

**Analysis of the January 2002 Performance Evaluation
HIV-1 Antibody Testing Results
Reported to the Centers for Disease Control and Prevention (CDC)
by Laboratories Participating in the Model Performance Evaluation Program**

This report is an analysis of results provided to the Centers for Disease Control and Prevention (CDC) by laboratories participating in the Model Performance Evaluation Program (MPEP) after they tested the human immunodeficiency virus type 1 (HIV-1) performance evaluation samples shipped to them in January 2002. Testing results were reported by 780 (90.3%) of 864 laboratories that received sample panels.

Samples used in the MPEP surveys are undiluted, defibrinated plasma obtained from individual donors who are HIV-1-infected (positive) or HIV-1-uninfected (negative). The samples were treated with an organic solvent-detergent mixture known to inactivate blood borne viruses including HIV-1, human T-lymphotropic virus, and hepatitis B and C. Before shipment, each donor sample was tested with two HIV-1 enzyme immunoassay (EIA) kits, two HIV-1/HIV-2 EIA kits and one rapid test (RT) kit (SUDS HIV-1) licensed by the Food and Drug Administration (FDA). Supplemental testing was performed with two FDA-licensed HIV-1 Western blot (WB) kits. Donor samples were not tested prior to shipment with any HIV-1 indirect immunofluorescence (IFA) test.

In pre-shipment testing, the strong-positive HIV-1 donor sample (Donor 5) was EIA repeatedly reactive with all of the HIV-1 EIA kits and the HIV-1/HIV-2 EIA kits. It was also WB reactive with the HIV-1 FDA-licensed WB kits. The negative donor sample (Donor 1) was EIA repeatedly non-reactive and demonstrated no bands with any FDA-licensed HIV-1 WB kit. Donor samples 3 and 4, obtained from individual donors recently infected with HIV-1, were positive for HIV-1 antibody and demonstrated EIA and WB reactivity with the FDA-licensed EIA, WB and RT kits used for preshipment testing. Testing information for sequential serum samples from Donors 3 and 4 demonstrated factors consistent with seroconversion such as a positive p24 antigen test, positive test for HIV-1 ribonucleic acid (RNA), rising HIV-1 antibody titers in all EIA tests, and WB reactivity changing from nonreactive (no bands) to indeterminate or reactive from one donation to the next.

Figure 1 shows the cumulative frequency of test result interpretations reported by participating laboratories, arranged according to donor reactivity, for the EIA, WB, and IFA methods. Of the 737 EIA interpretations reported for the HIV-1-negative sample, one (0.1%) was incorrectly reported as reactive. There were seven (0.2%) false-negative EIA interpretations among the 3,704 interpretations reported for the HIV-1-positive samples. Of 128 WB interpretations reported for the HIV-1-negative sample, two (1.6%) reactive and 10 (7.8%) indeterminate WB interpretations were reported. Among the 1,309 WB interpretations reported for the HIV-1-positive samples, there were two (0.2%) false-negative and six (0.5%) indeterminate interpretations reported. Among the 21 IFA interpretations reported for the HIV-1-negative sample, one (4.8%) indeterminate interpretation was reported. Of the 165 IFA interpretations reported for HIV-1-positive samples, there were five (3.0%) indeterminate and eight (4.8%) false-negative interpretations.

The types of laboratories that reported results to CDC are shown in Figure 2. Each laboratory type is listed, by decreasing frequency, for each of the test methods.

The combinations of test methods used by the laboratories and the frequency of use are shown in Figure 3. Of the 780 laboratories reporting results, 373 (47.8%) performed only EIA, 227 (29.1%) performed only EIA and a supplemental test, and six (0.8%) performed only a supplemental test. These numbers do not include the 174

(22.3%) laboratories that performed an “Other” test in addition to or instead of EIA, WB and IFA. The data for these “Other” tests are presented in Figure 10.

