CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, Sept. 1997, p. 598–603 Vol. 4, No. 5
1071-412X/97/$04.00+0

Synthetic-Peptide-Based Enzyme-Linked Immunosorbent Assay for Screening Human Serum or Plasma for Antibodies to Human Immunodeficiency Virus Type 1 and Type 2

LISETTE GONZALEZ,[1] ROSANNE W. BOYLE,[1] MEILUN ZHANG,[1] JONATHAN CASTILLO,[1] SUSAN WHITTIER,[2] PHYLLIS DELLA-LATTA,[2] LORRAINE M. CLARKE,[3] J. RICHARD GEORGE,[4] XINDE FANG,[1] JAMES G. WANG,[1] BARBARA HOSEIN,[1]* AND CHANG YI WANG[1]*

United Biomedical, Inc., Hauppauge, New York 11788[1]; Presbyterian Hospital, Columbia-Presbyterian Medical Center, New York, New York 10032[2]; State University of New York Health Science Center at Brooklyn, Brooklyn, New York 11203[3]; and Laboratory Investigations Branch, Division of HIV/AIDS, Centers for Disease Control and Prevention, Atlanta, Georgia 30333[4]

Received 25 February 1997/Returned for modification 23 April 1997/Accepted 27 June 1997

A synthetic-peptide-based enzyme-linked immunosorbent assay (EIA) capable of screening for antibodies to both human immunodeficiency virus type 1 (HIV-1) and HIV-2 has been developed for use in blood banks and diagnostic laboratories. Microtiter wells are coated with two synthetic peptides, one corresponding to the highly conserved envelope region of HIV-1 and another corresponding to the conserved envelope region of HIV-2. Overall, sensitivity was 100% in 303 individuals diagnosed with AIDS and 96 individuals diagnosed with AIDS-related complex, 14.8% in a study of 500 high-risk group members, 99.9% in 600 EIA repeatedly reactive (RR)-HIV-1 Western blot (WB)-positive repository specimens, and 100% for 222 geographically diverse HIV-1 specimens and 216 confirmed HIV-2-positive specimens evaluated. The specificity was determined to be 99.72% for a total of 13,004 serum and plasma samples from random volunteer donors evaluated across five blood banks. Forty donors who were found to be EIA RR-WB indeterminate but nonreactive on the United Biomedical, Inc., test (UBI HIV 1/2 EIA) were prospectively followed as an additional measure of specificity. None of the 40 low-risk cases evolved into a positive WB pattern at follow-up. The sensitivity and specificity of this new assay are comparable to those of other Food and Drug Administration-licensed HIV-1 and HIV-1–HIV-2 assays that are currently available in the United States. The UBI HIV 1/2 EIA affords laboratories another choice in the detection of antibodies for HIV-1 and HIV-2 with a test based on an alternative antigen format.

The utility of synthetic peptides in immunodiagnosis has been previously reported (15, 16). Synthetic peptides offer the advantage of eliminating nonspecific reactions resulting from cross-reactivity of antibodies in the specimen with host cell antigens which are copurified with cell-derived virus (8, 10, 13) or *Escherichia coli*-derived recombinant products as the immunoadsorbents (2, 5). The UBI human immunodeficiency virus type 1 (HIV-1)–HIV-2 enzyme-linked immunosorbent assay (EIA) is the first synthetic-peptide-based combination assay for the detection of HIV-1 and HIV-2 antibodies to be licensed by the Center for Biologics Evaluation and Research of the Food and Drug Administration. In this report, we present the results of the evaluation of the UBI HIV 1/2 EIA in the determination of the assay's sensitivity and specificity.

MATERIALS AND METHODS

Source and characterization of clinical materials. All samples were evaluated in parallel with a licensed HIV-1 EIA. Repeatedly reactive samples were further tested by HIV-1 Western blotting and/or radioimmunoprecipitation assay. Specimens that were nonreactive in both screening assays were not further characterized by supplemental tests. A total of 402 patient samples were selected on the basis of a documented diagnosis of AIDS ($n = 306$) or AIDS-related complex (ARC) ($n = 96$) and were unbiased with respect to results of the licensed HIV-1 EIA. High-risk group samples were obtained from 500 random individuals attending an AIDS prevention clinic at the State University of New York Health Science Center at Brooklyn. A total of 600 confirmed seropositive samples from volunteer blood donors were selected on the basis of presenting repeatedly reactive and positive results in a licensed HIV-1 EIA and an HIV-1 Western blot assay, respectively. Confirmed HIV-positive samples from geographically diverse areas were obtained from the Thai Red Cross, Bangkok, Thailand, and additional samples were evaluated at the Developmental Technology Section, Division of HIV/AIDS, Centers for Disease Control and Prevention, Atlanta, Ga. Twelve HIV-1 seroconversion panels were obtained from Boston Biomedica, Inc. (BBI; West Bridgewater, Mass.), and North American Biologicals, Inc. (NABI; Miami, Fla.), and were tested at United Biomedical, Inc., with the UBI HIV 1/2 EIA. Seroconversion data for other manufacturers' assays have been previously reported on the data sheets accompanying the panels.

