False-positive human immunodeficiency virus type 1 Western blot tests in noninfected blood donors

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Background: The manufacturers' criteria for a positive human immunodeficiency virus type 1 (HIV-1) Western blot (WB) test were recently revised to require reactivity to only two of the following bands: p24, gp41, and gp120/160. In a recent report, low-risk blood donors were identified in whom nonspecific reactivity to multiple *env* antigens in WB testing resulted in apparently false-positive WBs by these criteria. The present study was conducted to verify the existence of false-positive WBs among noninfected donors and to assess the extent of this problem.
Study Design and Methods: Four donors classified as WB-positive on the basis of *env*-only (3 cases) or p24/*env*-only (1 case) patterns were investigated. Index and/or follow-up specimens were tested by polymerase chain reaction (PCR), by overlapping recombinant *env* antigens and synthetic peptides in enzyme immunoassays, and by deglycosylated and denatured antigen WBs. WB records from American Red Cross blood centers were reviewed to determine the frequency of *env*-only and p24/*env*-only patterns, relative to all positive WBs, from 1988 through 1993.
Results: The four index-case donors denied risk and had stable WB reactivity during follow-up. HIV PCR was negative in all. *Env* reactivity was restricted to nonglycosylated gp41 epitopes; no gp120-specific reactivity was detected. For three of the four donors, *env* reactivity was mapped to a 20-amino acid N-terminal epitope of gp41. The rate of detecting WBs with these false-positive patterns increased from 0.6 percent of all positive WBs from 1988 to 1990 (4/776) to 8 percent in 1991 and 1992 (52/683), and then it declined to 6 percent in 1992 and 1993 (47/783). *Env*-only patterns predominated in 1991 and 1992, whereas p24/*env*-only patterns were more frequent following implementation of combined anti-HIV-1/HIV type 2 enzyme immunoassays in 1992.
Conclusion: Low-risk blood donors can have false-positive results on WB tests. Increased detection of *env*-only and p24/*env*-only WBs appears related to the enhanced sensitivity of newer enzyme immunoassays to gp41 and p24 antibodies. Donors with these patterns should undergo follow-up testing to document the presence or absence of HIV infection.

Abbreviations: CDC = Centers for Disease Control and Prevention; EIA(s) = enzyme immunoassay(s); FDA = Food and Drug Administration; HIV-1 = human immunodeficiency virus type 1; HIV-2 = HIV type 2; IFA = immunofluorescence assay; OD = optical density; PBS = phosphate-buffered saline; PCR = polymerase chain reaction; RIBA = strip recombinant immunoblot assay; WB(s) = Western blot(s).

THE WESTERN BLOT (WB) is the most widely employed supplemental assay for confirming human immunodeficiency virus (HIV) seropositivity of anti-HIV enzyme immunoassay (EIA)-reactive specimens.[1-4] Interpretative criteria for WBs have been reevaluated on several occasions[1,2,4] in response to 1) improvements in the sensitivity and specificity of EIA and WB reagents,[3,5,6] 2) increased understanding of the serologic patterns associated with evolving HIV seroconversion,[5-10] 3) experience with the patterns of nonspecific reactivity observed in low-risk[11-17] and high-risk[18] settings, and 4) determination of the serologic basis for nonspecificity.[19-25] According to currently recommended criteria,[2-4] EIA-repeatably reactive specimens are classified as WB-positive if reactivity is detected to gp41 and gp120/160 *env* bands or to either of these *env* bands plus the p24 *gag* band. These revised criteria differ from earlier recommendations[1] in that reactivity to a third gene product (e.g., p31 or p66*pol* bands) is not required, and reactivity to multiple *env* antigens alone is adequate for confirmation. These changes were adopted to reduce the number of HIV-infected persons, particularly those who seroconverted early and late-stage AIDS patients, who would be misclassified as indeterminate by earlier criteria.[2,3]

From Ortho Diagnostics Systems, Inc., Raritan, New Jersey; the Transmissible Diseases Laboratory and the National Reference Laboratory for Infectious Disease, American Red Cross, Rockville, Maryland; the Community Blood Center of Greater Kansas City, Kansas City, Missouri; and Irwin Memorial Blood Centers and the Department of Laboratory Medicine, University of California, San Francisco, California.

