

**Analysis of the August 17, 1998 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 17, 1998. Testing results were reported by 760 (91.1%) of 834 laboratories that were sent sample panels. One laboratory tested samples but failed to include information regarding the test kit they used; consequently, test results from this laboratory 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. Please note that the ASTPHLD has recently been renamed the Association of Public Health Laboratories (APHL).

In preshipment testing performed by CDC, the strongly positive HIV-1 antibody donor samples (Donors 4 - 7, 10 - 14, and 17 - 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 1-3, and 8 - 9, 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 these donors 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 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 sample reactivity, for the EIA, WB, and IIF methods. Of the 708 EIA interpretations reported for HIV-1 antibody-negative samples, 4 (0.56%) were incorrectly reported as reactive. There were 8 (0.23%) false-negative EIA interpretations among the 3,545 interpretations reported for the antibody-positive samples. Two of the false-negative EIA interpretations were reported for samples from Donor 18, a donor with strongly positive HIV-1 antibody. Of 131 WB interpretations reported for the HIV-1 antibody-negative samples, two (1.5%) indeterminate WB interpretation were reported. Among the 1,302 WB interpretations reported for the HIV-1 antibody-positive samples, there were no false-negative and 131 (10.1%) indeterminate interpretations. The weakly-reactive donor samples, particularly samples from Donors 1 and 3, accounted for all of the indeterminate WB interpretations reported for the HIV-1 antibody-positive samples. Among the 22 IIF interpretations reported for HIV-1 antibody-

negative samples, there were no false-positive or indeterminate interpretations reported. Of the 187 IIF interpretations reported for antibody-positive samples, there were 5 (2.7%) indeterminate and 9 (4.8%) false-negative interpretations. All 9 false-negative and 4 of the 5 indeterminate IIF interpretations were reported for the HIV-1 antibody weak-positive seroconversion samples from Donor 3.

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 (60.6%), while some laboratories performed both EIA and supplemental tests (36.9%), and others (2.5%) performed only supplemental tests. There were 81 laboratories that performed other tests in addition to EIA, WB and IIF, and 41 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, Murex ICE HIV 1.O.2 Detection, Innogenetics Innostest HIV-1/HIV-2, and Ortho Diagnostics HIV-1/HIV-2 Ab Capture EIA.

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**

The four false-positive EIA interpretations were reported equally for Donor 15 and Donor 16 by laboratories using EIA kits from two different manufacturers (Figure 5).

As indicated in Figure 5, there were 8 nonreactive EIA interpretations reported for the HIV-1 antibody-positive donor samples by laboratories using a variety of FDA-licensed EIA kits. Six of the 8 EIA false-negative interpretations were reported for the HIV-1 antibody weak-positive donor samples obtained from individuals during seroconversion (Donor numbers 1, 2, 3, 8, and 9). Some laboratories reported initially reactive EIA results but nonreactive repeat EIA results for these seroconversion samples.

### **WB Results**

Of the 760 laboratories reporting test results in this survey, only 261 (34.3%) performed WB testing. It is unclear why some laboratories performed WB testing on HIV-1 antibody-negative donor samples (Donors 15 and 16) that were nonreactive in EIA tests. However, there were 2 indeterminate WB interpretations reported for HIV-1 antibody-negative samples from Donor 15 by laboratories using the BioRad WB kit (Figure 6). One laboratory reporting the indeterminate WB interpretation noted the presence of only p24 and the other laboratory reported only a gp160 viral band for this HIV-1 antibody-negative sample. One laboratory, using an in-house WB kit, reported the presence of p24, p31, gp41, p51, p55, p66, and gp120, for their HIV-1 antibody-negative sample from Donor 16; however, this laboratory reported a negative WB interpretation for this sample.

All of the indeterminate WB interpretations reported for samples from the 14 HIV-1 infected donors (Donors

1-14) were reported for samples from the seroconverting donors (Donors 1, 2, 3, 8, and 9). Indeterminate WB interpretations were reported most often for Donor 1, 43 (53.1%) of 81 interpretations; Donor 3, 47 (66.2%) of 71 interpretations; and Donor 8, 18 (45.0%) of 40 interpretations. Indeterminate WB interpretations for the seroconversion samples were reported by laboratories using WB kits provided by six different manufacturers; however, the greatest frequency of indeterminate WB interpretations were reported by laboratories using a WB kit manufactured by Epitope/Organon Teknika, 60 (14.7%) of 409 interpretations (Figure 6). Indeterminate interpretations reported for these samples most often resulted from failure to detect antibody to envelope (env) antigens (e.g., gp41, gp160) 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 three FDA-licensed WB test kits, are shown in Table 2.

Of the 261 laboratories reporting WB test results, 242 indicated which WB criteria were used to interpret their WB tests. The ASTPHLD/CDC WB interpretive criteria was used by 199 (82.2%) of these 242 laboratories. The WB interpretive guidelines published by the FDA-licensed WB kit manufacturers are identical to the ASTPHLD/CDC HIV-1 WB interpretive criteria. Thirteen laboratories indicated they were using interpretive criteria different from that recommended by the kit manufacturer as approved 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 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.

