HIV-1 tests between the initial IWB and study enrollment, the higher proportion of group 2 cases compared to group 3 cases with high-risk sex partners since 1978 approached statistical significance ($P = .06$). This suggests that a factor associated with high-risk sexual contact may account for persistent EIA reactivity and IWBs. This provocative finding may reflect sampling bias or inadequate controlling for confounding but warrants further investigation.

On the basis of our study results, we propose the following algorithm for evaluating individuals with IWBs (figure 1). The first step is to reevaluate the individual's risk behaviors for possible exposure to HIV-1 and to repeat the EIA. Risk assessment, however, will not always accurately identify individuals with risk behaviors [44]; therefore, our recommendations incorporate reported history of risk behavior, persistence of the EIA reactivity, and the presence or absence of p24 antibodies on Western blot. The proportion of individ-

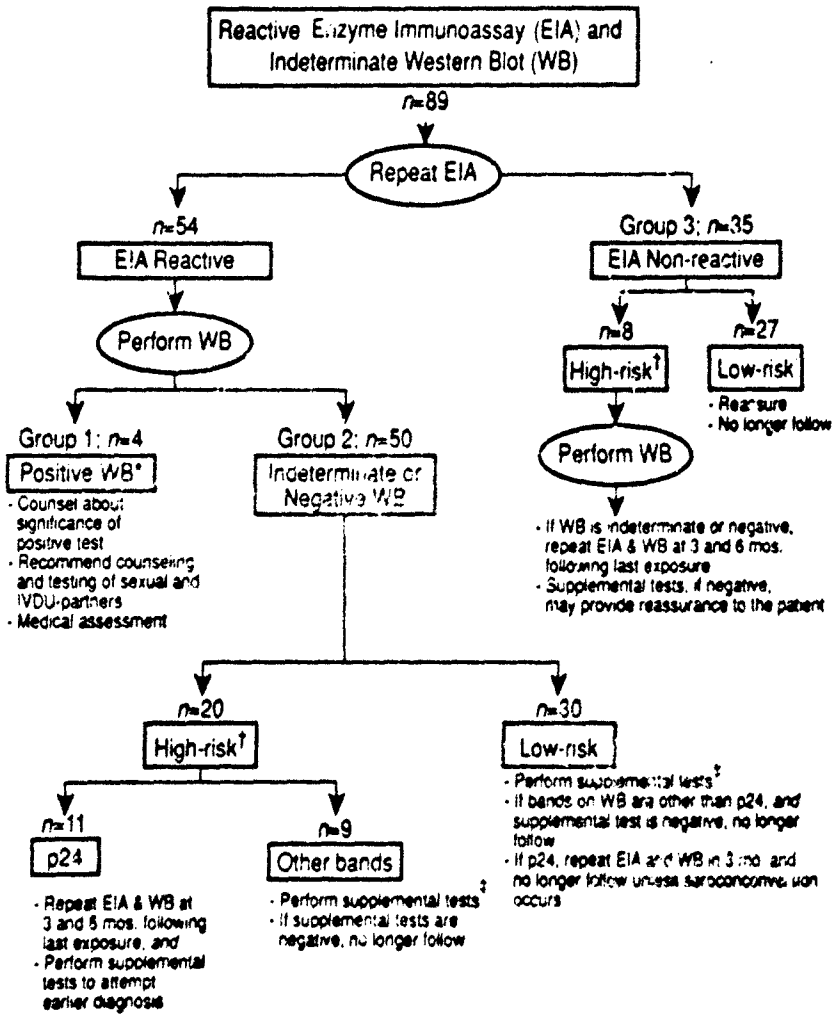


Figure 1. Algorithm for evaluation of individuals with indeterminate human immunodeficiency virus type 1 (HIV-1) Western blots (WBs). * A positive WB was defined as the presence of at least two of the following anti-HIV-1 antibodies: p24, gp41, or gp120/160 (Centers for Disease Control criteria). † High-risk individuals should be followed for at least 6 months after the last exposure (longer if they continue to engage in high-risk behavior). ‡ Supplemental tests include HIV-1 culture, polymerase chain reaction, or a recombinant envelope assay (e.g., ENV 9). If a supplemental test is positive, the HIV-1 EIA, WB, and supplemental test should be repeated in 3 and 6 months. IVDU = intravenous drug user.

uals in each group will vary according to the time between initial and repeat HIV-1 testing, HIV risk status of the population tested, and the use of different commercial sources of EIA and Western blot kits at repeat testing.

Repeat EIA and Western blot 1 month after the initial IWB will often detect the seroconverters, as was demonstrated in three of the four seroconverters in this sample and in all 18 seroconverters in the series by Wilbur et al. [14]. If the EIA is persistently reactive and the Western blot becomes positive, but infection seems implausible based on the individual's risk history, an EIA and Western blot should be repeated on a subsequent sample. Among those individuals with persistently reactive EIAs and IWBs who have not seroconverted upon repeat testing 1 month later, the risk of seroconversion is probably low. Nonetheless, we recommend that high-risk individuals be followed for at least 6 months after their last potential exposure to HIV-1, or longer if they still engage in high-risk behavior, with repeat EIAs and Western blots at 3- to 6-month intervals. Horsburgh et al. [45] have reported that 50% and 95% of individuals will seroconvert within 3 and 6 months after acquiring infection, respectively.

Low-risk individuals with persistent IWBs with p24 bands should be followed for at least 3 months in case they have denied existing risk behaviors, with EIA and Western blot repeated at 3 months. Although the sensitivity of supplemental tests in detecting the infrequent seroconversion in such individuals will be difficult to measure, negative supplemental tests may be useful in reassuring them, especially in situations such as pregnancy and applications for insurance and immigration. Low-risk individuals with bands on Western blot other than p24 antibodies or Western blots that are negative on repeat testing can be reassured that they are not infected and advised that they do not need further serologic follow-up.

High-risk individuals who revert to a negative Western

blot or have bands other than p24 or envelope bands should be followed for 6 months after their last high-risk behavior to exclude seroconversion. Negative supplemental tests may allow cautious reassurance, although again the sensitivity of such tests in this setting is uncertain. The eventual utility of supplemental tests for help in managing such persons with IWBs will be determined by further information from clinical epidemiologic studies that assess the sensitivity and predictive value of supplemental tests. The reasons for false-positive supplemental test results, like those for IWBs, require further study.

Addendum

Since submission of this manuscript, two additional high-risk cases have seroconverted. Both had a p24 band on initial Western blot and seroconverted within 1 month of their initial IWB.

Acknowledgments

We thank Todd Damrow and Delores Villareal (Washington State Public Health Laboratories) and Paul Swenson and June Nakata (Seattle–King County Department of Public Health Laboratory) for assistance and collaboration; Joan Dragavon (University of Washington Virology Laboratory) for HIV-1 EIAs and Western blots; Kim Chaloupka and the staff of the University of Washington Retrovirus Laboratory for HIV-1 cultures and p24 antigen assays; Nancy Siegel and Victory Murphy (Harborview Medical Center Sexually Transmitted Diseases Clinic) for interviewing study subjects; Margie Jones (Department of Biostatistics) for assistance; Marc Cooper (SmithKline Bio-Science Laboratories) for HIVAGEN assays; Regina Flagg (Biotech Laboratory) for HIV-1 RIPAs; Jeanne Neumann (Dupont Glasgow Research Laboratory) for collaborating with the ENV 9 assays; and Roberta Madej (Roche Diagnostic Laboratory), Shirley Kwok and John Sninsky (Department of Infectious Diseases, Cetus Corp.), and Thomas Calloway and Joseph Chimera (Roche Biomedical Laboratories) for polymerase chain reactions. We thank the following clinics and blood banks for referrals to the study: the Seattle–King County Department of Public Health AIDS Prevention Project and Sexually Transmitted Diseases Clinic at Harborview Medical Center, University of Washington and Harborview Medical Centers prenatal and women's clinics, Portland American Red Cross, and Puget Sound and Pierce County Blood Centers.

