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Evaluation of Two Commercial Human T-Cell Lymphotropic Virus Western Blot (Immunoblot) Kits with Problem Specimens

DANA GALLO,* JANICE L. DIGGS, AND CARL V. HANSON

Viral and Rickettsial Disease Laboratory, State of California Department of Health Services, Berkeley, California 94704

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We evaluated two commercial human T-cell lymphotropic virus (HTLV) Western blot (WB; immunoblot) kits, Cambridge Biotech Corp. (CBC) and Diagnostic Biotechnology Ltd. (DBL). Both methods employ HTLV type I (HTLV-I) viral lysate and rgp21. The DBL WB kit also distinguishes between HTLV-I and HTLV-II antibodies, using an HTLV-I-specific and an HTLV-II-specific recombinant. Fifty weakly reactive HTLV-II-positive plasma specimens which were falsely negative with the Abbott enzyme immunoassay (EIA) and 50 Ortho EIA false-positive samples were selected to determine sensitivity and specificity. The sensitivities of the CBC and the DBL WB kits were 90 and 68%, respectively. All positive samples reacted with rgp21 in both kits, but some did not display core bands. Five samples were typed as HTLV-I and four were typed as dual infection by the DBL WB kit. The specificities of the CBC and DBL kits were 48 and 70%, respectively. The most prevalent WB reaction with the negative samples was with the core protein, p19, followed by p24 and p28 for CBC and rgp21 and p28 for DBL. DBL had two false-positive interpretations, and CBC had none. rgp21 was the most sensitive antigen in both kits for the weakly reactive HTLV-II samples. If all samples not reacting with this protein were interpreted as WB negative, regardless of other bands, the specificity would improve to 90% for CBC and 86% for DBL.

It previously has been shown that human T-cell lymphotropic virus type I (HTLV-I) immunoblot (Western blot [WB]) antigens consisting of only viral lysate are not sensitive, especially for detection of antibody to HTLV-II (2). Although the addition of a recombinant transmembrane envelope protein, rgp21, greatly improves the sensitivity of the WB for detection of both HTLV-I and HTLV-II antibodies, nonspecific reactions with the recombinant protein, as well as with core proteins, also occur (6).

In this study, we evaluated two commercial HTLV WB kits, both of which employ rgp21 and viral lysate. Fifty weakly reactive HTLV-II samples that were falsely negative by enzyme immunoassay (EIA) were selected to compare the sensitivities of the two kits. Because the WB is usually employed to confirm EIA-reactive results, 50 HTLV EIA-false-positive samples were used to compare specificities.

MATERIALS AND METHODS

Specimens. The 50 HTLV-II-positive plasma samples were from injection drug users attending drug treatment centers in San Francisco, Calif. The samples were negative for HTLV antibody by an EIA (Abbott Laboratories, Abbott Park, Ill.) but were positive by immunofluorescence assay (IFA), in-house WB, and radioimmunoprecipitation assay (RIPA) and were typed as HTLV-II by IFA titration and by PCR. The results are shown in Table 1.

The 50 HTLV-negative plasma samples were from blood donors. The samples had been EIA reactive (Ortho Diagnostics Systems, Raritan, N.J.) at the blood bank but negative for HTLV antibody at this laboratory by Abbott EIA and by IFA and also negative for HTLV proviral DNA by PCR.

IFA. The specimens were reacted on HTLV-I (MT2)- and HTLV-II (clone 19)-infected slides, as previously described (3). A sample must react specifically with both antigens to be considered positive. For typing, specimens were titrated on both antigens, and the higher titer was indicative of the type (4).

WB. HTLV-I viral lysate (Hillcrest Biologicals, Cypress, Calif.) spiked with recombinant gp21 from Hoffmann-La-Roche, Inc., Nutley, N.J., was used for the in-house test, as previously described (2). A specimen was considered positive if it reacted with p19 or p24 and env.

