# Analysis of the January 27, 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 January 27, 1997. Testing results were reported by 801 (89%) of 900 laboratories that were sent sample panels. Additionally, result booklets were received from 11 laboratories more than three weeks after the cut-off date and their test results are not included in the analysis. Two laboratories reported results for the samples sent them in August, 1996 rather than results for the current panel, and six laboratories returned result booklets with no results.

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 done 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 result (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 1-4) 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 5-10) were EIA repeatedly negative and demonstrated no bands with any FDA-licensed WB kit.

Donor samples 11-18, obtained during seroconversion from individual donors recently infected with HIV-1, were HIV-1 antibody weakly-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 11-18 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 indirect immunofluorescence (IIF) methods. Of the 1,547 EIA interpretations reported for HIV-1 antibody-negative samples, 8 (0.52%) were incorrectly reported as reactive. False-negative EIA interpretations were reported for 94 (3%) of the 3,091 interpretations reported for the antibody-positive samples. One HIV-1 seroconversion sample (Donor 13) accounted for 87 (92.6%) of the 94 false-negative EIA interpretations reported. Of 252 WB interpretations reported for the HIV-1 antibody-negative samples, one false-reactive WB interpretation was reported and 16 (6.3%) indeterminate WB interpretations were reported. Among the 1,017 WB interpretations reported for the HIV-1 antibody-positive samples, there were 2 (0.2%) false-negative and 144 (14.2%) indeterminate interpretations. The seroconversion donor samples (Donors 11-18) accounted for all of the false-negative and 143 of the 144 indeterminate WB interpretations reported for the

HIV-1 antibody-positive samples. Among the 50 IIF interpretations reported for HIV-1 antibody-negative samples, there were no false-positive or indeterminate interpretations reported. Of the 157 IIF interpretations reported for antibody-positive samples, there were 4 (2.5%) indeterminate and 5 (3.2%) false-negative interpretations. All false-negative and indeterminate IIF interpretations were reported for the HIV-1 antibody weakly-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 (64%), while some laboratories performed both EIA and supplemental tests (34.6%), and others (1.4%) performed only supplemental tests. Forty laboratories performed other tests in addition to EIA, WB and IIF. Not represented in this figure are 31 laboratories that performed only tests other than EIA, WB, or IIF. Data for the other tests performed 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” EIA kits reported include Abbott HIV-1/HIV-2 3rd Generation PLUS (7 laboratories), Abbott AXSYM HIV-1/HIV-2 (3 laboratories), Innogenetics Innotest HIV-1/HIV-2 (3 laboratories), and Ortho Diagnostics HIV-1/HIV-2 Ab Capture EIA (3 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**

Among the 1,547 EIA interpretations reported for the HIV-1 antibody-negative samples (Donors 5-10) there were 8 false-positive interpretations (Figure 5). All of the false-positive EIA interpretations were reported for Donor 8. False-positive EIA interpretations were reported most by laboratories using the Abbott HIV1/HIV-2 rDNA kit (4 of 8) and the Abbott HIV-1 HIVAB kit (2 of 8). However, the overall EIA specificity calculated for the results reported by laboratories using these two Abbott EIA kits was 99.3% and 99.5%, respectively.

Among the HIV-1 antibody-positive donor samples, a total of 94 false-negative EIA interpretations were reported. There were three nonreactive EIA interpretations reported for the HIV-1 antibody strong-positive samples (Donors 1-4), one for Donor 2 and two for Donor 4. Laboratories reported 91 EIA nonreactive final interpretations for the HIV-1 antibody weak-positive donor samples obtained from individuals during seroconversion (Donor numbers 11-18). Some laboratories reported initially reactive EIA results but nonreactive repeat EIA results for these seroconversion samples. The 91 non-reactive EIA interpretations for seroconversion donor samples were reported by laboratories using eight different EIA kits provided by five different manufacturers. Donor 13 accounted for 87 (95.6%) of these 91 false-negative interpretations. Although the overall sensitivity calculated from the results of laboratories using the Abbott HIV-1/HIV-2 rDNA EIA kit was 94.8% (Figure 5), the greatest number of EIA false-negative interpretations for Donor 13, 60 (69%) of 87, were reported by laboratories using this Abbott kit. Please note that Donor 13 in this survey was the same Donor 13 used in five previous surveys. In these previous surveys,

the false-negative rate for Donor 13 samples, reported by laboratories using this Abbott kit were 17% (August 1994), 3.6% (January 1995), 21.4% (August 1995), 66.3% (January 1996), and 63.2% (August 1996). The reason(s) that laboratories using the Abbott HIV-1/HIV-2 rDNA EIA kit report higher false-negative rates for this particular seroconversion sample in the past three surveys are unknown.

