# Analysis of the August 18, 1997 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 August 18, 1997. Testing results were reported by 785 (89.6%) of 876 laboratories that were sent sample panels. Additionally, result booklets were received from 3 laboratories more than three weeks after the cut-off date and their test results are not included in the analysis.

Samples used in the MPEP surveys are undiluted, defibrinated plasma obtained from individual donors who are HIV-1 antibody-positive or HIV-1 antibody negative. The HIV-1 antibody-positive donor samples are heat treated. Before shipment, the CDC tested each donor sample with four HIV-1 and two HIV-1/HIV-2 enzyme immunoassay (EIA) kits licensed by the Food and Drug Administration (FDA). Supplemental testing was performed with three FDA-licensed HIV-1 Western blot (WB) kits and one HIV-2 WB kit. Donor samples were not tested by CDC with any HIV-1 indirect immunofluorescence (IIF) test.

The CDC sample reactivity shown in Figures 1, 5, 6, 7, 8, 9, and 10 is listed as negative or positive and was determined after composite EIA and WB testing with FDA-licensed kits and by using the WB interpretive criteria of the Association of State and Territorial Public Health Laboratory Directors/Centers for Disease Control (ASTPHLD/CDC) (MMWR 1989; 38, S-7: 1-7). The ASTPHLD/CDC WB interpretive criteria is the same criteria published in the package insert for all FDA-licensed HIV-1 WB test kits. In preshipment testing performed by CDC, the HIV-1 antibody strongly positive donor samples (Donors 17 and 18) were EIA repeatedly reactive with all of the HIV-1 and HIV-1/HIV-2 EIA kits and WB reactive with all HIV-1 FDA-licensed WB kits used by CDC. The negative donor samples (Donors 15 and 16) were EIA repeatedly non-reactive and demonstrated no bands with any FDA-licensed HIV-1 WB kit.

Donor samples 1-14, obtained from individual donors recently infected with HIV-1, were HIV-1 antibody weak-positive and demonstrated variable EIA and WB antibody reactivity with the FDA-licensed EIA and WB kits used for testing. Testing information for sequential serum samples from donors 1-14 demonstrated factors consistent with seroconversion such as a positive p24 antigen test, rising HIV-1 antibody titers in both lysate-based and recombinant antigen EIA tests with S/C ratios increasing as much as 10-fold between two bleeds, and WB reactivity changing from nonreactive (no bands) to reactive with the presence of antibody to p24 and gp120 and/or gp160 between bleeds.

Figure 1 shows the cumulative frequency of test result interpretations reported by participating laboratories, arranged according to sample reactivity, for the EIA, WB, and IIF methods. Of the 753 EIA interpretations reported for HIV-1 antibody-negative samples, 2 (0.27%) were incorrectly reported as reactive. False-negative EIA interpretations were reported for 39 (1.03%) of the 3,775 interpretations reported for the antibody-positive samples. One HIV-1 seroconversion sample (Donor 5) accounted for 15 (38.5%) of the 39 false-negative EIA interpretations reported. Of 138 WB interpretations reported for the HIV-1 antibody-negative samples, no false-reactive WB interpretations and only one indeterminate WB interpretation was reported. Among the 1,314 WB interpretations reported for the HIV-1 antibody-positive samples, there were 4 (0.3%) false-negative and 149 (11.3%) indeterminate interpretations. The weakly-reactive donor samples (Donors 1-14) accounted for all of the false-negative and indeterminate WB interpretations reported for the HIV-1 antibody-positive samples. Among the 27 IIF interpretations reported for

HIV-1 antibody-negative samples, there were no false-positive or indeterminate interpretations reported. Of the 201 IIF interpretations reported for antibody-positive samples, there were 17 (8.5%) indeterminate and 5 (2.5%) false-negative interpretations. All false-negative and indeterminate IIF interpretations were reported for the HIV-1 antibody weak-positive seroconversion samples.

