Analysis of the November 2001 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 in November 2001. Testing results for this analysis were provided by 186 (87.7%) of 212 laboratories sent sample panels. 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 1 and 4) and donors antibody-positive for either HTLV-I (donor number 3) or HTLV-II (donor number 2). All laboratories participating in this survey received identical samples. Before shipment, each donor sample was tested with two 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.

The cumulative frequency of test result interpretations reported by MPEP participant laboratories, arranged according to sample reactivity, for EIA, WB, and IIF methods are shown in Figure 1. There were no false-negative or false-positive interpretations among the 1,081 EIA results reported. Five indeterminate interpretations were among the 117 WB results reported. There were no false-negative or false-positive interpretations among the 18 Indirect Immunofluorescence test results reported. There was one indeterminate interpretation among the 62 test results reported using HTLV-I/II testing methods classified as Other.

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 (82.3%), while some laboratories performed both EIA and supplemental tests (11.8%). Six laboratories (3.2%) performed WB alone or WB in combination with testing methods classified as "Other."

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 68.2% of laboratories reporting EIA results, the Organon Teknika HTLV-I/II kit was used by 19.6% of laboratories, and a variety of other test kits were used. The Genelabs Diagnostic WB kit was used by 61.5% of laboratories reporting WB results, the Cambridge (Calypte) kit was used by 26.9% of laboratories, 7.7% of laboratories used WB test kits classified as Other, and one laboratory used a WB test kit manufactured in house.

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

All results were correctly reported for the HTLV-I/II antibody-negative and antibody-positive samples.

WB Results

Two indeterminate WB interpretations each were reported for the two HTLV-I/II antibody-negative samples (Donor numbers 1 and 4) by laboratories using WB test kits manufactured by Cambridge (Calypte). The CDC did not detect any bands in these HTLV-I/II antibody-negative samples using test kits manufactured by Genelabs and Cambridge (Calypte). One indeterminate interpretation was reported for the HTLV-II antibody-positive sample (Donor number 2) by a laboratory using a WB test kit manufactured by Genelabs.

Of the 26 participant laboratories reporting WB results, 24 (92.3%) provided information regarding the criteria used for WB interpretations. Thirteen of these (54.2%) used interpretive criteria contained in the insert of the manufactured WB kit they used for testing. Other laboratories used the interpretive criteria published by the World Health Organization, three (12.5%); the Association of Public Health Laboratories (APHL), two (8.3%); or "Other" criteria, five (20.8%). One laboratory indicated using the Public Health Service (PHS) Working Group criteria. The WB interpretive criteria of these organizations and the WB test kit manufacturers are described in the table on the following page.

CRITERIA FOR INTERPRETATION OF HTLV WESTERN BLOT TESTS

Source of Interpretative Criteria	Criteria for Positive Test
Public Health Service (PHS) Working Group	p24 **and** gp46 or gp61/68
Association of Public Health Laboratories (APHL)	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
Cambridge Biotech	p24 **and** gp46 or rp21e
Genelabs Diagnostics	**HTLV-I** p19 (with or without p24) **and** GD21 **and** rgp46-I **HTLV-II** p24 (with or without p19) **and** GD21 **and** rgp46-II

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

Excluding the three laboratories using test kits manufactured in-house or whose test kit manufacturer is described as "Other", eight laboratories are not using the WB interpretative criteria contained in the insert of the manufacturer's kit they used to test the performance evaluation samples. Two laboratories using the WHO WB interpretative criteria used the Genelabs WB test kit. One laboratory using the APHL guidelines used the Cambridge (Calypte) WB test kit. Four laboratories using the WB interpretative criteria described as "Other" used the Genelabs WB test kit. The one laboratory using the PHS criteria used a Cambridge (Calypte) WB test kit. In addition, one of the laboratories whose WB kit manufacturer is described as "Other" reported WB results using an Innogenetics Inno-LIA test kit and based their result interpretations on the APHL/CDC WB interpretative criteria. The Inno-LIA is not a Western blot test kit.

IIF Results

All results were correctly reported for the HTLV-I/II antibody-negative and antibody-positive samples.