Plasma and serum samples from 13,004 random volunteer donors at five regionally distinct blood banks were tested with the UBI HIV 1/2 EIA to determine the specificity of the assay. All initially reactive samples were retested in duplicate. Any samples reported as reactive in either one or both of the two repeat assays were considered repeatedly reactive and further tested by a licensed HIV-1 Western blot assay and, when indeterminate, by an investigational HIV-1 radioimmunoprecipitation assay.

A prospective follow-up of 40 blood donors previously determined to be HIV-1 EIA repeatedly reactive and HIV-1 Western blot indeterminate (index donation) was conducted to determine if any of the indeterminate Western blots would evolve into a positive blot pattern over time. Follow-up was undertaken by obtaining subsequent samples from each of the 40 donors studied across three regionally distinct blood banks. All 40 of the donors were found to be UBI HIV 1/2 EIA nonreactive on the index donation. Follow-up samples were evaluated by the laboratory test of record and HIV-1 Western blotting at the sites. The UBI HIV 1/2 EIA was performed at United Biomedical, Inc.

A panel of samples containing potentially interfering substances was assembled from commercially prepared sources and repositories held by several collaborators. Any samples resulting in an initially reactive determination were retested in duplicate. Positivity of all repeatedly reactive samples was confirmed by a licensed HIV-1 Western blot assay.

* Corresponding author. Mailing address: United Biomedical, Inc., 25 Davids Dr., Hauppauge, NY 11788. Phone: (516) 273-2828. Fax: (516) 273-1717.

TABLE 1. Detection of antibodies to HIV-1 in AIDS and ARC patient populations, high-risk groups, and confirmed-positive asymptomatic individuals

Group	Test[a]	Results of:					
		EIA reactivity[b]		HIV-1 Western blot analysis			Sensitivity (no. positive/total)
		RR	NR	Positive[c]	Ind.[d]	Negative	
AIDS	UBI HIV-1 EIA	69	0	69	0	0	69/69 (100%)
	Abbott HIV-1 EIA	237	0	234	3	0	237/237 (100%)
	UBI HIV 1/2 EIA	306	0	303 (99.0%)	3 (1.0%)	0	306/306 (100%)
ARC	UBI HIV-1 EIA	22	0	22	0	0	22/22 (100%)
	Abbott HIV-1 EIA	74	0	71	3	0	74/74 (100%)
	UBI HIV 1/2 EIA	96	0	93 (96.9%)	3 (3.1%)	0	96/96 (100%)
High risk[e]	CBC HIV-1 EIA	73	427				73/500 (14.6%)
	UBI HIV 1/2 EIA	74[f]	426	73 (14.6%)	0	0	74/500 (14.8%)
Repeatedly reactive, Western blot-positive asymptomatic individuals	OTC HIV-1 EIA	58	0	58	0	0	58/58 (100%)
	GS HIV-1 EIA	136	0	136	0	0	136/136 (100%)
	Abbott HIV-1 EIA	268	0	268	0	0	268/268 (100%)
	IAF HIV-1 EIA	54	0	54	0	0	54/54 (100%)
	CBC HIV-1 EIA	20	0	20	0	0	20/20 (100%)
	ENI HIV-1 EIA	7	0	7	0	0	7/7 (100%)
	GS HIV-1–HIV-2 EIA	52	0	52	0	0	52/52 (100%)
	Abbott HIV-1–HIV-2 EIA	5	0	5	0	0	5/5 (100%)
	UBI HIV 1/2 EIA	599[g]	1	600[h]	0	0	599/600 (99.8%)

[a] CBC, Cambridge Biotech; GS, Genetic Systems; OTC, Organon Teknika; IAF, IAF Biochem; ENI, Electronucleonics, Inc.
[b] Specimens were scored repeatedly reactive (RR) by the criteria indicated in the manufacturer's inserts. NR, nonreactive.
[c] Western blot-positive specimens were interpreted according to manufacturer's criteria.
[d] Western blot-indeterminate (Ind.) specimens are those presenting band patterns that did not correspond to a positive interpretation.
[e] High-risk group members included 500 attendees of an AIDS prevention clinic.
[f] The one sample that was HIV-repeatedly reactive by UBI HIV 1/2 EIA and nonreactive by licensed HIV-1 EIA was HIV-1 Western blot negative and nonreactive by licensed HIV-2 EIA.
[g] One sample yielded discordant HIV-1 EIA results at the facility using licensed test kits from two different manufacturers and was reported as Western blot positive. In subsequent testing, the sample demonstrated a negative result by licensed IFA. Follow-up specimens from this individual were not available.
[h] Includes five specimens that were originally reported as Western blot indeterminate and have been reinterpreted as Western blot positive according to current ASTPHLD-Centers for Disease Control and Prevention criteria.