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Recently, a group in Australia reported identifying low-risk, uninfected blood donors whose sera reacted nonspecifically with monomer (gp41) and oligomer (gp120/gp160) forms of gp41, which resulted in apparently false-positive WB interpretations.[26] Unfortunately, this study lacked follow-up specimens from donors and hence could not unequivocally prove the absence of HIV infection. We report here serologic and virologic studies on index donations and follow-up samples from four United States blood donors with similar reactivity, as well as data documenting the increasing frequency with which these patterns have been observed in the blood donor setting.

Case Reports

Case 1: KC

This 67-year-old woman was a 42-time blood donor at the time of the index donation in April 1991. She is a retired office worker who never married, and she denied sexual contact of any sort. She did volunteer hospital work but had no direct patient contact.

Case 2: IRW-1

This 27-year-old woman was a first-time donor at the time of the index donation in September 1993. She is married to a regular blood donor who has a negative testing history. She was asked to complete a Centers for Disease Control and Prevention (CDC) HIV risk factor questionnaire, and she denied all risk factors.

Case 3: IRW-2

This 27-year-old man had made one screen-negative donation prior to the index donation in June 1993. He is heterosexual, and no HIV risk factors were identified by the administration of a CDC HIV risk factor questionnaire.

Case 4: ARC-SEM

This 24-year-old man had made two screen-negative donations prior to the index donation in April 1995. He had no identifiable risk factors, according to his responses on a CDC HIV risk factor questionnaire.

Materials and Methods

Recombinant envelope antigen analysis

Five recombinant proteins derived from the envelope region of HIV-1 were used in EIA format to characterize the *env* specificity of all four donor samples. The recombinant proteins represent overlapping regions of the HIV-1 envelope genome from amino acid 272 through amino acid 767 (Fig. 1).[27] The specific sequences of each recombinant protein are as follows: *env* 4-5, 272-673; CBrE3, 350-674; *env* 9, 474-752; *env* 686, 474-752; and *env* 10, 548-767. We deleted the hydrophobic regions from CBrE3 and *env* 686 to improve specificity and allow for efficient vector expression of the protein. Seroreactivity to each protein was defined as an EIA optical density (OD) ≥0.200 units. *Env* 4-5 and *env* 10 were also available in strip recombinant immunoblot assay (RIBA) format: *env* 4-5 in RIBA-HIV-1[28] and *env* 10 in RIBA-HIV-1/2.[29]

Modified WB analysis

We examined reactivity to monomeric and multimeric forms of gp41 and to deglycosylated components of HIV-1 envelope by using modified WB strips.[30,31] HIV-1 IIIB viral lysate was treated with endoglycosidase-f (Endo-f type 2, Boehringer Mannheim, Indianapolis, IN) to remove the polysaccharide component. At the same time, we treated the viral lysate at 100°C for 5 minutes to disrupt the multimeric forms of gp41, thus obtaining only the deglycosylated monomer that migrates

