Potential of the Immune Complex Transfer Enzyme Immunoassay for Antigens and Antibodies to Improve the Sensitivity and Its Limitations

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In order to reduce the nonspecific signal of noncompetitive solid phase immunoassays and to improve their sensitivities, the immune complex transfer enzyme immunoassay has been developed. Antigens to be measured were reacted with 2,4-dinitrophenyl-biotinyl-antibody Fab' and antibody Fab'- β -D-galactosidase conjugate, and antibody IgGs to be measured were reacted with 2,4-dinitrophenyl-antigen and antigen- β -D-galactosidase conjugate. The immune complexes formed comprising the three components were trapped onto colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG. After washing, the immune complexes were eluted from the colored polystyrene beads with ϵ -N-2,4-dinitrophenyl-L-lysine, and the eluates were incubated with white polystyrene beads coated with streptavidin for antigens and coated with affinity-purified (anti-human IgG γ -chain) IgG for antibody IgGs to transfer the immune complexes. By this method, ultrasensitive enzyme immunoassays have been developed for HIV-1 p24 antigen and antibody IgGs to HIV-1 p17 and reverse transcriptase (RT). The nonspecific signals in the absence of the antigen and the antibody IgGs were reduced 300 to 15,000-fold by the immune complex transfer process, but the amounts of the immune complexes decreased only 1.8 to 3.1-fold by the immune complex transfer. As a result, the sensitivities for HIV-1 p24 antigen and antibody IgGs to HIV-1 p17 and RT were improved 100 to 5,600-fold by the immune complex transfer. The detection limit of HIV-1 p24 antigen by 20 hr assay of β -D-galactosidase activity (10 zmol) was 4,000 to 17,000-fold lower than those obtained with currently available com-

mercial kits. The improved sensitivities for antibody IgGs to p17 and RT by 20 hr assay of β -D-galactosidase activity were 1×10^5 to 3×10^5 -fold higher than those of Western blotting for p17 and p66 bands. However, the nonspecific signals in the absence of antigens and antibody IgGs were enhanced to various degrees by two factors. In order to transfer the immune complexes more efficiently within shorter periods of time, the colored polystyrene beads were incubated with the white polystyrene beads in the presence of ϵ -N-2,4-dinitrophenyl-L-lysine. Such direct contact between solid phases for trapping and transferring of the immune complexes significantly enhanced the nonspecific signals. In addition, the presence of human serum samples containing neither antigens to be measured nor antibody IgGs to be measured also enhanced the nonspecific signals to various extents. Namely, these two factors limited the effect of the immune complex transfer to improve the sensitivity by 20 hr assay of β -D-galactosidase activity. By 1 hr assay of β -D-galactosidase activity, the detection limit of HIV-1 p24 antigen using 10 μ l of serum samples (0.24 pg/ml) was 40 to 80-fold lower than those obtained with currently available commercial kits using 100 to 200 μ l of serum samples (10 to 20 pg/ml) and the detection limits of antibody IgGs to HIV-1 p17 and RT were 1×10^4 to 3×10^4 -fold lower than those by Western blotting for p17 and p66 bands. Finally, the immunoreactions involved in the immune complex transfer enzyme immunoassays—the formation, trapping, and transferring of the immune complexes—will be performed within 15 to 30 min. *J. Clin. Lab. Anal.* 12:154–161, 1998. © 1998 Wiley-Liss, Inc.

Key words: enzyme immunoassay; β -D-galactosidase; immune complex transfer; HIV-1 p24 antigen; antibody IgG to HIV-1 p17 antigen; antibody IgG to HIV-1 reverse transcriptase

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INTRODUCTION

Currently available immunoassays for antigens and antibodies may be divided into two groups. One uses various labels for measuring antigens and antibodies with ease and high sensitivity, and the other does not. The former may be divided into two groups: competitive and noncompetitive ones. Noncompetitive immunoassays with labels have been widely used for the last two decades and have been demonstrated to be more sensitive than other immunoassays. However, the sensitivity of noncompetitive immunoassays using labels is limited by the nonspecific signal, that is, the signal in the absence of antigens and antibodies to be measured. A decade ago, a method to markedly reduce the nonspecific signal in a conventional ELISA for antibody IgG was developed (1), and ultrasensitive enzyme immunoassays using this method, namely, the immune complex transfer enzyme immunoassays, have been developed for various antigens and antibodies (2–7). This article describes the potential of the immune complex transfer enzyme immunoassay to improve the sensitivity and its limitations, using HIV-1 p24 antigen and antibody IgGs to HIV-1 p17 and reverse transcriptase as examples.