The following two commercial blots were evaluated: HTLV-I WB kit from Cambridge Biotech Corp. (CBC), Worcester, Mass., which contains HTLV-I viral lysate and rgp21; and Diagnostic Biotechnology Ltd. (DBL) 2.3 blot, from Genelabs Diagnostics (Pte) Ltd., Singapore, which employs HTLV-I viral lysate, rgp21, a unique HTLV-I envelope recombinant protein (rgp46-I), and a unique HTLV-II envelope recombinant protein (rgp46-II). The tests were performed and interpreted according to the manufacturers' directions.

RIPA. The specimens were reacted with HTLV-I (MT2) and HTLV-II (clone 19) RIPA antigen labeled with ^{35}S-methionine and ^{35}S-cysteine, and the test was performed as previously described (2). A specimen was considered positive if it reacted with either gp68 of the HTLV-I antigen or gp67 of the HTLV-II antigen.

PCR. The PCR was performed as previously described (5) with primer pair SK110-SK111 and probes that were specific for HTLV-I (SK112) and HTLV-II (SK188). Primers and probes for HLA-DQa were also included to detect the presence of PCR inhibitors or lack of DNA.

RESULTS

A positive interpretation (for HTLV-I/II) with the CBC WB requires bands present at p24 and gp46 or rgp21. An HTLV-I/II positive with the DBL WB is defined as reactivity with gag (p19 or p24) and env (gp46 or rgp21). A specimen which reacts with p19 or p24 and rgp21 plus gp46 or rgp46-I is

* Corresponding author. Mailing address: California Dept. of Health Services, VRDL, 2151 Berkeley Way, Berkeley, CA 94704. Phone: (510) 540-2818. Fax: (510) 540-3430.

TABLE 1. Fifty EIA-false-negative HTLV-II samples

Specimen no.	EIA ratio[a]	IFA titer		WB band(s)	RIPA band(s)	PCR[b]
		HTLV-I	HTLV-II			
1	0.9	16	256	19 24 36 53 renv	68	II
2	0.9	32	128	19 24 renv	21 24 67[c]	II
3	0.9	16	256	19 24 renv	68	II
4	0.9	16	1,024	renv	68	II
5	0.9	32	128	24 renv	24 28 40 53 68	II
6	0.9	64	1,024	24 renv	68	II
7	0.9	4	64	24 renv	68	II
8	0.8	64	256	24 53 renv	68	II
9	0.8	64	1,024	19 24 53 renv	40 68	II
10	0.8	16	256	24 renv	21 38 67[c]	II
11	0.8	16	1,024	24 53 renv	24 28 40 68	II
12	0.8	16	256	19 24 renv	24 28 40 68	II
13	0.8	64	256	24 renv	40 68	II
14	0.8	64	1,024	19 24 renv	40 68	II
15	0.8	32	64	19 24 53 renv	21 38 53 67[c]	II
16	0.8	16	64	19 24 renv	28 68	II
17	0.8	8	16	24 renv	21 67[c]	II
18	0.8	16	256	19 24 53 renv	68	II
19	0.7	32	512	24 renv	40 68	II
20	0.7	32	256	19 24 53 renv	68	II
21	0.7	4	64	19 24 renv	68	II
22	0.7	16	256	19 24 renv	40 68	II
23	0.7	32	64	19 24 renv	68	II
24	0.7	8	32	24 renv	40 68	II
25	0.6	16	256	24 renv	40 68	II
26	0.6	16	1,024	24 renv	68	II
27	0.6	64	512	19 24 renv	28 40 51 53 68	II
28	0.6	32	128	24 renv	38 67[c]	II
29	0.6	4	32	24 renv	28 68	II
30	0.6	16	64	19 24 53 renv	68	II
31	0.6	16	256	24 renv	68	II
32	0.6	16	256	19 24 renv	21 24 38 67[c]	II
33	0.6	32	512	19 24 renv	68	II
34	0.6	4	64	24 renv	24 28 68	II
35	0.5	16	64	24 53 renv	21 38 67[c]	II
36	0.5	16	256	24 renv	28 68	II
37	0.5	16	1,024	19 24 renv	40 68	II
38	0.5	16	256	24 53 renv	24 28 40 51 53 68	II
39	0.5	16	256	24 53 renv	68	II
40	0.5	16	64	19 24 renv	40 68	II
41	0.5	8	64	24 renv	68	II
42	0.4	16	64	24 renv	21 67[c]	II
43	0.3	16	256	24 renv	40 68	II
44	0.3	16	64	24 renv	68	II
45	0.3	16	64	24 renv	40 68	II
46	0.3	64	256	24 renv	28 40 68	II
47	0.3	4	256	24 renv	68	II
48	0.2	8	16	24 renv	21 38 53 67[c]	II
49	0.2	4	256	19 24 renv	68	II
50	0.1	4	64	19 24 renv	28 68	II