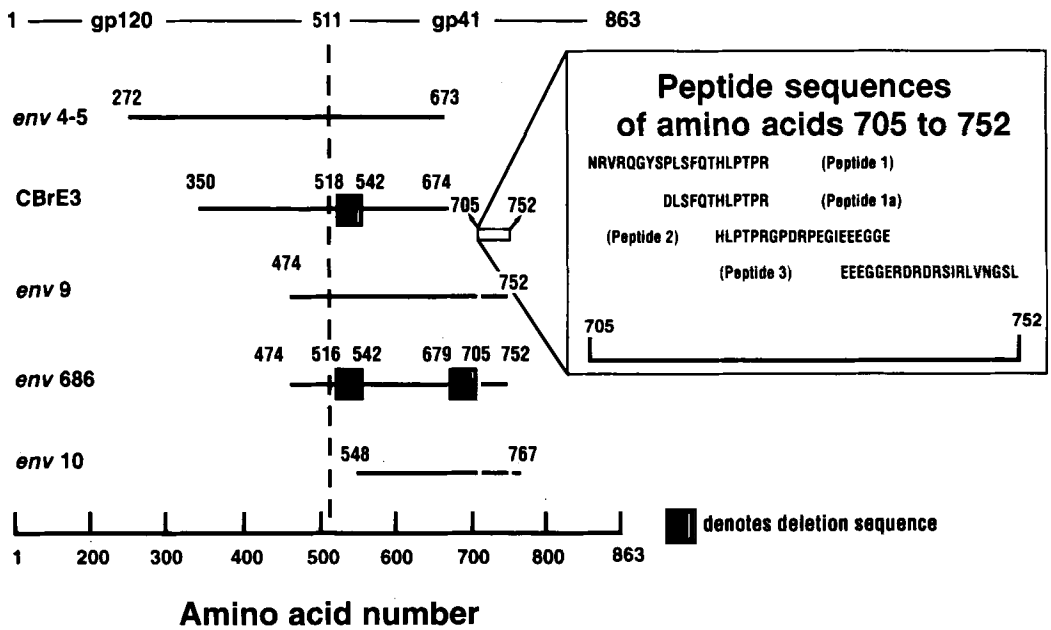

FIG. 1. Schematic representation of recombinant antigens (denoted at left) and synthetic peptides (detail) used in this study, relative to full length HIV-1 *env* precursor proteins (gp160).[27] Locations of gp120 and gp41 are indicated at top. Boxes in CBrE3 and *env* 686 indicate locations of deletions engineered into the recombinant antigens to enhance vector expression and specificity.

at 34 kDa. The treated antigen was electrophoresed and transblotted by standard procedures. We tested sera (from positive control and KC) or monoclonal antibody (anti-gp41 peptide) by the procedure for the Food and Drug Administration (FDA)-licensed WB assay (Cambridge Biotech, Worcester, MA).

Peptide analysis

Three synthetic peptides (Peptides 1, 2, and 3) were generated (ICI BioProducts, Wilmington, DE), corresponding to the HIV-1 envelope region to which there appeared to be donor sera reactivity, according to recombinant antigen EIA analysis (i.e., amino acids 705-752; see Results). The peptides were 20 amino acids long and overlapped each other by 6 amino acids (Fig. 1, detail). Peptides were dissolved in phosphate-buffered saline (PBS), with a final pH of 7.0. To improve solubilization, we initially dissolved basic peptides in 10-percent acetic acid before adding them to PBS; we initially dissolved acidic peptides in dilute ammonium hydroxide before adding them to PBS. The peptides were used in competition studies in which we mixed each donor or seroconversion sample (see below) with various concentrations of peptide or PBS and incubated the mixtures for 30 minutes at room temperature. The entire volume (sample and peptide) was then tested by EIA or WB. Concentrations of peptides used in competition experiments ranged from 21.5 µmol per mL to 215 pmol per mL. On the basis of test results with Peptide 1, an additional peptide (1A) was generated to define further the specific epitope within Peptide 1 to which the donor sera react. This peptide was diluted in PBS to a final concentration of 1 µg per mL and used in competition studies as described above.

Other assays

Immunofluorescence assay (IFA) testing of samples from KC, IRW-1, and IRW-2 was performed by using an FDA-licensed HIV-1 IFA (Fluorognost, Waldheim, Vienna, Austria), according to the manufacturer's directions. We performed p24 antigen testing on donor sera using an FDA-licensed antigen-capture EIA (Abbott Laboratories, Abbott Park, IL) according to manufacturer's directions. HIV-1 culture and polymerase chain reaction (PCR) assay were performed on peripheral blood mononuclear cells as described elsewhere.[17]

Seroconversion samples

We tested selected samples from commercial seroconversion panels to determine if the HIV-1 gp41 peptide (Peptide 1) that inhibited *env* reactivity of the donor sera also competes with specific HIV-1 *env* antibodies appearing during early seroconversion. These included samples BBI-K6, -C6, and -J3 (Boston Biomedical Inc, West Bridgewater, MA). We selected as the seroconversion sample the first sample that tested positive in the Cambridge Biotech HIV-1 WB by current interpretative criteria.

Analysis of donor HIV-1 WB data

Within the American Red Cross system, all donor samples found to be repeatably reactive for HIV in EIA are submitted to the American Red Cross National Reference Laboratory for Infectious Diseases (Rockville, MD). Each sample is evaluated by use of the Cambridge Biotech HIV-1 WB (distributed by Ortho Diagnostics, Raritan, NJ), according to the manufacturer's instructions. The results, including a notation of the observed band patterns, are maintained in a computerized database. We searched this database to determine the total number of samples with positive interpretations under current interpretive criteria and to determine the number of those samples displaying *env*-only or *env* p24-only diagnostic band patterns. The results were grouped according to screening EIAs employed at donor centers.

