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Comparison of Western Immunoblot Antigens and Interpretive Criteria for Detection of Antibody to Human T-Lymphotropic Virus Types I and II

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Twenty human T-lymphotropic virus type I (HTLV-I) antibody-positive sera from Japan, Hawaii, and the Marshall Islands and 15 HTLV type II (HTLV-II) antibody-positive sera from intravenous drug users in the United States were tested by immunoblotting with two recombinant HTLV-I proteins and three commercial kits to determine whether there were any differences in reactions between HTLV-I- and HTLV-II-positive sera by the Western immunoblot method and, also, to evaluate the ability of these reagents to detect HTLV-I- and HTLV-II-seropositive individuals by using the recommended Western blot interpretation. These sera were first extensively characterized by immunofluorescence, enzyme immunoassay, radioimmunoprecipitation assay, and Western blot using HTLV-I and HTLV-II viral lysates and an envelope (*env*) recombinant protein. Although both HTLV-I- and HTLV-II antibody-positive sera reacted with the *env* protein gp68, reactions with the gp46 *env* antigens appeared to be specific for HTLV-I. It was found that the use of either p19 or p24 core bands plus an *env* reaction instead of only the p24 plus *env* reaction (as presently recommended) increased the number of positive interpretations for HTLV-I but had no effect on the number of HTLV-II-positive interpretations.

There are research and Food and Drug Administration-approved enzyme immunoassay (EIA) kits commercially available for measurement of human T-lymphotropic virus type I (HTLV-I) antibody. HTLV type II (HTLV-II) is a closely related retrovirus which shares considerable genomic homology with HTLV-I (1), and the EIA cannot distinguish between antibodies to these two agents. The Centers for Disease Control (Atlanta, Ga.) recommends that sera that are reactive by EIA be confirmed by Western immunoblotting, radioimmunoprecipitation assay (RIPA), or both and that a serum specimen must react with both the p24 core antigen and an envelope antigen, either gp46 or gp61/gp68, to be considered positive. Western blotting is most sensitive for measurement of core antibody, and RIPA is most sensitive for detection of envelope antibody, which often necessitates the running of both tests for confirmation (2).

Although most diagnostic laboratories can test sera by Western blotting with a kit containing preblotted proteins, few are capable of performing RIPA, which requires polyacrylamide gel electrophoresis and frequent preparation and standardization of radiolabeled antigen. Thus, it is of great importance that envelope proteins reactive with both HTLV-I- and HTLV-II-positive sera be represented in the Western immunoblot antigen preparations so that the need for RIPA is eliminated.

In a previous HTLV-I and HTLV-II seroprevalence study of blood donors in Hawaii and the Marshall Islands, we found that 14 (10%) of 139 antibody-positive sera reacted with p19, but not with p24, in the Western blot when we used an HTLV-I viral lysate from Hillcrest Biologicals, Cypress, Calif. In addition, we have yet to find an HTLV-II-positive serum specimen that reacts with the gp46 in this antigen.

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In this study, 35 serum specimens that were previously tested for HTLV-I and HTLV-II antibody by immunofluorescence (IF), EIA, RIPA, and Western blot by using HTLV-I and HTLV-II viral lysates (Hillcrest Biologicals) and an *env* recombinant protein (Hoffmann-La Roche Inc., Nutley, N.J.) were used to evaluate two recombinant proteins and three HTLV-I Western blot kits, to determine the sensitivity and specificity of these reagents compared with those of the antigen from Hillcrest Biologicals.

MATERIALS AND METHODS

IF. The HTLV-I-transformed cell line MT2 and the HTLV-II-transformed cell line Mo-T were used for antibody determinations by IF, as described previously (4). Briefly, 1:10 dilutions of the sera were incubated on acetone-fixed cell spots for 20 min, the slides were washed in phosphate-buffered saline, and the working dilution of fluorescein-conjugated anti-human globulin conjugate was added. After 20 min of incubation, the slides were washed, mounted in 90% glycerol, and read on a fluorescence microscope (Zeiss). When the quantity of specimen permitted, the reactive sera were titrated in fourfold dilutions on both antigens, and the endpoint dilutions (1+ brightness) were determined.

