Analysis of the May 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 May 2001. Testing results for this analysis were provided by 186 (86.9%) of 214 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 was one false-negative among the 1,082 EIA interpretations reported. Five indeterminate interpretations were among the 121 WB results reported. There were two indeterminate interpretations among the 18 Indirect Immunofluorescence test results reported. There were one false-negative and two indeterminate interpretations among the 39 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 (81.7%), while some laboratories performed both EIA and supplemental tests (12.4%). 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 71.1% of laboratories reporting EIA results, the Organon Teknika HTLV-I/II kit was used by 17.8% of laboratories, and a variety of other test kits were used. The Genelabs Diagnostic WB kit was used by 60.7% of laboratories reporting WB results, the BioMerieux/Cambridge Biotech kit was used by 28.5% of laboratories, 7.1% 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

One laboratory using the Abbott HTLV-I/II EIA kit reported one false-negative EIA interpretation for Donor number 2.

WB Results

Two indeterminate WB interpretations were reported for one of the HTLV-I/II antibody-negative samples (Donor number 1) by laboratories using WB test kits manufactured by Genelabs. The CDC did not detect any bands in this HTLV-I/II antibody-negative sample using test kits manufactured by Genelabs and Cambridge Biotech. Three indeterminate interpretations were reported for the HTLV-II antibody-positive sample (Donor number 2) by laboratories using WB test kits manufactured by Genelabs (2 interpretations) and Cambridge Biotech (one interpretation).

Of the 28 participant laboratories reporting WB results, 26 (92.8%) provided information regarding the criteria used for WB interpretations. Fourteen of these (53.8%) 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 (19.2%); the Association of Public Health Laboratories (APHL), three (11.5%); or "Other" criteria, three (11.5%). 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**

Of the five laboratories using the WHO WB interpretative criteria, four used the Genelabs WB test kit, and one used the Cambridge Biotech WB test kit. Of the three laboratories using the APHL guidelines, one used the Cambridge Biotech WB test kit, one used the Genelabs WB test kit and one used a test kit manufactured in house. All three 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 Biotech WB test kit. These twelve 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

Two indeterminate interpretations were reported for the HTLV-I antibody positive sample (Donor number 3).

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. One laboratory using a test kit manufactured by Cambridge Biotech and using the APHL interpretative criteria reported a p19 bands and negative interpretations for both duplicate samples of the HTLV-I/II antibody-negative donor number 1. One laboratory using a test kit manufactured by Genelabs and using the manufacturer's interpretative criteria reported p19 and p21/22 bands and indeterminate interpretations for both duplicate samples of this same HTLV-I/II antibody-negative donor.

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 a particle agglutination test kit, Serodia-HTLV-I manufactured by Fujirebio, Inc., reported a false negative result for the HTLV-I positive donor sample. One laboratory using an in-house HTLV-I specific RIPA method reported positive reactivity for the HTLV-I antibody-positive sample (Donor 3) and indeterminate interpretations for the duplicate HTLV-II antibody-positive samples (Donor 2). One laboratory using a chemiluminescence assay, manufactured by Abbott, correctly identified all HTLV-I/II antibody-negative and antibody-positive samples it tested. Six 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.

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 180 laboratories reporting EIA test results, 174 laboratories responded to the question whether they used external EIA QC samples. Of these 174 laboratories, 135 (77.6%) indicated they used external EIA QC samples. Of the 135 affirmative external EIA QC responses, 103 (76.3%) indicated they obtained QC samples for EIA testing only from commercial sources. Twenty-five (18.5%) laboratories indicated they only used HTLV EIA QC samples obtained in house. Six laboratories use both in house and commercially prepared QC samples. One laboratory indicated it used QC samples, but did not indicate its source. Fifty-three (39.3%) of the 135 responses indicated the use of a single serum/plasma and 81 (60.0%) indicated the use of multiple sera/plasma. Seventy-one (52.6%) of the 135 responses indicated the use of a weakly positive external control. Eighty (59.3%) of the 135 responses indicated external EIA QC was used with each set/run of EIA plates and 43 (31.9%) of the 135 responses indicated external EIA QC was used with each plate.

Of the 27 laboratories responding to the question regarding the use of external QC samples in WB testing, 16 (59.3%) reported the use of external QC samples. Ten laboratories (62.5%) using external WB QC samples indicated they only used HTLV WB QC samples obtained in house. Five (31.3%) laboratories indicated they only used a commercial source for WB QC samples. One laboratory indicated it used WB QC samples obtained both commercially and in house. Nine (56.3%) of the 16 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.

Conclusion

Most of the laboratories participating in this survey correctly identified the HTLV-I/II antibody-positive and antibody-negative samples. The analytic sensitivity is 99.8%, the analytic specificity is 100% and the analytic performance is 99.9% for the EIA test. If indeterminate interpretations are considered correct for antibody-positive samples, the analytic sensitivity is 100%, the analytic specificity is 94.9%, and the analytic performance is 98.3% 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%.