

**Analysis of the January 2001 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 2001. Testing results were reported by 748 (86.7%) of 863 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 from HIV-1-infected donors 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 and two HIV-1/HIV-2 EIA kits licensed by the Food and Drug Administration (FDA). Supplemental testing was performed with four FDA-licensed HIV-1 Western blot (WB) kits. Donor samples were not tested prior to shipment with any HIV-1 indirect immunofluorescence (IIF) 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 all HIV-1 FDA-licensed WB kits. The negative donor sample (Donor 3) was EIA repeatedly non-reactive and demonstrated no bands with any FDA-licensed HIV-1 WB kit. Donor samples 1 and 2, obtained from individual donors recently infected with HIV-1, were positive for HIV-1 antibody and demonstrated variable EIA and WB reactivity with the FDA-licensed EIA and WB kits used for preshipment testing. Testing information for sequential serum samples from Donors 1 and 2 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 IIF methods. Of the 1,482 EIA interpretations reported for the HIV-1-negative sample, 16 (1.1%) were incorrectly reported as reactive. There were five (0.2%) false-negative EIA interpretations among the 2,988 interpretations reported for the HIV-1-positive samples. Of 249 WB interpretations reported for the HIV-1-negative sample, five (2.0%) reactive and 28 (11.2%) indeterminate WB interpretations were reported. Among the 1,060 WB interpretations reported for the HIV-1-positive samples, there were two (0.2%) false-negative and 82 (7.7%) indeterminate interpretations reported. Among the 38 IIF interpretations reported for the HIV-1-negative sample, there were no false-positive or indeterminate interpretations reported. Of the 125 IIF interpretations reported for HIV-1-positive samples, there were 11 (8.8%) indeterminate and 13 (10.4%) 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 748 laboratories reporting results, 414 (55.4%) performed only EIA, 241 (32.2%) performed only EIA and a supplemental test, and eight (1.1%) performed only a supplemental test. These numbers do not include the 85

(11.4%) laboratories that performed an “Other” test in addition to or instead of EIA, WB and IIF. 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 IIF 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 Murex HIV 1.2.O. (eight laboratories), Organon Teknika Vironostika HIV Uniform II Ag/Ab (six laboratories) and BioChem ImmunoSystems Detect HIV (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 IIF 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, the 16 false positive EIA interpretations were reported for Donor 3 by laboratories using nine different EIA kits from several manufacturers.

Of the five nonreactive EIA interpretations reported for HIV-1 positive samples, four were reported for the weak-positive samples (Donor 1 and 2) and one was reported for the strong-positive sample (Donor 5) by laboratories using 3 different EIA kits.

WB Results

Of the 748 laboratories reporting test results in this survey, 267 (35.7%) performed WB testing. Since laboratories are asked to test these performance samples as they would patient or donor samples, it is unclear why many laboratories performed WB testing on donor samples that they reported as nonreactive in EIA tests. Five reactive and 28 indeterminate WB interpretations were reported by 19 different laboratories for the HIV-1 uninfected donor sample (Donor 3), using five different WB kits.

Of the 82 indeterminate WB results reported for samples from the HIV-1-infected donors, 78 (95.1%) were reported for Donor 1, an HIV-1-infected seroconverting donor, and the other four were reported for Donor 2. Indeterminate WB interpretations were reported by laboratories using WB kits provided by seven different manufacturers (Figure 6). Laboratories using WB kits manufactured by BioRad accounted for 43 (52.4%) of the indeterminate WB results, and both of the false-negative interpretations.

The WB bands for the donor samples in this survey, as determined in pre-shipment testing with four 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.

Of the 267 laboratories reporting WB test results, 241 indicated which WB criteria were used to interpret their WB tests. The ASTPHLD/CDC WB interpretive criteria were used by 207 (85.9%) of these 241 laboratories. The

WB interpretive guidelines published by all the FDA-licensed WB kit manufacturers are identical to the ASTPHLD/CDC HIV-1 WB interpretive criteria. Please recall that the Association of State and Territorial Public Health Laboratory Directors (ASTPHLD) is now called the Association of Public Health Laboratories (APHL). Seven laboratories indicated they were using interpretive criteria different from that recommended by the kit manufacturer as licensed by the FDA.

WB Band Patterns

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. Note that 249 WB results were reported for the sample from an HIV-uninfected donor (Donor 3) although most laboratories do not normally include the testing of EIA-nonreactive donor samples in their routine algorithm for HIV antibody testing.