**WB Results**

Four laboratories incorrectly used the WB results form to report the results of line immunoassay tests (Liatek, Inno-Lia) although instructions indicated this type test was to be reported on the form for "Other" procedures. The results of the line immunoassay tests are not included in the WB results analysis.

Of the 801 laboratories reporting test results analyzed in this survey, only 260 (32.5%) performed WB testing. There were 16 indeterminate and one false-positive WB interpretations reported for the HIV-1 antibody-negative samples (Donors 5-10), shown in Figure 6. Indeterminate WB interpretations were reported most frequently for Donor 9, 8 (12%) of 68 interpretations. There were no EIA reactive interpretations reported for Donor 9 and, although laboratories are asked to test the MPEP samples as they would routine donor or clinical specimens, it is evident that some laboratories are performing WB supplemental testing on performance evaluation samples that are nonreactive in EIA screening tests. It is unclear why one laboratory reported a nonreactive WB interpretation for the Donor 8 sample in their panel, but also reported the presence of antibody to p24, p31, gp41, p66, gp120, and gp160 bands for this sample. Another laboratory, using a WB kit coded as "Other" manufacturer, reported one reactive (p24, p51, and gp41 bands) and one indeterminate (p24 and p51 bands) WB interpretation for the duplicated Donor 8 samples in their panel.

Among the 762 WB interpretations reported for samples from the 8 seroconverting donors (Donors 11-18), there were 2 (0.26%) false-negative and 143 (18.8%) indeterminate interpretations. A false-negative WB interpretation was reported for Donor 16 by one laboratory that used a BioRad WB kit and indicated the presence of weak (W, less than 1+ intensity) bands to p24 and gp160. Another laboratory using a BioRad WB kit reported p17, p24, and p55 bands and a nonreactive WB interpretation for their Donor 18 sample. Indeterminate WB interpretations were reported most often for Donor 13, 71 (60.2%) of 118 interpretations; Donor 16, 20 (37%) of 54 interpretations; and Donor 15, 23 (29.9%) of 77 interpretations. Indeterminate WB interpretations for the seroconversion samples were reported by laboratories using WB kits provided by seven 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 were reported by laboratories using kits manufactured by BioRad, 67 (20%) of 333, and Epitope/Organon Teknika, 34 (12.4%) of 275 interpretations (Figure 6).

Indeterminate interpretations reported for Donor samples 11-18 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, as indicated by reporting a 'W' for env band(s) in the WB results. For some samples, laboratories using FDA-licensed WB kits manufactured by BioRad, Cambridge Biotech, or Epitope/Organon Teknika, indicated the presence of gag, env, and frequently, pol bands with an 'X' which would indicate acceptable band intensity and a reactive WB test; however, they reported indeterminate WB interpretations for these samples. Therefore, it appears that some laboratories are reporting some bands with less than the required intensity with an 'X' rather than a 'W'. 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 260 laboratories reporting WB test results, 236 indicated which WB criteria were used to interpret their WB tests. The ASTPHLD/CDC WB interpretive criteria was used by 198 (83.9%) of these 236 laboratories. Two additional laboratories reported WB results interpreted by the ASTPHLD/CDC WB criteria except they indicated

that a nonreactive WB interpretation is assigned to a sample that produces no viral bands rather than no bands at all. 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.

**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 daily specimen 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 5-10 were negative for HIV-1 antibody; however, some laboratories reported major viral protein bands for Donors 8, 9, and 10. None of the HIV-1 antibody-negative donor samples demonstrated antibodies to any of the viral-specific proteins or non-viral proteins in preshipment testing with three FDA-licensed HIV-1 WB kits, on two different testing occasions.

For the HIV-1 antibody strongly positive samples (Donors 1-4), 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 individuals in early seroconversion, Donors 11-18, 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, donor numbers 5-10 (Figure 7). Among the 157 IIF interpretations reported for the HIV-1 antibody-positive samples, 5 (3.2%) false-negative and 4 (2.5%) indeterminate interpretations were reported. No indeterminate or false negative interpretations were reported for the HIV-1 antibody strongly-positive samples (Donors 1-4). For the antibody-weakly positive (seroconversion) samples (Donors 11 - 18), false-negative IIF interpretations were reported for Donor 13, 2 (12.5%) of 16 interpretations; Donor 15, 2 (20%) of 10 interpretations; and Donor 18, 1 (7.7%) of 13 interpretations. Indeterminate interpretations were reported for Donor 11, 1 (5.6%) of 18 interpretations; Donor 15, 1 (10%) of 10 interpretations; and Donor 18, 2 (15.4%) of 13 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 any of the HIV-1 antibody-negative samples (Donors 5 - 10). Most laboratories reported 2+ or greater fluorescence for the HIV-1 antibody strongly-positive samples (Donor numbers 1-

