NOTES

Detection of Anti-Human Immunodeficiency Virus Type 1 (HIV-1) Immunoglobulin G in Urine by an Ultrasensitive Enzyme Immunoassay (Immune Complex Transfer Enzyme Immunoassay) with Recombinant Reverse Transcriptase as an Antigen

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Anti-human immunodeficiency virus type 1 immunoglobulin G in urine was detected by an immunoassay with reverse transcriptase as the antigen and β-d-galactosidase as the label; this immunoassay was 30-fold more sensitive than the previous immunoassay with peroxidase as the label. The sensitivity and specificity were both 100%. The lowest signal for asymptomatic carriers was 20-fold higher than the highest signal for seronegative subjects.

Antibodies to human immunodeficiency virus type 1 (HIV-1) in urine have been detected by various methods, including the conventional enzyme-linked immunosorbent assay (ELISA) (2, 4, 5, 8), the gelatin particle agglutination test (5), the immunoglobulin G (IgG) antibody capture ELISA (3, 6, 10), the IgG antibody capture particle adherence test (3, 6), and the immune complex transfer enzyme immunoassay (ICTEIA) (5). In some of the above-described reports, the sensitivity and specificity were both 100% (5, 8). However, none of the methods appeared to be satisfactory for the diagnosis of HIV-1 infection. With the conventional ELISA, the ratio of signal to noise was not improved by concentration of urine samples (8). The specificities of the conventional ELISA and gelatin particle agglutination test were considerably lowered with concentrated urine samples because of enhanced nonspecific signals (5). For the IgG antibody capture ELISA and IgG antibody capture particle adherence test, sensitivity would not be improved with concentrated urine samples, as long as the level of IgG in the urine samples was low enough to saturate the anti-IgG binding sites in the assay (3). With ICTEIA, the lowest signal for asymptomatic carriers was only 1.5-fold higher than the highest signal for seronegative subjects, although the sensitivity and specificity were both 100% and the sensitivity was improved with concentrated urine samples (5). We describe the detection of anti-HIV-1 IgG in urine by a more sensitive ICTEIA, in which β-d-galactosidase from Escherichia coli was substituted for horseradish peroxidase in the previous ICTEIA (5).

Recombinant reverse transcriptase (RT) of HIV-1 (NL4.3) (1) was produced in E. coli transformed with expression plasmids carrying the corresponding cDNA and purified as described previously (9). p51 and p66 in the purified preparation were both found homogeneous by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. 2,4-Dinitrophenyl-bovine serum albumin-recombinant RT conjugate and recombinant RT–β-d-galactosidase conjugate

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FIG. 1. Effect of pH. Urine samples from a seropositive subject (○) and a seronegative subject (●) were incubated with the two RT conjugates at pH 5.0 to 8.0.
were prepared by reacting thiol groups introduced into recombinant RT molecules with maleimide groups introduced into 2,4-dinitrophenyl-bovine serum albumin molecules and maleimide groups introduced into β-D-galactosidase molecules (5).

Urine samples (100 μl) were incubated with 20 μl of nonspecific rabbit serum and 10 μl of buffer A (10 mmol of sodium phosphate buffer per liter [pH 7.0] containing 0.1 g of bovine serum albumin, 1.0 mmol of MgCl₂, and 1.0 g of NaCl per liter) containing 0.4 mol of NaCl per liter and 50 μg of inactive β-D-galactosidase (5) for 3 h. For testing serum (20 μl) and the effect of urine volumes, the total volume of the incubation mixture was adjusted to 130 μl with the buffer. For testing specificity, 15 or 150 pmol of recombinant RT was added. The reaction mixture (130 μl) was incubated with 20 μl of buffer A containing 0.4 mol of NaCl per liter and 100 fmol each of the two conjugates described above for 3 h. For testing the effect of pH, 1 μl of urine, 20 μl of nonspecific rabbit serum, and 129 μl of 10 mmol of sodium phosphate buffer per liter (pH 5.0 to 8.0), containing 50 μg of inactive β-D-galactosidase, 0.4 mol of NaCl per liter, 0.1 g of bovine serum albumin per liter, 1.0 mmol of MgCl₂ per liter, 1.0 g of NaCl per liter, and the two conjugates were incubated for 3 h. To the reaction mixture, two colored polystyrene balls coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG (5) were added, and incubation was continued overnight. The colored polystyrene balls were washed twice by the addition and aspiration of 2 ml of buffer A containing 0.1 mol of NaCl per liter and incubated with 150 μl of buffer A containing 0.1 mol of NaCl per liter and 1.0 mmol of εN-2,4-dinitrophenyl-t-lysine per liter and two white polystyrene balls coated with affinity-purified (anti-human IgG γ chain) IgG (5) for 1 h. The colored polystyrene balls were removed, and incubation was continued for 2 h. The white polystyrene balls were washed as described