Solid-phase EIA for the detection of antibodies to HIV-1 and HIV-2. The UBI HIV 1/2 EIA is a solid-phase assay in which synthetic peptide antigens are adsorbed onto the reaction microplate. Aliquots of specimen diluted 1:21 are added to the reaction microplate wells, and the plate is incubated at 37 ± 2°C for 30 ± 2 min. Antibodies to HIV-1 and/or HIV-2, if present, will bind to the specific peptide antigens. After a thorough washing of the plate has been performed to remove unbound antibodies and other specimen components, an aliquot of a standardized preparation of goat anti-human immunoglobulin G antibodies conjugated to horseradish peroxidase is added to each well. The reaction microplate is then returned to the incubator (37 ± 2°C) for 15 ± 1 min. This enzyme-antibody conjugate will bind to any antigen–anti-HIV-1 and antigen–anti-HIV-2 antibody complexes present. The wells are again washed to remove unbound enzyme-antibody conjugate, and a solution of *o*-phenylenediamine (0.67 mg/ml) containing hydrogen peroxide is added to the wells; then the plate is incubated at 37 ± 2°C for 15 ± 1 min. A yellow-orange color will develop with an intensity proportional to the amount of HIV antibodies present in the specimen. The enzyme–*o*-phenylenediamine reaction is stopped by the addition of stop solution (1.0 M sulfuric acid). The absorbance value of each microwell is read by an EIA plate reader at a wavelength of 492 ± 2 nm. Each run includes wells for two negative reactive controls, two HIV-1 strongly reactive controls, and two HIV-2 weakly reactive controls. The cutoff value used to determine seropositivity was calculated based on statistical analysis as follows: (0.1 × mean strongly reactive control value) + mean negative reactive control value. The total incubation time for the assay is 60 min, which is less than the 90 to 150 min required for other licensed HIV-1–HIV-2 assays. The total time needed to perform the assay is approximately 110 min.

Diagnostic assay kits. The Abbott (Abbott Park, Ill.) HIVAB HIV-1 assay is a whole-cell lysate EIA, and the Abbott HIVAB HIV-1–HIV-2 recombinant DNA (rDNA) EIA uses recombinant HIV-1 Env and Gag and HIV-2 Env proteins. The Recombigen (Env and Gag) HIV-1 recombinant EIA (Cambridge Biotech, Worcester, Mass.) uses a recombinant envelope protein, and the Genetic Systems (Redmond, Wash.) HIV-1–HIV-2 and HIV-2 EIAs use a whole viral lysate as the antigen. All assays were performed according to the manufacturers' instructions.

Western blotting. Western blotting for confirmation of the presence of anti-HIV-1 antibodies was performed with licensed HIV-1 immunoblotting kits from Bio-Rad Laboratories (Hercules, Calif.) or Cambridge Biotech. The investigational HIV-2 Western blot assay was from Genetic Systems. The manufacturers' instructions for the performance of immunoblotting assays were followed. Positivity of blots was determined according to manufacturers' criteria.

Statistics. Confidence intervals were determined through the use of the StatXact program.

RESULTS

Sensitivity. Antibodies to HIV-1 were detected by the UBI HIV 1/2 EIA in 306 of 306 patients with a clinical diagnosis of AIDS and in 96 of 96 patients with a clinical diagnosis of ARC (Table 1). These results were consistent with those of the licensed HIV-1 assays performed, the licensed UBI HIV-1 EIA and the Abbott HIVAB HIV-1 EIA. Specimens from three individuals diagnosed with AIDS and from three individuals diagnosed with ARC were reported as HIV-1 Western blot indeterminate. The remaining specimens were all confirmed to be HIV-1 Western blot positive.

The testing of a total of 500 samples from high-risk individuals attending an AIDS prevention clinic demonstrated that the UBI HIV 1/2 EIA was repeatedly reactive for all 73 specimens from this population that were repeatedly reactive by the licensed Cambridge Biotech HIV-1 EIA (Table 1). All 73 repeatedly reactive specimens were also HIV-1 Western blot positive. One additional sample was found to be repeatedly reactive with the UBI HIV 1/2 EIA and repeatedly nonreactive with the Cambridge Biotech HIV-1 EIA. This sample was reported as being HIV-1 Western blot negative and found to be nonreactive in the Genetic Systems HIV-2 EIA. No bands were reported for the investigational Cambridge Biotech HIV-2 Western blot. Follow-up samples were not available as this was a nonlinked study.