References

1. Reesink HW, Huisman JG, Gonsalves M, et al. Evaluation of six enzyme immunoassays for antibody against human immunodeficiency virus. Lancet 1986;2:483–6
2. Burke DS, Brundage JF, Redfield RR, et al. Measurement of the false positive rate in a screening program for human immunodeficiency virus infections. N Engl J Med 1988;319:961–4
3. Weiss SH, Goedert JJ, Sarngadharam MG, Bodne AJ, Gallo RC, Blattner WA. Screening tests for HTLV-III (AIDS agent) antibodies. JAMA 1985;253:221–5.
4. Ward JW, Grindon AJ, Feorino PM, Schable C, Parvin M, Allen JR. Laboratory and epidemiologic evaluation of an enzyme immunoassay for antibodies to HTLV-III. JAMA 1986;256:357–61.
5. Centers for Disease Control. Update: serologic testing for HIV-1 antibody—United States, 1988 and 1989. MMWR 1990;39:380–2.
6. Carlson JR, Bryant ML, Hinrich SH, et al. AIDS serology testing in low- and high-risk groups. JAMA 1988;253:3405–8.
7. Schwartz JS, Dans PE, Kinosian BP. Human immunodeficiency virus test evaluation, performance, and use. JAMA 1988;259:2574–9
8. Centers for Disease Control. Interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. MMWR 1989;38(S-7):1–7.
9. Damato JJ, O'Bryen BN, Fuller SA, et al. Resolution of indeterminate HIV-1 test data using the Department of Defense HIV-1 testing program. Lab Med 1991;22:107–13.
10. Detection of antibodies to HIV (Biotech/Dupont Western blot kit insert). Wilmington, DE: Dupont, 1987.
11. McDonald KL, Jackson JB, Bowman RJ, et al. Performance characteristics of serologic tests of human immunodeficiency virus type 1 (HIV-1) antibody among Minnesota blood donors. Ann Intern Med 1989;110:617–21.
12. Gaines H, Sonnerborg A, Czajkowski J, et al. Antibody response in primary human immunodeficiency virus infection. Lancet 1987;1:1249–53.
13. Ranki A, Krohn M, Allain JP, Franchini G, Valle SL, Antonen J. Long latency precedes overt seroconversion in sexually-transmitted human immunodeficiency virus infection. Lancet 1987;2:590–3.
14. Wilbur JC, Louie B, Soncuya E, Barnes J, Myers R, Back A. Evaluation of indeterminate results in HIV serology: distinguishing seroconversions from false positives [abstract 829]. In: Program and abstracts of the 27th Interscience Conference on Antimicrobial Agents and Chemotherapy (Los Angeles). Washington, DC: American Society for Microbiology, 1988.
15. Biggar RJ, Melbye M, Ebbesen P, et al. Variation in human T lymphotrophic virus III (HTLV-III) antibodies in homosexual men: decline before onset of illness related to acquired immune deficiency syndrome (AIDS). BMJ 1985;291:997–8.
16. Farzadegan H, Polis MA, Wolinsky SM, et al. Loss of human immunodeficiency virus type 1 (HIV-1) antibodies with evidence of viral infection in asymptomatic homosexual men. Ann Intern Med 1988;108:785–90.
17. Centers for Disease Control. Update: HIV-2 infection—United States. MMWR 1989;33:572–80.
18. Ranki A, Johansson E, Krohn K. Interpretation of antibodies reacting solely with human retroviral core proteins. N Engl J Med 1988;318:448–9.
19. Kleinman S, Fitzpatrick L, Secord K, Wilke D. Follow-up testing and notification of anti-HIV Western blot atypical (indeterminate) donors. Transfusion 1988;28:280–2.
20. Dock NL, Lamberson HV, O'Brien TA, Tribe DE, Alexander SS, Poiesz BJ. Evaluation of atypical human immunodeficiency virus immunoblot reactivity in blood donors. Transfusion 1988;28:412–8
21. Dock NL, Kleinman SH, Rayfield MA, et al. Human immunodeficiency virus infection and indeterminate Western blot patterns. Arch Intern Med 1991;151:525–30
22. Centers for Disease Control. Public Health Service guidelines for counseling and antibody testing to prevent HIV infection and AIDS. MMWR 1987;36:509–15
23. Jackson JB, Coombs RW, Sannerud K, Rhame FS, Balfour HH Jr. Rapid and sensitive viral culture method for human immunodeficiency virus type 1. J Clin Microbiol 1988;26:1416–8

24. Coombs RW, Collier A, Allain JP, et al. Plasma viremia in human immunodeficiency virus type-1 infection. N Engl J Med 1989; 321:1626–32.
25. Kenny C, Parkin J, Underhill G, et al. Human immunodeficiency virus antigen testing. Lancet 1987;1:565–6.
26. Lange JM, Paul DA, Juisman HG, DeWolf F, et al. Persistent HIV antigenaemia and decline of HIV core antibodies associated with transition to AIDS. BMJ 1986;315:9–12.
27. Farzadegan H, Taylor E, Hardy W, Odaka N, Polk FB. Performance of serological assays for early detection of human immunodeficiency virus type 1 seroconversion. J Clin Microbiol 1989;27:1882–4.
28. Kellogg DE, Kwok S. Detection of human immunodeficiency virus. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols. San Diego: Academic Press, 1990:337–47.
29. Higuchi R, Kwok S. Avoiding false positives with PCR. Nature 1989;339:237–8.
30. Scharf SJ, Horn GT, Ehrlich HA, et al. Direct cloning and sequence analysis of enzymatically amplified genomic sequences. Science 1986;233:1076–8.
31. Ng VL, Chiang CS, Debouck C, et al. Reliable confirmation of antibodies to human immunodeficiency virus type 1 (HIV-1) with an enzyme-linked immunoassay using recombinant antigens derived from the HIV-1 *gag*, *pol*, and *env* genes. J Clin Microbiol 1989;27:977–82.
32. Kenealy W, Reed D, Cybulski R, et al. Analysis of human serum antibodies to human immunodeficiency virus (HIV) using recombinant ENv and GAG antigens. AIDS Res Human Retrovirus 1987;3:95–104.
33. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680–5.
34. Jackson JB, MacDonald KL, Cadwell J, et al. Absence of human immunodeficiency virus infection in blood donors with indeterminate Western blot tests for antibody to HIV-1. N Engl J Med 1990;312:217–22.
35. Midthun K, Garrison L, Clements ML, et al. Frequency of indeterminate Western blot tests in healthy adults at low risk for human immunodeficiency virus infection. J Infect Dis 1990;162:1379–82.
36. Courouce AM. Following up indeterminate HIV-1 Western blots. Lancet 1989;2:1330–1.
37. Imagawa DT, Lee MH, Wolinsky SM, et al. Human immunodeficiency virus type-1 (HIV-1) infection in homosexual men who remain seronegative for prolonged periods. N Engl J Med 1989;320:1458–62.
38. Wolinsky SM, Rinaldo CR, Kwok S, et al. Human immunodeficiency virus type-1 (HIV-1) ...fection a median of 18 months before a diagnostic Western blot. A...n Intern Med 1989;111:961–72.
39. Rogers MF, Ou CY, Rayfield M, et al. Use of the polymerase chain reaction for early detection of the proviral sequences of human immunodeficiency virus in infants born to seropositive mothers. N Engl J Med 1989;320:1649–54.
40. Jackson JB, Kwok SY, Hopsicker JS, et al. Absence of HIV-1 infection in antibody-negative sexual partners of HIV-1 infected hemophiliacs. Transfusion 1989;29:265–7.
41 Thorn RM, Braman V, Stella M, et al. Assessment of HIV-1 screening test sensitivities using serially diluted positive sera can give misleading results. Transfusion 1989;29:78–80.
42. O'Bryen B, Fuller S, Damato J, et al. The Army HIV five year testing experience: 1985–90 [abstract SC-644]. In: Program and abstracts of the VI International Conference on AIDS, San Francisco, 1990.
43. Busch MP, Taylor PE, Lenes BA, et al. Screening of selected male blood donors for p24 antigen of human immunodeficiency virus type 1. N Engl J Med 1990;323:1308–12.
44. Barbacci MB, Dalabetta GA, Repke JT, et al. Human immunodeficiency virus infection in women attending an inner-city prenatal clinic: ineffectiveness of targeted screening. Sex Transm Dis 1990;17:122–6.
45. Horsburgh CR, Jason J, Longini IM, et al. Duration of human immunodeficiency virus infection before detection of antibody. Lancet 1989;2:637–9.

11655 JGIM Nov/Dec
Galley 177

#11655JGIM(Nov/Dec),Folder 16,msp.328,lb,8/13/92

PERSPECTIVES

Indeterminate HIV-1 Western Blots:

Implications and Considerations for Widespread HIV Testing

CONNIE L. CELUM, MD, MPH, ROBERT W. COOMBS, MD, PhD

ANTIBODY TESTS for the detection of human immunodeficiency virus type-1 (HIV-1) are being used for screening in the general population and are being advocated for screening of health care providers. Public health officials recommend HIV antibody testing of high-risk individuals (i.e., persons who have had blood-product transfusions between 1978 and 1985, injection drug use, male with male sexual contact, or sexual contact with a high-risk person or an HIV-infected partner, and infants born to HIV-infected mothers) in conjunction with counseling about HIV transmission and methods of risk reduction.[1] Following reports of zidovudine's efficacy in slowing the rate of disease progression in persons with asymptomatic HIV infection and mild to moderate symptomatic HIV infection with CD4 (T-helper lymphocytes) counts less than 500 cells per mm^3, HIV antibody testing of high-risk individuals is now widely advocated.[2, 3] Concerns over confidentiality and potential discrimination still persist but are balanced by the public health benefits of counseling and testing and by the medical benefits of antiretroviral therapy and *Pneumocystis carinii* prophylaxis when CD4 cell levels decline.

Received from the Departments of Medicine (CLC, RWC) and Laboratory Medicine (RWC), University of Washington, Seattle, Washington.

Supported by the Centers for Disease Control Family of Seroprevalence Studies, the Robert Wood Johnson Foundation, NIH grants AI-27757 and U01-AI-27664, and the U.S. Army Medical Research and Development Command under Grant No. DAMD17-90-Z-0042.

Address correspondence and reprint requests to Dr. Celum: Harborview Medical Center, Sexually Transmitted Diseases Clinic, University of Washington ZA-85, Seattle, WA 98104.

The opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the funding agencies.