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. Most laboratories performed only EIA (62.8%), while some laboratories performed both EIA and supplemental tests (35.9%), and others (1.3%) performed only supplemental tests. Thirty-nine laboratories performed other tests in addition to EIA, WB and IIF. Not represented in this figure are 30 laboratories that performed only tests other than EIA, WB, or IIF. The data for tests performed other than EIA, WB, or IIF are presented in Figure 10.

The types of 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 was no unique glossary code provided in the survey report form and these responses have been grouped as "Other" manufacturer. Some "Other" kits reported as being used for EIA include Abbott HIV-1/HIV-2 3rd Generation PLUS (8 laboratories), Abbott AXSYM HIV-1/HIV-2 (7 laboratories), Murex ICE HIV 1.O.2 Detection, (3 laboratories), Innogenetics Innotest HIV-1/HIV-2 (2 laboratories), and Ortho Diagnostics HIV-1/HIV-2 Ab Capture EIA (2 laboratories).

The results reported for the EIA, WB, and IIF methods, listed by kit manufacturer, for the positive and 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**

Both of the false-positive EIA interpretations were reported for Donor 15 by laboratories using the Abbott HIV- 1/HIV-2 rDNA kit (Figure 5). However, the overall EIA specificity calculated for the results reported by laboratories using this Abbott EIA kit was 99.3%.

Among the HIV-1 antibody-positive donor samples, there were 39 nonreactive EIA interpretations reported. There were three nonreactive EIA interpretations reported for Donor 17, an HIV-1 antibody strong-positive sample. Laboratories reported 36 EIA nonreactive final interpretations for the HIV-1 antibody weak-positive donor samples obtained from individuals during seroconversion (Donor numbers 1-14) and Donor 5 accounted for 15 (41.6%) of these false-negative results. Some laboratories reported initially reactive EIA results but nonreactive repeat EIA results for these seroconversion samples. The non-reactive EIA interpretations for HIV-1 antibody-positive donor samples were reported by laboratories using six different EIA kits provided by four different manufacturers.

**WB Results**

Of the 785 laboratories reporting test results in this survey, only 262 (33.4%) performed WB testing. There was only 1 indeterminate and no false-positive WB interpretations reported for the HIV-1 antibody-negative samples (Donors 15 and 16), shown in Figure 6. The indeterminate WB interpretation was reported for Donor 15.

All of the false-negative, 4 (0.51%), and indeterminate, 149 (18.9%), interpretations were among 788 WB interpretations reported for samples from the 14 HIV-1 infected, seroconverting donors (Donors 1-14). All the nonreactive WB interpretations were reported for samples from Donor 5 by laboratories using WB kits manufactured by Epitope/Organon Teknika. Indeterminate WB interpretations were reported most often for Donor 11, 17 (60.7%) of 28 interpretations; Donor 5, 30 (57.7%) of 52 interpretations; Donor 8, 28 (50.9%) of 55 interpretations, and Donor 9, 23 (50%) of 46 interpretations. Indeterminate WB interpretations for the seroconversion samples were reported by laboratories using WB kits provided by six different manufacturers as well as WB procedures developed "In House". Among the FDA-licensed WB kits, the greatest frequency of false-negative and indeterminate WB interpretations was reported by laboratories using a WB kit manufactured by Epitope/Organon Teknika, 75 (19.4%) of 386 interpretations (Figure 6).

Indeterminate interpretations reported for Donor samples 1-14 most often resulted from non-detection of antibody to envelope (env) antigens or detection of env-antibody reactivity resulting in bands with less than the required intensity. The WB bands (of greater than or equal to 1+ intensity) for these donor samples, as determined in preshipment testing by CDC with 3 FDA-licensed WB test kits, are shown in Table 2.