Table 1. *Summary of blood center testing data on four blood donors with suspected false-positive WBs*

Sample and date	Abbott HIV-1 S:C*	Abbott HIV-1/2 S:C	Genetic Systems HIV-1, -2, or -1/2 S:C	CBC† HIV-1 WB	RIBA HIV-1	RIBA HIV-1/2	IFA	p24 antigen	Culture	PCR
KC										
4/91	1.02	2.47	0.22 (HIV-1)	Pos‡ gp160/120,gp41	Neg§	Indll gp41	Neg	Neg	ND¶	ND
5/91	0.88	ND	0.22 (HIV-1)	Pos gp160/120,gp41	ND	ND	Neg	Neg	ND	ND
7/91	0.53	ND	0.14 (HIV-1)	Ind gp160/120	ND	ND	Neg	Neg	Neg	Neg
IRW-1										
9/93	ND	1.8	Neg (HIV-2)	Pos gp160/120,gp41,p24	ND	Ind gp41	Wk R**	ND	ND	ND
10/93	ND	1.5	ND	Pos gp160/120,gp41,p24	ND	ND	ND	ND	ND	Neg
5/95	ND		ND	Pos gp160/120,gp41,p24	ND	Ind gp41	ND	ND	ND	Neg
IRW-2										
6/93	ND	1.7	Neg (HIV-2)	Pos gp160/120,gp41	ND	Ind gp41	Wk R	ND	ND	ND
7/93	ND	1.2	ND	Pos gp160/120,gp41	ND	ND	ND	ND	ND	Neg
5/95	ND		ND	Pos gp160/120,gp41	ND	Ind gp41	ND	ND	ND	Neg
ARC-SEM										
4/95	ND	1.40	ND	Pos gp160,gp41,p17	ND	ND	ND	ND	ND	ND
5/95	ND	1.01	ND	Pos gp160,gp41,p17	ND	Neg	ND	Neg	ND	Neg

* Signal-to-cutoff ratio of EIA ODs.
† Cambridge Biotech Corporation. All bands were 1+ intensity or greater, unless indicated.
‡ Positive.
§ Negative.
ll Indeterminate.
¶ Not done.
** Weakly reactive.

Results

The four donors were identified by the individual blood centers as having possibly false-positive WBs, on the basis of the donors' denial of HIV risk factors and the restricted reactivity of WBs performed on both the index donations and follow-up specimens. Table 1 presents the results of all tests performed by the blood centers or blood center reference laboratories on serial samples from the four donors with suspected false-positive WBs. Figure 2 shows the Cambridge Biotech HIV-1 WB results on the index and follow-up specimens from the four blood donors.

Donor KC was identified in April 1991 after making a donation that reacted in a screening anti-HIV-1 EIA with a WB showing 1+ gp41 and gp120/160 bands. The index donation was subsequently tested with a licensed anti-HIV-1/2 EIA (Abbott), and it reacted. The sample showed discrepant reactivity to recombinant *env* antigens in RIBAs, failing to react to the *env* 4-5 antigen in RIBA HIV-1 while having strong reactivity to the *env* 10 antigen contained in RIBA HIV-1/2. This was the first suggestion that *env* reactivity may be restricted to the N-terminal region of gp41 (Fig. 1). Two follow-up specimens from KC showed declining EIA reactivity but persistent *env* reactivity in WB. All three serial specimens tested negative in the IFA and p24 antigen EIA. HIV culture and PCR were performed on the last follow-up specimen, and they were negative.

Donors IRW-1 and IRW-2 were identified in 1993 after making donations screened as EIA-reactive in anti-HIV-1/2 EIA; their WBs were interpreted as positive (gp160/120, gp41, and p24 and gp160/120 and gp41, respectively) and the anti-HIV-2 EIAs showed no reaction. Sera from these index donations tested weakly reactive in IFA and indeterminate in RIBA HIV-1/2 with a gp41 (*env*-10)-only pattern. Follow-up specimens collected approximately 4 weeks after the index donations had reactivity in anti-HIV-1/2 EIA and WB that was similar to that of the index donations. Peripheral blood mononuclear cells from these follow-up specimens tested negative for HIV DNA in PCR. Additional follow-up specimens collected more than 18 months after the index donations tested EIA-negative and PCR-negative but yielded persistent *env* reactivity on WB and RIBA HIV-1/2.

Donor ARC-SEM tested anti-HIV-1/2 EIA-reactive in April 1995. The donor was classified as WB-positive on the basis of 1+ reactivity to gp41 and gp160; 1+ reactivity to p17 was also observed. A follow-up specimen collected approximately 6 weeks after the index donation remained weakly reactive in anti-HIV-1/2 EIA and technically positive in WB, with 1+ gp160, gp41, and p17 bands. The follow-up specimen tested negative for HIV in p24 antigen EIA and PCR.