11655,LB177TO9 JAB 13:33 08-17-92 P0001 U3 C2243 0006

antibody tests as a part of all routine health care for all adults under the age of 60 years, regardless of reported risks for HIV exposure.[4] However, the widespread screening of populations with low HIV seroprevalence raises important issues about false-positive and indeterminate HIV serologic results. These issues pertain not only to the general population but also to the routine screening of health care workers for HIV-1 antibodies.[5,6]

The debate about false-positive HIV serologic results usually has centered on the probability of an individual's being falsely labeled as HIV-infected by both the HIV-1 enzyme-linked immunosorbent assay (EIA) and the usual confirmatory test, the Western blot assay.[7] Estimates of this combined false-positive rate have ranged from 0.0007% to 0.01%.[7-9] False-negative antibody test results also can occur but are thought to be relatively rare.[10-12] In short, the likelihoods of false-positive and false-negative tests have been overstated in the debate about HIV testing. In contrast, a repeatedly reactive EIA followed by an indeterminate HIV-1 Western blot (IWB) is approximately 100-fold more common than the estimated risk of a false-positive EIA and Western Blot.[7] Thus, the critical issues of the prevalence, significance, and psychological and economic costs of IWB results have often been minimized in the debate about the performance and utility of HIV-1 serologic tests for widespread HIV-1 screening.

Several blood banks have described the considerable anxiety generated among blood donors when they are notified about their IWB results.[13-15] Laboratories have reported difficulty in explaining the significance of IWB patterns to physicians, and physicians in clinical practice have also noted difficulty in counseling individuals with IWBs.[16-18] Expanded HIV-1 antibody testing will present primary care providers with more IWBs to interpret for their patients. It is necessary, therefore, to understand the laboratory evaluation of HIV-1 infection and the prevalence, etiology, follow-up, and possible implications of IWBs.

LABORATORY EVALUATION FOR HIV TESTING AND SCREENING PROGRAMS

The first laboratory step in HIV-1 screening is antibody detection by the EIA. The EIA has been reported to have sensitivities and specificities of ≥99%.[4,19] The EIA detects total serum antibodies directed against HIV-1 proteins for which the optical density reading is proportional to the amount of antibody present in the serum sample relative to positive and negative controls. All specimens that are repeatedly reactive by EIA are confirmed by a more specific supplemental test, typically the Western blot, or less commonly, immunofluorescence or radioimmunoprecipitation assays. The Western blot detects the serum antibodies directed against specific HIV-1 proteins of varying molecular weights following their separation by gel electrophoresis.

The Western blot detects antibodies to the following specific HIV-1 viral proteins: core (p17, p24, p55); polymerase (p35, p51, p66); and envelope (gp41, gp120/160) antigens. The HIV-1 Western blot has a reported analytic specificity of 97.8%.[19] The Western blot is interpreted as negative when no antibody-antigen band is present and positive when antibodies are present to core (p24) and envelope (gp41 or gp120/160) and, in some cases, polymerase proteins. The Centers for Disease Control (CDC) endorses interpre-

The Western blot is interpreted as indeterminate when one or more bands are present that do not meet the positive criteria. A fourth category, non-HIV bands, is also observed, but the interpretation of non-HIV bands is variable and is not consistently reported by all laboratories. For example, the CDC includes non-HIV bands as indeterminate in its interpretative criteria, which may account for the higher prevalence of indeterminate results using CDC criteria as compared with Red Cross and Food and Drug Administration (FDA) interpretative criteria.[20, 21] Differences among laboratories in their interpretative criteria and reporting formats have contributed to confusion among clinicians in understanding and explaining the HIV-1 antibody results to their patients.[22]

PREVALENCE OF IWBs

The prevalence of IWBs depends on the prevalence of HIV in the population being tested; the presence of other medical conditions and autoantibodies that may result in cross-reacting antibodies[23]; the cell lines used in the production of HIV-1 EIA and Western blots[15] and the inter-lot variability in the amount of antigen on the Western blot strips; the criteria used for the interpretation of Western blots[20, 21]; and the laboratory's experience in performing and interpreting Western blots. The prevalence of indeterminate blots among the initially screened population was 0.5% among Minnesota blood donations (13% of the EIA-reactive sera) and 0.3% among the military's screening of 5.5 million enlistees (10% of the EIA-reactive sera).[24, 25] The total number of individuals labeled thus far as indeterminate by EIA and Western blot screening is uncertain, but Jackson and coworkers estimate that 19,000–69,000 volunteer blood donors in the United States had indeterminate HIV-1 antibody tests between the institution of serologic screening of donors for HIV-1 infection in 1985 and 1990.[26]

ETIOLOGY OF IWBs

Indeterminate results occur in early HIV infection during the seroconversion window when only p24 antibody may be detected by Western blot and in late HIV infection when p24 antibody levels may decline.[27-29] The likelihood that an IWB reflects HIV infection is dependent on the prior probability of HIV infection (i.e., HIV risk factors) and the time to seroconversion. The risk of HIV infection among individuals with IWBs was 0% to 5% in four published blood donor cohorts.[13, 14, 25, 26] Several investigators have confirmed the lack of HIV infection and HIV transmission among low-risk blood donors with IWB, particularly those with p17 and polymerase bands.[15, 30-34] In contrast, 90% of a series of 20 individuals tested anonymously at San Francisco district health centers seroconverted, all of whom had p24 antibodies on their initial Western blots.[35] Seroconversion appears to be highest among those with a p24 band, based on our study in which 18% of high-risk individuals with p24 bands seroconverted and on other reports in the literature.[13, 14, 35-37]

The median time from known sexual exposure to development of a positive EIA and Western blot was estimated to be 2.1 months (SD ± 0.1 months), and 95% of exposed persons were estimated to develop a positive EIA and Western blot by six months.[38] This interval is referred to as the "window period." The median time from an IWB with a p24 band only to a positive Western blot was one month for the six seroconverters in our study [illegible]

Indeterminate HIV-1 Western blot due to cross-reactive antibodies to core or envelope antigens from other human or animal retroviruses (e.g., HIV-2 or bovine leukemia virus, BLV) was initially suggested by data from the New York Blood Center in Syracuse. This finding was not confirmed by supplemental testing or by other investigators.[14, 26, 39] Jackson and coworkers found no evidence of HIV-1 or HIV-2 infection in 99 Minnesota blood donors who had positive EIAs and IWBs.[26] Approximately 32 cases of HIV-2 infection in the United States have been reported to the CDC, all with a West African connection, although limited demographic information was available for four individuals tested anonymously.[40] Even though no blood donor has been identified as HIV-2-infected through ongoing surveillance studies, the FDA has required blood banks to screen for HIV-2 as of June 1, 1992. The FDA-licensed HIV-1-specific EIAs detect 8–91% of HIV-2 infections through cross-reacting antibodies to HIV-1 core and polymerase proteins, and 80–90% of HIV-2-infected persons have a positive or an indeterminate HIV-1 Western blot.[41-44]

With the FDA licensure of combination of HIV-1 and HIV-2 EIAs (HIV-1/2 EIAs), some reference laboratories will be likely to replace the HIV-1 EIA with the combination EIA. When using HIV-1/2 EIAs, the laboratory will perform a confirmatory HIV-1 Western blot in HIV-1/2 EIA-reactive sera, as well as a confirmatory HIV-2 Western blot or synthetic peptide assay in HIV-2 EIA-reactive, but HIV-1 Western blot-negative or indeterminate specimens.

The sera of patients with systemic lupus erythematosus, rheumatoid arthritis, and Hashimoto's thryoiditis may contain autoantibodies that cross-react with the Western blot.[24, 45] In our study, a potential immunologic cause for IWB, such as a positive rheumatoid factor or an antinuclear antibody, an autoimmune history, parity, or a recent tetanus booster, was found in two-thirds of 123 persons with reactive EIAs and IWBs.[46] Tissue culture-associated cellular proteins that comigrate with HIV-specific proteins during electrophoresis also may bind cross-reacting antibodies.[14, 47, 48]

IMPLICATIONS OF IWBs

The psychosocial consequences of being told that one has an indeterminate HIV-1 Western blot result have not been adequately addressed. Since March 1988 we have interviewed 236 individuals with IWBs referred to us from health department clinics, blood banks, prenatal clinics, and private providers in Washington and Oregon states.[18] The uncertainty engendered by an indeterminate result, the need for at least 6 months of follow-up, the deferral of these patients as blood donors, and discriminatory policies regarding insurability and immigration have been very distressing. Persons with IWBs referred to us have experienced difficulty in obtaining life or disability insurance (n = 7), elective surgery (n = 1), in vitro fertilization (n = 1), visas for foreign travel (n = 3), and U.S. citizenship (n = 4). Although these individuals were not infected with HIV, they were treated as if infected, sometimes for an indefinite period of time. Even after extensive pretest counseling, 56% of high-risk persons and 15% of low-risk persons with IWBs reported that they would not feel reassured even if they did not seroconvert after 6 months of serologic follow-up.

