

**Analysis of the January 29, 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 January 29, 1996. Testing results were reported by 826 (90.6%) of 912 laboratories that were sent sample panels.

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 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, using the ASTPHLD/CDC interpretive criteria, with all HIV-1 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 1605 EIA interpretations reported for HIV-1 antibody-negative samples, only 3 (0.19%) were incorrectly reported as reactive. False-negative EIA interpretations, however, were reported for 201 (6.2%) of the 3217 interpretations reported for the antibody-positive samples. The HIV-1 seroconversion samples (Donors 11-18) accounted for 200 of the 201 false-negative EIA interpretations reported. Of 269 WB interpretations reported for the HIV-1 antibody-negative samples, one false-reactive (0.4%) and 16 (6.0%) indeterminate WB interpretations were reported. Among the 1022 WB interpretations reported for the HIV-1 antibody-positive samples, there were 2 (0.19%) false-negative and 276 (27.0%) indeterminate interpretations. The seroconversion donor samples (Donors 11-18) accounted for all of the false-negative and indeterminate WB interpretations reported for the HIV-1 antibody-positive samples. Among the 48 IIF interpretations reported for HIV-1 antibody-negative samples, there were no false-positive or indeterminate interpretations reported. Of the 169 IIF interpretations reported for antibody-positive samples, there were 23 (13.6%) indeterminate and 29 (17.2%) false-negative interpretations. All of the false-negative and 22 (95.7%) of the 23 indeterminate IIF interpretations were reported for the HIV-1 antibody weakly-positive seroconversion samples (Donor numbers 11-18).

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 (65.0%), while some laboratories performed both EIA and supplemental tests (33.7%), and (1.2)% performed only supplemental tests. Not represented in this figure are 54 laboratories that performed only tests other than EIA, WB, or IIF. Information concerning 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 1605 EIA interpretations reported for the HIV-1 antibody-negative samples (Donor numbers 5-10) there were three false-positive interpretations (Figure 5). False-positive EIA interpretations were reported once for Donor 8 and twice for Donor 10.

Among the HIV-1 antibody strong-positive donor samples (Donors 1-4), there was one nonreactive EIA interpretation reported for Donor 1. Laboratories had more difficulty, however, in reporting EIA-reactive final interpretations for the eight antibody weak-positive donor samples obtained from individuals during seroconversion (Donor numbers 11-18). Nonreactive final interpretations for these samples were reported most frequently for Donor 13, 133 (33.0%) of 403; Donor 17, 29 (14.4%) of 202; and Donor 15, 22 (10.7%) of 206 final interpretations. The number of EIA false-negative interpretations, by manufacturer, are shown in the following table.

EIA False-negative interpretations/Total EIA interpretations

<u>EIA Kit Manufacturer</u>	<u>Donor 13</u>	<u>Donor 15</u>	<u>Donor 17</u>
Abbott Envacor	2/2	0/1	0/1
Abbott (HIV-1)	8/108	0/43	2/54
Abbott (HIV-1/HIV-2)	106/160	0/63	0/81
Behring Diagnostics	0/2	1/3	0/1
Cambridge Biotech	5/26	0/11	0/13
SYVA Microtrak	8/14	0/5	0/7
Murex Wellcozyme	1/6	0/3	0/3
Organon Teknika	0/46	16/33	14/23
Sanofi (Pasteur Diagnostics)	0/6	0/0	1/3
Sanofi (Genetic Systems) LAV	3/22	0/21	9/11
Sanofi (Genetic Systems) HIV -1/HIV-2	0/8	5/11	3/4

For this survey, plasma from Donor 13 was a duplicated sample in Panel P and plasma from Donors 15 and 17 were single samples in Panels M and P, respectively. In the previous survey (August 1995) these same donors provided single samples in panels tested by the same laboratories receiving them in this survey. However, in the previous survey, the aggregate false-negative rates for Donors 13, 15, and 17 were lower, as shown in the following table:

Aggregate EIA False-negative Rates in Replicate Sample Surveys		
<u>Donor Number</u>	<u>August 1995</u>	<u>January 1996</u>
13	12.7%	33.0%
15	9.5%	10.7%
17	9.8%	14.4%

Laboratories using the Abbott HIV-1/HIV-2 EIA kits in the previous survey reported EIA-nonreactive interpretations for Donor 13 for only 18 (21.4%) of 84 total EIA interpretations. The reason(s) for higher false-negative rates for these seroconversion samples in the present survey are unknown.

Some laboratories reported initially reactive EIA results but nonreactive repeat EIA results for these seroconversion samples. The 201 non-reactive EIA interpretations for donor samples 11-18 were reported by laboratories using eleven different EIA kits provided by eight different manufacturers. The greatest percentages of false-negative results were reported by laboratories using EIA kits manufactured by Abbott, Organon Teknika, and Sanofi (Genetic Systems). In examining the lot numbers of all EIA test kits used by laboratories reporting non-reactive EIA interpretations for Donor numbers 11-18, no specific lot number(s) appeared to be associated with these false-negative interpretations.