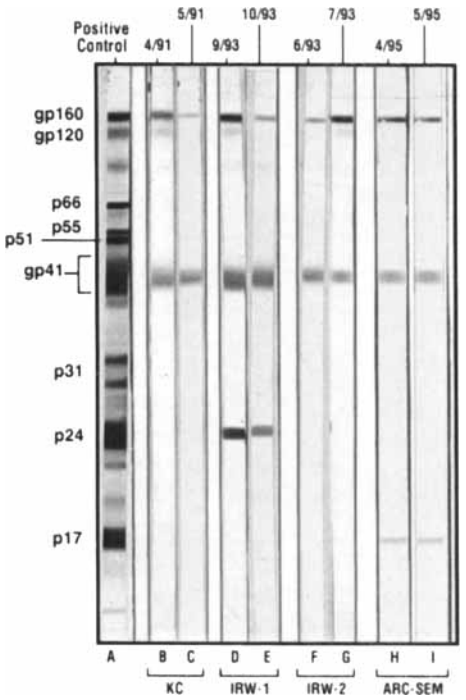

FIG. 2. HIV-1 WB results on serial specimens from each of the four blood donors with suspected false-positive WBs. Dates of specimen collection are indicated at the top. Designations of specific viral antigens are indicated to the left of corresponding bands on the positive control strip.

HIV-1 recombinant envelope antigen analysis

Table 2 summarizes the reactivity of the four index donation sera to five recombinant HIV-1 *env* antigens, as assayed by EIA. One donor sample (ARC-SEM) did not react to any recombinant proteins tested; the epitope to which this sample reacts therefore appears either to be located at the N-terminus of gp41 (i.e., beyond amino acid 767, the last amino acid found on *env* 10; see Fig. 1) or to require glycosylated or conformational determinants not expressed in the recombinant antigens. Three donor samples (from KC, IRW-1, and IRW-2) reacted to *env* 9, *env* 686, and *env* 10, but not to *env* 4-5 and CBrE3 recombinant proteins. As noted above, we also observed the reactivity of these three sera to *env* 10 in RIBA HIV-1/2 (Table 1). The epitope to which these three donor samples react therefore appears to be located between amino acids 705 and 752 (Fig. 1).

WB component analysis

We tested the sample from KC with WB strips prepared with deglycosylated and denatured HIV-1 antigen; it reacted only to the 34-kDa deglycosylated monomer form of gp41 (Fig. 3). No reactivity to the gp120 antigen was observed. Therefore, we attributed the apparent reactivity of this donor sample to gp160 and gp120 bands on standard WB strips to its reactivity to the monomer, trimer, and tetramer forms of gp41.

Inhibition analysis of gp41 peptide

To further map the epitope specificity of the three sera that reacted with the recombinant gp41 antigens, we generated three peptides spanning amino acids 705 to 752 and used them in competition studies employing the *env* 686 and *env* 10 EIAs. Incubation with one of the three gp41 peptides (Peptide 1) inhib-

Table 2. *Reactivity to recombinant HIV envelope antigen EIAs of index donation sera from four donors with* env*-only or* env*/p24-only WBs*

	EIA reactivity*				
Sample	*env* 4-5	CBrE3	*env* 9	*env* 686	*env* 10
KC	0.030	0.033	0.441†	0.636†	0.567†
IRW-1	0.055	0.025	0.771†	0.865†	0.896†
IRW-2	0.007	0.006	0.674†	0.621†	0.738†
ARC-SEM	0.007	0.027	0.017	0.027	0.048

* Values are in absorbance units. Reactivity is defined as ≥0.200 units.
† Reactive.