Nevertheless, in posttest counseling, individuals with indeterminate blots often need reassurance about the very low likelihood of seroconversion in low-risk individuals, possible causes for the inconclusive result, the need for safe sexual and drug-use practices pending resolution of the test result, and the CDC recommendations for evaluation and follow-up.[20]

Pregnant women with IWBs present the most difficult clinical problem that we have yet encountered.[18] Fifteen (13%) of the 123 women referred to our IWB study were tested during prenatal care, seven of whom reported some risk behavior since 1978. Health care providers are encouraged to screen all pregnant women for HIV risk factors and, if risk factors are present, to encourage HIV antibody testing.[1, 49] Unfortunately in our experience, HIV screening is sometimes being incorporated into routine prenatal screening for rubella, hepatitis B, and alpha-fetoprotein testing, often with inadequate HIV-1 pretest counseling. Upon learning of their indeterminate test results, pregnant women are faced with difficult decisions about pregnancy termination, making the six-month follow-up period especially stressful.

Some of the psychological and economic consequences of IWBs may reflect both society's and the medical profession's expectations of unambiguous results from screening programs. Similar issues have been previously described with hypertension screening among workers and alpha-fetoprotein screening in pregnancy.[50-53] Often little provision is made for "indeterminate" results. While positive HIV test results are reportable by name in 24 states[54] and issues around discrimination still persist, only one state has required reporting of individuals with indeterminate results (personal communication, Susan Mottice, PhD, 1991). In that state, issues around confidentiality and discrimination were encountered through the reporting of persons with IWB, the majority of whom were at low risk for HIV.

FOLLOW-UP OF PERSONS WITH IWBs

The CDC recommends that low-risk individuals with an IWB pattern be followed with repeat serologic testing for six additional months; if the indeterminate Western blot pattern persists or turns negative, then the individual can be reassured that he or she is not infected with HIV, based on the seroconversion "window" of six months.[20] In contrast, additional diagnostic follow-up is recommended for higher-risk persons, such as those with a history of possible exposure to HIV or symptoms compatible with HIV infection. This diagnostic strategy includes serial testing by EIA and Western blot, assessment of immune function (i.e., lymphocyte count and T-cell subset analysis), and HIV testing of the person's sexual and needle-sharing partners. The different recommendations for follow-up based on risk assessment can be problematic for physicians and HIV counselors. Even with obtaining a good risk history, providers will encounter individuals of intermediate or unknown risk, for example, heterosexuals who are unsure of their sexual partners' drug use or sexual histories or health care workers with percutaneous needlestick exposures of unknown inoculum sizes from high-risk persons of unknown HIV status.

11655 JGIM Nov/Dec
Galley 182

Follow-up of persons with IWB is labor-intensive and involves longitudinal follow-up, risk assessment and counseling, additional clinical evaluation, and supplemental assays, if available. Limited data exist about the use of supplemental virologic evaluation of individuals with IWB, such as HIV-1 culture, serum p24 antigen, recombinant or radioimmunoprecipitation assays, or selective HIV-1 proviral DNA amplification utilizing the polymerase chain reaction (PCR). The U.S. Army reported that only 0.004% of samples in its HIV-1 screening program from 1985 to 1990 remained nondiagnostic for HIV infection when a recombinant envelope EIA and radioimmunoprecipitation were used in indeterminate sera.[55] In our prospective study of high- and low-risk persons with IWB, we found the specificities of HIV-1 culture, serum p24 antigen, PCR, and recombinant ENV 9 EIA to be 100%, 100%, 98.6%, and 94.4%, respectively.[37] Subsequent analysis of the FDA-licensed recombinant Syva HIV-1 Microtrak assay was promising; the Syva Microtrak showed a sensitivity of 100% in four of the seroconverters in our study and 100% among a panel of 17 seroconverter sera, with a specificity of 99.2% among 128 EIA-reactive, IWB nonseroconverters.[56] The clinical utility of these supplemental tests remains to be determined, since the negative and positive predictive values necessitate estimates of the test's sensitivity in seroconversion.

Considering the above data, we summarize our recommendations for evaluation of persons with IWB in Table 1. These recommendations are consistent with those proposed by Kleinman regarding the counseling and evaluation of blood donors with IWB.[15]

RECOMMENDATIONS REGARDING IWBs

We recognize that widespread screening for HIV is inevitable given the current climate surrounding the HIV epidemic. Relative to the total number of individuals tested for HIV, IWBs will most likely continue to be seen in small numbers. However, those numbers will increase as testing criteria widen and more low- and high-risk individuals are tested. The costs of serial Western blot testing and further virologic and immunologic evaluation can be considerable. Furthermore, IWBs identified during routine screening of health care workers could result in the temporary disruption of practices where invasive procedures are performed, such as surgery and dentistry. Finally, the psychosocial consequences of IWB are considerable.[18]

XXX

11655;LB180TO2 JAB 13:33 08-17-92 P0003 U3 C9439 0021

#11655 JGIM(Nov/Dec),Folder 16,msp.337,lb,8/13/92
12

We make the following recommendations to help clinicians counsel and evaluate persons with IWB:

1. Health care providers should differentiate between HIV screening and testing. We support the CDC recommendations for HIV antibody testing of those individuals with a history of possible exposure to HIV.[1] In addition, for some worried low-risk individuals, the benefits of documenting a negative test result may outweigh the risk of false-positive or indeterminate results.
2. HIV-1 testing programs should train health care providers to evaluate and counsel individuals with IWBs, based on the currently available data about the natural history of IWB. In addition, counselors should describe the possibility of both false-positive and indeterminate results in pretest counseling.
3. The most important factor in the interpretation of indeterminate HIV-1 Western blots is knowledge of the individual's risk status and the HIV seroprevalence of the population being tested for HIV antibody. A low prior probability of HIV infection after careful risk assessment and the absence of a p24 band on Western blot allow the clinician to reassure patients with IWB.
4. Laboratories should adhere to the interpretative criteria recommended by the CDC, and efforts should be made to standardize reporting formats. We would, however, recommend not designating non-HIV bands alone on Western blot as indeterminate. Laboratory reports should provide an interpretation of the Western blot result as well as the CDC recommendations for duration and type of follow-up on laboratory reports. Laboratory quality-assurance programs should be developed for new HIV-1 supplemental assays.
5. Insurance companies, blood banks, and other institutions that require HIV testing should establish a clear and consistent policy regarding individuals with IWBs. In particular, low-risk individuals with indeterminate results and appropriate follow-up should not be considered to be infected with HIV[15, 57]; nevertheless, the FDA requires their deferral as blood donors at this time.[57] High-risk individuals should be considered potentially infected, counseled accordingly, and tested over six months or longer if they have ongoing risk for HIV infection.

In conclusion, we agree with the general recommendation that high-risk individuals be counseled and encouraged to be tested for HIV antibodies and that

11655 JGIM Nov/Dec
Galley 184

The authors gratefully acknowledge William E. Lafferty, MD, Michael Grey, MD, MPH, Victory Murphy, ARNP, and H. Hunter Handsfield, MD, for their review of the manuscript and their many helpful comments, and Joan Dragavon, MT, Todd Damrow, PhD, MPH, and Delores Villareal, MT, for technical assistance.

REFERENCES

1. Public Health Service guidelines for counseling and antibody testing to prevent HIV infection and AIDS. MMWR. 1987; 36:509-15.
2. Volberding PA, Lagakos SW, Koch MA, et al. Zidovudine in asymptomatic human immunodeficiency virus infection. N Engl J Med. 1990;322:941-9.
3. Recommendations for zidovudine: early infection. State-of-the-Art Conference on AZT, National Institutes for Health, March 3–4. JAMA. 1990;263:1606-7.
4. Rhame FS, Maki DG. The case for wider use of testing for HIV infection. N Engl J Med. 1989;320:1248-51.
5. Angell M. A dual approach to the AIDS epidemic. N Engl J Med. 1991;324:1448-50.
6. Brennan TA. Transmission of the human immunodeficiency virus in the health care setting—time for action. N Engl J Med. 1991;324:1504-9
7. Meyer KB, Pauker SG. Screening for HIV: can we afford the false-positive rate? N Engl J Med. 1987;317:238-41.
8. Schwartz DS, Dans PE, Kinosian BP. Human immunodeficiency virus test evaluation, performance, and use. JAMA. 1988; 259:2574-9.
9. Update: serologic testing for antibody to human immunodeficiency virus. MMWR. 1988;36:833-45.
10. Imagawa DT, Lee MH, Wolinsky SM, et al. Human immunodeficiency virus type-1 (HIV-1) infection in homosexual men who remain seronegative for prolonged periods. N Engl J Med. 1989;320:1458-62.
11. Wolinsky SM, Rinaldo CR, Kwok S, et al. Human immunodeficiency virus type 1 (HIV-1) infection a median of 18 months before a diagnostic Western blot. Ann Intern Med. 1989; 111:961-72.
12. Imagawa D, Detels R. HIV-1 in seronegative men [letter]. N Engl J Med. 1991;325:1250-1.
13. Kleinman S, Fitzpatrick L, Secord K, Wilke D. Follow-up testing and notification of anti-HIV Western blot atypical (indeterminant) donors. Transfusion. 1988;28:280-2.
14. Dock NL, Lamberson HV, O'Brien TA, Tribe DE, Alexander SS, Poiesz BJ. Evaluation of atypical human immunodeficiency virus immunoblot reactivity in blood donors. Transfusion. 1988; 28:412-8.
15. Kleinman S. The significance of HIV-1 indeterminate Western blot results in blood donor populations. Arch Pathol Lab Med. 1990;114:298-303.
16. Calabrese LH. Indeterminate reactors to tests for human immunodeficiency virus (HIV). Ann Intern Med. 1989;111:95.
17. Nuttall KL, Ashwood ER. Wider use of testing for HIV infection [letter]? N Engl J Med. 1989;321:1266.