EIA. The HTLV-I EIA (Organon Teknika, Durham, N.C.) was performed according to the directions of the manufacturer.

RIPA. The cells from 160 ml of MT2 cell suspension were incubated for 16 h in 10 ml of methionine- and cysteine-free Eagle minimum essential medium in Earles balanced salt solution containing 1% dialyzed fetal bovine serum and 1 mCi each of [^{35}S]methionine and [^{35}S]cysteine (Dupont, NEN Research Products, Wilmington, Del.). The cell suspension was centrifuged at 500 × *g* for 5 min, and the cell pellet was washed once in cold phosphate-buffered saline. The sera were tested by the RIPA-polyacrylamide gel electrophoresis method as described previously (7). Briefly, a

soluble cell lysate was prepared by disruption of the antigen with RIPA buffer (0.15 M NaCl, 0.05 M Tris [pH 7.2], 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate) containing 0.01 M phenylmethylsulfonyl fluoride and 100 kIU of aprotinin per ml and centrifugation at 100,000 × *g* for 30 min. One milliliter of lysate was absorbed with 12.5 mg of protein A-Sepharose beads (Sigma Chemical Co., St. Louis, Mo.) for 20 min and reacted with 5 μl of serum for 60 to 90 min at 4°C. Five milligrams of protein A-Sepharose beads was added to bind the immune precipitates, and after 30 min the beads were washed three times with cold RIPA buffer and the complexes were eluted by heating them at 80°C for 5 min in sample buffer containing 0.1 M Tris (pH 6.8), 2% sodium dodecyl sulfate, 10% glycerol, 0.05% bromphenol blue, and 0.1 M dithiothreitol. Samples were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (5), the gel was dried and exposed to sensitive X-ray film (XAR; Eastman Kodak Co., Rochester, N.Y.) for several days, the film was developed, and the precipitin bands were measured.

Western blot. The HTLV-I and HTLV-II viral lysates from Hillcrest Biologicals and the HTLV-I recombinant protein from Hoffmann-La Roche, as well as the two recombinant proteins evaluated in the present study, p40x and *env* from Triton Biosciences, Inc., Alameda, Calif., were electrophoresed, blotted, and reacted with the specimens as described previously (4). The Western blotting was performed with three kits (Biotech Research Laboratories, Inc., Rockville, Md.; Epitope, Inc., Beaverton, Oreg.; and Pan-Data Systems, Inc., Rockville, Md.) according to the directions of the manufacturers. Each kit contained a photocopy or a diagram of a strip showing the bands present on the strips and their molecular weights, and the band reactions of the panel containing serum specimens were compared with those of the reference strip and recorded according to the directions of each manufacturer. The antigens in the kits were viral lysates, and the lysate from Biotech Research Laboratories was spiked with an *env* recombinant protein, 21E.

Specimen selection. We have been testing sera for HTLV-I and HTLV-II antibody by IF since 1983, by Western blotting since 1986, and by RIPA since 1987. The 35 serum specimens, which were coded and thus unlinked from any personal identifiers, were selected because they were representative of the various reactions we have encountered using these methods. All the sera were IF positive for HTLV-I antibody, HTLV-II antibody, or both and were also reactive with the *env* recombinant protein from Hoffmann-La Roche by Western blotting. Twenty of the positive serum specimens were presumed to result from HTLV-I infections because they were from HTLV-I endemic areas. Nine of these were from blood donors from either Hawaii or the Marshall Islands, and the other 11 were from patients from Japan with T-cell leukemia.

Fifteen serum specimens were from intravenous drug users in the United States, and nine of these have been identified as HTLV-II by the polymerase chain reaction (PCR) technique (4a). The other six serum specimens were also presumed to be from HTLV-II-infected individuals because of their IF and Western blot reactions, which are discussed below.