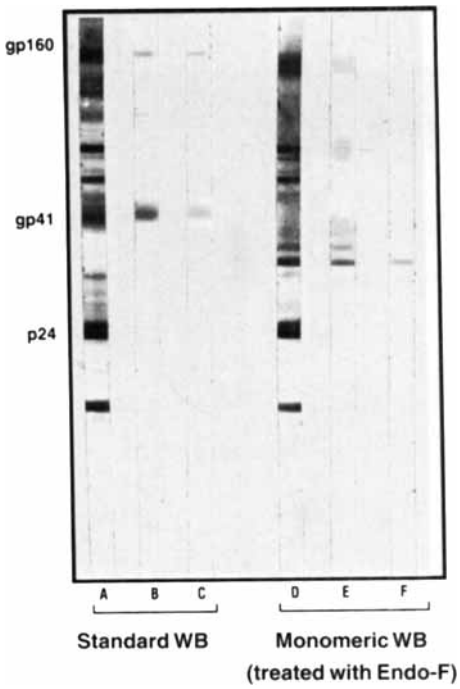

FIG. 3. Reactivity of KC's and control's antisera to WB strips prepared with standard versus Endo-F-treated and denatured HIV-1 (monomeric) viral lysate. Lanes A and D, positive control sera; Lanes B and E, antisera to gp41 peptide; Lanes C and F, Donor KC. Note that the sample from KC yielded reactivity similar to that of the gp41 peptide antisera on the deglycosylated and denatured WB strips, that is, a single 34-kDa band corresponding to deglycosylated monomer.

ited *env* reactivity of the samples from KC, IRW-1, and IRW-2 (Table 3). In contrast, incubation of the sample from KC with the other two peptides resulted in no depression of *env* 686 or *env* 10 EIA signal at any concentration tested. For the sample from KC, reactivity to *env* 9 and whole viral lysate EIAs was also significantly reduced (85-95% OD depression) at Peptide 1 concentrations of 2.15 μ*M* (not shown). In competition studies involving WBs, *env* reactivity of the samples from KC, IRW-1, and IRW-2 was reduced to negative at Peptide 1 concentrations of 2.15 to 21.5 μ*M* (Fig. 4). It should be noted that, for the sample from IRW-2, the coexisting nonspecific reactivity to p24 was not affected by peptide incubation. We saw no reduction in *env* band intensity when we tested three early seroconversion samples (BBI C-8, F-3, and K-8 in Fig. 4) by WB after incubation with Peptide 1 at 21.5 μ*M* concentrations, which indicates that the early immune response to HIV infection is directed at epitopes of gp41 other than those represented by Peptide 1.

Prevalence of env*-only and* env*/p24-only WBs among blood donors*

To estimate the rate of identification of nonspecific *env*-reactive and *env*/p24-reactive blood donors over time, we compiled HIV-1 WB data from the American Red Cross National Reference Laboratory for Infectious Diseases. Throughout this period, a single, FDA-licensed WB kit (Cambridge Biotech) was employed for confirmatory testing; this test has not been modified since its initial licensing in 1988 (although interpretative criteria have changed). Table 4 presents these results by time periods defined by the EIA employed in donor screening. The

Table 3. *Competition studies employing gp41 peptides (20 amino acids) to further define the epitope specificity of donor sera reactivity with recombinant antigens* env *686 and* env *10*

Donor sample	Peptide		EIA reactivity	
	Number	Concentration (μ*M*)	*env* 686	*env* 10
KC	None		0.636	0.567
	1	2.15	0.080†	0.078†
	1	21.5	0.035†	0.057†
	2	2.32	0.547	0.549
	2	23.2	0.538	0.751
	3	2.29	0.619	0.592
	3	22.9	0.594	0.574
IRW-1	None		QNS*	0.616
	1	2.15	QNS	0.145†
	1	21.5	QNS	0.045†
IRW-2	None		0.890	0.598
	1	2.15	0.533	0.323
	1	21.5	0.143†	0.063†

* Quantity not sufficient.
† Results showing inhibition below cutoff (0.200 OD units).

marked increase in the rate of detection of donations with *env*-only (gp41 plus gp120/160) and, to a lesser extent, *env*/p24-only patterns in 1991 coincided with implementation of a modified viral lysate EIA (enriched with viral lysate-derived p24 and gp41 antigens [3A11, Abbott Laboratories]) in routine donor screening.

The increased rate of donations with *env*/p24-only band patterns in mid-1992 coincided with introduction of a recombinant DNA-derived, p24 and gp41 antigen-based HIV-1/2 EIA (3A77, Abbott Laboratories). Thus, increased detection of donor sera with these limited reactivity patterns on WB appears to be related to changes in the screening EIAs, rather than to changes in the WB. During the most recent period, approximately 3.8 percent of all positive WB results had restricted WB reactivity that was consistent with possible false-positive patterns. These numbers may overestimate the rate of false-positive results, however, because these patterns are also seen during early HIV seroconversion, and follow-up data were not available for these donors.