PRINCIPLE OF THE IMMUNE COMPLEX TRANSFER ENZYME IMMUNOASSAY

The immune complex transfer enzyme immunoassay was developed initially for antibody IgG to minimize the nonspecific signal in the conventional ELISA, in which antigen-coated solid phase is reacted with antibody IgG to be measured in serum samples and, after washing, with (anti-IgG) antibody-enzyme conjugate (1–6). In the immune complex transfer enzyme immunoassay initially developed, antibody IgG to be measured in serum samples was reacted with 2,4-dinitrophenyl-biotinyl-antigen, and the immune complex formed was trapped onto colored polystyrene beads coated with (anti-2,4-dinitrophenyl group) IgG. After washing to remove most of the nonspecific IgG, the immune complex was eluted with excess of *N*-2,4-dinitrophenyl-L-lysine and was transferred onto clean polystyrene beads coated with avidin to remove nonspecific IgG more completely, followed by reaction with (anti-IgG) Fab'-enzyme conjugate. The nonspecific signal in the absence of antibody IgG to be measured is markedly lowered by the immune complex transfer, whereas it was very high due to nonspecific IgG adsorbed on the antigen-coated solid phase in the conventional ELISA.

The initially developed immune complex transfer enzyme immunoassay was modified in various ways (2–6). The most sensitive immune complex transfer enzyme immunoassay for antibodies among those developed so far is shown in Fig. 1 (2–7). Antibodies to be measured are reacted simultaneously with 2,4-dinitrophenyl-antigens and antigen-enzyme conjugates, and the immune complexes formed comprising the three components are trapped onto solid phase coated with (anti-2,4-dinitrophenyl group) IgG. After washing, the immune complexes

are eluted with excess of *N*-2,4-dinitrophenyl-L-lysine and are transferred to solid phase coated with anti-immunoglobulin IgG. By substituting 2,4-dinitrophenyl-biotinyl-antigen and streptavidin-coated solid phase for 2,4-dinitrophenyl-antigen and anti-immunoglobulin IgG-coated solid phase, antibodies of all classes can be measured (5,6). The use of β -D-galactosidase from *Escherichia coli* provides much more sensitive immunoassays than that of horseradish peroxidase (8).

For antigens, labeled antibody IgGs or Fab's are substituted for labeled antigens in the above immune complex transfer enzyme immunoassay for antibodies (Fig. 2) (5). Antigens to be measured are reacted simultaneously with 2,4-dinitrophenyl-biotinyl-antibody Fab' and antibody Fab'-enzyme conjugate, and the immune complex formed comprising the three components is trapped onto solid phase coated with (anti-2,4-dinitrophenyl group) IgG. After washing, the immune complex is eluted and is transferred to solid phase coated with streptavidin as described above.

POTENTIAL OF THE IMMUNE COMPLEX TRANSFER ENZYME IMMUNOASSAY TO IMPROVE THE SENSITIVITY

By the immune complex transfer, the nonspecific signal, that is, the signal in the absence of antigens and antibodies to

Fig. 1. Immune complex transfer enzyme immunoassay for antibodies. **DNP:** 2,4-dinitrophenyl group. **Ag:** antigen. **Ab:** antibody. **Enz:** enzyme. **Ig:** immunoglobulin.

Fig. 2. Immune complex transfer enzyme immunoassay for antigens. **DNP:** 2,4-dinitrophenyl group. **Ag:** antigen. **Enz:** enzyme.

be measured is reduced to greater extents than the specific signal due to the presence of the analytes, and the detection limit of the analytes is lowered to various extents from case to case. Three examples shown in Tables 1–3 are HIV-1 p24 antigen (9,10) and antibody IgGs to HIV-1 p17 and reverse transcriptase (RT) antigens (8,11–18).

In Table 1, effect of the immune complex transfer on the detection limit of HIV-1 p24 antigen is shown. The antigen was incubated with 2,4-dinitrophenyl-biotinyl-bovine serum albumin-affinity-purified rabbit anti-p24 Fab' conjugate and monoclonal mouse anti-p24 Fab'- β -D-galactosidase conjugate in a total volume of 150 μ l for 3 hr to form the immune complex comprising the three components. The reaction mixture was incubated with two colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG for 1 hr to trap the immune complex. After washing, the colored polystyrene beads were incubated with *N*-2,4-dinitrophenyl-L-lysine for 0.5 hr to elute the immune complex. The eluate was incubated with two white polystyrene beads coated with streptavidin for 1 hr to transfer the immune complex. β -D-Galactosidase activity bound to the white polystyrene beads was assayed by fluorometry using 4-methyl-umbelliferyl- β -D-galactoside as a substrate.

