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Sensitivities of Radioimmunoprecipitation Assay and PCR for Detection of Human T-Lymphotropic Type II Infection

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Five hundred forty-eight uncoagulated blood specimens from intravenous drug users infected with human T-lymphotropic virus type II (HTLV-II) were used to evaluate the sensitivities of the radioimmunoprecipitation assay (RIPA) and PCR for detecting HTLV-II-infected people. The sensitivities of both RIPA and PCR were found to be dependent on the HTLV-II antibody titer, as determined by the immunofluorescence assay. Neither of these recommended confirmatory methods was as sensitive for detecting weakly reactive HTLV-II specimens as the immunofluorescence assay, Western blotting (immunoblotting), or a modified licensed enzyme immunoassay. Use of RIPA and PCR to determine the reliabilities of other tests may sometimes give erroneous results.

Antibodies to human T-lymphotropic virus type I (HTLV-I) and HTLV-II are usually detected by enzyme immunoassay (EIA) and are confirmed by Western blotting (WB; immunoblotting). Because of the sensitivity and specificity issues concerning WB, the radioimmunoprecipitation assay (RIPA) and PCR are recommended for resolving questionable HTLV serologic results (1).

In the study described here we evaluated the reliabilities of RIPA and PCR for detecting HTLV antibody and HTLV-II DNA, respectively, in 548 HTLV-II-positive specimens.

MATERIALS AND METHODS

Specimens. Uncoagulated blood specimens were collected from intravenous drug users attending local drug treatment centers by using acid citrate dextrose-treated Vacutainers. The plasma samples, which were stored at 4°C, were screened by EIA and immunofluorescence assay (IFA) for HTLV antibodies. Specimens reactive in the screening assays were further characterized by WB and RIPA and were typed as HTLV-I or HTLV-II by IFA titration and PCR. The specimens in the study, which were selected consecutively, were typed as HTLV-II by IFA, were positive by WB, and were positive by RIPA or PCR or both methods.

EIA. The specimens were tested with a licensed HTLV-I EIA, which uses an HTLV-I viral lysate (Abbott Laboratories, Abbott Park, Ill.), according to the directions of the manufacturer. Selected samples were also tested with a more sensitive licensed HTLV-I viral lysate EIA available since February 1993 (Abbott).

IFA. All specimens were reacted at a 1:10 dilution on HTLV-I (MT2)- and HTLV-II (clone 19)-infected slides as described previously (4). A sample must react specifically with both antigens to be considered positive. For typing, specimens were titrated on both antigens by using fourfold dilutions, and the higher titer was indicative of the type (5).

WB. An in-house test was used. The test used either HTLV-I viral lysate (Hillcrest Biologicals, Cypress, Calif.) spiked with recombinant gp21 from Hoffmann-La Roche Inc., Nutley, N.J. (3), or commercial WB strips (Cambridge Biotech Corp., Worcester, Mass.) containing HTLV-I viral lysate and recombinant gp21. A specimen was considered positive if it reacted with envelope proteins and either p19 or p24.

RIPA. The specimens were reacted with HTLV-I (MT2) and HTLV-II (clone 19) RIPA antigen containing ^{35}S-labeled methionine and ^{35}S-labeled cysteine. Clone 19 is an HTLV-II-transformed cell line established in this laboratory from an intravenous drug user from California infected with HTLV-II (5). The test was performed as described previously (3). A specimen was considered positive if it reacted with either the gp68 band with the HTLV-I antigen or the gp67 band with the HTLV-II antigen.