WB Results

Six laboratories used the WB results form to report the results of line immunoassay tests (Liatek, Inno-Lia) although instructed to report this type test on the form for “Other” procedures. The results of the line immunoassay tests are not included in the WB results analysis.

Of the 826 laboratories reporting test results in this survey, only 263 (31.8%) performed WB testing. Among the WB interpretations reported for the HIV-1 antibody-negative samples, one false-reactive WB interpretation was reported for Donor 8 by a laboratory reporting the presence of p24, gp41, p66, gp120, and gp160 bands. The 16 indeterminate interpretations for the HIV-1 antibody-negative donor samples (Donors 5-10) were reported by laboratories using five different WB kits (Figure 6). All of the indeterminate WB interpretations for the HIV-1 antibody-negative samples were accompanied by reports of a single gag band (p17 or p24). Indeterminate interpretations were reported for Donor number 9, 8 (12.7%) of 63 WB test results, and Donor number 10, 8 (11.1%) of 72 WB interpretations. There were two reports of a weak (‘W’) p17 band for Donor 8 and non-reactive WB interpretations. 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’. There were no EIA reactive interpretations reported for Donor 9, and only two EIA reactive interpretations reported for Donor 10. 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.

There were no false-negative or indeterminate WB interpretations among the 264 WB interpretations reported for the HIV-1 antibody strongly positive donor samples (Donors 1-4). Among the 756 WB interpretations reported for samples from the 8 seroconverting donors (Donors 11-18), there were 2 (0.26%) false-negative and 276 (36.5%) indeterminate interpretations. Both false-negative WB interpretations were reported for Donor 18. Indeterminate interpretations were reported most often for Donor 15, 50 (64.9%) of 77 total interpretations; Donor 13, 65 (62.5%) of 104; Donor 17, 28 (57.1%) of 49; and Donor 11, 65 (38.0%) of 171 WB interpretations. Indeterminate WB interpretations were reported by laboratories using 9 different WB kits. The greatest frequency of false-negative and indeterminate WB interpretations were reported by laboratories using WB kits manufactured by BioRad,

165 (44%) of 375, and Genelabs Diagnostics, 13 (29.5%) of 44 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 of less than the required intensity with an 'X' rather than a 'W'. A table, on the second page following the title page of the graphic report, lists the WB bands with 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.

Of the 263 laboratories reporting WB test results, 251 indicated which WB criteria were used to interpret their WB tests. The ASTPHLD/CDC WB interpretive criteria was used by 197 (78.5%) of these 251 laboratories. Four additional laboratories reported WB results interpreted by "other" WB criteria described as the criteria published in the manufacturer's inserts of the FDA-licensed Organon Teknika (Epitope), Cambridge Biotech, and BioRad WB kits. However, the WB interpretive criteria published by these manufacturers are identical to the ASTPHLD/CDC WB interpretive criteria.

WB Band Patterns

The protein band patterns 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.

Donor samples 5-10 were negative for HIV-1 antibody; however, one laboratory reported a single p24 band (Donor 10), and one laboratory reported p24, gp41, gp120 and gp160 bands for Donor 8. None of the HIV-1 antibody-negative donor samples demonstrated antibodies to any viral-specific 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, caused much more difficulty. Most of the false-negative and 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 in the table on page 2 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 169 IIF interpretations reported for the HIV-1 antibody-positive samples, 29 (17.2%) false-negative and 23 (13.6%) indeterminate interpretations were reported. Only one indeterminate and no false negative interpretations were reported for the HIV-1 antibody strongly-positive samples (Donors 1-4).

The greatest frequencies of false-negative interpretations for the seroconversion samples (Donors 11 - 18) were reported for Donor 15, 5 (55.6%) of 9 interpretations; Donor 17, 5 (45.5%) of 11 interpretations; Donor 18, 4 (40.0%) of 10 interpretations; and Donor 11, 7 (31.8%) of 22 interpretations. Indeterminate interpretations were reported at least once for Donors 11-18. Non-reactive and indeterminate IIF interpretations were reported for the samples from these HIV-1 seroconverting donors by laboratories using IIF kits from identified commercial sources, unidentified (other) commercial sources, and in-house IIF procedures and reagents. However, a laboratory using IIF

reagents from a noncommercial source such as a state health department reported no false-negative or indeterminate IIF interpretations. The greatest frequencies of indeterminate interpretations were reported for Donor 11, 8 (36.4%) of 22 interpretations and Donor 12, 4 (22.2%) of 18 interpretations. A lack of agreement was noted in IIF test interpretation among laboratories using IIF kits from the same manufacturer. For example, laboratories using IIF kits manufactured by Waldheim Fluorognost reported 15 reactive, 6 nonreactive, and one indeterminate IIF interpretations for Donor 13.