In the presence of HIV-1 p24 antigen, the signal, that is,

the fluorescence intensity for β -D-galactosidase activity bound to the white polystyrene beads was 3.1-fold lower than that bound to the colored polystyrene beads. Namely, the fraction of the immune complex transferred was 32%. By contrast, in the absence of HIV-1 p24 antigen, the signal for β -D-galactosidase activity bound to the white polystyrene beads was 300-fold lower than that bound to the colored polystyrene beads. Namely, the nonspecific signal in the absence of HIV-1 p24 antigen was reduced 300-fold by the immune complex transfer process. As a result, by the immune complex transfer, the detection limit of HIV-1 p24 antigen was lowered 100-fold to 0.1 amol when β -D-galactosidase activity was assayed for 1 hr and to 10 zmol when assayed for 20 hr. The detection limit by 1 hr assay of β -D-galactosidase activity was 400 to 1,700-fold lower than those obtained with currently available commercial kits and that by 20 hr assay was 4,000 to 17,000-fold lower.

In Table 2, effect of the immune complex transfer on the detection limit of antibody IgG to HIV-1 p17 antigen is shown. Serum from an HIV-1 seropositive subject was diluted 1×10^4 -fold with a buffer, and 10 μ l of the diluted serum was incubated with 2,4-dinitrophenyl-maltose binding protein-recombinant p17 (rp17) fusion protein and rp17- β -D-galactosidase conjugate in a total volume of 150 μ l for 0.5 hr to form the immune complex comprising the three components. The reaction mixture was incubated with two colored polystyrene beads coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG for 1 hr to trap the immune complex. The immune complex was transferred to two white polystyrene beads coated with affinity-purified (anti-human IgG γ -chain) IgG as described above.

The signal in the presence of antibody IgG to HIV-1 p17 antigen was reduced 2.2-fold by the immune complex transfer. By contrast, the nonspecific signal in the absence of antibody IgG to HIV-1 p17 antigen was reduced 720-fold by the immune complex transfer process. As a result, the detection limit of antibody IgG to HIV-1 p17 antigen was lowered approximately 300-fold by the immune complex transfer process. This was 3×10^4 -fold more sensitive than Western blotting for p17 band when β -D-galactosidase activity was assayed for 1 hr and was 3×10^5 -fold more sensitive when assayed for 20 hr.

In Table 3, effect of the immune complex transfer on the detection limit of antibody IgG to reverse transcriptase (RT) is shown. Serum from an HIV-1 seropositive subject was diluted 2×10^3 -fold with a buffer and 10 μ l of the diluted serum was incubated with 2,4-dinitrophenyl-bovine serum albumin-recombinant RT (rRT) conjugate and rRT- β -D-galactosidase conjugate in a total volume of 150 μ l for 4 hr to form the immune complex comprising the three components. The immune complex was trapped and was transferred as described above for antibody IgG to HIV-1 p17 antigen.

Two different preparations of rRT- β -D-galactosidase conjugate—less polymerized and highly polymerized ones—were

TABLE 1. Effect on Signals in the Absence and Presence of HIV-1 p24 Antigen of Immune Complex Transfer

Addition	Signal for anti-p24 Fab'-β-D- galactosidase conjugate used	Signal in the absence of p24 antigen			Signal in the presence of p24 antigen (100 amol)		
		Before transfer of the immune complex	After transfer of the immune complex		Before transfer of the immune complex	After transfer of the immune complex	
			Contact between colored and white beads			Contact between colored and white beads	
			No	Yes		No	Yes
None	1.28 × 10 ⁶ (2.13 × 10 ⁴) (6.4 × 10 ⁶)	60 (1.0) (300)	0.20 — (1.0)	0.23 — (1.15)	358 — (3.1)	116 — (1.0)	144 — (1.24)
Serum (10 µl) from HIV-1 seronegative subjects	1.28 × 10 ⁶	—	—	0.18–0.5 (n=10)	—	—	—