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 samples obtained from HIV-1 infected individuals during seroconversion appeared to cause more difficulty. Most of the indeterminate WB interpretations reported for the seroconversion samples (Donors 1, 2, 3, 8, and 9) resulted from inability 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 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 antibody-negative donor samples (Figure 7). Among the 187 IIF interpretations reported for the HIV-1 antibody-positive samples, 9 (4.8%) false-negative and 5 (2.7%) indeterminate interpretations were reported only for the seroconversion samples (Donors 1,2,3,8 ,and 9). All of the false-negative and 3 of 5 (60%) indeterminate interpretations were reported for Donor 3 samples.

### **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 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 either of the HIV-1 antibody-negative samples (Donors 15 and 16).

### 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. Forty-one (33.6%) of the 122 laboratories reporting results of "Other" types of tests did not report results of EIA, WB or IIF tests. The procedures used by 60 (49.2%) of these 122 laboratories can be described as "rapid" microfiltration EIA procedures (e.g., SUDS HIV-1, Testpack HIV-1/HIV-2, MultiSpot HIV-1/HIV-2, and HIV-Spot HIV 1+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. Eighteen laboratories tested samples using a gelatin particle agglutination test (Fujirebio Serodia HIV) and one laboratory used a latex agglutination test (bioMerieux/Cambridge Biotech Capillus). Results of "Line or Strip Immunoassay" tests such as Liatek (Organon Teknika), INNO-LIA (Innogenetics) and RIBA (Chiron) were appropriately reported on the "Other Test" results form by twelve laboratories. Note that most laboratories using the Abbott AXSYM or PRISM systems correctly reported their results as "Other" test type since these tests are based on microparticle capture and chemiluminescence measurements and differ from the traditional microtiter-format EIA tests.

Of the 122 laboratories reporting results on the form for "Other" types of tests, 53 (43.4%) are laboratories within the United States. Each of these 53 laboratories reported results using the FDA-approved Murex SUDS HIV-1 test.

Among the 121 final interpretations reported for HIV-1 antibody-negative samples (Donors 15 and 16) tested by procedures other than EIA, WB, and IIF, one false-positive interpretation was reported by a laboratory using the Fujirebio Serodia HIV particle agglutination test and one indeterminate interpretation was reported by another laboratory using a test described as "passive particle agglutination", but not coded as Fujirebio Serodia HIV. The false-positive interpretations and the indeterminate interpretation was reported for Donor 15.

Among the 646 interpretations reported for the HIV-1 antibody-positive samples tested by procedures other than EIA, WB, or IIF, there were two false-negative interpretations and two indeterminate interpretations. The false-negative interpretations were reported once each for seroconversion samples from Donors 1 and 9 by laboratories using "rapid" tests. The indeterminate interpretations were reported for samples from Donors 4 and 11 by a single laboratory using the Serodia passive particle agglutination test; however, this laboratory reported a reactive test for the duplicated Donor 4 sample in their panel.

### 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, only 418 (59.6%) indicated they used quality control samples other than those provided with the manufactured test kit. Of these 418 laboratories, 239 (57.2%) used samples obtained commercially, 157 (37.6%) used QC samples from in-house sources, and 20 (4.8%) 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 either weak-positive or weak-positive and negative serum/plasma with each set or run of EIA plates.

Of the 261 laboratories reporting WB test results, only 77 (29.5%) laboratories described their external QC samples. Of these 77 laboratories, 50 (64.9%) used samples prepared in-house, 24 (31.2%) used QC samples obtained commercially, and 3 (3.9%) 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 38 laboratories reporting IIF results, only 12 (31.6%) used IIF external QC samples. Of these, 11 (91.7%) used samples from in-house sources and 1 (8.3%) used QC samples obtained commercially. The majority indicated that both a strong positive and a negative QC samples were included with each set/run of slides.

Of the 122 laboratories reporting results of tests other than EIA, WB or IIF, only 40 (32.8%) indicated the use of external QC samples. Of these, 24 (60%) used samples from in-house sources, 13 (32.5%) used samples from commercial sources and 3 (7.5%) indicated using QC material obtained from both of these sources. The majority indicated that a strong-positive and a negative QC sample was 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. No laboratories reported false-negative WB results and only a few laboratories reported false-negative EIA (0.23%) or false-negative IIF (4.8%) results for the HIV-1 antibody-positive samples (Donor numbers 1-14 and 17-18). False-positive EIA (0.56%) and indeterminate WB (1.5%) results were reported infrequently 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 99.8% and 99.4%, respectively. When indeterminate and reactive WB interpretations are combined, the WB analytic sensitivity was 100%. If indeterminate interpretations are considered incorrect for HIV-1 antibody-negative samples, the WB analytic specificity was 98.5%. When indeterminate and reactive IIF interpretations are combined for the HIV-1 antibody-positive samples, the IIF analytic sensitivity was 95.2%; the IIF analytic specificity was 100% for this survey. The analytic sensitivity and specificity of the test procedures other than EIA, WB, and IIF vary greatly, depending on which test method results are analyzed (Figure 10). 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 99.76%, 99.9%, and 95.7% respectively.