1655;LB183TO5 JAB 13:34 08-17-92 P0002 U3 C5809 0020

1655 JGIM Nov/Dec
alley 185

. Pollack A, Celum CL, Murphy V, et al. Indeterminate Western blots: psychological impact from notification [Abstract 6271]. Florence, Italy: VII International Conference on AIDS, June 16–21, 1991.
. Update: serologic testing for HIV-1 antibody — United States, 1988 and 1989. MMWR. 1990;39:380-2.
. Interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. MMWR. 1989;38(suppl 7):1-7.
. Condie T, Mottice S. Western blot interpretation: major reporting shift from negative to indeterminate using the new ASTPHLD criteria [Abstract VII-45]. Kansas City, MO: American State and Territorial Public Health Laboratory Directors Fifth Consensus Conference on Testing for Human Retroviruses, 1990.
. Benenson AS, Peddecord M, Hofheerr LK, et al. Reporting the results of human immunodeficiency virus testing. JAMA. 1989; 262:3435-8.
. Detection of antibodies to HIV (Biotech/Dupont Western blot kit insert). Wilmington, DE: Dupont Company, 1987.
. Damato JJ, O'Bryen BN, Fuller SA, et al. Resolution of indeterminate HIV-1 test data using the Department of Defense HIV-1 testing program. Lab Med. 1991;22:107-13.
McDonald KL, Jackson JB, Bowman RJ, et al. Performance characteristics of serologic tests of human immunodeficiency virus type 1 (HIV-1) antibody among Minnesota blood donors. Ann Intern Med. 1989;110:617-21.
Jackson JB, MacDonald KL, Caldwell J, et al. Absence of human immunodeficiency virus infection in blood donors with IWB tests for antibody to HIV-1. N Engl J Med. 1990;322:217-22.
Ranki A, Krohn M, Allain J-P, Panchini G, Valle SL, Antonen J. Long latency precedes overt seroconversion in sexually-transmitted human immunodeficiency virus infection. Lancet. 1987;ii:590-3.
Farzadegan H, Polis MA, Wolinsky SM, et al. Loss of human immunodeficiency virus type 1 (HIV-1) antibodies with evidence of viral infection in asymptomatic homosexual men. Ann Intern Med. 1988;108:785-90.
Biggar RJ, Melbye M, Ebbesen P, et al. Variation in human T lymphotropic virus III (HTLV-111) antibodies in homosexual men: decline before onset of illness related to acquired immune deficiency syndrome (AIDS). BMJ. 1985;291:997-8.
Lefrere JJ, Courouce AM, Lucotte G, et al. Follow-up of subjects with isolated and persistent anti-core (anti-p24 or anti-p17) antibodies to HIV. AIDS. 1988;7:287-90.
van der Poel CL, Lelie PN, Reesink HW, et al. Blood donors with indeterminate anti-p24 reactivity in HIV-1 Western blot: absence of infectivity to transfused patients and in virus culture. Vox Sang. 1989;56:162-7.
Leitman SF, Klein HG, Melpolder JJ, et al. Clinical implications of positive tests for antibodies to human immunodeficiency virus type 1 in asymptomatic blood donors. N Engl J Med. 1989;321:917-24.
Mozzi F, Zanella A, Bellobuono A, et al. Clinical and laboratory follow-up of asymptomatic blood donors with only anti-HIV 'core' antibodies. Vox Sang. 1988;54:188-9.

5;LB183TO5 JAB 13:34 08-17-92 P0003 U3 C8828 0031

34. Genesca J, Jett BW, Epstein JS, et al. What do Western blot indeterminate patterns for human immunodeficiency virus mean in EIA-negative blood donors? Lancet. 1989;ii:1023-25.
35. Wilbur JC, Louie B, Soucuya E, Barnes J, Myers R, Beck A. Evaluation of indeterminate results in HIV serology: distinguishing seroconversions from false-positives [Abstract 829]. Los Angeles, CA: Twenty-eighth Interscience Conference on Antimicrobial Agents and Chemotherapy, October 1988.
36. Dock NL, Kleinman SH, Rayfield MA, et al. Human immunodeficiency virus infection and indeterminate Western blot patterns. Arch Intern Med. 1991;151:525-30.
37. Celum CL, Coombs RW, Lafferty WE, et al. Indeterminate HIV-1 Western blots: risk of seroconversion, specificity of supplemental tests, and an algorithm for evaluation. J Infect Dis. 1991; 164:656-64.
38. Horsburgh CR, Jason J, Longini IM, et al. Duration of human immunodeficiency virus infection before detection of antibody. Lancet. 1989;ii:637-9.
39. Perroni P, Tagger A, Pasquali M, et al. HIV antibody screening and confirmatory testing of Italian blood donors. Vox Sang. 1988; 55:143-7.
40. O'Brien TR, George JR, Holmberg SD. Human immunodeficiency virus type 2 infection in the United States: epidemiology, diagnosis, and public health implications. JAMA. 1992; 267:2775-9.
41. DeCock KM, Porter A, Kouadio J, et al. Cross-reactivity on Western blots in HIV-1 and HIV-2 infections. AIDS. 1991;5:859-60.
42. Myers RA, Patel JD, Joseph JM. Identifying HIV-2 seropositive individuals by reevaluating HIV-1 indeterminate sera. J Acquir Immune Defic Syndr. 1992;5:417-23.
43. George JR, Rayfield MA, Phillips S, et al. Efficacies of U.S. Food and Drug Administration-licensed HIV-1 screening enzyme immunoassays for detecting antibodies to HIV-2. AIDS. 1990; 4:321-6.
44. Schumacher RT, Howard J, Ayres L. Cross-reactivity of anti-HIV-2 positive serum in U.S. FDA licensed screening tests for anti-HIV-1 [Abstract C 745]. San Francisco, CA: Presented at the Sixth International Conference on AIDS, June 20–23, 1990.
45. Drabick JJ, Horning VL, Lennox JL, et al. A retrospective analysis of diseases associated with indeterminate HIV Western blot patterns. Milit Med. 1991;156:93-6.
46. Celum CL, Coombs RW, Murphy VM, et al. Etiologies of indeterminate HIV-1 Western blots: autoantibodies, alloimmunization, an' HIV-1 seroconversion [Abstract 48*9]. Amsterdam, The Netherlands: VIII International Conference on AIDS, July 1992.
47. Handsfield HH, Wandell M, Goldstein L, Shriver K, Cooperative Study Group. Screening and diagnostic performance of EIA for antibodies to LAV. J Clin Microbiol. 1987;25:879-84.

55 JGIM Nov/Dec
ley 187

layers MH, Beatty PG, Hansen JA. HLA antibodies as a cause of false-positive reactions in screening enzyme immunoassays for antibodies to human T-lymphotropic virus type III. Transfusion. 1986;26:113-5.
Working group on HIV testing of pregnant women and newborns, HIV infection, pregnant women, and newborns. JAMA. 1990;264:2416-20.
Yelkin D, Tancredi L. Dangerous diagnostics: the social power of biological information. New York: Basic Books, 1989.
Haynes RB, Sackett DL, Taylor DW, Gibson ES, Johnson AL. Increased absenteeism from work after detection and labeling of hypertensive patients. N Engl J Med. 1978;244:741-4.
Johnson ML, Gibson ES, Terry CW, et al. Effects of labelling on income, work, and social function among hypertensive employees. J Chron Dis. 1984;37:417-23.
Kurton BK, Dillard RG, Clark EN. The psychological impact for false-positive elevations of maternal serum alpha-fetoprotein. Am J Obstet Gynecol. 1985;151:77-82.
Public health uses of HIV-infection reports — South Carolina, 1986–1991. MMWR. 1992;41:245-9.
Roberts CR. Laboratory diagnosis of retroviral infections. Dermatol Clin. 1991;9:1-12.
Murphy VM, Celum CL, Roberts CR, et al. Recombinant antigen and synthetic peptide assays as supplemental tests for indeterminate HIV-1 Western blots [Abstract 4888]. Amsterdam, The Netherlands: VIII International Conference on AIDS, July 1992.
Recommended testing protocol to clarify status of donors with a reactive anti-HIV screening test. Washington, DC: Office of Biologics Research and Review, Center for Drugs and Biologics, U.S. Food and Drug Administration, 1987.

TABLE 1
Recommendations for the Evaluation of Persons with HIV-1 Indeterminate Western Blots (IWBs)

Repeat HIV-1 enzyme immunoassay (EIA) one month after the initial IWB with reassessment of risk history at that time
Six-month follow-up of any persons with reported or suspected high-risk behavior
Three-month follow-up for low-risk persons with a p24 or envelope (gp41 or gp120/160) band on Western blot
No further follow-up for low-risk persons who are EIA-nonreactive on the repeat test or EIA-reactive with a band other than p24 or an envelope band
A negative supplemental test may provide earlier and additional reassurance to those persons with a p24 or an envelope band

11655;LB186TO8 JAB 13:35 08-17-92 P0003 U3 C5368 0020

SCIENCE challenging AIDS

FORM

96

SEVENTH INTERNATIONAL CONFERENCE ON AIDS
FLORENCE, ITALY
16-21 JUNE 1991

Secretariat Space RA _____ Date Received _____ Abst # _____

Abstracts must be submitted on this form and accompanied by 10 photocopies.

