Diagnosis of Human Immunodeficiency Virus (HIV) Infection: Multicenter Evaluation of a Newly Developed Anti-HIV 1 and 2 Enzyme Immunoassay

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A new anti-human immunodeficiency virus type 1 and 2 (anti-HIV 1 and 2) test is described. It uses recombinant p24 and peptides covering gp32, gp41, and gp120 to identify HIV-1 and HIV-2 infections. This test has been shown to be specific (99.5%) and sensitive (99.8%). In this respect, the assay was equal or superior to anti-HIV 1 and 2 tests run as references. The test was able to discriminate sera from patients with HIV infections from those from uninfected individuals with excellence; it also exerted high intra- and interassay precisions. The “modular” concept of the test allows the use of single components (gp32 or gp41) to separate between HIV-2 and HIV-1 infections, respectively.

Two types of human immunodeficiency virus (HIV) have been identified; they are designated HIV type 1 (HIV-1) and HIV-2. While HIV-2 is mainly restricted to West Africa or to individuals originating from that area or having contact with individuals from West Africa, the spread of HIV-1 continues worldwide (1, 4, 6, 7, 9, 11, 12, 16, 17, 19).

Control of HIV has been shown to be difficult. Sexual transmission of HIV and spread of the virus through illicit drug abuse can be influenced to some extent by educational programs (3, 8).

An important tool that can be used to reduce the spread of HIV is the early and reliable recognition of the HIV infection by sensitive tests (15). The anti-HIV 1 and 2 test has been shown to reduce significantly the risk of HIV infection by blood and blood products (2, 15, 18). The test is also able to identify HIV-infected persons, allowing them to adjust their behaviors. Thus, sensitive and reliable anti-HIV 1 and 2 tests also contribute significantly to a reduction in the spread of HIV infection (2, 15, 18).

Here we report on a newly developed anti-HIV 1 and 2 test which also allows the serological discrimination between HIV-1 and HIV-2. In addition, components of the test can be used to monitor HIV infection and thereby add to the staging of the HIV disease.

MATERIALS AND METHODS

Methods. Five different antigens were selected for use in recognizing antibodies to HIV-1 and HIV-2. p24 is a recombinant protein that was generated as described previously (13); all other antigens were peptides and were derived from gp32/36 (HIV-2) and gp41 and gp120 (HIV-1).

Recombinant antigens and peptides were labelled as described previously (10).

Test concept. Streptavidin-coated tubes were manufactured as described previously (5, 10). Biotin-labelled antigens were allowed to bind to streptavidin for 60 min. After removal of unbound antigen, 20 μl of serum was added to the tube and was allowed to react with the antigens for 60 min. Specifically bound immunoglobulins were recognized by an antibody against immunoglobulin G obtained from sheep and labelled with peroxidase as described previously (14).

The peroxidase that was bound to the tube was visualized by using ABTS substrate and was read with a photometer at a wavelength of 405 nm. Tests were performed on automated systems (ES 22, ES 300, and ES 600; Boehringer Mannheim GmbH, Mannheim, Germany). The cutoff of the Enzymun test was calculated from 2,130 HIV-negative serum samples, 476 anti-HIV-1-positive serum samples, and 200 anti-HIV-2-positive serum samples according to the formula 0.07 × E positive + E negative, where E is extinction. For routine use, the positive and negative controls were run in triplicate, and the mean was determined for the cutoff determination.

The following anti-HIV tests were used for comparison: Abbott recombinant HIV-1 and HIV-2 enzyme immunoassay (EIA), Abbott recombinant HIV-1, Behring Enzymnost Anti-HIV 1 and 2, Organon Teknika Vironostika HIV-1, Pasteur Rapid’Elavia HIV-1, and Pasteur Elavia II. All tests were performed and validated according to the instructions of the manufacturers.