PCR. PCR was performed on all HTLV antibody-positive samples. Peripheral blood mononuclear cells were isolated from approximately 14 ml of uncoagulated blood by centrifugation through Ficoll-Hypaque. One dry cell pellet and two vials of cells containing 10% fetal bovine serum and 10% dimethyl sulfoxide were stored at −70°C. PCR was performed on the thawed pellet as described previously (8) with primer pair SK110-SK111, which amplifies across regions that are conserved as well as regions that are divergent between HTLV-I and HTLV-II. Cellular DNAs from HTLV-I (MT2) and HTLV-II (clone 19) served as positive controls. Reagent controls were also included in each run. Amplified products were detected by oligomer hybridization with ^{32}P-labeled probes specific for HTLV-I (SK112) and HTLV-II (SK188). Some amplified products were also probed with pol 2.2, which is specific for HTLV-II (6). Primers GH26-GH27 and probe GH64 for HLA-DQa were included to detect the presence of PCR inhibitors or the lack of DNA.

Because we used PCR to type antibody-positive specimens, negative PCRs were considered erroneous, and further efforts to demonstrate HTLV DNA were attempted. If the first PCR run gave a positive reaction for HLA but no evidence of HTLV DNA, the PCR was repeated with another aliquot of the same lysate. Further attempts to detect HTLV DNA included repetition of PCR with new lysate prepared from a frozen cell suspension, concentration by ethanol precipitation, and repetition of PCR with the product from the SK110-SK111 primer pair by using HTLV-I pol 1.1-pol 1.3 primers and the SK112 probe and HTLV-II pol 1.2-pol 3.2 primers and the pol 2.2

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TABLE 1. HTLV-II-positive samples with negative RIPA or PCR results

IFA titer for HTLV-II	No. of specimens	No. (%) of samples					
		RIPA[a]			PCR[b]		
					Repeat test result		
		HI^-, HII^+	HI^-, HII^-	Total HI^-	Positive[c]	Negative	Total negative[b]
≥4,096	128	1	0	1 (0.8)	7	0	7 (5.5)
1,024–2,048	219	5	0	5 (2.3)	24	1	25 (11.4)
256–512	140	6	4	10 (7.1)	25	9	34 (24.3)
16–128	61	18	8	26 (42.6)	13	7	20 (32.8)

[a] HI^-, HII^+, no *env* (gp68) reaction with HTLV-I antigen, but the *env* band (gp67) was present with HTLV-II antigen; HI^-, HII^-, no reaction with either antigen; Total HI^-, samples negative by RIPA with the HTLV-I antigen.
[b] Samples negative in the first PCR run.
[c] No HTLV-II DNA was detected in the first PCR run but was detected in a subsequent run.

probe. This nested primer procedure has been shown to increase the sensitivity of HTLV PCR with some samples (6). If neither HLA nor HTLV DNA was detected, further efforts included purification of the sample with chloroform (9) or Chelex 100 (7), dilution of the purified product, and repetition of PCR with new lysate.

RESULTS

Seventy (12.8%) of the 548 positive samples were negative by the Abbott EIA but were detected by IFA and were confirmed by WB and RIPA or PCR. Although some of these EIA-negative specimens had relatively high IFA titers with the HTLV-II antigen, they all displayed HTLV-I titers of 1:64 or less. In our experience, the sensitivity of this EIA is dependent on the anti-HTLV-I titer of the specimen. We have not experienced a false-negative EIA reaction in a specimen with an HTLV-I IFA titer of ≥1:128.

In Table 1 the samples are arranged by descending HTLV-II IFA antibody titer. One hundred twenty-eight specimens had HTLV-II IFA titers of ≥1:4,096. In this group, one sample reacted only with the HTLV-II RIPA antigen. The percentage of false-negative RIPA reactions with the HTLV-I and HTLV-II antigens increased as the antibody titer decreased. Thirty samples failed to react with the HTLV-I RIPA antigen but were positive with the HTLV-II RIPA antigen. Twelve other samples were negative with both antigens. Forty-two percent of the HTLV-II-positive samples with IFA titers of ≤1:128 were RIPA negative with the HTLV-I antigen.

A similar pattern was seen by PCR. Only seven (5.5%) of 128 specimens with IFA titers of ≥1:4,096 were negative in the first PCR run, but the percentage of samples that had to be repeat tested increased as the antibody titer decreased. Thirty-two percent of the samples in the group with the lowest antibody titer were PCR negative for HTLV-II DNA the first time that they were tested. Seventeen samples were negative even after repeat testing.

