Analysis of the May 2002 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 testing the human T-lymphotropic virus types I and II (HTLV-I/II) performance evaluation samples shipped in May 2002. Of the 212 laboratories which were sent sample panels, testing results were provided by 187 (88.2%). 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.

Methods

Samples in this shipment consisted of plasma from donors who were HTLV-I/II antibody-negative (donor numbers 1 and 2) and donors antibody-positive for either HTLV-I (donor number 3) or HTLV-II (donor number 4). 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.

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 on page 2 of the accompanying aggregate results report. There were 4 false-negative and 7 false-positive interpretations among the 1,110 EIA results reported. There was one indeterminate interpretation among the 110 WB results reported. There were no false-negative or false-positive interpretations among the 18 indirect immunofluorescence test results reported. There were two indeterminate interpretations among the 76 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 on page 3 of the accompanying aggregate results report. 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 on page 3 of the accompanying aggregate results report. Most laboratories performed only EIA (81.8%), while some laboratories performed both EIA and supplemental tests (10.7%). Additionally, eight laboratories (4.3%) performed EIA in combination with testing methods classified as "Other." 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 on page 4 in the accompanying aggregate results report. The Abbott HTLV-I/HTLV-II EIA kit was used by 68.5% of laboratories reporting EIA results, the Organon Teknika HTLV-I/II kit was used by 22.7% of laboratories, and a variety of other test kits were used. The Genelabs Diagnostic WB kit was used by 92.0% of laboratories reporting WB results, and the Cambridge (Calypte) kit was used by one laboratory.

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 on pages 5-7 in accompanying aggregate results booklet.

EIA Results

As can be seen in Figure 5, for Donor number 1 (HTLV-I/II antibody-negative), four false-positive interpretations were reported by laboratories using EIA test kits manufactured by Pasteur Diagnostics (Sanofi) and one false positive interpretation was reported by a laboratory using an EIA test kit classified as "Other." For Donor number 2 (HTLV-I/II antibody-negative), two false positive interpretations were reported by laboratories using test kits manufactured by Abbott. For Donor number 3 (HTLV-I antibody-positive), one false-negative interpretation was reported by a laboratory using an EIA test kit classified as "Other." For Donor number 4 (HTLV-II antibody-positive), false negative interpretations were reported by laboratories using an EIA test kit manufactured by Ortho (one interpretation) and by laboratories using EIA test kits classified as "Other" (two interpretations).

WB Results

As can be seen in Figure 6, one indeterminate WB interpretation was reported for Donor 2 (HTLV-I/II antibody-negative) by a laboratory using a WB test kit 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 (Calypte).

Of the 25 participant laboratories reporting WB results, 21 (84.0%) provided information regarding the criteria used for WB interpretations. Fourteen of these (66.7%) used interpretive criteria contained in the insert of the manufactured WB kit they used for testing. Two (9.5%) laboratories used the interpretive criteria published by the World Health Organization, and five (23.8%) used "Other" criteria. The WB interpretive criteria of several 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, p24**

Of the seven laboratories 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 criteria and four laboratories using a WB criteria classified as "Other" used test kits manufactured by Genelabs, and one laboratory using a test kit manufacturer classified as "Other" used a WB criteria classified as "Other."

IIF Results

As shown in Figure 7, 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 on page 8 of the accompanying aggregate results booklet. 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 3-4), the participating laboratories detected antibodies to most of the native viral-specific proteins (e.g., p19, p24, p32/33, and gp46). One laboratory using a test kit manufactured by Genelabs, and using the manufacturer's interpretative criteria, reported a p24 band and an indeterminate interpretation for the HTLV antibody-negative Donor 2.

IIF Fluorescence Intensity

The fluorescence intensity patterns of HTLV-infected cells, as reported by participant laboratories, are shown in Figure 9 on page 9 of the accompanying aggregate results booklet. 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-I antibody-positive sample (Donor 3) and 2+ or greater for the HTLV-II antibody positive sample (Donor 4). No IIF reactivity was reported for any of the HTLV-I/II antibody-negative samples (Donors 1 and 2) 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 on page 10 of the accompanying aggregate results booklet. One laboratory using an in-house HTLV-I specific RIPA method reported two indeterminate interpretations for the duplicate HTLV-II antibody-positive samples (Donor 4). 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 they tested. Eight 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 on page 11 of the aggregate results booklet. One laboratory reported a HTLV-I/II antigen specific line (p19 I/II) for one of the HTLV-I/II antibody-negative donor specimens (Donor 2).

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 181 laboratories reporting EIA test results, 172 responded to the question whether they used external EIA QC samples. Of these 172 laboratories, 139 (80.8%) indicated they used external EIA QC samples. Of the 139 affirmative external EIA QC responses, 103 (74.1%) indicated they obtained QC samples for EIA testing only from commercial sources, 26 (18.7%)

laboratories indicated they only used HTLV EIA QC samples obtained in house, and 10 laboratories (7.2%) use both in house and commercially prepared QC samples. Forty-nine (35.3%) of the 139 responses indicated the use of a single serum/plasma and 89 (64.0%) indicated the use of multiple sera/plasma. Seventy-one (51.1%) of the 139 responses indicated the use of a weakly positive external control. Seventy-eight (56.1%) of the 139 responses indicated use of external EIA QC with each set/run of EIA plates, and 48 (34.5%) indicated use of external EIA QC with each plate.

Of the 25 laboratories reporting WB results, 23 laboratories (92.0%) responded to the question regarding the use of external QC samples, and 13 (56.5%) of these 23 laboratories reported the use of external QC samples. Of the 13 laboratories reporting the use of external QC samples in WB testing, 8 (61.5%) indicated they only used HTLV WB QC samples obtained in house, 4 (30.8%) laboratories indicated they only used a commercial source for WB QC samples, and one laboratory indicated it used WB QC samples obtained both commercially and in house. Seven (53.8%) of the 13 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 multiple sera/plasma QC samples and ran the samples with each set/run of IIF determinations.

Conclusions

Most of the laboratories participating in this survey correctly identified the HTLV-I/II antibody-positive and antibody-negative samples. In this survey, the analytic sensitivity was 99.3%, the analytic specificity was 98.7%, and the analytic performance was 99.0% for the EIA test. In this survey, the analytic sensitivity was 100%, the analytic specificity was 97.1%, and the analytic performance was 99.1% for the WB test. The analytic sensitivity, analytic specificity and analytic performance for the IIF test were all 100% in this survey.