For the HIV-1 antibody strong-positive sample (Donor 5) and one seroconversion sample (Donor 2), laboratories had no difficulty in detecting antibodies to gag, pol, and env antigens regardless of the HIV-1 WB kit used. The donor samples obtained from the other seroconversion sample (Donor 1) appeared to cause more difficulty. Indeterminate interpretations reported for this sample most often resulted from failure to detect antibody to envelope (env) antigen gp160, or from failure to detect antibody to env antigen at sufficient intensity to be determined reactive. Six laboratories reported indeterminate results, even though the band patterns appeared to fit the reported criteria for reactive results. The CDC WB test results are shown in Table 2 of the results report accompanying this analysis.

IIF Results

No false-positive or indeterminate IIF interpretations were reported for the HIV-1-negative donor sample (Figure 7). Among the 125 IIF interpretations reported for the HIV-1-positive samples, 13 (10.4%) false-negative and 11 (8.8%) indeterminate interpretations were reported. All but one false-negative and all indeterminate interpretations were reported for the samples from seroconverting Donors 1 and 2. Eight (61.5%) of the false-negative interpretations were reported for samples from Donor 1.

Fluorescence Intensity Patterns

The IIF 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 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. No fluorescence intensity was reported for the sample from HIV-1-uninfected Donor 3. Eight (25.8%) of the 31 IIF reports received for samples from Donor 1 indicated no fluorescence observed and six (9.7%) of the 62 IIF reports received for Donor 2 samples indicated that no fluorescence was observed. One IIF report for the HIV-1-infected Donor 5 indicated no fluorescence observed.

Other Tests Performed

Figure 10 provides information on the test results and interpretations provided by 85 laboratories that performed HIV-1 antibody tests in addition to or other than microtiter-format EIA, WB or IIF. 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. Twenty-seven (31.8%) of the 85 laboratories reporting results of "Other" types of tests did not report results using EIA, WB or IIF tests.

The procedures used by 30 (35.3%) of these 85 laboratories can be described as "rapid tests". Of these, 13 (43.3%) laboratories tested samples using Fujirebio Serodia HIV, and eight (26.7%) laboratories reported using Abbott Determine. Results of "Line or Strip Immunoassay" tests such as Innogenetics INNO-LIA, Organon Teknika Liatek and Chiron RIBA were appropriately reported on the "Other Test" results form by 19 laboratories. Note that all laboratories using the Abbott AXSYM or PRISM systems correctly 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 186 final interpretations reported for HIV-1-negative sample (Donor 3) tested by laboratories using these "Other" procedures, six false-positive interpretations were reported from four laboratories using four different test systems. One indeterminate interpretation was reported.

Among the 418 interpretations reported for the HIV-1-positive samples tested by procedures other than EIA, WB, or IIF, there was one false-negative interpretation and one indeterminate interpretation. Both were reported for HIV-antibody strongly reactive samples from Donor 5.

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 705 laboratories that reported EIA test results, 463 (65.7%) indicated they used quality control samples other than those provided with the manufactured test kit. Of these 463 laboratories, 281 (60.7%) used samples obtained commercially, 161 (34.8%) used QC samples from in-house sources, and 23 (5.0%) used QC material from both commercial and in-house sources. Two 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 EIA plates.

Of the 267 laboratories reporting WB test results, 86 (32.2%) laboratories used external QC samples. Of these 86 laboratories, 59 (68.6%) used samples prepared in-house, 24 (27.9%) used QC samples obtained commercially, and two (2.3%) 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 a weakly-positive serum/plasma included in each set/run of WB strips.

Of the 33 laboratories reporting IIF results, nine (27.3%) used IIF external QC samples. Of these, six (66.7%) used

samples from in-house sources, and three (33.3%) used QC samples obtained commercially. Most of the nine laboratories included at least a weakly-positive external QC sample with each set/run of slides.

Of the 85 laboratories reporting results of tests other than EIA or WB or IIF, 37 (43.5%) indicated the use of external QC samples. Of these, 23 (62.2%) used samples from in-house sources and 14 (37.8%) used samples from commercial sources. The majority of these laboratories indicated they used at least a weakly-positive QC sample with each set/run.

Conclusion

Most participant laboratories performed well in testing the HIV-1 donor samples in this shipment. However, some false-negative results were reported for EIA (0.2%), WB (0.2%) and IIF (10.4%) with HIV-1-positive samples. Also, a few false-positive EIA (1.1%) and WB (2.0%) results were reported for samples negative for HIV-1 antibody (Donor 3). No false-positive IIF results were reported in this survey.

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 98.9%, 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 86.8%. When indeterminate and reactive IIF interpretations are combined for the HIV-1-positive samples, the IIF analytic sensitivity was 89.6%. The IIF analytic specificity was 100% 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 IIF 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 IIF procedures was 99.5%, 97.3%, and 92.0% respectively.

Please note that we plan to ship the next panel of MPEP HIV-1 antibody samples to participating laboratories on July 10, 2001, to laboratories located outside the United States and on July 24, 2001, to laboratories located within the United States.