Analysis of the May 5, 1997 Performance Evaluation Testing Results for Human T-lymphotropic Virus Types I and II Antibody Reported to the Centers for Disease Control and Prevention (CDC) by Participant Laboratories in the Model Performance Evaluation Program

This report is an analysis of results provided to the Centers for Disease Control and Prevention (CDC) by participant laboratories in the Model Performance Evaluation Program (MPEP) after they tested the human T-lymphotropic virus types I and II (HTLV-I/II) performance evaluation samples shipped to them on May 5, 1997. Testing results for this analysis were provided by 185 (86.4%) of 214 laboratories sent sample panels. Two laboratories returned result booklets with no testing results, and two laboratories returned results more than three weeks past the cutoff date. Data from these 4 laboratories are not included in this report. The testing results reported by the participant laboratories reflect their testing performance using manufactured kits to test performance evaluation samples and do not necessarily reflect an evaluation of these manufactured kits. Samples in this shipment consisted of plasma from donors who were HTLV-I/II antibody-negative (donor numbers 9-16) and donors antibody-positive for either HTLV-I or HTLV-II (donor numbers 1-8). Not all laboratories participating in this survey received identical samples, but each laboratory did receive the same number of HTLV-I/II antibody-positive and antibody-negative samples. Before shipment each donor sample was tested with three HTLV lysate-based enzyme immunoassay (EIA) kits licensed by the Food and Drug Administration (FDA) and with two HTLV Western blot (WB) kits. Additionally, each HTLV-I/II antibody-positive donor sample was tested by radioimmunoprecipitation assay (RIPA) and with an indirect immunofluorescent (IIF) antibody assay that can differentiate antibodies specific for HTLV-I or HTLV-II. Donor sample reactivity was determined by the CDC based on composite EIA, WB, and RIPA testing. The CDC MPEP interpretation of WB reactivity for each donor sample was consistent with the kit manufacturers' criteria for interpretation of WB results.

Figure 1 shows the cumulative frequency of test result interpretations reported by MPEP participant laboratories, arranged according to sample reactivity, for EIA, WB, and IIF methods. Two false-positive interpretations were among the 545 EIA interpretations reported for the HTLV-I/II antibody-negative samples, and no false-negative EIA interpretations were among the 546 EIA interpretations reported for the HTLV-I/II antibody-positive samples. There were no false-positive WB interpretations reported for the 8 HTLV-I/II antibody-negative samples tested. Two false-negative WB interpretations were included in the 81 WB interpretations reported for the HTLV-I/II antibody-positive samples. No false-negative or indeterminate IIF interpretations were reported among the 12 IIF interpretations for HTLV-I/II antibody-negative samples. Two indeterminate IIF interpretations were reported among the 12 IIF interpretations for HTLV-I/II antibody-positive samples.

Shown in a supplemental figure (Figure 10) are the results reported by two laboratories that correctly identified each of the HTLV antibody-positive samples, and the results of one of these laboratories that also tested and correctly identified the antibody-negative samples in their panels using a particle agglutination test kit (Serodia-HTLV-I) manufactured by Fujirebio, Inc. Also shown are the results of two laboratories that correctly identified all samples in their panel using

a strip immunoblot assay (RIBA HTLV-I/HTLV-II SIA) manufactured by Chiron. Another laboratory, using an in-house method correctly identified one of the HTLV-I/HTLV-II antibody positive samples, but incorrectly identified the other two HTLV-I/II antibody-positive samples as indeterminate.

The types of laboratories that reported HTLV antibody testing results to CDC are shown in Figure 2. Each laboratory type is noted, by decreasing frequency, for each of the test methods. The "Other" category includes, for example, research laboratories, organ procurement laboratories, drug screening/toxicology laboratories, and sexually transmitted diseases clinics.

The combinations of EIA, WB and IIF test methods used by laboratories and frequency of use are shown in Figure 3. Most laboratories performed only EIA (84.8%), while some laboratories performed both EIA and supplemental tests (13.6%). Three laboratories (1.6%) performed only supplemental tests (IIF or WB).

The types of test kits used, by manufacturer, for the EIA, WB, and IIF methods are shown, by decreasing frequency, in Figure 4. One laboratory reported EIA results using an in-house method. An HTLV-I/II WB kit that is capable of distinguishing HTLV-I from HTLV-II infection (Genelabs Diagnostics) was used by 14 (53.8%) of the 26 laboratories reporting WB results.

The results reported for the EIA, WB, and IIF methods, listed by kit manufacturer, for the CDC HTLV-I/II survey samples are shown in Figures 5, 6, and 7.

EIA Results

There were eight donor samples used in this shipment that were composite tested by EIA, WB, and RIPA and interpreted as HTLV-I/II antibody negative (Donors 9-16). False-positive EIA final interpretations were reported once each for donor 9 and donor 16, by laboratories using test kits manufactured by Organon Teknika (Figure 5).

The eight samples that were composite tested by EIA, WB, and RIPA and interpreted as HTLV-I/II antibody-positive included donors antibody-positive for either HTLV-I or HTLV-II. Donors 1-4 were infected with HTLV type I, while donors 5-9 were infected with HTLV type II. There were no false-negative EIA final interpretations reported for any of these samples (Figure 5).

WB Results

There were no false-positive or indeterminate WB interpretations reported for the HTLV antibody-negative donor samples (Donors 9-16).

Of the 8 HTLV antibody-positive donor samples, there were two false-negative WB interpretations reported, both for donor 7, by a laboratory using an in-house WB method. Positive bands were reported by this laboratory, along with both false-negative interpretations for p19, p24, gp21, gp22, and 46II.