In the evaluation of a panel of previously screened specimens from asymptomatic individuals that were repeatedly reactive by a licensed HIV-1 EIA and positive by a Western blotting assay, the UBI HIV 1/2 EIA was repeatedly reactive for all but one Western blot-positive sample (Table 1). The one Western blot test-of-record-positive donor sample demonstrated discordant HIV-1 EIA results when tested with licensed kits from two different manufacturers and was negative by the licensed Fluorognost HIV-1 immunofluorescence assay (IFA). Follow-up specimens from this individual for both Western blotting and additional EIA test analyses were not available.

The UBI HIV 1/2 EIA was reactive for all samples in a study of 222 Western blot-confirmed HIV-1-seropositive individuals representing the following diverse geographical areas: Southeast Asia (n = 96), South America (n = 48), Central America (n = 45), and South Africa (n = 33).

A critical parameter in the determination of the sensitivity of an assay is its ability to identify specimens derived from individuals with early infection. The UBI HIV 1/2 EIA and three licensed HIV-1 EIAs were evaluated to determine their relative sensitivities in detecting early HIV-1 infections (Table 2). Sequential samples, collected at short intervals from eight individuals who seroconverted during the period, were tested in all four EIAs. Specimens were tested by the HIV-1 Western blot assay, and the results were interpreted by using the AST-PHLD-Centers for Disease Control and Prevention criteria (4). The UBI HIV 1/2 EIA performed substantially equivalently to the other licensed HIV-1 EIA test kits.

Studies on samples from commercially available seroconversion panels that demonstrate predominantly Gag reactivity on HIV-1 Western blots (Table 3) were conducted to evaluate the sensitivity of the test in view of the absence of the p24 antigen as one of the solid-phase antigens. The UBI HIV 1/2 EIA was found to be reactive with seroconversion panel members that in a licensed Western blot assay showed only a p24 band as the earliest parameter of seroconversion. To further clarify the sensitivity issue for the synthetic envelope antigen, the data for a total of 106 specimens for which there was no indication of the presence of antibody to gp41 on HIV-1 Western blots were extracted from the complete data set reported in the evaluation of the UBI HIV 1/2 EIA. The UBI HIV 1/2 EIA was repeatedly reactive for all 106 confirmed-positive specimens which lacked a gp41 band when subjected to a licensed Western blot assay.

A total of 216 HIV-2-seropositive specimens from West Africa, an area where HIV-2 infection is endemic, were evaluated. These samples were repeatedly reactive in the licensed Genetic Systems HIV-2 EIA and were confirmed by reactivity in an investigational HIV-2 immunoblot assay. All 216 samples tested were repeatedly reactive with the UBI HIV 1/2 EIA.

Specificity. The UBI HIV 1/2 EIA in the random-donor population yielded an initially reactive rate of 0.43% when a total of 13,004 voluntarily donated blood samples were tested across five blood banks. Of the initially reactive samples, 36 (64%) were repeatedly reactive, giving rise to a repeatedly reactive rate of 0.28% (Table 4). The specificity was thus 99.72%, with a 95% confidence interval of 99.62 to 99.81%.

A prospective follow-up study of 40 low-risk, nonautologous blood donors was conducted to evaluate the ability of the UBI HIV 1/2 to discriminate samples from noninfected individuals among sera that were indeterminate by HIV-1 Western blotting. These 40 blood donors were from three geographically distinct blood banks; 20, 18, and 2 were repeatably reactive by Cambridge Biotech Recombigen, Abbott HIVAB HIV-1–HIV-2 EIA, and Abbott HIVAB HIV-1 EIA, respectively. All 40 repeatably reactive samples were found to be negative by the UBI HIV 1/2 EIA and were indeterminate by HIV-1 Western blotting. These donors were followed for between 5 and 37 months, with a mean follow-up interval of 12.4 months. After the completion of the follow-up period, 27 and 13 donors were repeatedly reactive and nonreactive, respectively, in a licensed HIV EIA. The Western blot assay results for three of the donors repeatedly reactive in the Abbott HIVAB HIV-1–HIV-2 EIA, five of the donors nonreactive in the Abbott HIVAB HIV-1–HIV-2 EIA, and one of the donors nonreactive in the Abbott HIVAB HIV-1 EIA became negative by the end of the follow-up period. The same licensed EIA used to test the index sample was used throughout the follow-up interval for each donor. For all 40 cases, there was no evolution to a positive Western blot pattern and the UBI HIV 1/2 EIA remained negative at the end of the follow-up interval. Of the 31 samples that were Western blot indeterminate at the end of follow-up, 26 had Gag-only patterns, one had a Pol-only pattern, 3 had Gag and Pol bands, and one (followed for 23.5 months) had only a faint, atypically sharp gp41 Env band.