Data for 50 consecutive weakly reactive HTLV-II specimens that were negative by EIA but reactive by IFA, WB, RIPA, and PCR are presented in Table 2. A modified licensed Abbott EIA, which contains more antigen on the bead and a lower cutoff value, has been available since February 1993. Thirty-five of the 50 specimens were reactive by this improved procedure. These 35 samples gave the highest ratios in the previous test.

Nine (18%) of these 50 samples displayed no reaction with gp68, and thus were negative with the HTLV-I RIPA antigen, and 16 (32%) were negative for HTLV-II DNA in the first PCR run.

DISCUSSION

In the study described here the sensitivities of EIA, RIPA, and PCR were dependent on the IFA antibody titer. We screened all samples for HTLV antibody by EIA and IFA, and 12.8% of these HTLV-II-positive samples were detected only by IFA. Although IFA is in general use in Japan for the detection of HTLV-I antibody, it is not often used in the United States. Thus, it may be difficult for laboratories in the United States to select a similar panel of weakly reactive samples to determine the sensitivities of their RIPAs and PCRs.

Five hundred nineteen (94.7%) of the 548 HTLV-II-positive samples were positive by both RIPA and PCR, 12 samples were PCR positive but RIPA negative with both antigens, and 17 samples were RIPA positive but PCR negative. Thirty additional HTLV-II-positive samples would have been negative in the RIPA if only the HTLV-I antigen had been used (Table 1). Our HTLV-II antigen, clone 19, was selected as the antigen for IFA and RIPA because it was found to be more sensitive than the HTLV-I strain MT2 for detecting HTLV-II antibody by these two methods. Mo-T, the HTLV-II prototype strain, is very insensitive for detecting glycoprotein antibody by RIPA, even in HTLV-II-positive samples, and most laboratories performing RIPA use only the HTLV-I antigen. It might be assumed that these laboratories would determine that these 42 samples that were unreactive with the HTLV-I RIPA antigen were truly negative and that screening tests that found them to be positive lacked specificity.

This same problem applies to the use of PCR to confirm positive reactions. Eighty-six of the HTLV-II-positive samples were PCR negative in the first run (Table 1). We have had considerable experience with the IFA for HTLV antibody detection and have found this test to be very reliable (2–5). Thus, as described above, when a specimen is positive for HTLV antibody by IFA but negative for HTLV antibody by PCR, we feel that the PCR is in error and further attempts to demonstrate HTLV DNA are made. In the present study, repeat testing detected HTLV-II DNA in an additional 69 samples (Table 1). However, investigators who rely on PCR to determine the specificity of a positive serologic result would have no reason to repeat the test for a sample that gave a negative PCR result.

We have previously shown that the licensed EIAs missed between 10 and 40% of HTLV-II infections (2). Table 2 illustrates an example of the improved sensitivity of an EIA procedure obtained by the product from one manufacturer. The modified Abbott EIA detected 35 of the 50 samples missed by the previous EIA. Other manufacturers are also

TABLE 2. Results for 50 weakly reactive HTLV-II samples negative by previous Abbott EIA

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

[a] Abbott EIA before 1993 modification.
[b] Reactive with modified Abbott EIA.
[c] renv, recombinant *env*.
[d] HTLV-II DNA was detected in third PCR run.
[e] No *env* reaction with HTLV-I antigen, but the *env* band was present with the HTLV-II antigen.

working to improve the reliability of their EIA kits. However, only 28 of the 50 weakly reactive specimens in Table 2 reacted with the *env* band of the HTLV-I antigen in RIPA and were also PCR positive in the first run. Presumably, many of these samples would be deemed negative for HTLV antibody in other laboratories. Developmental tests for the detection of HTLV antibody may suffer from undeserved low specificities because they are more sensitive than the RIPA and the PCR used to determine these values.

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