RESULTS

Table 1 contains the IF, EIA, RIPA, and Western blot (Hillcrest Biologicals and Hoffmann-La Roche) results used to characterize the 35 serum specimens. The sera usually fluoresced more brightly or exhibited higher titers in the IF test and reacted with more bands in the Western blot test with the homologous antigen, and in some cases they did not cross-react. Of the 20 HTLV-I serum specimens, 17 were reactive by EIA and all but 1 displayed at least gp68 in the RIPA. Specimens 1 to 3 reacted with both gp46 and gp68 with the Hillcrest HTLV-I lysate, specimens 4 to 6 reacted with gp68, specimens 7 to 9 reacted with gp46, and specimens 10 to 20 did not react with either *env* protein. The HTLV-II lysate did not possess glycoprotein, and the reactions observed were only to core antigens, as determined by monoclonal antibodies to *gag* proteins. Eight specimens reacted with HTLV-I p19 but not with p24.

Of the 15 HTLV-II serum specimens, 13 were EIA reactive. Both the negative specimens, 26 and 35, were positive by PCR for HTLV-II. Specimens 21 to 23 reacted with multiple proteins in the RIPA and in the Western blot, 9 serum specimens reacted with p24 only with the HTLV-I antigen, specimens 29 and 30 were nonreactive with both antigens but were positive by PCR, and 1 of the 5 RIPA-negative specimens was positive by PCR. None of the HTLV-II-positive serum specimens reacted with either of the *env* proteins in the HTLV-I lysate from Hillcrest Biologicals.

Table 2 displays the bands that were present on testing with the three Western blot kits. Of the 20 HTLV-I serum specimens, 19 reacted with gp46 in the kit from Epitope, 11 reacted with gp68, and 8 reacted with p19 but not p24. Only the first 8 serum specimens reacted with the gp46 protein from Biotech Research Laboratories, but 19 specimens reacted with the recombinant *env* antigen, 21E, which banded below p19, and 5 reacted with p19 but not with p24. Nine serum specimens reacted with gp46 in the kit from Pan-Data Systems, and 3 of these also displayed a gp68 band. Five sera reacted with the p19 but not with the p24 band. Of the 20 HTLV-I-positive serum specimens, 12 reacted with the Triton p40x recombinant. A total of 9 of the first 10 specimens reacted with the *env* protein from Triton, but the sera with lower antibody titers did not.

None of the HTLV-II-positive serum specimens reacted with the gp46 protein in antigens from any of the Western immunoblot kits. Four serum specimens displayed a gp68 band with the kit from Epitope, all 15 reacted with the *env* recombinant in the kit from Biotech Research Laboratories, and 2 reacted with the gp68 protein in the kit from Pan-Data Systems. None of the HTLV-II-positive serum specimens reacted with the *env* protein from Triton, and only the first two specimens reacted with the p40x recombinant.

DISCUSSION

In a recent study to determine interpretive criteria for serologic confirmation of HTLV-I infection, seven laboratories evaluated 142 specimens by Western blotting and RIPA (1). These samples were chosen because they were seroreactive for HTLV-I by EIA or gel agglutination and were IF positive or displayed at least one viral band by Western blotting. One hundred thirty-seven samples were positive by RIPA for envelope antibody, and they also were positive by Western blotting for the p24 antibody. No specimens were p24 negative but p19 and envelope positive.

In our panel of serum specimens, we chose not only strongly reactive sera but also seven HTLV-I-positive serum specimens which displayed gp68 by RIPA and p19 but not p24 with the Western blot antigen from Hillcrest Biologicals.