4) with all commercial, noncommercial, and in-house IIF kits used. The IIF intensity reported for the weakly-positive samples (Donors 11-18) frequently was greater than 2+, 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 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. In addition to the 71 laboratories reporting "Other" types of HIV tests on the correct result form, there were 4 laboratories incorrectly using the WB result form to report results of line-immunoassay tests (Inno-Lia and Liatek) manufactured by Innogenetics. Of the 71 laboratories reporting results on the form for "Other" types of tests, 37 were laboratories within the United States. Thirty-one (43.7%) of the 71 laboratories reporting results of "Other" tests did not report results of EIA, WB or IIF tests. The "Other" procedures used by 46 (64.8%) of these 71 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.

Fifteen 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 six laboratories. Among the 137 final interpretations reported for HIV-1 antibody-negative samples (Donors 5 - 10) tested by procedures other than EIA, WB, and IIF, there were eight false-positive and five indeterminate interpretations reported by laboratories using the Fujirebio gelatin particle agglutination test, the Murex SUDS rapid microfiltration test, or a procedure coded as "Other" and described as Abbott PRISM Chemiluminescent Immunoassay. False-positive interpretations were reported twice each for Donors 7, 8, 9 and 10. Indeterminate reactions were reported twice each for Donors 7 and 9 and once for Donor 10.

Among the 303 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 six indeterminate interpretations. False-negative and indeterminate interpretations were reported only for the seroconversion samples (Donors 11-18). False-negative interpretations were reported five times for Donor 13 and once each for Donors 11 and 17. Indeterminate interpretations were reported three times for Donor 13 and once each for Donors 14, 15 and 17.

**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 763 laboratories that reported EIA test results, only 355 (46.5%) indicated they used quality control samples other than those provided with the manufactured test kit. Of these 355 laboratories, 214 (60.3%) used samples obtained commercially, 138 (38.9%) used QC samples prepared in-house, and 3 (0.85%) used QC material from both commercial and in-house sources. The majority indicated the use of a single, weakly positive serum/plasma with each set or run of EIA plates. Of the 260 laboratories reporting WB test results, only 70 (26.9%) laboratories described their external QC samples. The

majority used at least a single weakly-positive serum/plasma obtained in-house, and included this sample in each set/run of WB strips. Of the 41 laboratories reporting IIF results, only 11 (26.8%) used IIF QC samples and the majority indicated that a single weakly-positive sample prepared in-house was included with each set of slides.

## Conclusion

Most participant laboratories performed well in testing the HIV-1 donor samples in this shipment. However, some laboratories reported false-positive EIA (0.5%) and false-positive or indeterminate (6.7%) WB results for samples that CDC tested and found negative for HIV-1 antibody in both EIA and WB tests. Additionally, some laboratories reported false-negative EIA (3.0%), false-negative WB (0.2%), and false-negative IIF (3.2%) results for the HIV-1 antibody-positive samples (Donor numbers 1-4 and 11-18).

Please note that the information in this report 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 97% and 99.5%, 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 was 93.3%. When indeterminate and reactive IIF interpretations are combined for the HIV-1 antibody-positive samples, the IIF analytic sensitivity was 96.8%; the IIF analytic specificity was 100% for this survey. Combining indeterminate and reactive interpretations, the overall analytic sensitivity of the procedures other than EIA, WB, and IIF was 97.7%. The analytic specificity of these other procedures was 97.7%. 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, IIF and “Other” procedures was 97.8%, 98.5%, 97.6%, and 95.5% respectively.

For this survey, the EIA analytic sensitivity decreased to 97.0% compared to 98.4% in the previous survey; the WB sensitivity was 99.8% compared to 99.6% in the previous survey, and the IIF sensitivity decreased for this survey to 96.8% compared to 98.3% for the previous survey. The overall analytic sensitivity reported for “Other” types of HIV tests decreased in this survey to 97.7% compared to 99.3% in the previous survey. The decreased analytic specificity, and consequently overall analytic performance of laboratories reporting results for EIA, IIF and “Other” procedures may be due to the presence of duplicated HIV-1 seroconversion samples in the panels for this survey compared to the inclusion of duplicated HIV-1 antibody strong-positive samples in the panels of the previous survey.