The types of test kits used, by kit manufacturer, for the EIA, WB, and IFA methods are shown, by decreasing frequency, in Figure 4. For each test method, some laboratories indicated using test kits for which there were no unique manufacturer codes provided in the survey report form and these responses have been grouped as “Other” manufacturer kits. Some “Other” kits reported as being used for EIA include Bio-Rad Genscreen Plus HIV Ag-Ab (five laboratories), Abbott HIV-1/2 gO EIA (four laboratories) and Innogenetics Innostest HIV-1/HIV-2 Ab (four laboratories). Some laboratories, located outside the United States, used the Abbott AXSYM system or the Abbott PRISM analyzer and reported results as S/CO (sample/cutoff ratio). Since the S/CO data can not be entered correctly on the MPEP EIA result form, the data from laboratories using either AXSYM or PRISM systems are reported with “Other” tests in Figure 10.

The results reported for the EIA, WB, and IFA methods, listed by kit manufacturer, for the HIV-1-positive and HIV-1-negative samples are shown in Figures 5, 6, and 7. Results reported by the participant laboratories reflect their testing performance using manufactured kits to evaluate MPEP samples and do not necessarily reflect an evaluation of these manufactured kits.

EIA Results

In Figure 5, one false positive EIA interpretation was reported for Donor 1.

Of the seven nonreactive EIA interpretations reported for HIV-1 positive samples, four were reported for Donor 3, with four different test kits. The remaining three false negative interpretations were reported for Donors 4 and 5 by laboratories using two different test kits.

WB Results

Of the 780 laboratories reporting test results in this survey, 265 (34.0%) performed WB testing. Two reactive and 10 indeterminate WB interpretations were reported by 12 different laboratories for the HIV-1 uninfected donor sample (Donor 1), using seven different WB kits.

Of the six indeterminate WB results reported for samples from the HIV-1-infected donors, four were reported for Donor 4 and two for Donor 3, both HIV-1-infected seroconverting donors. Indeterminate WB interpretations were reported by laboratories using two different WB kits (Figure 6). The two nonreactive WB interpretations were reported by one laboratory for Donor 3 and Donor 5, using a test kit from a manufacturer classified as “Other”.

Of the 265 laboratories reporting WB test results, 234 indicated which WB criteria were used to interpret their WB tests. The APHL/CDC WB interpretive criteria were used by 197 (84.2%) of these 234 laboratories. The WB interpretive guidelines published by all the FDA-licensed WB kit manufacturers are identical to the APHL/CDC HIV-1 WB interpretive criteria. Most laboratories are using the APHL/CDC (formerly ASTPHLD/CDC) interpretive criteria.¹ Please be reminded that according to this interpretive criteria, a positive test result is defined by the presence of any two of the following bands: p24, gp41, and gp120/160. (Distinguishing the gp120 band from the gp160 band is often very difficult. These two glycoproteins can be considered as one reactant for purposes of

¹CDC. Interpretation and use of the western blot assay for serodiagnosis of human immunodeficiency virus type 1 infections. MMWR 1989;38:1-7.

interpreting WB test results). Four laboratories indicated they were using interpretive criteria different from that recommended by the kit manufacturer as licensed by the FDA.

WB Band Patterns

The WB bands for the donor samples in this survey, as determined in pre-shipment testing with two FDA-licensed WB test kits, are shown in Table 2. Only bands scoring greater than or equal to 1+ intensity are listed in Table 2.

The protein band patterns for the major viral proteins, as reported by participant laboratories for each donor sample, are shown in Figure 8. The frequency of a reported band is listed above the column. The number of WB reports received for the donor sample is indicated in the far right column. This figure does not include WB bands reported as 'W', or “weak”, indicating intensity less than that of the designated band of the weak positive control provided in the WB kit, nor does it include bands of greater than 1+ intensity reported for p15, p17, p51, p55, or p66. Five laboratories that reported indeterminate results reported non-viral bands. Note that 128 WB results were reported for the sample from an HIV-uninfected donor (Donor 1) although most laboratories do not normally include the testing of EIA-nonreactive donor samples in their routine algorithm for HIV antibody testing. Ten of the 12 laboratories that reported indeterminate or reactive WB results for the HIV-1 nonreactive donor reported non-reactive results with EIA testing for this donor.