Sera. Sera were obtained from different sources. They included sera from 2,834 unselected blood donors, 81 dialysis patients, and 38 pregnant women and 405 serum samples from HIV-1-infected individuals as well as from 200 HIV-2-infected individuals. Anti-HIV-positive samples were confirmed by Western blotting (immunoblotting; Dupont, Pasteur, Bio-Rad) and were rated as prescribed by each manufacturer. In addition, serial samples from 15 individuals who seroconverted to anti-HIV status were analyzed. Addi-
tional sera included samples from patients with autoimmune disorders, different liver diseases, and tumors, and rheumatoid factor-positive individuals. Furthermore, selected samples from patients with sexually transmitted diseases and blood donors (panel II) and from African and Asian patients (panel III) were analyzed. Moreover, sera with selected bands on Western blots were tested.

Panel I contained 35 serum samples; 21 samples had a single p24 band, 12 samples had a single gp160 band, and 2 samples had a single gp41 band on Western blotting. Panel II comprised 56 samples; 32 samples had a single p24 band, 16 samples had a single gp160 band, 4 samples had a single p55 band, and 3 samples had both p24 and p55 bands on Western blotting. None of these serum samples was positive for HIV antigen, as assessed by a commercial enzyme-linked immunosorbent assay (Abbott). All patients were followed for at least 6 months; none of them converted to anti-HIV status with a positive Western blot result.

The latter two categories of sera were selected and designated "tricky samples."

RESULTS

Optimization of the anti-HIV 1 and 2 test. A total of 200 anti-HIV-1 and 50 anti-HIV-2-positive serum samples as well as 600 serum samples from healthy humans were used to optimize the anti-HIV 1 and 2 test.

Individual HIV-1 and HIV-2 antigens were diluted in incubation buffer, and the anti-HIV test was performed as described above. The individual antigen concentration that revealed 95% of the maximum extinction was considered the optimum antigen concentration (data not shown). The actual antigen concentration was reduced by 20% in order to avoid antigen excess in the tube. Different sample volumes were also evaluated. The sensitivity of the test was identical irrespective of the sample volume (10, 20, or 40 μl) (data not shown). Samples and conjugates were incubated for between 30 and 120 min; an incubation time exceeding 30 min did not improve the sensitivity or specificity of the test (data not shown).

Additional features that we analyzed included incubation temperature, analysis after different storage times (up to 3 weeks), and use of different hardware (Boehringer Mannheim Enzymun Test Systems ES 33 and ES 600). None of the experiments resulted in a reduction of sensitivity (data not shown).

As a result of the series of experiments described above, a 20-μl sample volume was chosen for the standard test; incubation times for sample and substrate of 180 min at room temperature were chosen.

The test is distributed under the trademark Enzymun-Test anti-HIV 1 and 2.

Performance of the anti-HIV 1 and 2 test. (i) Sensitivity. A total of 495 anti-HIV-positive serum samples originating from different European countries (Italy, Spain, Germany, and Belgium) confirmed by Western blotting were analyzed by the anti-HIV 1 and 2 test; 494 serum samples were positive (sensitivity, 99.79%) and serum sample 1 was negative; identical results were obtained with the different reference tests.

Two hundred anti-HIV-2-positive serum samples that were collected in Portugal and West Africa were recognized by the anti-HIV 1 and 2 test, revealing a sensitivity of 100%. Identical results were obtained with the Pasteur Elavi II test.

A series of serum samples was available from a total of 15 individuals who seroconverted to anti-HIV-1 status. The anti-HIV 1 and 2 test result turned positive at the same time or earlier than Western blotting or reference anti-HIV tests (Abbott HIV 1, Abbott HIV-1/HIV-2 EIA). Figure 1 shows the results of the anti-HIV 1 and 2 test in comparison with the results of Western blotting and a second anti-HIV test (Abbott HIV-1/HIV-2 EIA) for serial serum samples.

(ii) Specificity. A total of 2,834 serum samples from unselected blood donors were tested; 2,822 of 2,834 tested negative (specificity, 99.5%); the reference test (Abbott HIV-1/HIV-2 EIA) resulted in negative results for 2,828 of 2,834 serum samples (specificity, 99.8%). Positive serum samples were analyzed by Western blotting and were found to be negative; additional samples of these sera were not available. In addition, sera from 81 patients on dialysis and
38 pregnant women were tested; they were negative for HIV in the test.