Additional evidence of test specificity was obtained by testing samples from persons with medical conditions unrelated to AIDS or HIV infection and samples containing potentially interfering substances. Among 228 such samples, including those from individuals with autoimmune conditions, viral diseases other than HIV, and compromised specimens (e.g., hemolyzed), 5 specimens were repeatedly reactive with the UBI HIV 1/2 EIA. Four of five repeatedly reactive specimens, one each reactive for anti-hepatitis B core, syphilis, lymphadenopathy, and lymphoma, were confirmed positive in a licensed HIV-1 Western blot assay. The one repeatedly reactive, HIV-1 Western blot-negative specimen had a signal-to-cutoff ratio (S/CO) of 1.76 in the UBI HIV 1/2 EIA and was positive for anti-human T-cell lymphotropic virus type 2 antibodies. Thus, the UBI HIV 1/2 EIA demonstrated a specificity of 99.6% (223/224) for samples from persons with medical conditions other than HIV infection and for samples containing potentially interfering substances.

DISCUSSION

The utility of synthetic peptides in immunodiagnosis has been previously reported (15, 16). The UBI HIV 1/2 EIA is the first combination assay for the detection of both HIV-1 and HIV-2 antibodies that solely utilizes synthetic peptides targeting the immunodominant regions of the HIV-1 and HIV-2 envelopes. There are only two other combination assays licensed for use in blood banks and diagnostic laboratories, the Abbott HIVAB HIV-1–HIV-2 (rDNA) EIA and the Genetic Systems HIV-1–HIV-2 EIA. These assays use rDNA and a whole-cell viral lysate, respectively, as the antigens adsorbed

TABLE 2. Detection of HIV-1 antibodies from seroconversion panels

Panel	Interval (days)	Results of licensed HIV-1 Western blot assay[b,c]	S/CO ratio determined by[a]:			
			UBI HIV-1–HIV-2 EIA	Abbott HIVAB-1 EIA[b]	Cambridge Recombigen HIV-1 EIA[b]	Organon Vironostika HIV-1 EIA[b]
SV0031 A-N	1	Negative	0.20	0.312	0.072	0.470
	13	Negative	0.16	0.230	0.097	0.404
	27	Negative	0.07	0.225	0.044	0.447
	47	Ind. (gp120-gp160)	0.13	0.795	0.267	0.820
	49	Ind. (gp160 ±)	0.48	0.830	0.722	0.691
	54	Positive	6.93	2.925	1.430	1.619
	56	Positive	9.33	3.343	1.311	1.592
SV0161 A-F	1	Negative	NT[d]	0.254	0.035	0.165
	5	Negative	0.10	0.231	0.039	0.391
	8	Negative	0.14	0.240	0.052	0.396
	12	Ind. (p24)	1.34	0.965	0.583	0.719
	16	Positive	7.27	3.038	0.864	1.583
	19	Positive	7.75	3.867	0.919	1.765
SV0111 A-G	1	Negative	0.12	0.270	0.164	0.261
	2	Ind. (gp120-gp160)	0.20	0.254	0.163	0.255
	8	Positive	1.37	1.145	1.581	0.529
	16	Positive	17.89	10.093	3.427	1.098
	20	Positive	17.89	10.502	3.427	5.020
SV0091 A-I	1	Negative	0.14	0.206	0.662	0.281
	3	Ind. (p24 ±)	0.14	0.166	0.743	0.608
	7	Ind. (p24, gp120 ± gp160 ±)	0.31	0.744	1.926	1.124
	10	Positive	3.46	1.942	2.782	1.124
	17	Positive	14.82	3.417	3.058	3.837
SV0081 A-F	1	Ind. (p24 ±)	0.13	0.299	0.176	0.271
	6	Ind. (p24 ±)	0.15	0.293	0.216	0.225
	23	Ind. (p24 ±)	0.11	0.306	0.203	0.265
	48	Positive	9.67	4.763	3.031	2.846
	61	Positive	10.19	7.947	2.612	4.856
SV0061 A-I	1	Negative	0.18	0.315	0.703	0.399
	3	Negative	0.17	0.261	0.643	0.419
	8	Ind. (p24)	1.02	0.608	1.346	0.649
	10	Positive	2.94	1.194	1.458	1.120
	16	Positive	8.92	1.680	1.506	1.854
SV0051 A-F	1	Ind. (p24 ±)	0.26	0.753	0.319	0.403
	7	Negative	0.20	0.341	0.234	0.442
	9	Ind. (p17 ±, p24 ±)	0.34	0.463	0.580	0.480
	14	Positive	>13.60	2.722	2.365	2.994
	16	Positive	>13.60	3.983	2.235	3.932
PRB 903 panel C	0	Negative	0.2	0.2	0.1	0.4
	7	Negative	0.2	0.2	0.6	0.4
	9	Ind. (fp24)	0.5	0.3	1.3	0.4
	14	Positive	6.1	0.6	2.1	1.0
	16	Positive	11.2	0.7	1.9	1.2
	21	Positive	17.2	1.6	1.6	2.1

[a] Positive results are boxed.
[b] Data for SV series panels were provided by NABI; data for the PRB panel were provided by BBI.
[c] Ind., indeterminate; antigens in parentheses are those evident on blot.
[d] NT, not tested.

onto the solid phase. In this report, we have presented the results of the evaluation of the UBI HIV 1/2 EIA in the determination of the assay's sensitivity and specificity.

