Analysis of the May 15, 2000 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 15, 2000. Testing results for this analysis were provided by 204 (88.3%) of 231 laboratories sent sample panels. Of these, two laboratories supplied testing information (test kit manufacturer, quantitative testing results, etc.), but did not provide interpretations (positive, negative, indeterminate). One laboratory provided testing results using a test kit specific for detection of antibody to human immunodeficiency virus (HIV). Results from these three laboratories are not included in this aggregate 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 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-positive or false-negative interpretations among the 1,198 EIA interpretations reported. One laboratory provided testing information (kit manufacturer and lot number, testing absorbance values, S/C ratios, etc.), but did not provide EIA interpretations. Nine indeterminate interpretations were among the 130 WB results reported. There was one indeterminate interpretation among the 24 Indirect Immunofluorescence test results reported. There were 6 indeterminate interpretations among the 31 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 (83.2%), while some laboratories performed both EIA and supplemental tests (13.9%). Two laboratories (1.0%) 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 75.3% of laboratories reporting EIA results, the Organon Teknika HTLV-I/II kit was used by 17.2% of laboratories, and a variety of other test kits were used. The Genelabs Diagnostic WB kit was used by 55.2% of laboratories reporting WB results, the BioMerieux/Cambridge Biotech kit was used by 34.5%, and two laboratories used test kits manufactured in house. Three laboratories reported using IIF kits 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

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

Of the 29 participant laboratories reporting WB results, 28 (96.6%) provided information regarding the criteria used for WB interpretations. Fifteen of these (53.5%) 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, five (17.9%); the Association of Public Health Laboratories (APHL), three (10.7%); or "Other" criteria, four (14.3%). 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 or p24 **and** gp56 or rgp46-I **and** rgp21 **HTLV-II** p24 **and** rgp46-II **and** rgp21

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

Of the five laboratories using the WHO WB interpretative criteria, three used the Genelabs WB test, one used the Cambridge Biotech, and one used a kit manufacturer classified as Other. Of the three laboratories using the APHL guidelines, one used the Cambridge Biotech WB test, one used the Genelabs WB test, and one used a WB kit manufactured in house. Of the four laboratories using the WB interpretative criteria described as "Other", three laboratories used the Genelabs WB test and one used the Cambridge Biotech WB test. The one laboratory using the PHS criteria used a Cambridge Biotech WB test kit. Excluding laboratories using test kits manufactured in house or classified as Other, eleven 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.

IIF Results

All results were correctly reported for the HTLV-antibody-negative samples. The one indeterminate interpretation reported for the HTLV-I antibody-positive sample (Donor 3) was reported by a laboratory using a test kit manufactured in house and reported a fluorescent intensity of 4+ for both uninfected and infected cells.

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). The presence of recombinant gp46-type I (r46I) was correctly reported for the HTLV-I antibody-positive sample, and recombinant gp46-type II (r46II) was correctly reported for the HTLV-II antibody-positive sample by the laboratories using commercially available WB strips designed to detect these bands. Four laboratories using test kits manufactured by Genelabs reported a p19 band for the HTLV-I/II antibody-negative donor number 1. Two laboratories using test kits manufactured by Genelabs reported a r21e band for this sample. One laboratory using a WB test kit manufactured by Genelabs reported p19, r21e, and p24 bands for the HTLV-I/II antibody-negative donor number 4.

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. Generally, laboratories reported 1+ or greater fluorescence intensity in HTLV-infected cells for the HTLV-II antibody-positive samples (Donor 2) and 4+ 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. Two laboratories using a particle agglutination test kit, Serodia-HTLV-I manufactured by Fujirebio, Inc., correctly identified 4 of 6 HTLV-I/II antibody-positive and 4 of 6 HTLV-I/II antibody-negative samples. A laboratory using an in-house HTLV-I specific RIPA method correctly identified the HTLV-I antibody-positive sample (Donor 3) and reported indeterminate results for the duplicate HTLV-II antibody-positive sample (Donor 2). This laboratory used an in-house HTLV-II specific RIPA and correctly reported positive results for the HTLV-II antibody-positive samples (Donor 2). Two laboratories using a chemiluminescence assay, manufactured by Abbott, correctly identified all HTLV-I/II antibody-negative and antibody-positive samples. Two laboratories using a line immunoassay (Inno-LIA HTLV-I/II, manufactured by Innogenetics) correctly identified all HTLV-I/II antibody-positive samples. One laboratory using the Inno-LIA HTLV-I/II test kit provided testing information (kit lot number, viral specific bands detected, etc.), but did not provide interpretations of their testing results.

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.

Of the 198 laboratories reporting EIA test results, 195 laboratories responded to the question whether they used external EIA QC samples. Of these 195 laboratories, 133 (68.2%) indicated they used external EIA QC samples. Of the 133 affirmative external EIA QC responses, 112 (84.2%) indicated they obtained QC samples for EIA testing from commercial sources. Fifty-one (38.3%) of the 133 responses indicated the use of a single serum/plasma and 82 (61.7%) indicated the use of multiple sera/plasma. Sixty-seven (50.4%) of the 133 responses indicated the use of a weakly positive external control. Seventy-five (56.4%) of the 133 responses indicated external EIA QC was used with each set of EIA plates and 49 (36.8%) of the 133 responses indicated external EIA QC was used with each plate run.

Of the 29 laboratories responding to the question regarding the use of external QC samples in WB testing, 13 (44.8%) reported the use of external QC samples. Eleven laboratories (84.6%) using external WB QC samples indicated they obtained HTLV WB QC samples in house. In addition, four (30.8%) laboratories also used a commercial source for WB QC samples. Four (30.8%) of the 13 laboratories reported using external QC material with each set or run of WB strips.

One (33.3%) of three laboratories reporting IIF results reported using external QC samples.

Conclusion

Most of the laboratories participating in this survey correctly identified the HTLV-I/II antibody-positive and antibody-negative samples. Since no incorrect EIA interpretations were reported, the analytic sensitivity, analytic specificity and analytic performance for this test are 100%. If indeterminate interpretations are considered correct for antibody-positive samples, the analytic sensitivity is 100%, the analytic specificity is 89.1%, and the analytic performance is 96.2% for the WB test. If indeterminate interpretations are considered correct for antibody-positive samples, the analytic sensitivity, analytic specificity and analytic performance for the IIF test are 100%.