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 2-3), the participating laboratories detected antibodies to most of the native viral-specific proteins (e.g., p19, p24, p32/33, and gp46). Two laboratories using test kits manufactured by Cambridge Biotech (one using the APHL interpretative criteria and the other using the PHS interpretative criteria) each reported a p19 band and an indeterminate interpretation for the HTLV-I/II antibody-negative donor number 1. One laboratory using a test kit manufactured by Cambridge Biotech and using the PHS interpretative criteria reported a p19 band and an indeterminate interpretation for the HTLV-I/II antibody-negative donor number 4. One laboratory using a WB test kit manufactured by Genelabs, and using a interpretative criteria described as "Other", reported p19, p21, p24, p36, rgp46-II, and p53 bands and an indeterminate interpretation for the HTLV-II antibody-positive donor number 2.

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. Generally, laboratories reported 1+ or greater fluorescence intensity in HTLV-infected cells for the HTLV-II antibody-positive samples (Donor 2) and 2+ or greater for the HTLV-I antibody positive samples (Donor 3). No IIF reactivity was reported for any of the HTLV-I/II antibody-negative samples (Donors 1 and 4) tested.

Results derived from testing methods classified as Other

The results reported by laboratories using testing methods classified as Other are shown in Figure 10. One laboratory using an in-house HTLV-I specific RIPA method reported an indeterminate interpretation for the duplicate HTLV-II antibody-positive samples (Donor 2). Five laboratories using a particle agglutination test kit, Serodia-HTLV-I manufactured by Fujirebio, Inc., correctly identified all HTLV-I/II antibody-negative and antibody-positive samples they tested. Two laboratories using a chemiluminescence assay, manufactured by Abbott, correctly identified all HTLV-I/II antibody-negative and antibody-positive samples it tested. Five laboratories using the line immunoassay (Inno-LIA HTLV-I/II, manufactured by Innogenetics) correctly identified all HTLV-I/II antibody-negative and antibody-positive samples they tested.

The antigen line results reported by laboratories using the Inno-LIA HTLV-I/II assay are shown in Figure 11. One laboratory reported a HTLV-II specific antigen line (rgp-46II) for the HTLV-I donor specimen (Donor 3).

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 manufacturer's 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.

All 179 laboratories reporting EIA test results responded to the question whether they used external EIA QC samples. Of these 179 laboratories, 134 (74.9%) indicated they used external EIA QC samples. Of the 134 affirmative external EIA QC responses, 99 (73.9%) indicated they obtained QC samples for EIA testing only from commercial sources. Twenty-five (18.7%) laboratories indicated they only used HTLV EIA QC samples obtained in house. Nine laboratories (6.7%) use both in house and commercially prepared QC samples. One laboratory indicated it used QC samples, but did not indicate its source. Forty-nine (36.6%) of the 134 responses indicated the use of a single serum/plasma and 84 (62.7%) indicated the use of multiple sera/plasma. Seventy-four (55.2%) of the 134 responses indicated the use of a weakly positive external control. Seventy-two (53.7%) of the 134 responses indicated external EIA QC was used with each set/run of EIA plates and 49 (36.6%) of the 134 responses indicated external EIA QC was used with each plate.

Of the 24 laboratories responding to the question regarding the use of external QC samples in WB testing, 15 (62.5%) reported the use of external QC samples. Nine of the 15 laboratories (60.0%) using external WB QC samples indicated they only used HTLV WB QC samples obtained in house. Four (26.7%) laboratories indicated they only used a commercial source for WB QC samples. Two laboratories (13.3%) indicated they used WB QC samples obtained both commercially and in house. Seven (46.7%) of the 15 laboratories reported using external QC material with each set or run of WB strips.

One (50.0%) of two laboratories reporting IIF results reported using external QC samples. The laboratory used in-house prepared QC samples and ran the samples with each set/run of IIF determinations.

Conclusion

Most of the laboratories participating in this survey correctly identified the HTLV-I/II antibody-positive and antibody-negative samples. The analytic sensitivity, analytic specificity, and analytic performance is 100.0% for the EIA test. If indeterminate interpretations are considered correct for antibody-positive samples, the analytic sensitivity is 100%, the analytic specificity is 89.7%, and the analytic performance is 96.6% for the WB test. The analytic sensitivity, analytic specificity and analytic performance for the IIF test are 100%.