AN ALGORITHM FOR EVALUATION OF INDETERMINATE HIV-1 WESTERN BLOTS

Celum, Connie,* Coombs RW*, Lafferty WE**, Fisher LD*, Murphy VM*, Inui TS*, Corey L*, Holmes, KK*. *Univ of Washington, **DSHS Office on HIV/AIDS, Seattle, WA, USA

Objective: To determine the risk of seroconversion and clinical utility of supplemental tests in persons repeatedly reactive by HIV-1 EIA and indeterminate by Western blot (indeterminate WB).
Methods: Eighty-nine cases (with prior repeatedly reactive HIV-1 EIA and indeterminate WB) were referred from HIV testing sites in Washington and Oregon and followed prospectively for 6-9 months. Subjects were tested by HIV-1 EIA and WB (Epitope & Dupont) at study enrollment and every 3 months. At the first study visit, we performed polymerase chain reaction (PCR), HIV-1 mononuclear and plasma culture, serum p24 antigen, ENV 9 recombinant envelope antigen, and radioimmunoprecipitation assay.
Results: Thirty-two (36%) of the cases reported prior HIV risk behaviors. Four of the 89 cases (4.5%; 95% CI= 1.2%,11.1%) seroconverted within 1-10 months after the initial indeterminate WB. All 4 seroconverters had HIV risk factors and p24 antibodies on initial WB. Thirty-five (39%) cases were no longer repeatedly reactive by EIA at the first study visit, 7 of whom were negative by Western blot. The remaining 50 cases had reactive EIA and indeterminate WB, primarily with core reactivity on WB (60% with p24 bands). Specificities of HIV-1 culture, PCR, ENV 9, and p24 antigen were 100%, 99%, 94%, and 100% among the 85 cases who did not seroconvert by WB in ≥ 6 months follow-up.
Conclusions: Risk of seroconversion was low (4.5%) and was seen only among high-risk cases with p24 bands on initial indeterminate WB. Specificities of supplemental HIV-1 tests (HIV-1 culture, PCR, ENV 9, and p24 antigen) were high. An algorithm is proposed based on HIV risk history, repeatability of EIA reactivity, and presence of p24 bands on WB. Low-risk persons with nonreactive EIAs on repeat testing or WB without p24 bands do not need further follow-up. High-risk persons should be followed at least 6 months beyond their last risk behavior. Supplemental tests (PCR, HIV-1 culture, or a recombinant envelope antigen, e.g., ENV 9) may be useful in exclusion or early detection of HIV infection in persons with p24 bands on WB.

ABSTRACTS RECEIVED AFTER 28 JANUARY 1991 CANNOT BE CONSIDERED

1. Presentation Preference (check one): Oral Presentation _____ Poster Presentation X
2. Important: Indicate below the Track and Category code (e.g., A5. D12) in which you believe your abstract should be programmed (see track/Category list on reverse side for codes)

Choice 1 Track/Category (letter and number)	Choice 2 Track/Category (letter and number)
C7	C6

3. I certify that: 1) this abstract has not been published elsewhere or submitted for presentation at another national or international meeting; and 2) this is the only abstract I have submitted to 7th International Conference on AIDS on which I am the presenting author. Presenting Author's Signature Connie L. Celum

Print Presenting Author's Full Name CONNIE L. CELUM

Airmail or Express this original Abstract Form plus 10 photocopies (along with one copy of the Abstract Information Form plus four photocopies) in the self-addressed blue-corner envelope provided to: *7th International Conference on AIDS. General Secretariat. Laboratory of Virology. Istituto Superiore di Sanità. Viale Regina Elena 299. 00161 Rome.*

FOR REVIEWERS USE: A _____ R _____ Score _____ Oral _____ Poster _____ Rejected _____
COMMENTS. _____

SCIENCE challenging AIDS

ABSTRACT FORM

97

SEVENTH INTERNATIONAL CONFERENCE ON AIDS
FLORENCE, ITALY
16-21 JUNE 1991

Secretariat Space #A _____ Date Received ____ Abst # _____

Abstracts must be submitted on this form and accompanied by 10 photocopies

INDETERMINATE WESTERN BLOTS: PSYCHOLOGICAL IMPACT OF NOTIFICATION

Pollack Amy*; Celum C.L.*; Murphy V.*; Inui T.S.*; Fisher L.L.*; Vitaliano P.*, Jones M.*
*University of Washington, Seattle, Washington, USA.

Objective: The HIV-1 Western blot has been reported as "indeterminate" in 4%-20% of sera repeatedly reactive by HIV-1 enzyme immunoassay (EIA). Notification of an indeterminate Western blot (IWB) may result in anxiety due to uncertainty of the significance of the test result, the need for six months follow-up and repeat HIV testing, and potential impact on insurability and immigration. The purpose of this study was to assess the psychological impact of notification of an IWB test result.
Methods: Study subjects were participants in a case-control study designed to determine etiologies of IWB; 101 cases (prior repeatedly reactive HIV-1 EIA and an IWB) and 73 controls (EIA negative in the past three months and frequency matched by test site) were recruited from test sites in Washington and Oregon. At the intake interview all cases and controls completed a 13-item Beck Depression Inventory (BDI), Symptom Checklist-90 (SCL-90) Anxiety Subscale, Life Event Scale, and a short psychological questionnaire specifically about the IWB. HIV testing sites and Ob/Gyns in Washington State are also currently being surveyed about their practices regarding HIV counseling and testing and IWB.
Results: Univariate analysis showed no significant difference in measured anxiety and depression between the cases and controls overall. However, 14% of cases compared to 6% of controls reported suicidal ideation ($p=0.058$). Discriminant analysis indicated that younger age, single marital status, elective testing for HIV, prior risk behavior, prior psychiatric treatment, and having an IWB were independent predictors of anxiety.
Discussion: Notification of an IWB is associated with increased anxiety and suicidal ideation in some cases. With increased use of HIV tests for widespread screening, it is important to recognize the impact of notification of an IWB result and the need for counseling guidelines.

ABSTRACTS RECEIVED AFTER 28 JANUARY 1991 CANNOT BE CONSIDERED

1. Presentation Preference (check one): Oral Presentation _____ Poster Presentation _____
2. Important. Indicate below the Track and Category code (e.g. A5, D12) in which you believe your abstract should be programmed (see track/Category list on reverse side for codes)

Choice 1	Choice 2
Track/Category (letter and number) _____	Track/Category (letter and number)

3. I certify that: 1) this abstract has not been published elsewhere or submitted for presentation at another national or international meeting; and 2) this is the only abstract I have submitted to 7th International Conference on AIDS on which I am the presenting author. Presenting Author's Signature _____

Print Presenting Author's Full Name _____

Airmail or Express this original Abstract Form plus 10 photocopies (along with one copy of the Abstract Information Form plus four photocopies) in the self-addressed blue-corner envelope provided to: *7th International Conference on AIDS, General Secretariat, Laboratory of Virology, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome.*

FOR REVIEWERS USE: A _____ R _____ Score _____ Oral _____ Poster _____ Rejected _____

COMMENTS: _____

Secretariat Space RA _____ Date Received _____ Abstract # _____
Abstracts must be submitted on this form and accompanied by ten (10) photocopies.

ETIOLOGIES OF INDETERMINATE HIV-1 WESTERN BLOTS: AUTOANTIBODIES, ALLOIMMUNIZATION, AND HIV-1 SEROCONVERSION

Celum, Connie; Coombs RW; Murphy VM; Jones M, Fisher L, Holmes KK, Inui TS
University of Washington, Seattle, Washington, USA.

Objectives: To determine etiologies of indeterminate HIV-1 Western blots in low- and high-risk persons with previous reactive HIV-1 EIAs (EIA PR) and indeterminate Western blots (IWB), including seroconversion, cross-reactivity with other retroviruses, and autoantibodies.

Methods: 238 cases (EIA PR and IWB) were frequency-matched with 145 EIA nonreactive controls recruited from the same testing sites and enrolled in a case-control study. 79 cases referred their current sexual partner. General medical and HIV risk histories were obtained and antinuclear antibodies, rheumatoid factor, lymphocyte subsets, serologies for HIV-1, HTLV-1, HIV-2, bovine (BIV) and feline immunodeficiency (FIV) viruses were performed. Cases were followed prospectively for 6-9 months to rule out HIV seroconversion.

Results: Six of 161 (3.7%) cases with $\geq$ 6 months follow-up seroconverted; all had high-risk behavior and a p24 band initially. Of the 232 nonseroconverter cases, 132 (57%) were EIA repeatedly reactive (EIA RR) at the first study visit and 147 (62%) had indeterminate Epitope blots. All controls were EIA nonreactive and 29 (23%) had IWB. Five (6%) of cases' sexual partners were EIA RR, 3 (4%) were HIV positive and 17 (22%) had IWB. Among the 232 nonseroconverter cases and 145 controls, autoantibodies (O.R. 1.9; 95% CI 1.1,3.1), sex with a prostitute (O.R. 3.3; 95% CI 1.1,10), and a tetanus booster in the past 2 years (O.R. 2.0; 95% CI 1.1,3.9) were independently associated with an IWB. Among women, parity (O.R. 1.3; 95% CI 1.1,1.5) and viral illness in the past 3 months (O.R. 2.0; 95% CI 1.1,3.6) were independently associated with an IWB. No cross-reactivity was seen with HIV-2, HTLV-1, FIV, or BIV.