TABLE 1. Serologic characterization of HTLV-I- and HTLV-II antibody-positive panel

Serum specimen source and no.	IF titer[a]		EIA[b]	RIPA[c]	Western blot		
					Hillcrest biologicals[c]		Hoffmann-La Roche *env*
	I	II			HTLV-I	HTLV-II	
HTLV-I sera[d]							
1	2,560	640	≥3.6	24, 28, 40, 50, 68	15, 19, 24, 46, 53, 68	21, 45, 58	+
2	4[e]	3	≥3.6	24, 28, 40, 50, 68	19, 24, 46, 53, 68	21, 24, 45, 51, 58	+
3	4[e]	2	≥3.6	40, 68	19, 24, 46, 53, 68	21, 24, 51, 58	+
4	4[e]	3	≥3.6	24, 28, 40, 50, 68	15, 19, 24, 45, 53, 68	21, 24, 45, 51, 58	+
5	160	40	≥3.6	24, 40, 68	15, 19, 24, 45, 53, 68	21, 24, 45, 51, 58	+
6	4[e]	3	≥3.6	28, 40, 50, 68	19, 24, 45, 53, 68	24, 45, 58	+
7	4[e]	4	≥3.6	24, 28, 40, 50, 68	15, 19, 24, 46, 53	21, 24, 45, 51, 58	+
8	3[e]	3	≥3.6	40, 68	19, 24, 46, 53	24, 58	+
9	160	<10	≥3.6	40, 50, 68	19, 46, 53	No bands	+
10	4[e]	<10	≥3.6	28, 40, 68	19, 24, 53	21, 24, 58	+
11	2[e]	<10	0.22	68	19, 24	No bands	+
12	160	<10	2.01	40, 50, 68	19	24	+
13	160	<10	≥3.6	68	19	21, 24, 51, 58	+
14	160	10	≥3.6	68	19	24	+
15	160	10	2.46	68	19	24	+
16	3[e]	1	2.67	68	19	No bands	+
17	2[e]	<10	≥3.6	68	19	No bands	+
18	40	10	0.70	40, 68	No bands	No bands	+
19	160	10	1.82	68	No bands	No bands	+
20	40	<10	0.21	No bands	19	No bands	+
IVDU sera[f]							
21[g]	640	2,560	≥3.6	24, 40, 50, 68	15, 19, 24, 28, 42, 45, 51, 53	15, 21, 24, 42, 45, 51, 58	+
22[g]	640	2,560	≥3.6	24, 28, 40, 50, 68	15, 19, 24, 28, 42, 45, 51, 53	15, 21, 24, 42, 45, 51, 58	+
23[g]	160	640	≥3.6	24, 28, 40, 50, 68	15, 19, 24, 45, 51	15, 21, 24, 42, 45, 51, 58	+
24[g]	40	160	1.79	40, 68	24	15, 21, 24, 42, 45, 51, 58	+
25	40	160	≥3.6	68	24	21, 24, 45, 51, 58	+
26[g]	10	40	0.46	68	24	24, 42, 45, 51, 58	+
27[g]	10	40	1.94	68	24	21, 58	+
28	10	160	2.71	68	24	24	+
29[g]	<10	40	1.24	68	No bands	No bands	+
30[g]	40	40	1.07	68	No bands	No bands	+
31	1[e]	1	3.30	No bands	19, 24	21, 24, 42, 45, 51, 58	+
32	10	160	1.70	No bands	24	24, 51, 58	+
33	10	40	2.20	No bands	24	24	+
34	40	160	3.12	No bands	24	21, 24, 45, 51, 58	+
35[g]	10	10	0.15	No bands	24	21	+

[a] Values are endpoint titers for the HTLV-I antigen and (I) and the HTLV-II antigen (II).
[b] Values are the ratios of the optical density of the specimen/optical density cutoff; a ratio of ≥1.00 is reactive.
[c] Values are molecular weights (in thousands) of the reactive protein bands.
[d] Serum specimens were from patients in Japan with T-cell leukemia and blood donors from Hawaii and the Marshall Islands.
[e] Degree of fluorescence at a 1:10 dilution (1 to 4+) (quantity not sufficient for titration).
[f] Serum specimens were from intravenous drug users (IVDU) who were presumed to be HTLV-II positive or were confirmed to be positive by PCR.
[g] HTLV-II DNA detected in cells by PCR.