The sensitivity of the UBI HIV 1/2 EIA was estimated to be 100% (402/402) with a 95% confidence interval of 99.3 to 100% in the AIDS and ARC groups. The results observed with the UBI HIV 1/2 EIA for samples from the high-risk population were comparable to those observed with the licensed lab-

TABLE 3. Detection of antibodies to HIV-1 in seroconversion panels with earliest seroconversion to Gag reactivity

Panel	Interval (days)	Results of licensed HIV-1 Western blot assay		S/CO ratio determined by[a]:			
		Band pattern[b]	Interpretation[c]	UBI HIV 1/2 EIA	Abbott HIVAB-1 EIA[d]	Cambridge Recombigen HIV-1 EIA[d]	Genetic Systems HIV-1–HIV-2 EIA[d]
PRB914 panel N	0	f24	Ind	2.5	1.9	1.3	0.8
	4	24, vf55	Ind	2.7	2.1	1.1	1.1
	7	24, f55	Ind	2.4	2.8	1.0	1.4
	25	18, 24, 55, vf160	Pos	5.8	5.4	1.4	2.8
	31	18, 24, 55, vf160	Pos	4.9	5.2	1.5	3.0
PRB911 panel K	6	No bands	Neg	0.3	0.3	0.2	0.1
	8	No bands	Neg	0.3	0.3	0.2	0.1
	13	24	Ind	0.9	0.3	1.2	0.2
	15	24	Ind	2.8	0.4	2.7	0.4
	20	24, 160	Pos	6.8	0.9	4.0	1.1
	22	24, f41, f55, 160	Pos	7.3	1.5	3.6	1.5
PRB917 panel Q	62	vf24	Ind	0.3	0.2	0.1	0.1
	65	vf24	Ind	0.4	0.2	0.1	0.1
	70	vf24	Ind	1.8	1.6	2.0	0.3
	72	f24	Ind	4.3	2.5	2.8	0.3
	77	24, vf41, vf160	Pos	14.2	4.7	3.7	1.2
PRB918 panel R	0	No bands	Neg	0.2	0.2	0.1	0.1
	2	No bands	Neg	0.2	0.2	0.2	0.1
	7	vf24	Ind	1.6	0.7	2.3	0.5
	13	24, 160	Pos	18.2	3.0	2.7	2.5
	15	24, 160	Pos	16.2	3.9	2.7	3.1

[a] Values in boxes are positive results.
[b] f, faint; vf, very faint.
[c] Ind, indeterminate; Pos, positive; Neg, negative.
[d] Data provided by BBI.

oratory test of record (14.8% versus 14.6%). In the sensitivity studies for the panel of specimens from asymptomatic individuals that were previously screened by a licensed HIV-1 EIA and found to be repeatedly reactive and Western blot positive, the UBI HIV 1/2 EIA yielded an estimated sensitivity of 99.9% (672/673) with a 95% confidence interval of 99.2 to 100%. The one donor sample which was negative by the UBI HIV 1/2 EIA demonstrated discordant HIV-1 EIA results with licensed test kits from two different manufacturers and was negative by a licensed IFA. Follow-up specimens from this individual were not available, and were thus unable to resolve the conflicting serology results for this specimen observed not only with the UBI HIV 1/2 EIA but with other licensed assays.

Genetic diversity has been reported to exist among HIV-1 isolates for which multiple distinct genotypes or subtypes have been documented in the literature (12). Diversity may be evidenced by changes in the envelope glycoprotein and in the *gag* gene. These changes may ultimately influence the presentation of a humoral immune response. Due to the increase in international travel and the immigration of people from many different countries to the United States, an assay must be sensitive enough to detect the presence of antibodies to HIV-1

TABLE 4. Detection of antibodies to HIV-1 and HIV-2 in plasma and serum samples from random blood donors

Clinical facility	Sample source	No. of specimens tested	No. (%) of specimens that were:		No. of specimens that were HIV-1 Western blot[a]:		
			Initially reactive	Repeatedly reactive	+	I[b]	−[c]
Blood bank 1	Plasma	3,000	8 (0.27%)	6 (0.20%)	0	2	4
	Serum	1,000	3 (0.30%)	2 (0.20%)	0	0	2
Blood bank 2	Plasma	1,022	2 (0.20%)	1 (0.10%)	0	0	1
Blood bank 3	Plasma	2,062	11 (0.53%)	8 (0.39%)	0	2	6
Blood bank 4	Plasma	3,000	13 (0.43%)	7 (0.23%)	0	0	7[d]
Blood bank 5	Plasma	2,920	19 (0.65%)	12 (0.41%)	0	0	12
Total		13,004	56 (0.43%)	36 (0.28%)	0	4	32