For the HIV-1 antibody strong-positive sample (Donor 5) and the seroconversion samples (Donor 3 and Donor 4), most laboratories had no difficulty in detecting antibodies to gag, pol, and env antigens. Indeterminate and nonreactive interpretations reported for these samples most often resulted from failure to detect antibody to envelope (env) antigen gp120, or from failure to detect antibody to env antigen at sufficient intensity to be determined reactive. Two laboratories reported six indeterminate results, even though the band patterns appeared to fit the reported criteria for reactive results.

IFA Results

One indeterminate IFA interpretation was reported for the HIV-1-negative donor sample (Figure 7). Among the 165 IFA interpretations reported for the HIV-1-positive samples, eight (4.8%) false-negative and five (3.0%) indeterminate interpretations were reported. Six (75.0%) of the false negatives and four (80.0%) of the indeterminate results were reported for the samples from Donor 3.

The IFA intensity patterns for HIV-1 infected cells, as reported by participating laboratories, are shown in Figure 9. The frequency of reports of fluorescence intensity for each donor is listed in the far right column. A scoring of fluorescence intensity is not required for interpretation of seroreactivity with the FDA-licensed Sanochemia (formerly know as Waldheim) Fluorognost HIV-1 IFA kit; therefore, some laboratories provided interpretation, but did not score fluorescent intensity. Data from these laboratories were included in Figures 1 and 7, but cannot be included in Figure 9. Two (10.0%) of the 20 IFA reports received for samples from HIV-1-uninfected Donor 1 indicated observed fluorescence. Six (18.2%) of the 33 IFA reports received for samples from Donor 3 indicated no fluorescence observed and two (3.0%) of the 66 IFA reports received for Donor 4 samples indicated that no fluorescence was observed.

Other Tests Performed

Figure 10 provides information on the test results and interpretations provided by 174 laboratories that performed

HIV-1 antibody tests in addition to or other than traditional EIA, WB or IFA. The first graphic of this figure shows manufacturers of the "Other" types of tests and frequency of use. The rest of this figure shows the results reported by laboratories after testing the HIV-1-negative and HIV-1-positive samples in this shipment. Sixty-one (35.1%) of the 174 laboratories reporting results of "Other" types of tests did not report results using EIA, WB or IFA tests. The procedures used by 113 (64.9%) of these 174 laboratories can be described as "rapid tests". Of these, 78 (69.0%) laboratories reported using Murex/Abbott SUDS-HIV-1, 17 (15.0%) tested samples using Fujirebio Serodia HIV, and seven (6.2%) laboratories reported using Abbott Determine. Results of "Line or Strip Immunoassay" tests such as Innogenetics INNO-LIA, Organon Teknika Liatek and Chiron RIBA were reported by 18 laboratories. Note that all laboratories using the Abbott AXSYM or PRISM systems reported their results on the "Other" test type result form since these tests are based on microparticle capture and chemiluminescence measurements, and differ from the traditional microtiter-format EIA tests.

Among the 179 final interpretations reported for HIV-1-negative sample (Donor 1) tested by laboratories using these "Other" procedures, 13 false-positive interpretations were reported by 12 laboratories using five different test systems. Seven (53.8%) of the false positive interpretations were from laboratories using the Murex/Abbott SUDS-HIV-1 test, and three (23.1%) were from laboratories using the Fujirebio Serodia HIV. Three indeterminate interpretations were reported by laboratories using three different test systems.

Among the 972 interpretations reported for the HIV-1-positive samples tested by procedures other than EIA, WB, or IFA, there were 14 (1.4%) false-negative and six (0.6%) indeterminate interpretations. Seven (50.0%) of the false-negatives and four (66.7%) of the indeterminates were reported for samples from Donor 3. Seven (50.0%) of the false negative results were reported by four of the 78 laboratories using the SUDS HIV-1 test, and four (28.6%) additional false negative results were reported by a laboratory using the Abbott AXSYM HIV-1/HIV-2. Four laboratories reported six indeterminate interpretations using four different test kits.