Discussion

There are a number of possible causes of false-positive WB results. First, sample labeling errors are known to occur; such errors are the primary source of reports of so-called seroreversion.[32] A second possible cause is cross-contamination of a negative sample with a positive sample during blood processing or serum testing.[33] Some WB-positive samples can be diluted in excess of 1 in 1,000,000 and still show reactivity to the gp160 band in WB (Sayre KR, unpublished observations, 1991). Such samples would usually be classified as indeterminate on the basis of isolated gp160/120 reactivity. However, because there is approximately 15-percent probability that an HIV-negative sample will evidence nonspecific reactions to p24 on WB,[13,28] a proportion of such contaminated samples could present a gp160/120-plus-p24 banding pattern consistent with early seroconversion. Concern

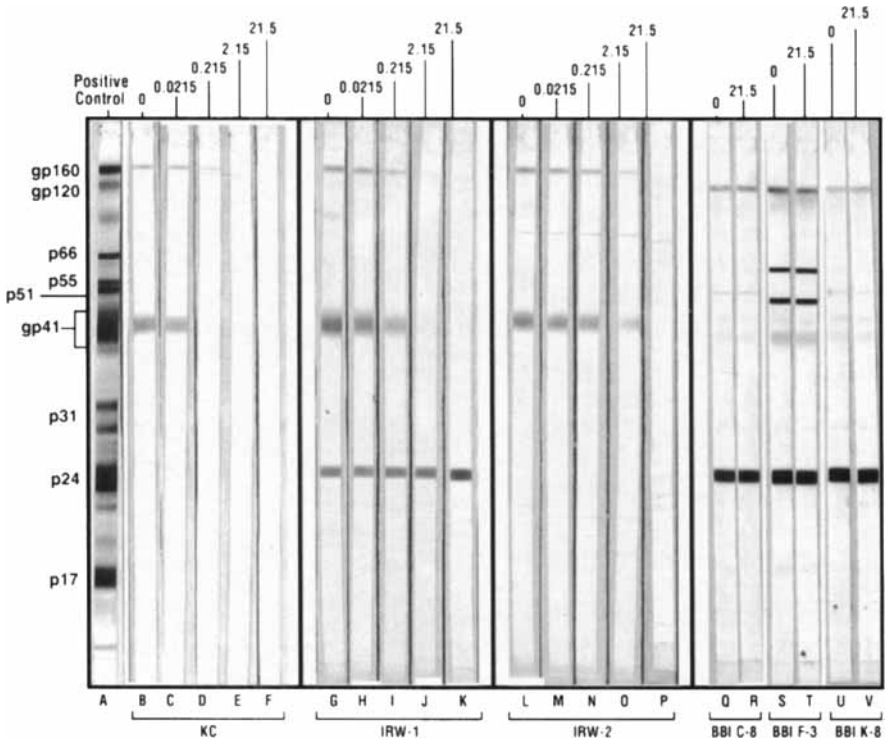

FIG. 4. Competition study involving incubation of donor and control sera with synthetic Peptide 1, followed by standard WB analysis. Concentrations of Peptide 1 are indicated above each strip. BBI C-8, F-3, and K-8 are early HIV-1 seroconversion samples. Complete inhibition of *env* reactivity at higher peptide concentrations is noted for the sera from donors with false-positive WB results, whereas no inhibition of *env* reactivity is observed with the HIV-1 seroconversion samples.

about the two previous causes of false-positive HIV WBs has led the Association of State and Territorial Public Health Laboratory Directors to recommend that a dedicated second or follow-up sample be requested and tested to verify the result whenever a donor or patient exhibits a first-time HIV-positive WB.[34] We strongly endorse this recommendation.

A third cause of false-positive WBs is the misinterpretation of bands. In particular, samples with strong nonspecific reactivity to *gag* antigens can present multiple bands on WB, including p17, p24, p32, p46 (a sharp band just above the diffuse gp41 band), and p55.[35] Such reactivity can be misinterpreted as p17, p24, p31, gp41, and p55 bands, and this results in an overall positive interpretation by current criteria. Laboratorians using commercial kits and experienced in WB interpretation are aware that the gp160 and gp120 multimeric bands are more sensitive to anti-gp41 than is the gp41 monomeric band and that gp160 and/or gp120 bands are, therefore, always visualized prior to a gp41 band during early seroconversion (see strips Q, S, and U in Fig. 4). Thus, the presence of a sharp band in the vicinity of gp41 and in the absence of gp160 or gp120 bands should alert workers to the likely false-positive nature of such seroreactivity.

