Analysis of the May 4, 1998 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 4, 1998. Testing results for this analysis were provided by 196 (97.5%) of 201 laboratories sent sample panels. Three laboratories returned result booklets with no testing results, and two laboratories returned results more than three weeks past the cutoff date. Data from the 2 laboratories returning late results 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 (donor numbers 1-4) or HTLV-II (donor numbers 5-8). 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. Four false-positive and three false-negative interpretations were among the 1158 EIA interpretations reported. No false-negative and no false-positive WB interpretations were among the 133 results reported. Two indeterminate interpretations were reported.

Results reported by a two laboratories that correctly identified all HTLV-I/II antibody-positive samples and HTLV-I/II antibody-negative using a particle agglutination test kit (Serodia-HTLV-I) Manufactured by Fujirebio, Inc. are shown in figure 10. The results of two laboratories using in-house methods are also shown in figure 10. One laboratory correctly identified all three HTLV-I/II antibody-positive samples, while another laboratory reported false negative results for two of the positive samples.

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 (84.1%), while some laboratories performed both EIA and supplemental tests (14.4%). Three laboratories (1.5%) 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. The Abbott HTLV I/ HTLV II EIA kit was used by 77% of laboratories reporting EIA results, the Organon Tecknika HTLV-I/II kit was used by 17% of laboratories, and a variety of other test kits were used.

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). Four false positve interpretations were reported for these samples. Three false negative interpretations were reported for the eight HTLV-I/II antibody-positive samples (Donors 1-8).

WB Results

Two indeterminate interpretations were reported for the HTLV antibody-positive sample, CDC donor #1 by a laboratory using the Genelabs Diagnostics/Diagnostic Biotech WB kit. This sample was HTLV I antibody-positive. All other WB results were reported correctly.

All of the 29 participant laboratories reporting WB results provided information regarding the criteria used for WB interpretations. Eighteen of these (62%) 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; now known as the Association of Public Health Laboratories, APHL), 4 (14%); or "Other" criteria, 6 (21%). One laboratory indicated using the Public Health Service (PHS) Working Group criteria. No laboratories reported using the criteria of either the Consortium for Retrovirus Serology (CRSS) or the World Health Organization (WHO). 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

All interpretations by laboratories using IIF technologies were reported correctly. The two laboratories reporting IIF results used 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 1-8), 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 donors #6 and #8. Both of these samples were HTLV-II antibody-positive. The presence of recombinant gp46 type I (r46I) was correctly reported for all HTLV-I antibody-positive samples, and or recombinant gp46-type II (r46II) was correctly reported for all HTLV-II antibody-positive samples by some laboratories using commercially available WB strips designed to detect these bands.

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. Two 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. 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 192 laboratories reporting EIA test results, 101 (52.6%) indicated they used external EIA QC samples. Of the 101 laboratories using external EIA QC, 73 (72%) indicated they obtained QC samples for EIA testing from commercial sources. Forty-five percent of the 101 laboratories used a single serum/plasma and 54% reported using multiple sera/plasma. Fifty-two percent (53/101) of the laboratories used a weakly positive external control while eighty-nine percent (90/101) used external EIA QC with each EIA plate or each set of plates.

Of the 29 laboratories reporting WB results, 13 (44.8%) reported the use of external QC samples in WB testing. Of the 13 laboratories using external WB QC samples, 10 (77%) indicated they obtained HTLV WB QC samples in house while 2 (15%) used a commercial source for WB QC samples. Six (46%) of the 13 laboratories reported using external QC material with each set or run of WB strips.

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
9805	0.69%	0.52%	0.00%	0.00%

Indeterminate	
Positive samples	Negative samples
2.30%	0.0%

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
9805	99.5%	99.3%	99.4%	97.7%	100.00%	98.6%