Quality Control Testing

Information was sought on the use of quality control (QC) samples other than the controls provided in various test kits. Positive and negative samples included in manufactured kits are internal kit control material used to validate the test run, calculate test run cut-off values, and may not validate the analytic testing process which may include testing problems such as faulty pipettors, inadequate incubation conditions, or kit lot sensitivity. Most laboratories completing the QC section of the form adhered to the instructions pertaining to this section and described only external QC samples used in their HIV testing procedures.

Of the 701 laboratories that reported EIA test results, 503 (71.8%) indicated they used quality control samples other than those provided with the manufactured test kit. Of these 503 laboratories, 309 (61.4%) used samples obtained commercially, 157 (31.2%) used QC samples from in-house sources, and 27 (5.4%) used QC material from both commercial and in-house sources. Ten laboratories did not indicate the source of their external QC samples. The majority indicated the use of weakly-positive and negative serum/plasma with each set/run of plates or each EIA plate.

Of the 265 laboratories reporting WB test results, 93 (35.1%) laboratories used external QC samples. Of these 93 laboratories, 57 (61.3%) used samples prepared in-house, 31 (33.3%) used QC samples obtained commercially, and four (4.3%) used QC material from both commercial and in-house sources. One laboratory did not indicate the source of external QC samples used in WB. Most laboratories used weakly-positive serum/plasma with each set/run of WB strips.

Of the 33 laboratories reporting IFA results, 11 (33.3%) used IFA external QC samples. Of these, seven (63.6%) used samples from in-house sources, and four (36.4%) used QC samples obtained commercially. Most of the 11 laboratories included weakly-positive and negative serum/plasma with each set/run of slides or kit.

Of the 174 laboratories reporting results of tests other than EIA or WB or IFA, 48 (27.6%) indicated the use of external QC samples. Of these, 24 (50.0%) used samples from in-house sources and 20 (41.7%) used samples from commercial sources. Two (4.2%) used samples from both commercial and in-house sources. The majority of these laboratories indicated they used at least a weakly-positive QC sample with each set/run.

Conclusion

The MPEP provides challenging samples for participant laboratories to perform HIV-1 antibody testing. Most participant laboratories performed well in testing the HIV-1 donor samples in this shipment. Some false-negative results were reported for traditional EIA (0.2%), WB (0.2%) and IFA (4.8%) with HIV-1-positive samples. Also, one false-positive EIA (0.1%) and two false-positive WB (1.6%) results were reported for samples negative for HIV-1 antibody (Donor 1). No false-positive IFA results were reported in this survey. Some false-positive and false-negative results were reported by laboratories that performed testing using “Other” test systems, primarily by laboratories using rapid test kits.

The following information regarding overall analytic performance, analytic sensitivity, and analytic specificity is determined from the results reported by laboratories testing performance evaluation samples and is not intended to reflect the actual sensitivity and specificity of the manufactured test kits. For this survey, the overall EIA analytic sensitivity and specificity was 99.8% and 99.9%, respectively. When indeterminate and reactive WB interpretations are combined, the WB analytic sensitivity was 99.8%. If indeterminate interpretations are considered incorrect for HIV-1 antibody-negative samples, the WB analytic specificity for this survey was 90.6%. When indeterminate and reactive IFA interpretations are combined for the HIV-1-positive samples, the IFA analytic sensitivity was 95.2%. If indeterminate interpretations are considered incorrect for HIV-1 antibody-negative samples, the IFA analytic specificity was 95.2% for this survey. The analytic sensitivity and specificity of the “Other” test procedures vary greatly, depending on which test method results are analyzed (Figure 10). When indeterminate WB and IFA interpretations for the HIV-1 antibody-positive samples are combined with reactive interpretations, the overall analytic performance for laboratories testing these performance evaluation samples by EIA, WB, and IFA procedures was 99.8%, 99.0%, and 95.2% respectively.

Please note that we plan to ship the next panel of MPEP HIV-1 antibody samples to participating laboratories on July 9, 2002 to laboratories located outside the United States, and on July 23, 2002 to laboratories located within the United States.