Our results further document a fourth source of false-positive HIV-1 WB results, which is the reproducible but nonspecific reactivity to monomeric and multimeric forms of gp41, with or without coincidental nonspecific reactivity with p24.[26,36] The four donors we studied all lacked HIV risk factors and were proven by HIV PCR and, in two cases, culture and p24 antigen analyses not to be infected. In three of four samples studied, *env* reactivity was restricted to an epitope located within amino acids 705 to 752 of gp41. In the fourth sample, the reactive epitope may be located at the extreme N-terminus of gp41. Preliminary studies suggest that the basis for this cross-re-

Table 4. *Frequency of identification of blood donors with probable false-positive WB patterns*

Anti-HIV EIA	Years	Number of positive WBs observed*	Probable false-positive WBs (%)		
			env-only	*env*/p24-only	Total
HIV-1 lysate	1988-1990	776	2 (0.3)	2 (0.2)	4 (0.5)
HIV-1 lysate gp41-enhanced	1991-1992	683	41 (6.0)	11 (1.6)	55 (8.0)
HIV-1/2 p24/gp41 rDNA	1992-1993	783	17 (2.2)	30 (3.8)	47 (6.0)

* According to revised interpretative criteria.

activity with HIV-1 gp41 epitopes may be infection by paramyxoviruses, naturally occurring carbohydrate antibodies in human sera, or autoantibodies against cellular proteins.[37-39]

We documented an increase in the rate of observation of *env*-only and *env*/p24-only WB results over the past 6 years, which appears to be related to the implementation of new donor-screening EIAs (e.g., second-generation anti-HIV-1 and third-generation anti-HIV-1/2 screening assays, Abbott Laboratories). These assays have been shown to have significantly increased sensitivity to *env* and *gag* antibodies, such that infected persons are identified earlier during seroconversion.[5,6] Unfortunately, this enhanced sensitivity appears also to have resulted in the detection of specimens with nonspecific reactivity, evidenced by *env*-only and *env*/p24-only patterns on WB. The enhanced sensitivity of the new EIAs to early HIV-1 infection is critical for transfusion safety and counterbalances the problem of inadvertent detection of samples with false-positive WB patterns documented here. Nonetheless, it is important that WB interpretative criteria be revised so that the patterns discussed herein are classified as indeterminate rather than positive, or that measures be introduced to identify possible false-positive band patterns so that proper donor notification occurs. After reviewing the findings of the present study with CDC and FDA scientists, we decided that a revision of WB interpretative criteria is not warranted at present. The rationale is that the public health benefits of correct classification of a large number of infected persons as positive under the revised criteria (rather than their misclassification as indeterminate under the earlier criteria) outweigh the rare occurrence of false-positive WBs. On the other hand, an approach for "flagging" possible false-positive patterns was widely endorsed.

We therefore recommend that subjects with WB patterns associated with false positivity (i.e., those lacking p31 *or* gp120/160 band reactivity) be informed that, although the results on their specimen were technically positive, these patterns have been seen in low-risk blood donors who have been determined through follow-up testing not to be infected with HIV. For such persons, further testing should be performed on a specimen collected at least 2 weeks after the index specimen. Serum or plasma from this follow-up specimen should be tested for anti-HIV by licensed WB in parallel with the retesting of an aliquot of frozen sera from the index donation. Additional tests performed on the index and/or follow-up specimen, such as p24 antigen EIA, virus culture, or PCR for HIV-1 RNA or DNA, may also be useful, if available. Because the IFA may react with *env* false-positive samples, this test is of less utility in resolving these cases. If testing of follow-up samples indicates no change or loss of bands in WB (i.e., an absence of seroconversion), as well as an absence of HIV antigen, nucleic acids or virus (if such tests are performed), the donor should be notified that the earlier results probably represent a false-positive finding. A recommendation for final follow-up testing at 6 months should be given, consistent with CDC recommendations.[2] In contrast, if parallel WB testing shows an increase in the number of bands (including p31 and gp120/160) and a marked increase in band intensities with the follow-up sera relative to the index donation sera, or if direct virus assays are positive, the donor should be notified of the probability of recent HIV infection, with evolving seroconversion. An additional follow-up sample, obtained 4 to 6 weeks after the first follow-up sample (that is, 8-12 weeks after the index donation) and retested as above, should be used to corroborate a final diagnosis of HIV infection.

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