[a] Abbott EIA.
[b] Primer pair SK110-SK111 and probes SK112 and SK188 were used. II, HTLV-II.
[c] No env with HTLV-I; these are bands present with HTLV-II antigen.

typed as HTLV-I. An HTLV-II positive is defined as reactivity with p24, rgp21, and rgp46-II.

Thirty-two (64%) of the 50 weakly reactive HTLV-II-positive samples were correctly identified as positive by the CBC WB and typed as HTLV-II by the DBL WB. Table 2 displays the discrepant WB results: two samples were positive with the DBL WB but did not react with the HTLV-II typing recombinant, rgp46-II; five reacted with rgp46-I and were mistyped as HTLV-I; four were typed as dual infections; and seven were indeterminate. Five of these were also indeterminate with the CBC WB. Although these indeterminate samples reacted with the recombinant env proteins, they did not display the core bands required for a positive interpretation. Five of the seven samples with indeterminate results in one or both tests gave EIA ratios of 0.5 or less (Table 1).

Only 22 (44%) of the 50 negative samples were negative by both commercial immunoblots. Eleven samples were interpreted as indeterminate by both methods, 13 were indeterminate by CBC but negative by DBL, 2 were negative by CBC but indeterminate by DBL, and 2 were indeterminate by CBC and positive by DBL (Table 3). The most prevalent nonspecific band with both methods was p19, followed by p24 and p28 for

TABLE 2. HTLV-II positive samples with discrepant WB results

Specimen no.	CBC		DBL	
	Band(s)	Interpretation	Band(s)	Interpretation
21	rgp21 19 24 28	Positive	rgp21 19 24	Positive
28	rgp21 24 28	Positive	rgp21 19 24	Positive
6	rgp21 24	Positive	rgp21 24 rgp46-I	HTLV-I
15	rgp21 19 24 28	Positive	rgp21 24 rgp46-I	HTLV-I
17	rgp21 24	Positive	rgp21 24 rgp46-I	HTLV-I
29	rgp21 24	Positive	rgp21 24 rgp46-I	HTLV-I
40	rgp21 19 24 28 38x	Positive	rgp21 24 rgp46-I	HTLV-I
16	rgp21 19 24 28	Positive	rgp21 19 24 rgp46-I&II	Dual infection
18	rgp21 24 38x	Positive	rgp21 19 24 rgp46-I&II	Dual infection
23	rgp21 19 24	Positive	rgp21 19 24 rgp46-I&II	Dual infectin
37	rgp21 19 24	Positive	rgp21 19 24 rgp46-I&II	Dual infection
4	rgp21 24	Positive	rgp21 rgp46-II	Indeterminate
42	rgp21 24	Positive	rgp21 rgp46-I&II	Indeterminate
24	rgp21 38x	Indeterminate	rgp21 rgp46-II	Indeterminate
35	rgp21	Indeterminate	rgp21 rgp46-II	Indeterminate
46	rgp21	Indeterminate	rgp21	Indeterminate
48	rgp21	Indeterminate	rgp21	Indeterminate
50	rgp21	Indeterminate	rgp21 rgp46-I	Indeterminate

CBC and rgp21 and p28 for DBL. Nonspecific reactions with p24 contributed to all but 3 of the 13 CBC-indeterminate but DBL-negative results. There were five false-positive reactions with rgp21 with CBC and seven false-positive reactions with DBL. Specimens 27 and 28 reacted similarly with the two kits. However, these reactions are interpreted differently by the two manufacturers.