Both p19 and p24 bands were present with the more strongly reactive HTLV-I-positive sera, and p19 but not p24 reactions were usually accompanied by lower EIA ratios and fewer band reactions (Tables 1 and 2). The difference in results between the two studies probably reflects the differences in antibody titers of the specimens that were used.

In Table 3, the number of antibody-positive sera based on p24 and *env* versus p19 or p24 and *env* reactions by Western blotting and blotting plus RIPA are listed by commercial antigen source.

Eight specimens were positive with the kit from Biotech Research Laboratories when we used either the p19 or p24 core plus gp46 interpretation. Seven more specimens which reacted with p24 but not with gp46 by Western blotting were positive by combining blot and RIPA results, and an additional four specimens were positive when we combined p19 and RIPA results for a total of 15 positive specimens by using p24 + *env* criterion and 19 positive specimens by using p19 or p24 and *env*. A total of 15 specimens were p24 and *env* positive and 19 were p19 or p24 and *env* positive when recombinant *env* in the kit from Biotech Research Laboratories was used for interpretation. The RIPA results did not increase the number of positive specimens, since specimen 20, which was p21E negative by Western blotting, was also RIPA negative.

Nine HTLV-I-positive serum specimens were positive with the kit from Epitope when the p24 and *env* criterion was used, and 17 were positive when p19 and *env* reactions were added. Since *env* was demonstrated in all 20 specimens, RIPA did not increase the number of positive specimens. The three specimens that were not positive, 11, 18, and 19, did not display either core band.

Eight specimens were positive on immunoblotting with the kit from Pan-Data Systems, 5 more were p24 and RIPA positive, and 9 were p19 or p24 and RIPA positive. Eight sera were p24 and *env* positive with the Hillcrest antigen, and 9 were p19 or p24 and *env* positive. Two were p24 and