Conclusions: The risk of seroconversion was low (3.7%) and limited to persons with recent high-risk behavior. The reactivity detected by EIA and Western blot was transient in 40% of cases. Autoantibodies and nonspecific immune stimulation may explain the IWB among 69% of nonseroconverters. We found no cross-reactivity with other human or animal retroviruses. Indeterminate blots are equally common in EIA nonreactive controls and cases' sexual partners, reflecting nonspecific cross-reactivity detected by Western blot.

Abstracts received after 2 March 1992 will not be considered
1. Presentation preference (check one): Oral presentation _X_ Poster presentation _____; 2. Indicate below two choices for track and category codes (e.g., A10, A15 or D2, D25) in which you believe your abstract should be classified. See "Categories for Abstract Submission" on page vi or on the other side of this form for codes. *Choice 1:* Track/Category (letter and number) C27 ; *Choice 2:* Track/Category (letter and number) D27 ; 3. Rule of One Statement: I certify that this is the only abstract I have submitted to the VIII International Conference on AIDS/III STD World Congress on which I am the presenting author.

Presenting Author's Signature Connie L Celum

Print Presenting Author's Full Name Connie L. Celum, MD, MPH
Air Mail or Express Mail this original Abstract Form, with ten (10) photocopies, and the original Abstract Information Form, with one (1) photocopy, in the provided self-addressed *orange* envelope to: VIII International Conference on AIDS/III STD World Congress, Abstract and Registration Administration, CONGREX Holland BV, P.O. Box 302, 1000 AH Amsterdam, The Netherlands.
Overnight Mail Delivery Address: VIII International Conference on AIDS/III STD World Congress, Abstract and Registration Administration, CONGREX Holland BV, Keizersgracht 782, 1017 EC Amsterdam, The Netherlands.

For reviewers use: A _____ R _____ Score _____ Oral _____ Poster _____ Publish _____ International Merit _____

Comments: _____

Abstract Form

A World United Against AIDS 99
VIII International Conference on AIDS/III STD World Congress
Amsterdam, the Netherlands 19-24 July 1992

Secretariat Space RA _____ Date Received _____ Abstract # _____
Abstracts must be submitted on this form and accompanied by ten (10) photocopies.

RECOMBINANT ANTIGEN AND SYNTHETIC PEPTIDE ASSAYS AS SUPPLEMENTAL TESTS FOR INDETERMINATE HIV-1 WESTERN BLOTS

Murphy VM*; Celum CL*; Roberts CR**; Rosencranz L*, Lee W*, Jones M*, Coombs RW*. *University of Washington, Seattle, Washington;**Walter Reed Army Institute of Research, Rockville, MD, USA.

Objectives: To determine the sensitivity, specificity, and clinical utility of two recombinant antigen and a synthetic peptide enzyme immunoassay (EIA) in differentiating false positives from early seroconverters among persons with previous reactive HIV-1 EIAs (EIA PR) and indeterminate Western blots (IWB).

Methods: 238 cases with EIA PR and IWB were enrolled in a prospective study to determine the risk of seroconversion. HIV-1 EIA and Epitope Western blots were performed at three month intervals over at least 6 months. The Syva Microtrak and Cambridge BioSciences Recombugen (recombinant p24 and gp 41/gp120 EIAs) and Genetic Systems GENIE (a synthetic peptide HIV-1 gp41 and HIV-2 gp31 EIA) were performed on serum from 172, 55, and 84 study subjects, respectively. The assays were also tested on a commercial panel of seroconverter sera (Boston Biomedica).

Results: The 3 supplemental assays were positive in four seroconverters from our study, all of whom initially had a p24 band only on Western blot. The supplemental assays were positive 12 to 14 days prior to the conventional Genetic Systems EIA in the commercial seroconverter panel. The specificities of the three supplemental assays were 98-99%, respectively. The 3 assays were negative on samples from 3 low-risk persons with false positive Western blots (p24 and envelope bands), confirmed by negative HIV-1 culture and polymerase chain reaction.

Conclusions: The sensitivities of these recombinant antigen and synthetic peptide EIA assays were 100% and the specificities were 98-99% among persons with HIV-1 EIA PR and IWB. These assays were rapid and accurate methods of identifying persons who were seroconverting and provided reassurance to noninfected persons with IWB, especially those with p24 bands or envelope bands on Western blot.

Abstracts received after 2 March 1992 will not be considered
1. Presentation preference (check one): Oral presentation _____ Poster presentation X; 2. Indicate below two choices for track and category codes (e.g., A10, A15 or D2, D25) in which you believe your abstract should be classified. See "Categories for Abstract Submission" on page vi or on the other side of this form for codes. *Choice 1:* Track/Category (letter and number) C27; *Choice 2:* Track/Category (letter and number) D27; 3. Rule of One Statement: I certify that this is the only abstract I have submitted to the VIII International Conference on AIDS/III STD World Congress on which I am the presenting author.

Presenting Author's Signature Victory M Murphy

Print Presenting Author's Full Name Victory M. Murphy

Air Mail or Express Mail this original Abstract Form, with ten (10) photocopies, and the original Abstract Information Form, with one (1) photocopy, in the provided self-addressed *orange* envelope to: VIII International Conference on AIDS/III STD World Congress, Abstract and Registration Administration, CONGREX Holland BV, P.O. Box 302, 1000 AH Amsterdam, The Netherlands.
Overnight Mail Delivery Address: VIII International Conference on AIDS/III STD World Congress, Abstract and Registration Administration, CONGREX Holland BV, Keizersgracht 782, 1017 EC Amsterdam, The Netherlands.

For reviewers use: A _____ R _____ Score _____ Oral _____ Poster _____ Publish _____ International Merit _____

Comments: _____

AN "INDETERMINATE" HIV-1 WESTERN BLOT: WHAT DOES IT MEAN?

Q: What are the HIV-1 (AIDS) antibody tests?

A: To screen for HIV-1 antibodies, the laboratory first performs an ELISA or EIA test. This is a very accurate test, but occasionally it picks up antibodies that are not caused by HIV and which cause false-positive EIAs. False-positive EIAs occur because the immune system produces countless antibodies during the process of fighting off diseases, which can "cross-react" with the EIA.

To determine whether a positive EIA is a true or false-positive, a confirmatory Western blot is performed which detects antibodies to individual characteristic proteins that make up the HIV virus. The Western blot is called positive if several antibodies are present, negative if no antibodies are present, and indeterminate if bands representing antibodies are present that don't meet the criteria for a positive Western blot. The Western blot is also a very accurate test, but occasionally it too can detect "cross-reacting" antibodies.

Q: How common is an indeterminate Western blot (abbreviated as IWB)?

A: Among low-risk persons who are reactive by HIV-1 EIA, 10% will be indeterminate by Western blot.

Q: What causes indeterminate Western blots?

A: It appears that many indeterminate Western blots are due to cross-reacting antibodies which may be found in some healthy individuals as well as others with medical conditions such as lupus or rheumatoid arthritis. While these conditions may cause an IWB, it is important to note that an IWB itself does not indicate or diagnose other conditions. Women with previous pregnancies may also have cross-reacting antibodies present.

Occasionally an IWB can be seen very early in HIV infection in the first few months after an individual has been infected with the virus. In the University of Washington IWB study, we found that approximately 4% of the individuals referred to us with IWB were infected with HIV but hadn't yet formed all the antibodies initially needed to call the test positive.

Q: What is the likelihood someone with an IWB is infected with HIV?

A: It depends on whether that individual recently had high-risk sexual contacts or intravenous drug use. In a recent University of Washington study, individuals with an IWB and no reported risk factors for HIV infection were not infected with HIV.

Q: How can someone find out if the IWB is due to HIV infection?

A: The current recommendation from the Centers for Disease Control is to repeat the EIA and Western blot several times over six months to see whether the Western blot becomes positive. If the IWB stays indeterminate or turns negative, the person is not HIV-infected.

Q: How will an IWB affect my eligibility to donate blood or obtain life insurance?

A: Currently the Food and Drug Administration (FDA) requires that blood banks not allow blood donors with IWB to donate blood because of the small chance that they could be recently infected with HIV. The FDA is understandably taking a cautious approach to avoid infecting any transfusion recipients. You can find out whether the blood center policy about donors with IWB has changed by calling your blood center. Occasionally individuals have been temporarily deferred from obtaining life insurance because of an IWB until follow-up tests indicate they are not HIV-infected.

Q: What should I do now?

A: Discuss your concerns about the IWB with your health care provider, including a thorough discussion about possible HIV risk factors. It is very important to be fully honest with your health care provider about your sexual and drug-related behaviors so that he or she can decide how long to follow you and whether to perform additional tests. It is advisable to observe safe sexual practices (eg., using condoms and avoiding anal intercourse) and to avoid sharing needles, in the case of persons who inject drugs. For both low- and high-risk persons, some anxiety about the test result is understandable. We hope the information in this brochure, combined with discussions with your health care provider, will help reassure you.