DISCUSSION

The sensitivity of the CBC WB in this study was 90%. The sensitivity of the DBL WB (68%) was based on the number of HTLV-II-positive samples that were interpreted as either positive or typed as HTLV-II. The five HTLV-I and four dual results with the DBL blot are puzzling. As previously men-

TABLE 3. HTLV-negative samples with discrepant WB results

Specimen no.	CBC		DBL	
	Band(s)	Interpretation	Band(s)	Interpretation
1	19	Indeterminate	19	Indeterminate
2	19	Indeterminate	19	Indeterminate
3	19 28	Indeterminate	19 24 28 53	Indeterminate
4	19 28	Indeterminate	19 26 28 36 53	Indeterminate
5	19 28	Indeterminate	19	Indeterminate
6	19 28	Indeterminate	19 28 53	Indeterminate
7	19 28	Indeterminate	19 26 28	Indeterminate
8	24 28	Indeterminate	24 28	Indeterminate
9	rgp21	Indeterminate	rgp21	Indeterminate
10	rgp21	Indeterminate	rgp21	Indeterminate
11	rgp21 38x	Indeterminate	rgp21	Indeterminate
12	24 53	Indeterminate	None	Negative
13	42	Indeterminate	None	Negative
24	24	Indeterminate	None	Negative
15	24	Indeterminate	None	Negative
16	24	Indeterminate	None	Negative
17	24 28	Indeterminate	None	Negative
18	24	Indeterminate	None	Negative
19	42	Indeterminate	None	Negative
20	19 24 28	Indeterminate	None	Negative
21	24	Indeterminate	None	Negative
22	19 24	Indeterminate	None	Negative
23	24 42	Indeterminate	None	Negative
24	28 42	Indeterminate	None	Negative
25	None	Negative	rgp21	Indeterminate
26	None	Negative	rgp21	Indeterminate
27	rgp21 19	Indeterminate	rgp21 19	Positive
28	rgp21 19 28	Indeterminate	rgp21 19 26 28 36 53	Positive

tioned, these specimens were typed as HTLV-II by IFA titration and PCR. In addition, one would expect HTLV-I positives to display a p19 core band rather than p24. It appears that the 11 reactions with the rgp46-I band shown in Table 2 are nonspecific.

All samples reacted with rgp21 in both kits. However, although a p24 band could be demonstrated in all but one of these specimens with our in-house WB, the amount of p24 antigen in these commercial kits was insufficient to detect p24 antibody in five samples with CBC and in seven samples with DBL.

The apparent greater amount of p24 in the CBC antigen was a disadvantage when determining specificity with the 50 EIA false-positive specimens. Although the numbers of negative specimens reacting nonspecifically with p19 were comparable (11 versus 9) for the CBC and DBL kits, there were 11 false-positive p24 reactions with CBC versus only 2 with DBL. There were 24 negative interpretations with the CBC WB and 35 with the DBL kit, giving specificities of 48 and 70% for CBC and DBL, respectively. Differences in the manufacturers' recommendations for a positive reaction resulted in two false-positive interpretations for DBL, although the WB reactions of these two samples were similar with both kits.

Specificities and sensitivities of the licensed EIA kits vary (1). One way to minimize indeterminate WB results with negative specimens is to choose the most specific as well as the most sensitive screening test available. As mentioned previously, all of these Ortho EIA-false-positive samples were negative with the Abbott EIA. It is not usually necessary to perform the WB on a specimen that is EIA negative.

The rgp21 protein is very sensitive. In the past 4 years, we have performed IFA titration, in-house WB, RIPA, and PCR on over 450 positive specimens. We have never experienced an HTLV-positive sample that did not react with rgp21 in our WB. Thus, we interpret rgp21-negative reactions as WB negative, regardless of the presence of other bands, since rgp21 is the most sensitive antigen in our HTLV WB. We have found this to be a reliable method which greatly reduces the number of WB-indeterminate interpretations on negative samples.

In the present study, we chose 50 of our weakest HTLV-II-positive specimens to compare the sensitivities of these two commercial WB kits. Both methods detected antibody to rgp21 in all 50 specimens. Thus, it might be assumed that if a sample did not react with rgp21 in these immunoblots, it could be interpreted as WB negative, regardless of reactions with other bands. This would improve the specificities of the CBC and DBL kits to 90 and 86%, respectively, without affecting the sensitivity.

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