TABLE 2. Panel results with three Western blot kits and two recombinant proteins

Serum specimen source and no.	Results with the following HTLV-I Western blot kit[a]:			Triton	
	Epitope	Biotech	Pan-Data	40×	*env*
HTLV-I sera[b]					
1	19, 21, 24, 29, 34, 36, 46, 53, 55, 68	21[c], 19, 24, 26, 28, 32, 36, 42, 46, 53	15, 19, 22, 24, 29, 33, 36, 40, 46, 53, 68	+	+
2	19, 21, 24, 29, 34, 36, 46, 53, 55, 68	21, 19, 24, 26, 28, 32, 36, 42, 46, 53	15, 19, 22, 24, 29, 33, 36, 40, 46, 53	+	+
3	19, 21, 24, 29, 34, 36, 46, 53, 55, 68	21, 19, 24, 26, 28, 32, 36, 42, 46, 53	15, 19, 22, 24, 29, 33, 36, 40, 46, 53	+	+
4	19, 21, 24, 29, 34, 36, 46, 53, 55, 68	21, 19, 24, 26, 28, 32, 36, 42, 46, 53	15, 19, 22, 24, 29, 33, 36, 40, 46, 53, 68	+	+
5	19, 21, 24, 29, 34, 36, 46, 53, 55, 68	21, 19, 24, 26, 28, 32, 36, 42, 46, 53	15, 19, 22, 24, 29, 36, 40, 53	+	+
6	19, 21, 24, 29, 34, 36, 46, 53, 55, 68	21, 19, 24, 26, 28, 32, 36, 42, 46, 53	19, 24, 29, 33, 36, 40, 46, 53, 68	+	+
7	19, 21, 24, 29, 34, 36, 46, 53, 55, 68	21, 19, 24, 26, 28, 32, 36, 42, 46, 53	15, 19, 22, 24, 29, 33, 36, 40, 46, 53	+	+
8	19, 21, 24, 29, 34, 36, 46, 53, 55, 68	21, 19, 24, 26, 28, 32, 36, 42, 46, 53	15, 19, 24, 29, 36, 40, 46, 53	−	+
9	19, 21, 29, 34, 46, 53, 55	21, 19, 26, 28, 32, 36, 42	19, 29, 36, 40	−	−
10	19, 21, 24, 29, 34, 36, 46, 53, 55, 68	21, 19, 24, 26, 28, 32, 36, 42	19, 24, 29, 36, 40, 46	+	+
11	46, 53, 55	21, 19, 24, 36, 42	19, 24	+	−
12	19, 21, 29, 34, 46, 53, 55	21, 19, 36, 42	19	−	−
13	19, 21, 29, 34, 46, 53, 55	21, 19, 26, 28, 36, 42	19	−	−
14	19, 21, 29, 34, 46, 53, 55	21, 19, 24, 26, 28, 36, 42	19, 24, 29, 33	+	−
15	19, 21, 29, 34, 46, 53, 55	21, 19, 24, 26, 28, 36, 42	19, 33	−	−
16	19, 21, 29, 34, 46, 53, 55, 68	21, 19, 24, 26, 28, 36, 42	19, 24	+	−
17	19, 21, 29, 34, 36, 46, 53, 55	21, 19, 24, 26, 28, 36	19, 24, 29, 40	−	−
18	29, 46, 53, 55	21, 24, 28	No bands	−	−
19	29, 46, 53, 55	21, 19, 36, 42	33, 46	+	−
20	19, 21, 29, 45, 53, 55, 68	19	19	−	−
IVDU sera[d]					
21	19, 21, 24, 29, 34, 36, 45, 53, 55, 68	21, 19, 24, 26, 28, 36, 42, 53	15, 19, 22, 24, 36, 40, 53, 68	+	−
22	19, 24, 29, 36, 45, 53, 55, 68	21, 19, 24, 26, 28, 36, 42, 53	15, 19, 22, 24, 36, 40, 53, 68	+	−
23	19, 24, 29, 36, 45, 53, 55, 68	21, 19, 24, 26, 36, 42	15, 19, 22, 24, 40, 53	−	−
24	24, 29, 36, 45, 53, 55	21, 24, 42	24	−	−
25	24, 29, 36, 45, 53, 55, 68	21, 19, 24, 28, 36, 42, 53	19, 24, 36	−	−
26	24, 29, 36, 45, 53, 55	21, 24, 42	24	−	−
27	24, 45	21, 24, 42	24	−	−
28	24, 45, 53, 55	21, 24, 42	No bands	−	−
29	24, 45, 53, 55	21, 24, 42	24	−	−
30	19, 24, 29, 45, 53, 55	21, 19, 24, 42	19, 24	−	−
31	24, 36, 45, 53, 55	21, 19, 24, 42	19, 24	−	−
32	19, 24, 36, 45, 53, 55	21, 19, 24, 42	19, 24	−	−
33	24, 45, 53, 55	21, 24	24	−	−
34	24, 29, 36, 45, 53, 55	21, 19, 24, 42	24	−	−
35	24, 45	21, 24, 42	No bands		

[a] Values are molecular weights (in thousands) of the reactive protein bands.
[b] Serum specimens were from patients in Japan with T-cell leukemia and individuals from Hawaii and the Marshall Islands.
[c] The value 21 represents the recombinant *env* band, which has a molecular weight of 21,000.
[d] Serum specimens were from intravenous drug users (IVDU) who were presumed to be HTLV-II positive and were confirmed to be positive by PCR.