[a] Results of licensed HIV-1 Western blot assay were interpreted according to the manufacturer's criteria.
[b] Western blot indeterminate specimens are those with HIV-1 banding patterns other than those specified by the manufacturer as being positive or negative.
[c] HIV-1 western blot negative samples were evaluated by a licensed HIV-2 EIA and investigational HIV-2 Western blot assay and were found to be negative.
[d] Three of the seven samples were not tested by licensed HIV-2 EIA due to insufficient sample volume.

strains that are genetically distinct from those present in the United States. The UBI HIV 1/2 EIA was evaluated with confirmed positive specimens from areas where genetic diversity of HIV-1 isolates is known to occur, i.e., Thailand, Brazil, Central America, and Africa. It was determined that there was no impact on the results of testing for anti-HIV-1. The sensitivity in this population was reported to be 100%. The UBI HIV 1/2 EIA was also tested in May 1994 as part of a World Health Organization Global Programme on AIDS evaluation of operational characteristics of commercially available assays to determine antibodies to HIV-1 and/or HIV-2 in human serum (unpublished data). In this evaluation, the UBI HIV 1/2 EIA was found to be reactive with all confirmed HIV-positive specimens from Africa, Asia, Europe, and Latin America (14). These data further support the calculated sensitivity of 100% for the detection of HIV infection in geographically diverse regions with the UBI HIV 1/2 EIA and the conserved nature of the region represented by the synthetic peptide antigens.

Since the HIV-1 antigen employed in the UBI HIV 1/2 EIA consists of highly antigenic epitopes within the envelope region, studies of sensitivity were conducted on seroconversion panel samples that demonstrate predominantly Gag reactivity on HIV-1 Western blots. These samples were evaluated to demonstrate that the UBI HIV 1/2 EIA has high sensitivity for samples with predominantly anti-Gag reactivity on Western blots, despite the absence of antigen corresponding to Gag in the immunoassay. The results observed for the UBI HIV 1/2 EIA were comparable to those observed for previously licensed HIV-1 and HIV-1–HIV-2 EIAs, all of which contained Gag antigens. Detection of anti-HIV in these Gag-predominant specimens by the UBI HIV 1/2 EIA further supports the previously published data demonstrating that antibodies to Env can be detected earlier than antibodies to p24 (Gag) in confirmatory assays other than Western blotting (1, 7). For some confirmed positive specimens, Western blots do not indicate the presence of antibodies to gp41 (17). The gp160 precursor Env glycoprotein band may in many cases represent a multimer of the gp41 transmembrane protein (3). A gp120-gp160 Western blot band may be present in the absence of a gp41 band because of the lower degree of sensitivity of the latter, often due to its diffuse banding pattern (1). The fact that the UBI HIV 1/2 EIA was repeatedly reactive for all 106 confirmed positive specimens which lacked a gp41 band upon Western blotting by a licensed procedure further attests to the assay's sensitivity. The sensitivity of the assay for confirmed HIV-2-positive specimens was calculated to be 100%.

The specificity in the random-donor population, assuming an anti-HIV-1 and anti-HIV-2 antibody prevalence rate of zero, was calculated to be 99.72% with a 95% confidence interval of 99.62 to 99.81%. The specificities for the Genetic Systems and Abbott Recombinant combination HIV-1–HIV-2 EIAs were reported to be 99.70 and 99.90%, respectively.

Forty random blood donors were prospectively followed for between 5 and 37 months, with a mean follow-up interval of 12.4 months. For all 40 cases in which the Western blot failed to evolve into a positive pattern, the UBI HIV 1/2 EIA remained negative at the end of the follow-up interval. This study was carried out as an additional measure of the assay's specificity in the low-risk population. Thus, the samples with discordant results, i.e., nonreactive on the UBI HIV 1/2 EIA but reactive on previously licensed EIAs, did not represent HIV-infected individuals. The observations from this prospective study are consistent with observations previously published by other investigators (6, 7, 11) wherein donors in a low-prevalence population demonstrating persistently indeterminate Western blots and having no evidence of HIV exposure or clinical manifestations of HIV infection do not represent a risk for transmission of HIV.

Thus, as demonstrated by the data presented in this report, the UBI HIV 1/2 EIA exhibits a high degree of sensitivity for the detection of HIV-1 and HIV-2 in confirmed seropositive specimens. It is comparable to previously licensed assays in the detection of HIV-1 seroconversion and in specificity. It allows the laboratory an additional alternative in the selection of a sensitive and specific assay for the detection of anti-HIV-1 and anti-HIV-2.