Of the 26 participant laboratories reporting WB results, 25 of them provided information regarding the criteria used for WB interpretations. Twelve of these (48%) used WB interpretive criteria published by the manufacturer of the WB kit they used for testing. Other laboratories used the interpretive criteria published by the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD), 7 (28%); the World Health Organization, 1 (4%); or "Other" criteria, 5 (20%). No laboratories indicated using the HTLV WB criteria of the Consortium for Retrovirus Serology Standardization (CRSS) or of the Public Health Service (PHS) Working Group. The criteria of these organizations is described in the following table:

CRITERIA FOR INTERPRETATION OF HTLV WESTERN BLOT TEST

Organization	Criteria for Positive Test
Public Health Service (PHS) Working Group	p24 **and** gp46 or gp61/68
Association of State and Territorial Public Health Laboratory Directors (ASTPHLD)	p19 or p24 **and** one env* band
Consortium for Retrovirus Serology Standardization (CRSS)	p19 or p24 **and** gp46 or gp61/68
World Health Organization (WHO)	One gag** **and** one env band

*** env bands = gp21, gp46, gp61/68**
**** gag bands = p15, p19, and p24**

IIF Results

No false-positive or indeterminate IIF interpretations were reported for HTLV-I/II antibody-negative donors by the three laboratories that performed IIF using reagents and procedures developed in house or obtained from a noncommercial source (Figure 7). Indeterminate results, however, were reported twice, both for donor 2, an HTLV-I antibody-positive donor.

Western Blot Band Patterns

The percentage and frequency of WB protein bands reported are shown in Figure 8. The frequency of a reported band is shown above or within the column, and the number of reports is listed in the far right column. For the HTLV-I/II antibody-positive donor samples (donor numbers 1-8), the participating laboratories detected antibodies to most of the native viral-specific proteins (e.g., p19, p24, p32/33, and gp46,) with the exception of p32/33 and gp46 for donor 8. The presence of recombinant gp46 type I (r46I) or recombinant gp46-type II (r46II) and/or recombinant gp21 (r21e or GD21) proteins were detected for all positive donors by some laboratories using commercially available WB strips designed to detect these bands.

Donor samples 9-16 were negative for antibody to HTLV-I/II and no laboratories reported WB bands for any of these donors.

IIF Fluorescence Intensity

The fluorescence intensity patterns of HTLV-infected cells, as reported by participant laboratories, are shown in Figure 9. The number of reports received for each donor sample is listed in the far right column. Three laboratories reported IIF results. Some laboratories, however, reported only interpretations, and therefore, intensity data is not available for all donor samples. Generally, laboratories reported 2+ or greater fluorescence intensity in HTLV-infected cells for the HTLV-I/II antibody-positive samples for which intensity information was collected (donor numbers 1, 4, and 5). A fluorescence intensity of 1+ was reported twice for donor 8; the reactivity of the sample was correctly reported as positive. No fluorescence was detected in uninfected control cells for any of these HTLV-I/II antibody-positive samples.

No IIF reactivity was reported for any of the HTLV-I/II antibody-negative samples (Donors 9-16) tested.

Quality Control Testing

Although information was requested on the use of quality control (QC) materials not included with the manufacturer's kits, some laboratories continue to describe the kit controls as their only QC material. Positive and negative samples included in manufactured kits are internal kit control materials used to verify each lot's performance, calculate EIA test run cutoff values, and provide visual guidelines for determining band intensity for reading WB test results. They are often of limited value in assessing test performance over multiple lots of reagents. To verify the performance specifications of a test method and confirm that the accuracy and precision of a procedure are adequate, laboratories would benefit from testing external positive and negative QC samples, that is, samples which closely mimic patient specimens and are independent of the manufacturers' kit controls. An analysis of external control values over time allows a more accurate detection of shifts and trends in an analytic testing process resulting from testing problems such as faulty pipettors, inadequate incubation conditions, or kit lot sensitivity.

Of the 181 laboratories reporting EIA test results, 77 (42.5%) indicated they used external EIA QC samples. Of the 77 laboratories using external EIA QC, 55 (71.4%) indicated they obtained QC samples for EIA testing from commercial sources. Thirty-five percent of the 77 laboratories used a single serum/plasma and 57% described the single serum/plasma as weakly positive. Fifty-four percent of laboratories who reported using external QC used multiple sera/plasma, and 82% used external EIA QC with each EIA plate or each set of plates.

Of the 26 laboratories reporting WB results, 10 (38.5%) reported the use of external QC samples in WB testing. Of the 10 laboratories using external WB QC samples, 7 (70%) indicated they obtained HTLV WB QC samples in house while 3 (30%) used a commercial source for WB QC samples. Five (50%) of the 10 laboratories reported using external QC material with each set or run of WB strips.

The use of external IIF QC samples was reported by one of the three laboratories reporting HTLV IIF results. This laboratory used a strong-positive single serum/plasma control with each set of IIF samples.

Conclusion

Most of the laboratories participating in this survey correctly identified the HTLV-I/II antibody-positive and antibody-negative samples. The following table shows the relative frequencies of false-positive and false-negative EIA interpretations and indeterminate WB interpretations for these donor samples.

	EIA Interpretation		WB Interpretations		
Survey	**False-positive**	**False-negative**	**False-positive**	**False-negative**	
9705	0.37%	0.00%	0.00%	2.47%	
			Indeterminate		
			Positive samples	**Negative samples**	
			0.00%	0.00%	

A comparison of the EIA and WB analytic sensitivity, analytic specificity, and overall analytic performance for this sample survey is shown in the following table:

	Enzyme Immunoassay		Analytic	Western Blot		Analytic
Survey	**Sensitivity**	**Specificity**	**Performance**	**Sensitivity**	**Specificity**	**Performance**
9705	100%	99.6%	99.8%	97.5%	100%	98.5%