

**Analysis of the August 19, 1996 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 19, 1996. Testing results were reported by 829 (89.7%) of 924 laboratories that were sent sample panels. However, 14 laboratories returned their result booklets more than a week after the cut-off date indicated in the cover letter accompanying the results booklet, 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-inactivated. 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 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 1,562 EIA interpretations reported for HIV-1 antibody-negative samples, 13 (0.83%) were incorrectly reported as reactive. False-negative EIA interpretations were reported for 49 (1.6%) of the 3,130 interpretations reported for the antibody-positive samples. One HIV-1 seroconversion sample (Donor 13) accounted for 38 (77.6%) of the 49 false-negative EIA interpretations reported. Of 267 WB interpretations reported for the HIV-1 antibody-negative samples, no false-reactive WB interpretations were reported. However, 13 (4.9%) indeterminate WB interpretations were reported. Among the 1,015 WB interpretations reported for the HIV-1 antibody-positive samples, there were 4 (0.4%) false-negative and 117 (11.5%) indeterminate interpretations. The seroconversion donor samples (Donors 11-18) accounted for 50% of the false-negative and all of the indeterminate WB interpretations reported for the HIV-1 antibody-positive samples. Among the 62 IIF interpretations reported for HIV-1 antibody-negative samples, there were no false-positive or indeterminate interpretations reported. Of the 178 IIF interpretations reported for antibody-positive samples, there were 7 (3.9%) indeterminate and 3 (1.7%) 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 (64.9%), while some laboratories performed both EIA and supplemental tests (33.5%), and others (1.5%) performed only supplemental tests. Not represented in this figure are 31 laboratories that performed only tests other than EIA, WB, or IIF. Information regarding these "Other" tests performed is 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.

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 1,562 EIA interpretations reported for the HIV-1 antibody-negative samples (Donor numbers 5-10) there were 13 false-positive interpretations (Figure 5). False-positive EIA interpretations were reported at least once for all HIV-antibody negative Donors except Donor 7. False-positive EIA interpretations were reported most by laboratories using the Abbott HIV1/HIV-2 rDNA kit (5 of 13) and the Abbott HIV-1 HIVAB kit (4 of 13). However, the overall specificity of these two Abbott EIA kits was 99.2% and 99%, respectively.

Among the HIV-1 antibody strong-positive donor samples (Donors 1-4), a total of 49 false-negative EIA interpretations were reported. There were six nonreactive EIA interpretations reported, one for Donor 1 and five for Donor 4. Laboratories reported 43 EIA nonreactive final interpretations for the eight 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 43 non-reactive EIA interpretations for seroconversion donor samples were reported by laboratories using eleven different EIA kits provided by eight different manufacturers. Donor 13 accounted for 38 (77.6%) of the 49 false-negative interpretations. Although the overall specificity of the Abbott HIV-1/HIV-2 rDNA EIA kit was 97.5% (Figure 5), the greatest number of EIA false-negative interpretations for Donor 13, 24 (63.2%) of 38, were reported by laboratories using this Abbott kit. Please note that Donor 13 in this survey was the same Donor 13 used in four previous surveys. Laboratories using the Abbott HIV-1/HIV-2 EIA kit to test samples from Donor 13 in the previous survey (January 1996) indicated EIA-nonreactive interpretations for 106 (66.3%) of 160 total EIA interpretations. Prior to these two surveys, the false-negative rate for Donor 13 samples, reported by laboratories using this Abbott kit were 17% (August 1994), 3.6% (January 1995), and 21.4% (August 1995). 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 two surveys are unknown.

WB Results

Four laboratories 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 815 laboratories reporting test results analyzed in this survey, only 256 (31.4%) performed WB testing.