FIG. 2. Lack of cross-reaction of the present ICTEIA with serum and urine samples from HTLV-I-infected subjects. Circles and triangles indicate results in the present ICTEIA and the ICTEIA for anti-HTLV-I IgG, respectively.

FIG. 3. Dilution curves for urine samples from seropositive subjects in the present ICTEIA with β-D-galactosidase as the label (O) and the previous ICTEIA with peroxidase as the label (●). Urine samples from three seropositive subjects were serially diluted with urine from a seronegative subject.
above. All of the above-described processes were performed at room temperature. β-D-Galactosidase activity bound to the white polystyrene balls was assayed at 30°C for 2.5 h by fluorometry with 4-methylumbelliferyl-β-D-galactoside as a substrate, and the fluorescence intensity was measured relative to that of 10⁻⁸ mol of 4-methylumbellifereone per liter (5).

Urine samples were collected from 100 seronegative subjects aged 24 to 68 years and 70 seropositive subjects (49 asymptomatic carriers aged 14 to 47 years, 13 patients with AIDS-related complex and aged 10 to 56 years, and 8 patients with AIDS and aged 21 to 60 years). Negative and positive sera were discriminated by gelatin particle agglutination, and seronegativity was confirmed by Western blotting (immunoblotting) (5). The urine samples collected were mixed with a 1/100 volume each of 10 g of bovine serum albumin per liter and 5 g of thimerosal per liter and stored at −20°C. Before use, thawed urine samples were briefly centrifuged to remove precipitates.

Up to 100 μl of urine samples could be used with only slight interference. The signal was maximal at pH 7.5 for incubation with the two conjugates and 30% lower at pH 5.0 (Fig. 1). For urine samples showing three different levels of signal (12, 213, and 2,407 for within assay and 11, 166, and 1,782 for between assay), the coefficients of variation for within assay and between assay were 2.8 to 7.2% (n = 10) and 6.0 to 9.7% (n = 10), respectively. No significant reaction was observed with serum and urine samples from 10 human T-cell lymphotropic virus type I (HTLV-I)-infected subjects, whose sera were strongly positive in the gelatin particle agglutination test with HTLV-I as the antigen and in the ICTEIA with Cys-env gp46(188-224) of HTLV-1 as the antigen (7) (Fig. 2). The sensitivity was 30-fold higher than that of the previous ICTEIA with peroxidase as the label (5) (Fig. 3).

All signals for the 70 seropositive subjects were unequivocally higher than those for the seronegative subjects (Fig. 4). The lowest signals for asymptomatic carriers and patients with AIDS-related complex and AIDS were 20-, 11- and 7.2-fold, respectively, higher than the highest signal for the seropositive subjects. Levels of anti-HIV-1 RT IgG were as high in all but 2 of the 21 patients with AIDS-related complex and AIDS as in asymptomatic carriers. With a mixture of 30 fmol each of recombinant RT, p17, and p24 conjugates (instead of 100 fmol each of the recombinant RT conjugates described above), low signals for some seropositive subjects were enhanced to various degrees, with little change in the signal for the seronegative subjects. The combined use of RT and other antigens, including env proteins, may discriminate seropositive from seronegative subjects more easily.

All signals for the 70 seropositive subjects were lowered to the levels of those for the seronegative subjects by preincubation of urine samples with an excess of recombinant RT of HIV-1 (Fig. 4). This result indicated that all signals for the seropositive subjects were not due to unknown or nonspecific reactions but were due to the presence of IgG antibody to recombinant RT. The possibility that IgG antibody to some protein(s) other than recombinant RT in the recombinant RT preparation used was detected was unlikely, since recombinant RT was purified to homogeneity.

Finally, specificity for the diagnosis of HIV-1 infection may
be improved to increasing degrees by separately demonstrating antibodies to increasing numbers of different antigens or epitopes of HIV-1; therefore, ICTEIAAs with recombinant p17, p24, and env proteins are being developed.

REFERENCES