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 (Donor numbers 11-18) rarely showed fluorescence intensity greater than 2+, and frequently 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 classical EIA, WB or IIF. The top part of this figure shows 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 54 laboratories reporting "Other" types of HIV tests on the correct result form, there were 6 laboratories incorrectly using the WB result form to report results of line-immunoassay tests (Inno-Lia and Liatek). Of the 54 laboratories reporting results on the form for "Other" types of tests, 31 were laboratories within the United States. Twenty (37.0%) of the 54 laboratories only reported results from "Other" tests and did not report results of EIA, WB or IIF tests. The "Other" procedures used by 35 (64.8%) of these 54 laboratories can be described as "rapid" microfiltration enzyme immunoassay procedures (e.g., SUDS HIV-1, HIVSpot HIV1+2, 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.

Twelve laboratories tested samples using a gelatin particle agglutination test (Fujirebio Serodia HIV) and two laboratories used a latex agglutination test (Cambridge Biotech). Results of "Line Immunoassay" tests were correctly reported on this form by two laboratories using the Liatek test manufactured by Innogenetics and marketed by Organon Teknika. Three laboratories indicated "in-house" as manufacturer and all of these did a radioimmunoprecipitation (RIPA) assay.

Of the 98 final interpretations reported for HIV-1 antibody-negative samples (Donors 5 - 10) tested by "Other" procedures, there were 4 (4.1%) false-positive and 3 (3.1%) indeterminate interpretations. False-positive interpretations were reported twice each for Donor 8 and Donor 10 samples that were duplicated in Panels N and R, respectively. Indeterminate reactions were reported twice for Donor 9 and once for Donor 10 samples. Indeterminate and/or false-positive interpretations were reported most frequently by laboratories using the Fujirebio gelatin particle agglutination test.

Among the 210 final interpretations reported for the HIV-1 antibody-positive samples tested by "Other" procedures, there were 18 (8.6%) false-negative interpretations and 8 (3.8%) indeterminate interpretations. False-negative and indeterminate interpretations were reported only for the seroconversion samples (Donors 11-18). False-negative interpretations were reported most frequently for Donor 18, 8 (57.1%) of 14 final interpretations, and Donor 17, 2 (22.2%) of 9 final interpretations, by laboratories using the Murex SUDS HIV-1 test. Indeterminate interpretations

were reported most often for Donor 11, 4 (13.3%) of 30 reports, and Donor 15, 2 (12.5%) of 16 reports, and were reported in equal number by laboratories using the Murex SUDS and the Fujirebio Serodia HIV tests.

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 796 laboratories that reported EIA test results, only 348 (43.7%) indicated they used quality control samples other than those provided with the manufactured test kit. Of these 348 laboratories, 202 used samples obtained commercially and 150 used QC samples prepared in-house. The majority indicated the use of a single, weakly positive serum/plasma with each set or run of EIA plates. Of the 263 laboratories reporting WB test results, 72 (27.4%) laboratories described their external QC samples. The majority described a single weakly-positive serum/plasma obtained in-house, and used with each set/run of WB strips. Of the 44 laboratories reporting IIF results, only 9 (20.5%) 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 (0.2%) and both reactive (0.4%) and indeterminate (5.9%) 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 (6.2%), nonreactive WB (0.3%), and nonreactive IIF (17.2%) results for the HIV-1 seroconversion samples (Donor numbers 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 93.6% and analytic specificity was 99.8%. 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 93.7%. When indeterminate and reactive IIF interpretations are combined, the IIF analytic sensitivity was 82.8%; the IIF analytic specificity was 100% for this survey. For the HIV-1 antibody strongly-positive samples only, analytic sensitivity for EIA, WB, and IIF was 99.9%, 100%, and 100% respectively. Combining indeterminate and reactive interpretations, the analytic sensitivity and specificity of the "Other" procedures was 91.4% and 92.9%, respectively.

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 95.8%, 98.5%, 86.6%, and 91.9% respectively.

The samples mailed in this survey were replicate samples of those received by laboratories participating in the August 1995 MPEP HIV-1 survey. Each laboratory participating in both surveys would have tested the same samples in each of them. However, for this survey, the EIA sensitivity was 93.6% compared to 97.7% in the previous survey, and the IIF sensitivity fell to 82.8% for this survey compared to 87.2% for the previous survey. The overall analytic performance of laboratories reporting "Other" types of HIV test results improved in this survey to 91.9% compared to 87.4% in the previous survey. Sensitivity, specificity and overall analytic performance for WB was comparable in the two surveys.