There were 13 indeterminate WB interpretations reported for the HIV-1 antibody-negative samples (Donors 5-10). Indeterminate WB interpretations were reported for Donor 6 by seven laboratories indicating the presence of antibody to p55 and one laboratory reporting p66 plus gp120/160 bands. Indeterminate WB interpretations for Donor 9 were reported by five laboratories that indicated the presence of antibody to p15 and/or p17. There were four EIA reactive interpretations reported for Donor 6, and only three EIA reactive interpretations reported for Donor 9. 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. Eleven of the 13 indeterminate interpretations for the HIV-1 antibody-negative donor samples were reported by laboratories using BioRad WB kits.(Figure 6). Four laboratories reported weak ('W') bands (> 1+ intensity) and non-reactive WB interpretations for some of these HIV-negative samples. Instructions for reporting WB bands on the MPEP WB results form indicate that WB bands present at an intensity equal to or greater than the intensity of a specified band in the test kit weak positive control should be marked with an 'X', while bands present at an intensity less than that of the specified control band should be marked with a 'W'. .

One laboratory indicated the presence of antibody to all major HIV viral proteins for duplicate HIV-1 antibody strongly-positive samples in their panel (Donor 3); however, final interpretations of "N" (nonreactive) were indicated on the result form for these samples. Among the 506 WB interpretations reported for samples from the 8 seroconverting donors (Donors 11-18), there were 2 (0.39%) false-negative and 117 (23.1%) 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) bands to p24 and gp160. Another laboratory using an Epitope WB kit reported no bands and a nonreactive WB interpretation for this same donor sample. Indeterminate WB interpretations were reported most often for Donor 13, 38 (69.1%) of 55; Donor 16, 24 (40%) of 60 total interpretations; Donor 17, 20 (33.3%) of 60; and Donor 15, 23 (31.1%) of 73 WB interpretations. Indeterminate WB interpretations were reported by laboratories using 7 different WB kits. 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, 55 (16%) of 343, and Epitope/Organon Teknika, 37 (13%) of 285 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 on page 4 of the figures accompanying this report.

Of the 256 laboratories reporting WB test results, 244 indicated which WB criteria were used to interpret their WB tests. The ASTPHLD/CDC WB interpretive criteria was used by 195 (79.9%) of these 244 laboratories. Six additional laboratories reported WB results interpreted by "other" WB criteria described as the criteria published in the manufacturer's inserts of the FDA-licensed Epitope/Organon Teknika, Cambridge Biotech, and BioRad WB kits. Apparently some laboratories are not aware that the WB interpretive criteria published by these manufacturers are identical to the ASTPHLD/CDC 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, one laboratory reported p66, gp120 and gp160 bands for Donor 6. None of the HIV-1 antibody-negative donor samples demonstrated antibodies to any of the viral-specific proteins or non-viral protein in preshipment testing with three FDA-licensed HIV-1 WB kits, on two different testing occasions, and with one HIV-2 WB kit.

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 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 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 on page 4 of the figures 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 178 IIF interpretations reported for the HIV-1 antibody-positive samples, 3 (1.7%) false-negative and 7 (3.9%) 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, 1 (11.1%) of 9 interpretations; Donor 15, 1 (9.1%) of 11 interpretations; and Donor 17, 1 (10.0%) of 10 interpretations. Indeterminate interpretations were reported for Donor 15, 3 (27.3%) of 11 interpretations, Donor 16, 2 (18.2%) of 11 interpretations, and Donor 18, 2 (14.3%) of 14 interpretations. Non-reactive and indeterminate IIF interpretations were reported for the samples from these HIV-1 seroconverting donors by laboratories using IIF kits from two identified commercial sources, unidentified (other) commercial sources, non-commercial sources, and in-house IIF procedures and reagents. In this survey, unlike the January 1996 survey, a better agreement was shown in IIF test interpretation among laboratories using IIF kits from the same manufacturer. For example, laboratories using IIF kits manufactured by Waldheim Fluorognost were unanimous in reporting reactive IIF interpretations for all of the seroconversion samples except Donor 15 for which six reactive and three indeterminate IIF interpretations were reported.