Of the 262 laboratories reporting WB test results, 233 indicated which WB criteria were used to interpret their WB tests. The ASTPHLD/CDC WB interpretive criteria was used by 197 (84.5%) of these 233 laboratories. Some laboratories continue to indicate they use the WB interpretive guidelines described by the manufacturer of the WB kit they use and apparently are not aware that the WB interpretive guidelines published by the FDA- licensed WB kit manufacturers are identical to the ASTPHLD/CDC HIV-1 WB interpretive criteria. Five laboratories using the WB kit manufactured by BioRad indicated they were using interpretive criteria different from that recommended by the kit manufacturer as approved by 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 WB results include the testing of EIA-nonreactive donor samples, which most laboratories do not normally include in their algorithm of routine testing. The frequency of a reported band is listed above the column. The number of band pattern reports is listed in the far right column. This figure **does not** include WB bands reported as 'W', 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.

Donor samples 15 and 16 were negative for HIV-1 antibody; however, one laboratory reported a gp160 viral protein band for Donor 15. None of the HIV-1 antibody-negative donor samples demonstrated antibodies to any of the viral-specific proteins or non-viral proteins in CDC preshipment testing with three FDA-licensed HIV-1 WB kits.

For the HIV-1 antibody strong-positive samples (Donors 17 and 18), laboratories had no difficulty in detecting antibodies to gag, pol, and env antigens with any HIV-1 or HIV-1/HIV-2 WB kit used. The donor material obtained from HIV-1 infected individuals during seroconversion, Donors 1-14, appeared to cause more difficulty. Most of the indeterminate WB interpretations reported for the seroconversion samples resulted from the laboratory failing to detect antibody to viral envelope antigen and, infrequently, to gag antigen in these donor samples. These findings are consistent with the CDC WB test results as indicated in Table 2 of the graphics accompanying this analysis.

**IIF Results**

No false-positive or indeterminate IIF interpretations were reported for the HIV-1 antibody-negative donor samples (Figure 7). Among the 201 IIF interpretations reported for the HIV-1 antibody-positive samples, 5 (2.5%) false-negative and 17 (8.5%) indeterminate interpretations were reported. No indeterminate or false negative interpretations were reported for the HIV-1 antibody strong-positive samples (Donors 17 and 18). For the seroconversion samples (Donors 1 - 14), false-negative and indeterminate interpretations were reported most frequently for Donor 13, 2 (50%) of 4 interpretations; Donor 9, 3 (43%) of 7 interpretations; Donor 5, 8 (40%) of 20 interpretations, and Donor 4, 5 (35.7%) of 14 interpretations.

**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 for fluorescence intensity patterns 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 show 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 either of the HIV-1 antibody-negative samples (Donors 15 and 16). Most laboratories reported 3+ or greater fluorescence for the HIV-1 antibody strongly-positive samples (Donor numbers 17 and 18) with all commercial, noncommercial, and in-house IIF kits used. The IIF intensity reported for the weak-positive samples (Donors 1-14) frequently was greater than 1+, but occasionally no fluorescence (antibody) was reported for HIV-1 infected cells.

**Other Tests Performed**

Figure 10 provides information on the test results and interpretations provided by laboratories that do 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 antibody-negative and antibody-positive samples in this shipment. Of the 69 laboratories reporting results on the form for “Other” types of tests, 34 are laboratories within the United States. These 34 laboratories reported results using only the FDA-approved Murex SUDS HIV-1 test. Thirty (43.5%) of the 69 laboratories reporting results of “Other” types of tests did not report results of EIA, WB or IIF tests. The procedures used by 44 (63.8%) of these 69 laboratories can be described as "rapid" microfiltration EIA procedures (e.g., SUDS HIV-1, Testpack HIV-1/HIV-2, HIV-Spot HIV 1+2, and MultiSpot HIV-1/HIV-2). These tests are generally provided as kits that use microparticles, such as latex, coated with purified lysate, synthetic, or recombinant HIV-1, and sometimes HIV-2 antigens.