REFERENCES

1. **Allain, J. P., D. A. Paul, Y. Laurian, D. Senn, and Members of the AIDS Haemophilia French Study Group.** 1986. Serological markers in early stages of human immunodeficiency virus infection in haemophiliacs. Lancet **ii:** 1233–1236.
2. **Cabradilla, C. D., J. E. Groopman, J. Lanigan, M. Renz, L. A. Lasky, and D. J. Capon.** 1985. Serodiagnosis of antibodies to the human AIDS retrovirus with a bacterially synthesized polypeptide. Bio/Technology **4:**1228–1231.
3. **Cambridge Biotech Corporation.** Product insert. Cambridge Biotech HIV-1 Western blot kit for detection of antibodies to HIV-1. Cambridge Biotech Corporation, Worcester, Mass.
4. **Centers for Disease Control.** 1989. Interpretation and use of the Western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. Morbid. Mortal. Weekly Rep. **38:**1–7.
5. **Chang, N. T., P. K. Chanda, A. D. Varone, S. McKinney, D. P. Rhodes, S. H. Tam, C. W. Shearman, J. Huang, T. W. Chang, R. C. Gallo, and F. Wong-Staal.** 1985. Expression in *Escherichia coli* of open reading frame gene segments of HTLV-III. Science **228:**93–96.
6. **Dock, N. L., S. H. Kleinman, M. A. Rayfield, C. A. Schable, A. E. Williams, and R. Y. Dodd.** 1991. Human immunodeficiency virus infection and indeterminate Western blot patterns. Prospective studies in a low prevalence population. Arch. Intern. Med. **151:**525–530.
7. **Gaines, H., A. Sönnerborg, J. Czajkowski, F. Chiodi, E. M. Fenyö, M. VonSydow, J. Alpert, P. O. Pehrson, L. Moberg, B. Åsjö, and M. Forsgren.** 1987. Antibody response in primary human immunodeficiency virus infection. Lancet **i:**1249–1253.
8. **Henderson, L. E., R. Sowder, T. D. Copeland, S. Oroszlan, L. O. Arthur, W. G. Robey, and P. Fischinger.** 1987. Direct identification of class II histocompatibility DR proteins in preparations of human T-cell lymphotropic virus type III. J. Virol. **61:**629–632.
9. **Jackson, J. B., K. L. MacDonald, J. Caldwell, C. Sullivan, W. E. Kline, M. Hanson, K. J. Sannerud, S. L. Stramer, N. J. Fildes, S. Y. Kwok, J. J. Sninsky, R. J. Bowman, H. F. Polesky, H. H. Balfour, and M. T. Osterholm.** 1990. Absence of HIV infection in blood donors with indeterminate Western blot tests for antibody to HIV-1. N. Engl. J. Med. **322:**217–222.
10. **Kühnl, P., S. Seidl, and G. Holzberger.** 1985. HLA DR4 antibodies cause positive HTLV-III antibody ELISA results. Lancet **ii:**1222–1223.
11. **Leitman, S. F., H. G. Klein, J. J. Melpolder, E. J. Read, J. J. Esteban, E. M. Leonard, L. Harvath, J. W.-K. Shih, R. Nealon, J. Foy, F. Darr, and H. J. Alter.** 1989. Clinical implications of positive tests for antibodies to human immunodeficiency virus type 1 in asymptomatic blood donors. N. Engl. J. Med. **321:**917–924.
12. **Louwagie, J., F. E. McCutchan, M. Peeters, T. P. Breenan, E. Sander-Buell, G. A. Eddy, G. van der Groen, K. Fransen, G.-M. Gershy-Damet, R. Deleys, and D. S. Burke.** 1993. Phylogenetic analysis of *gag* genes from 70 international HIV-1 isolates provides evidence for multiple genotypes. AIDS **7:**769–780.
13. **Sayers, M. H., P. G. Beatty, and J. A. Hausen.** HLA antibodies as a cause of false-positive reactions in screening enzyme immunoassays for antibodies to human T-lymphotropic virus type III. 1986. Transfusion **26:**113–115.
14. **Vercuateren, G.** 1994. Personal communication.
15. **Wang, C. Y.** 1988. Synthetic-peptide-based immunodiagnosis of retrovirus infections: current status and future prospects. Adv. Biotechnol. Processes **10:**131–148.
16. **Wang, J. J. G., S. Steel, R. Wisniewolski, and C. Y. Wang.** 1986. Detection of antibodies to human T-lymphotropic virus type III by using a synthetic peptide of 21 amino acid residues corresponding to a highly antigenic segment of gp41 envelope protein. Proc. Natl. Acad. Sci. USA **83:**6159–6163.
17. **Zaaijer, H. L., P. van Exel-Oehlers, T. Kraaijeveld, E. Altena, and P. N. Lelie.** 1992. Early detection of antibodies to HIV-1 by third-generation assays. Lancet **340:**770–772.