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). The HIV-1 antibody strongly-positive samples (Donor numbers 1-4) showed 2+ or greater fluorescence intensity with all commercial, noncommercial, and in-house IIF kits used. The weakly-positive samples (Donors 11-18) frequently showed fluorescence intensity greater than 2+, but occasionally demonstrated no fluorescence (antibody) in 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 top part 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 68 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). Of the 68 laboratories reporting results on the form for "Other" types of tests, 41 were laboratories within the United States. Of the 68 laboratories reporting results from "Other" tests, 31 (45.6%) did not report results of EIA, WB or IIF tests. The "Other" procedures used by 44 (64.7%) of these 68 laboratories can be described as "rapid" microfiltration enzyme immunoassay procedures (e.g., SUDS HIV-1, Testpack HIV-1/HIV-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 Immunoassay" tests (Innogenetic Liatek and INNO-LIA and Chiron RIBA) were correctly reported on this form by four laboratories. Of the final interpretations reported for HIV-1 antibody-negative samples (Donors 5 - 10) tested by "Other" procedures, there were eight false-positive and three indeterminate interpretations reported by five laboratories using the Fujirebio gelatin particle agglutination test. False-positive interpretations were reported three times for Donor 6, twice each for Donor 8 and Donor 10, and once for Donor 9. Indeterminate reactions were reported once each for Donors 5, 6 and 8.

Among the interpretations reported for the HIV-1 antibody-positive samples tested by "Other" procedures, there were two false-negative interpretations and four indeterminate interpretations. False-negative and indeterminate interpretations were reported only for the seroconversion samples (Donors 11-18). False-negative interpretations were reported once each for Donors 13 and 16. Indeterminate interpretations were reported twice for Donor 13 and once each for Donors 15 and 16.

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 of the 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 772 laboratories that reported EIA test results, only 363 (47%) indicated they used quality control samples other than those provided with the manufactured test kit. Of these 363 laboratories, 222 used samples obtained commercially, 141 used QC samples prepared in-house, and 5 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 256 laboratories reporting WB test results, only 68 (26.6%) laboratories described their external QC samples. The majority used at least a single weakly-positive serum/plasma obtained in-house, and included with each set/run of WB strips. Of the 46 laboratories reporting IIF results, only 12 (26%) used IIF QC samples and the majority indicated that a single weakly-positive sample prepared in-house would be run 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 reactive EIA (0.8%) and indeterminate (4.9%) WB results for samples that CDC tested and found negative for HIV-1 antibody in both EIA and WB tests. Additionally, some laboratories reported nonreactive EIA (1.6%), nonreactive WB (0.4%), and nonreactive IIF (1.7%) 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 performance results reported by laboratories testing performance evaluation samples and does not reflect the actual sensitivity and specificity of the manufactured test kits. For this

survey, the EIA analytic sensitivity was 98.4% and analytic specificity was 99.2%. When indeterminate and reactive WB interpretations are combined, the WB analytic sensitivity was 99.6%. If indeterminate interpretations are considered incorrect for HIV-1 antibody-negative samples, the WB analytic specificity was 95.1%. When indeterminate and reactive IIF interpretations are combined for the HIV-1 antibody-positive samples, the IIF analytic sensitivity was 98.3%; the IIF analytic specificity was 100% for this survey. Combining indeterminate and reactive interpretations, the analytic sensitivity of the "Other" procedures was 99.3%. The analytic specificity of "Other" tests, with the exception of Fujirebio Serodia, was 100%. If indeterminate WB, IIF and "Other" test 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" tests was 98.7%, 98.5%, 98.7%, and 96.8% respectively.

For this survey, the EIA sensitivity increased to 98.4% compared to 93.6% in the previous survey; the WB sensitivity was 99.6% compared to 99.8% in the previous survey, and the IIF sensitivity increased for this survey to 98.3% compared to 82.8% for the previous survey. The analytic sensitivity reported for "Other" types of HIV tests improved in this survey to 99.3% compared to 91.4% in the previous survey. The increased specificity reported for EIA and IIF procedures may be due to the presence of duplicate HIV-1 antibody strong-positive samples in the panels for this survey compared to the inclusion of duplicate seroconversion samples in the panels of the previous survey.