Analysis of the November 4, 1996 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 November 4, 1996. Testing results for this analysis were provided by 181 (81.2%) of 223 laboratories sent sample panels. Four laboratories returned result booklets with no testing results, one laboratory tested these HTLV-I/II samples using a test kit intended for detecting human immunodeficiency virus (HIV) antibody and six laboratories returned results more than three weeks past the cutoff date. Data from these 11 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 13 and 14) and donors antibody-positive for either HTLV-I or HTLV-II (donor numbers 1 - 10). 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, the CDC tested each donor sample 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 CDC after 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. No false-positive interpretations were among the 179 EIA interpretations reported for the HTLV-I/II antibody-negative samples, and no false-negative EIA interpretations were among the 894 EIA interpretations reported for the HTLV-I/II antibody-positive samples. There were no false-positive WB interpretations reported for the 20 HTLV-I/II antibody-negative samples tested. Four (3.2%) indeterminate WB interpretations were included in the 125 WB interpretations reported for the HTLV-I/II antibody-positive samples. No false-negative, false-positive or indeterminate IIF interpretations were reported.

Shown in a supplemental figure (Figure 10) are the results reported by one laboratory that correctly identified each of the HTLV antibody-positive and antibody-negative samples in their panels using a particle agglutination test kit (Serodia-HTLV-I) manufactured by Fujirebio, Inc., and the results of a second laboratory that correctly identified all samples in their panel using a strip immunoblot assay (RIBA HTLV-I/HTLV-II SIA) manufactured by Chiron.
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 test methods used by laboratories and frequency of use are shown in Figure 3. Most laboratories performed only EIA (86.2%), while some laboratories performed both EIA and supplemental tests (12.2%). Three laboratories (1.7%) 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. An HTLV-I/II WB kit that is capable of distinguishing HTLV-I from HTLV-II infection (Genelabs Diagnostics) was used by 10 (41.7%) of the 24 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 only two donor samples (Donors 13 and 14) used in this shipment that CDC composite tested by EIA, WB, and RIPA and interpreted as HTLV-I/II antibody negative. No false-positive EIA final interpretations were reported for either of these two samples (Figure 5).

The ten samples that CDC 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. Donor numbers 1 - 4 and donors 8 - 10 were infected with HTLV type I while donor numbers 5, 6, and 7 were infected with HTLV type II. There were no false-negative EIA final interpretations reported for any of these samples.

WB Results

There were no false-positive or indeterminate WB interpretations reported for the HTLV antibody-negative donor samples (Donors 13 and 14).

There were no false-negative WB interpretations reported for the 10 HTLV antibody-positive donor samples. However, two indeterminate WB interpretations were reported for Donor 7 samples, duplicated in Panel K, by a laboratory using a BioMerieux Vitek (Cambridge Biotech) WB kit and reporting p19, p24 and p21env bands. Two additional indeterminate WB interpretations were reported for Donor 10 samples by a laboratory using an in-house WB procedure and reporting p19, p24 and gp21 bands.

Of the 24 participant laboratories reporting WB results, 12 (50%) 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), 6 (25%); the Public Health Service (PHS) Working Group, 2

(8.3%); the World Health Organization, 1 (4.2%); or "Other" criteria, 3 (12.5%). No laboratories indicated using the HTLV WB criteria of the Consortium for Retrovirus Serology Standardization (CRSS). 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, false-negative, or indeterminate IIF interpretations were reported by the three laboratories that performed IIF using reagents and procedures developed in-house or obtained from a noncommercial source (Figure 7).

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 1-10), the participating laboratories detected antibodies to most of the native viral-specific proteins (e.g., p19, p24, p32/33, and gp46,) and recombinant gp46 type I (r46I) or recombinant gp46-type II (r46II) and/or recombinant gp21 (r21e or GD21) proteins present on some commercially available WB strips.

Donor samples 13 and 14 were negative for antibody to HTLV-I/II and no laboratories reported WB bands for either one 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. Only three laboratories reported IIF results; therefore, data is

available for only 8 of the 12 total donor samples that comprised the sample panels for this shipment. With one exception, laboratories reported 2+ or greater fluorescence intensity in HTLV-infected cells for the six HTLV-I/II antibody-positive samples tested (donor numbers 2, 3, 6, 7, 9 and 10). No fluorescence was detected in uninfected control cells for any of these HTLV-I/II antibody-positive samples.

No IIF reactivity was reported for either of the HTLV-I/II antibody-negative samples (Donors 13 and 14) 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 should run external positive and negative QC samples, that is, samples which closely mimic patient specimens and are independent from 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 178 laboratories reporting EIA test results, 70 (39.3%) indicated they used external EIA QC samples. Of the 70 laboratories using external EIA QC samples, 48 (68.6%) indicated they obtained QC samples for EIA testing from commercial sources. Fifty-four percent used a single serum/plasma, 54% described the single serum/plasma as weakly positive, and 81% described the use of this EIA QC sample with each EIA plate or each set of plates.

Of the 24 laboratories reporting WB results, 11 (45.8%) reported the use of external QC samples in WB testing. Of the 11 laboratories using external WB QC samples, 7 (63.6%) indicated they obtained HTLV WB QC samples in house while 4 (36.4%) used a commercial source for WB QC samples. Nine (81.8%) of the 11 laboratories reported using external QC material each set or run of WB strips.

The use of IIF external QC samples was reported by one of the three laboratories reporting HTLV IIF results. This laboratory used a strong-positive and a negative QC sample, obtained commercially, 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 same donor samples tested in this survey were tested previously by participating laboratories in the May 1994 (9405), November 1994 (9411), May 1995 (9505), November 1995 (9511) and May 1996 (9605) HTLV panels. The following

table shows the relative frequencies of false-positive and false-negative EIA interpretations and indeterminate WB interpretations for these donor samples over the six survey periods.

	EIA Interpretation		WB Indeterminate	
Survey	**False-positive**	**False-negative**	**Positive samples**	**Negative samples**
9405	0.0%	0.0%	16%	2.0%
9411	0.2%	1.2%	0.0%	3.1%
9505	0.0%	1.3%	3.8%	6.8%
9511	0.0%	1.0%	0.0%	0.0%
9605	0.8%	0.5%	0.0%*	0.0%*
9611	0.0%	0.0%	3.3%	0.0%

* One false-positive and one false-negative WB interpretation were reported for this survey

A comparison of the EIA and WB analytic sensitivity, analytic specificity, and overall analytic performance for the same six replicate-sample surveys is shown in the following table:

	Enzyme Immunoassay			Western Blot		
Survey	**Sensitivity**	**Specificity**	**Analytic Performance**	**Sensitivity**	**Specificity**	**Analytic Performance**
9405	100 %	100 %	100 %	98.4%	98.0%	98.2%
9411	98.8%	99.8%	99.1%	100 %	96.9%	99.2 %
9505	98.7%	100 %	98.8%	96.2%	93.5%	95.5 %
9511	99.0%	100%	99.5%	100 %	100 %	100 %
9605	99.5%	99.2%	99.4%	99.1 %	97.5 %	98.6 %
9611	100 %	100 %	100 %	100 %	96.7%	97.2 %