Provided by
University of Washington
Dept. of Laboratory Medicine,
Division of Virology and the
Indeterminate Western Blot Study

- For additional information, contact your health care provider -

FOR PROVIDERS

Additional Information about Indeterminate Western Blots

The serum sample submitted from this individual was repeatedly reactive by HIV-1 enzyme-linked immunosorbent assay (EIA) and indeterminate by Western blot. Both clinicians and patients have reported difficulty in understanding the significance of an indeterminate HIV-1 Western blot (IWB).

This uncertainty prompted the University of Washington to conduct a study on the causes, risk of seroconversion, specificity of supplemental tests, and psychological impact of IWB. The study enrolled individuals with IWB from April 1988 through April 1991. Since the study is no longer open for new enrollments, the study investigators have written these brochures for clinicians to summarize the current state of our knowledge and to aid in counseling and evaluating persons with IWB.

How common is an indeterminate Western blot (IWB)?

The prevalence of IWBs depends on the prevalence of HIV infection, other medical conditions, and autoantibodies in the population being tested; inter-lot variability in the amount of HIV-1 antigen on the Western blot strips; and the laboratory's experience in performing and interpreting Western blots. The published prevalence of indeterminate results has ranged from 10% in EIA reactive military recruits to 13% of EIA reactive Minnesota blood donations (1).

What causes indeterminate Western blots?

Indeterminate results occur in HIV infection in the seroconversion window, when only core antibody (e.g., p24) may be detected by Western blot, and late in AIDS when core antibody levels decline (2,3). Autoantibodies may account for cross-reactivity with the Western blot, as in systemic lupus erythematosis and among some healthy individuals. Among women, alloimmunization due to current or past pregnancies may be associated with IWB (unpublished data, Univ of Wash study, 1991). Cross-reactivity with HIV-2 can occur, but cross-reactivity does not occur with HTLV-1 (4,5).

What is the likelihood someone with an IWB is infected with HIV?

The risk of true HIV infection among individuals with IWB is determined by the rate of seroconversion to positive results. This rate was 0-5% in the published blood donor cohorts, 90% among a San Francisco cohort of 20 individuals with p24 antibodies on initial Western blots, and 4% in the University of Washington study (4,6-9). The likelihood that an IWB reflects HIV infection is dependent on HIV risk factors and the time to seroconversion. The highest risk of seroconversion appears to be for those with a p24 band.

What are the potential adverse consequences of an IWB?

We have interviewed over 200 individuals with IWB referred to us from health department clinics, blood banks, prenatal clinics, and private providers. The uncertainty engendered by an indeterminate result, the need for at least 6 months follow-up, and discriminatory policies regarding insurability and immigration have been very distressing to both low- and high-risk individuals in our study population. For example, several persons with IWB referred to us have experienced difficulty in obtaining life or disability insurance, elective surgery, in vitro fertilization, visas for foreign travel, and U.S. citizenship. Although none of these individuals were infected with HIV, they were treated as if they were infected, sometimes for an indefinite period of time.

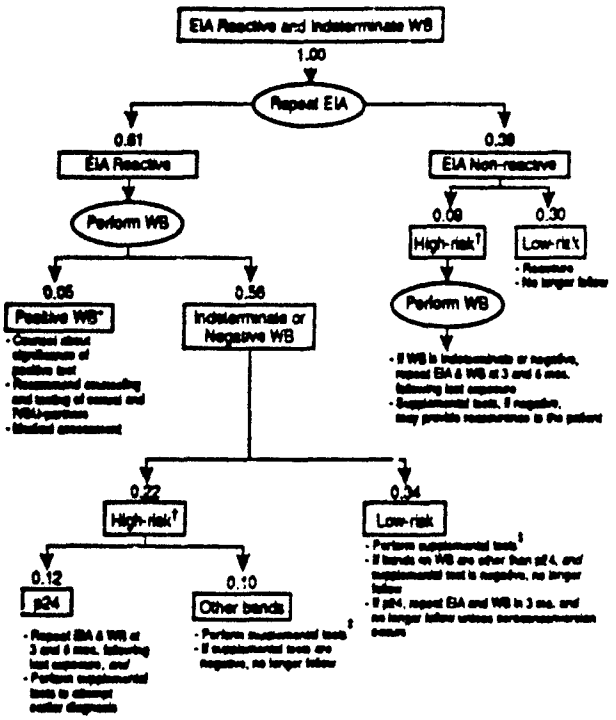
When counseling individuals with IWB, we recommend discussing the following:

- the absence of seroconversion in low-risk individuals
- possible etiologies for the inconclusive result (e.g., cross-reacting antibodies, autoantibodies, and in high-risk persons, possible early seroconversion)
- the need for safe sexual and drug-use practices pending resolution of the test result for high-risk individuals
- recommendations for evaluation, as described below

How should an individual with an IWB be followed?

The CDC recommends that low-risk individuals with an IWB pattern be followed for at least six months; if the Western blot pattern persists as indeterminate or turns negative, then the individual can be reassured that he or she is not infected with HIV (10). Additional diagnostic follow-up is recommended for high-risk persons, including serial testing by Western blot, assessment of immune function, and HIV testing of the person's sexual and needle-sharing partners. Limited data exist about the use of supplemental virologic evaluation of individuals with indeterminate results. Such an evaluation may include HIV-1 culture, recombinant or radioimmunoprecipitation assays, or selective HIV-1 proviral DNA amplification utilizing PCR, if available.

Based upon the University of Washington study of IWB, a sequential process for evaluation was proposed that would provide more rapid determination of the HIV status of an individual with an IWB (9) (see next page):



KEY POINTS

- Risk assessment is very important
- Repeat EIA and WB after 1 month
- Follow high-risk persons with repeat EIA & WB at 3 and 6 months
- No need to follow low-risk persons with bands other than p24 or blots that turn negative

Legend:

* WB = Western blot. The numbers on the algorithm indicate the proportion of cases in the University of Washington study who were in different groups, based on their ELISA and WB results at the first study visit (N = 89 cases).

† High-risk includes recipients of blood products between 1978 and 1985, intravenous drug users (IVDU), homosexual and bisexual men, and sexual partners of IVDU and homosexual and bisexual men.

‡ In most cases supplemental tests will be optional and an individual's HIV status will be clarified by serologic follow-up over three-six months. If available, supplemental tests to consider performing are HIV-1 culture, PCR, or a recombinant envelope antigen assay.

Selected References

1. McDonald KL, Jackson JB, Bowman RJ, et al. Performance characteristics of serologic tests of human immunodeficiency virus type 1 (HIV-1) antibody among Minnesota blood donors. Ann Intern Med 1989; 110: 617-21.
2. Ranki A, Krohn M, Allain J-P, Franchini G, Valle SL, Antonen J. Long latency precedes overt seroconversion in sexually-transmitted human immunodeficiency virus infection. Lancet 1987; ii: 590-3.
3. Farzadegan H, Polis MA, Wolinsky SM, et al. Loss of human immunodeficiency virus type 1 (HIV-1) antibodies with evidence of viral infection in asymptomatic homosexual men. Ann Intern Med 1988; 108: 785-90.
4. Dock NL, Lamberson HV, O'Brien TA, Tribe DE, Alexander SS, Poiesz BJ. Evaluation of atypical human immunodeficiency virus immunoblot reactivity in blood donors. Transfusion 1988; 28: 412-8.
5. Update: HIV-2 infection--United States. MMWR 1989; 33: 572-80.
6. Jackson JB, MacDonald KL, Cadwell J, et al. Absence of human immunodeficiency virus infection in blood donors with indeterminate Western blot tests for antibody to HIV-1. N Engl J Med 1990; 322: 217-22.
7. Kleinman S, Fitzpatrick L, Secord K, Wilke D. Follow-up testing and notification of anti-HIV Western blot atypical (indeterminant) donors. Transfusion 1988; 28: 280-2.
8. Wilbur JC, Louie B, Soncuya E, Barnes J, Myers R, Back A. Evaluation of indeterminate results in HIV serology: Distinguishing seroconversions from false-positives. [Abstract 829] 28th ICAAC. Los Angeles, CA, October 1988.
9. Calum CL, Coombs RW, Lafferty WE, et al. Indeterminate HIV-1 Western blots: Seroconversion risk, specificity of supplemental tests, and an algorithm for evaluation. J Infect Dis: in press.
10. Interpretation and use of the Western blot assay for serodiagnosis of HIV type 1 infections. MMWR 1989; 38 (S-7):1-7.

- For additional information, please call the U. W. Center for AIDS Research at (206) 720-4298 -

Provided by the University of Washington Department of Laboratory Medicine, Division of Virology and the Indeterminate Western Blot Study

APPENDIX A

Scores for All ACCES Measures

CATEGORY G: GENERAL MEASURES

MEASURES			DAY					
NUMBER	TITLE		1	2	3	4	5	AGGREGATE
G.1.0	Plan Duration (median in hours) [time the plan ends minus time the plan is implemented]							
	CP:	DMAIN	-	0.8	3.2	4.8	-	3.8
		DREAR	-	6.9	-	-	-	6.9
		Division	-	3.9	3.2	4.8	-	4.2
G.1.1	Mission Duration (median in hours) [time mission assignments changed minus time mission assignments established]							
	CP:	DMAIN	-	-	13.3	-	-	13.3
G.1.2	Task Organization Duration (median in hours) [time task organization changed minus time task organization established]							
	CP:	DMAIN	-	11.9	3.2	4.0	-	4.0