TABLE 3. Number of HTLV-I- and HTLV-II antibody-positive sera based on p24 and *env* compared with either p19 or p24 and *env* reactions by Western blot and RIPA

Serum specimen and antigen	No. of specimens					
	Western immunoblot		Western immunoblot and RIPA[a]		Total positive[b]	
	p24 and *env*[c]	p19/p24 and *env*	p24 and gp68	p19/24 and gp68	p24 and *env*	p19/p24 and *env*
HTLV-I sera (n = 20)						
Biotech Research Laboratories	8	8	7	11	15	19
core and *env*[d]	15	19	0	0	15	19
Epitope	9	17	0	0	9	17
Pan-Data Systems	8	8	5	9	13	17
Hillcrest Biologicals	8	9	2	8	10	17
HTLV-II sera (n = 15)						
Biotech Research Laboratories	0	0	10	10	10	10
core and *env*[d]	15	15	0	0	15	15
Epitope	6	6	6	6	12	12
Pan-Data Systems	2	2	7	7	9	9
Hillcrest Biologicals	0	0	8	8	8	8

[a] Sera exhibiting only core bands in Western blot plus gp68 in RIPA.
[b] Combination of Western blot and RIPA.
[c] gp46, gp68, or both.
[d] Recombinant *env*.

RIPA positive, and six more were positive by blotting and RIPA when p19 or p24 and *env* were used.

The addition of p19 to the positive criterion increased the number of HTLV-I antibody-positive specimens. In contrast, the addition of p19 to the positive criterion did not increase the total number that were positive in the HTLV-II group, since all positive specimens reacted with p24. Five of the HTLV-II specimens were chosen because they were RIPA negative, which was a disadvantage for the kits from Hillcrest Biologicals and Pan-Data Systems, whose *env* antigens did not react with the HTLV-II-positive sera.

The commercial Western blot antigens that we evaluated were not lacking in envelope proteins, and the differences observed in *env* reactions appeared to be due to serologic specificity. RIPA was not needed for a positive interpretation of the HTLV-I-positive sera when the kit from Epitope was used, nor was it helpful for either the HTLV-I- or the HTLV-II-positive sera interpretations with the kit from Biotech Research Laboratories when the recombinant antigen was used for the *env* interpretation.

It is often stated that HTLV-I and HTLV-II infections can not be differentiated serologically. As shown in this study, that is not strictly true. We found that the gp68 proteins in the kits from Epitope and Pan-Data Systems and the recombinant *env* proteins in the kits from Hoffmann-La Roche and Biotech Research Laboratories reacted with both HTLV-I- and HTLV-II-positive sera, while the reactions of gp68 from Hillcrest Biologicals; the *env* from Triton; and gp46 from Hillcrest Biologicals, Epitope, Biotech Research Laboratories, and Pan-Data Systems were specific for HTLV-I.

This may be due, in part, to the fact that only two strongly reactive HTLV-II-positive sera were included in the panel. The *env* recombinant from Triton did not appear to be very sensitive, even with the HTLV-I specimens, and we have previously demonstrated reactions of gp68 from Hillcrest Biologicals with high-titer sera from HTLV-II-infected patients. However, to date, no PCR-identified HTLV-II-positive sera have reacted with gp46. Thus, it seems that if a serum specimen reacts with gp46, it is HTLV-I positive, and if it does not, it is either weakly HTLV-I positive or HTLV-II positive. We are continuing to test specimens to determine whether this is always the case. Dual infections, however, have been detected by PCR (3), and this method would not distinguish them. Ideally, the Western blot antigen should contain a protein specific for HTLV-I and a second, distinguishable protein specific for HTLV-II.

When accompanied by an *env* reaction, it appears that p19 should be included as a valid core reaction when interpreting Western blots. Admittedly, low-risk, negative sera often exhibit a nonspecific p19 band in the Western blot for HTLV-I (9), but a similar problem is encountered with p24 in the Western blot for human immunodeficiency virus. As with the interpretation of the immunoblot for human immunodeficiency virus, the envelope reaction in the Western blot for HTLV-I determines the specificity of the core band reaction.

The impact of the deficiencies in the present HTLV-I Western blot interpretive criterion in the United States is mitigated by the finding that the HTLV-I- and HTLV-II-positive results in the United States are mainly due to HTLV-II infections (4a, 6, 8, 10) (and thus, the p24 core band is usually demonstrable). Nevertheless, the present criterion unnecessarily confuses serologic studies, especially in areas endemic for HTLV-I.

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