Analysis of the November 15, 1999 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 15, 1999. Testing results for this analysis were provided by 200 (86.2%) of 232 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.

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. There was one false-negative interpretation among the 1,187 EIA interpretations reported. Ten indeterminate interpretations were among the 139 WB results reported. No false-positive or false-negative results were reported using the Indirect Immunofluorescence test.

Results reported by two laboratories that correctly identified all HTLV-I/II antibody-positive and 5 of 6 HTLV-I/II antibody-negative samples using a particle agglutination test kit, Serodia-HTLV-I, manufactured by Fujirebio, Inc., are shown in figure 10. Also shown in this figure are: results of a laboratory using an in-house RIPA method which correctly identified two of three HTLV-I/II antibody-positive samples; results of a laboratory using a chemiluminescence assay, manufactured by Abbott, which correctly identified all HTLV-I/II antibody-negative and antibody-positive samples; and results from three laboratories using a line immunoassay (Inno-LIA HTLV-I/II, manufactured by Innogenetics) which correctly identified all HTLV-I/II antibody-negative and antibody-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 (83.9%), while some laboratories performed both EIA and supplemental tests (13.6%). Five laboratories (2.5%) 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 76.8% of laboratories reporting EIA results, the Organon Teknika HTLV-I/II kit was used by 18.6% of laboratories, and a variety of other test kits were used. The Genelabs Diagnostic WB kit was used by 60.0% of laboratories reporting WB results, the BioMerieux/Cambridge Biotech kit was used by 36.7%, and one laboratory used a test kit 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 samples. One false-negative result was reported for a HTLV-I antibody-positive sample by a laboratory using an Abbott HTLV-I/II EIA kit.

WB Results

Of the seven indeterminate WB interpretations reported for the HTLV-I/II antibody-negative samples (Donor numbers 1 and 4), three were reported by laboratories using WB test kits manufactured by Cambridge Biotech, three were reported by laboratories using WB test kits manufactured by Genelabs, and one was reported by a laboratory using a test kit manufactured in house. Of the three indeterminate interpretations reported for the HTLV-II antibody-positive sample (Donor number 2), two were reported by laboratories using WB test kits manufactured by Genelabs and one was reported by a laboratory using a test kit manufactured by Cambridge Biotech.

Of the 30 participant laboratories reporting WB results, 28 (93.3%) provided information regarding the criteria used for WB interpretations. Nineteen of these (67.9%) 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 (10.7%); the Association of Public Health Laboratories (APHL), two (7.1%); or "Other" criteria, three (10.7%). 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 following table:

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 State and Territorial Public Health Laboratory Directors (ASTPHLD, now Association of Public Health Laboratories or 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**

All three laboratories using the WHO WB interpretative criteria used the Genelabs WB test. Of the two laboratories using the APHL guidelines, one used the Cambridge Biotech WB test and one used the Genelabs WB test. Of the three laboratories using the WB interpretative criteria described as "Other", two 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. These nine 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 (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 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. Two laboratories using a test kits manufactured by Cambridge and two laboratories using test kits manufactured by Genelabs reported a p19 band for the HTLV-I/II antibody-negative donor number 1. One laboratory using a test kit manufactured by Genelabs reported a p24 band for this sample. Two laboratories using WB test kits manufactured by Cambridge reported a p19 band for the HTLV-I/II antibody-negative donor number 4. One laboratory using a test kit manufactured in house reported a p24 band for this sample.

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 2+ or greater fluorescence intensity in HTLV-infected cells for the HTLV-I/II antibody-positive samples for which intensity information was collected No IIF reactivity was reported for any of the HTLV-I/II antibody-negative samples (Donors 1 and 4) 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 194 laboratories reporting EIA test results, 182 laboratories responded to the question whether they used external EIA QC samples (185 responses, 3 laboratories reported results using 2 EIA kits). Of these 185 responses, 130 (70.3%) indicated they used external EIA QC samples. Of the 130 affirmative external EIA QC responses, 105 (80.8%) indicated they obtained QC samples for EIA testing from commercial sources. Fifty-six (43.1%) of the 130 responses indicated the use of a single serum/plasma and 74 (56.9%) indicated the use of multiple sera/plasma. Sixty-six (50.8%) of the 130 responses indicated the use of a weakly positive external control. Seventy-four (56.9%) of the 130 responses indicated external EIA QC was used with each set of EIA plates and 49 (37.7%) of the 130 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, 9 (31.0%) reported the use of external QC samples. All 9 laboratories (100%) using external WB QC samples indicated they obtained HTLV WB QC samples in house. In addition, two (22.2%) laboratories also used a commercial source for WB QC samples. Three (33.3%) of the 9 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. The sensitivity, specificity, and analytic performance of the EIA test are, respectively, 99.8%, 100%, and 99.9%. If indeterminate interpretations are considered correct for antibody-positive samples, the sensitivity is 100%, the specificity is 85.7%, and the analytic performance is 95.0% for the WB test. Since no incorrect IIF interpretations were reported, the sensitivity, specificity and analytic performance for this test are 100%.