Tricky sera as described in Materials and Methods and outlined in Table 1 were also analyzed. The specificity of the anti-HIV 1 and 2 test varied between 89.3 and 97.1%; this was superior to the specificities of the reference tests (Table 1).

In addition, the intra- and interassay precisions were determined. The intraassay precision was assessed by using one negative and two positive serum samples. In order to analyze the interassay precision, five serum samples (one negative and four positive serum samples) were tested in seven consecutive runs. Precision in all experiments varied between 3.9 and 6.9%.

(iii) **Discrimination between HIV-1 and HIV-2 infection by EIA.** Five HIV-1- and HIV-2-positive serum samples each were analyzed by the anti-HIV 1 and 2 test. Figure 2 shows the results for these positive samples. The same sera were then introduced into the anti-HIV 1 and 2 selective test by using either gp41 (HIV-1) or gp32 (HIV-2). Anti-HIV-1-positive sera were readily recognized by gp41, but not by gp32; similarly, anti-HIV-2-positive sera tested positive with gp32 as the antigen but not with gp41 as the antigen (Fig. 2).

The data indicate that components of the anti-HIV 1 and 2 test can be used to discriminate between HIV-1 and HIV-2 infections if the anti-HIV 1 and 2 test reads positive.

**DISCUSSION**

This report described a new anti-HIV 1 and 2 test. The test achieved specificity and sensitivity similar to those of the tests used as references in the present trial (2). In some experiments, it appeared that the test was even superior to the reference tests. This became evident in selected serum samples from individuals from Africa and Asia, but also when serum samples from individuals who seroconverted were tested. In the seroconversions described here, one anti-HIV test became positive only when the Western blot analysis already showed a nearly complete Western blot pattern. This is unusual, because, in general, enzyme-linked immunoassays detect seroconversion several weeks earlier than Western blot assays do. The variable performance of anti-HIV 1 and 2 tests may depend predominantly on the antigens that are selected for use and the variable antibody responses of the individuals. Also, the level of the initial immunoglobulin M response may have an impact on the early outcome of an anti-HIV test.

The specificity of the anti-HIV 1 and 2 test appeared to be lower when blood samples from healthy donors were tested. Whether this is in fact the case is open to question. In fact, it has been demonstrated that the anti-HIV 1 and 2 test is highly sensitive; sera from some of the blood donors may have been weakly positive, but anti-HIV-1 or anti-HIV-2 was not recognized by other enzyme-linked immunosorbent assays or Western blotting. Whether this consideration remains an option cannot be defined because sequential serum samples were not available for testing. This would have allowed an estimate of whether some of the false-positive sera in fact represented early seroconversion probes.

An additional important advantage of the anti-HIV 1 and 2 test is that it is run on a highly automated system. This automation limits to a minimum the mistakes that can be made by human hands; in addition, it significantly reduces the risk of mixing samples, which is of high significance in anti-HIV testing. It also has improved precision and thereby
helps to reduce false-positive and, to a limited extent, false-negative readings.

The test concept presented here has an additional advantage; the modular concept facilitates the use of a single antigen. This can be used to discriminate between HIV-1 and HIV-2-infected individuals and may, in fact, save expensive and time-consuming Western blot analyses in case each serum that reads positive in the anti-HIV 1 and 2 test is considered a candidate for double infection with HIV-1 and HIV-2. Also, the cross-reactivity of HIV-1 and HIV-2 on the Western blot level may facilitate misinterpretation as to the type of HIV infection. As shown here, gp32 reliably recognizes HIV-2 infections but not HIV-1 infections; similarly, gp41 detects HIV-1 but not HIV-2 infections.

Thus, the data that we obtained suggest that the discrimination assay can be used to select samples for Western blot analysis and to reassess HIV results by Western blotting with respect to cross-reactivity between HIV-1 and HIV-2.

In summary, the new anti-HIV 1 and 2 test is highly suited for anti-HIV screening. The flexibility of the test concept allows discrimination between HIV-1 and HIV-2; thus, the newly developed anti-HIV 1 and 2 test has the additional features of being able to diagnose HIV infection and may contribute to a reduction in the spread of this deadly disease (2, 15, 18).

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