Thirteen laboratories tested samples using a gelatin particle agglutination test (Fujirebio Serodia HIV) and one laboratory used a latex agglutination test (Cambridge Biotech). Results of “Line or Strip Immunoassay” tests Liatek (Organon Teknika), INNO-LIA (Innogenetics) and RIBA (Chiron) were appropriately reported on the “Other Test” results form by 7 laboratories. Among the 63 final interpretations reported for HIV-1 antibody-negative samples (Donor 15 and 16) tested by procedures other than EIA, WB, and IIF, false-positive interpretations were reported by laboratories using the Fujirebio gelatin particle agglutination test or the Murex SUDS test; one indeterminate interpretation was reported by a laboratory using the Organon Teknika Liatek test. The one indeterminate and three of the four false-positive interpretations were reported for Donor 16.

Among the 357 interpretations reported for the HIV-1 antibody-positive samples tested by procedures other than EIA, WB, or IIF, there were seven false-negative interpretations and eleven indeterminate interpretations. False-negative and indeterminate interpretations were reported only for the seroconversion samples (Donors 1-14), and were reported most frequently, 12 (40%) of 30 reports, for 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 745 laboratories that reported EIA test results, only 380 (51%) indicated they used quality control samples other than those provided with the manufactured test kit. Of these 380 laboratories, 227 (59.7%) used samples obtained commercially, 144 (37.9%) used QC samples prepared in-house, and 10 (2.6%) used QC material from both commercial and in-house sources. The majority indicated the use of either weak-positive or weak-positive and negative serum/plasma with each set or run of EIA plates. Of the 262 laboratories reporting WB test results, only 71 (27.1%) laboratories described their external QC samples. Of these 71 laboratories, 43 (60.6%) used samples obtained commercially, 26 (36.6%) used QC samples prepared in-house, and 2 (2.8%) used QC material from both commercial and in-house sources. Most laboratories used at least a weak-positive serum/plasma and included this sample in each set/run of WB strips. Of the 42 laboratories reporting IIF results, only 12 (28.6%) used IIF external QC samples. Of these, 10 (83.3%) used samples from in-house sources and 2 (16.7%) used QC samples obtained commercially. The majority indicated that a single weak-positive sample was included with each set of slides.
Of the 69 laboratories reporting results of tests other than EIA, WB or IIF, only 19 (27.5%) used external QC samples. Of these, 12 (63.2%) used samples from in-house sources and the majority indicated that a combination of strong-positive, weak-positive and negative QC samples were included with each run or at least with each new kit lot.

**Conclusion**

Most participant laboratories performed well in testing the HIV-1 donor samples in this shipment. Only a few laboratories reported false-negative EIA (1.0%), false-negative WB (0.3%), and false-negative IIF (2.5%) results for the HIV-1 antibody-positive samples (Donor numbers 1-14 and 17-18) and only rarely were false-positive EIA (0.3%) or indeterminate (0.7%) WB results reported for samples that CDC tested and found negative for HIV-1 antibody in both EIA and WB tests (Donors 15 and 16).

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% and 99.7%, respectively. When indeterminate and reactive WB interpretations are combined, the WB analytic sensitivity was 99.7%. If indeterminate interpretations are considered incorrect for HIV-1 antibody-negative samples, the WB analytic specificity was 99.3%. When indeterminate and reactive IIF interpretations are combined for the HIV-1 antibody-positive samples, the IIF analytic sensitivity was 97.5%; the IIF analytic specificity was 100% for this survey. The analytic sensitivity and specificity of the test procedures other than EIA, WB, and IIF varied greatly, depending on which test results are analyzed (Table 10). When indeterminate and reactive interpretations are combined for the HIV-1 antibody-positive samples, analytic sensitivity of 100% was

calculated from results reported by laboratories using seven procedures other than EIA, WB, or IIF. Analytic specificity of 100% was determined from results reported by laboratories using five of these "Other" procedures. If indeterminate 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 99.1%, 99.7%, and 97.6% respectively.

Although there were the same number of HIV-1 weak-positive antibody samples in this survey as in the January, 1997 MPEP survey, the EIA analytic sensitivity increased to 99% compared to 97% in the previous survey; the WB sensitivity was 99.7% compared to 99.8% in the previous survey, and the IIF sensitivity increased for this survey to 97.5% compared to 96.8% for the January, 1997 survey.