^aThe antigen, p24, (100 amol) was incubated with 5 fmol of monoclonal anti-p24 Fab' β-D-galactosidase conjugate, 100 fmol of 2,4-dinitrophenyl-biotinyl-bovine serum albumin-affinity-purified rabbit anti-p24 Fab' conjugate, 10 µl of nonspecific rabbit serum and 50 µg of inactive β-D-galactosidase in a total volume of 150 µl for 3 hr. The reaction mixture was incubated with two colored polystyrene beads (3.2 mm in diameter) coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG for 1 hr. The buffer used was 10 mmol/L sodium phosphate buffer, pH 7.0, containing 0.4 mol/L NaCl, 1 mmol/L MgCl₂, 1 g/L bovine serum albumin and 1 g/L NaN₃. The colored polystyrene beads were incubated with 150 µl of 1 mmol/L εN-2,4-dinitrophenyl-L-lysine for 0.5 hr, and the eluate was incubated with two white polystyrene beads (3.2 mm in diameter) coated with streptavidin for 1 hr (Contact between colored and white beads—No). Alternatively, the colored polystyrene beads were incubated with the white polystyrene beads in 150 µl of 1 mmol/L εN-2,4-dinitrophenyl-L-lysine for 1 hr (Contact between colored and white beads—Yes). The signals in the absence of HIV-1 p24 antigen and the signal for monoclonal anti-p24 Fab'-β-D-galactosidase conjugate added are the fluorescence intensities for β-D-galactosidase activity assayed for 20 hr, and the signals in the presence of HIV-1 p24 antigen are the fluorescence intensities for β-D-galactosidase activity assayed for 1 hr.

^bValues in parentheses are the ratios of signals to those for β-D-galactosidase activity bound to the colored polystyrene beads before transfer of the immune complex and the white polystyrene beads after transfer of the immune complex without contact between the colored and white polystyrene beads.

TABLE 2. Effect on Signals in the Absence and Presence of Antibodies to p17 Antigen of Immune Complex Transfer

Addition	Signal for rp17- β-D- galactosidase conjugate used	Signal in the absence of antibodies to p17 antigen			Signal in the presence of antibodies to p17 antigen		
		Before transfer of the immune complex	After transfer of the immune complex		Before transfer of the immune complex	After transfer of the immune complex	
			Contact between colored and white beads			Contact between colored and white beads	
			No	Yes		No	Yes
None	1.98 × 10 ⁷ (9.2 × 10 ⁴) (6.6 × 10 ⁷)	216 (1.0) (720)	0.30 — (1.0)	0.60 — (2.0)	1027 — (2.2)	462 — (1.0)	568 — (1.2)
Serum (10 μl) from HIV-1 seronegative subjects	1.98 × 10 ⁷	234	0.63 0.55 1.15 0.35 0.35 0.45 0.45	1.37 0.95 1.55 0.65 0.65 0.65 0.55	1039	427 — — — — — — —	522 — — — — — — —

^aSerum from an HIV-1 seropositive subject was diluted 1×10^4 -fold with buffer and 10 μ l of the diluted serum was incubated with 100 fmol each of 2,4-dinitrophenyl-maltose binding protein-recombinant p17 (rp17) fusion protein and rp17- μ β -D-galactosidase conjugate in the presence of 50 μ g of inactive β -D-galactosidase in a total volume of 150 μ l for 0.5 hr and with two colored polystyrene beads (3.2 mm in diameter) coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG for 1 hr. The buffer used was 10 mmol/L sodium phosphate buffer, pH 7.0, containing 0.4 mol/L NaCl, 1 mmol/L MgCl₂, 1 g/L bovine serum albumin and 1 g/L NaN₃. After washing, the colored polystyrene beads were incubated with 150 μ l of 1 mmol/L *ε*N-2,4-dinitrophenyl-L-lysine for 0.5 hr. The eluate was incubated with two white polystyrene beads coated with affinity-purified (anti-human IgG γ -chain) IgG for 1 hr (Contact between colored and white beads—No). Alternatively, the colored polystyrene beads were incubated with the white polystyrene beads in 150 μ l of 1 mmol/L *ε*N-2,4-dinitrophenyl-L-lysine for 1 hr (Contact between colored and white beads—Yes). The signals in the absence of antibodies to p17 antigen and the signal for rp17- β -D-galactosidase conjugate added are the fluorescence intensities for β -D-galactosidase activity assayed for 22.5 hr, and the signals in the presence of antibodies to p17 antigen is the fluorescence intensities for β -D-galactosidase activity assayed for 1 hr.

^bValues in parentheses are the ratios of signals to those for β -D-galactosidase activity bound to the colored polystyrene beads before transfer of the immune complex and the white polystyrene beads after transfer of the immune complex without contact between the colored and white polystyrene beads.

TABLE 3. Effect on Signals in the Absence and Presence of Antibodies to RT of Immune Complex Transfer

β-D-Galactosidase conjugate no.	Signal for rRT-β-D-galactosidase conjugate used	Signal in the absence of antibodies to RT			Signal in the presence of antibodies to RT		
		Before transfer of the immune complex	After transfer of the immune complex		Before transfer of the immune complex	After transfer of the immune complex	
			Contact between colored and white beads			Contact between colored and white beads	
			No	Yes		No	Yes
1	1.87 × 10 ⁷ (3.1 × 10 ⁴) (5.8 × 10 ⁷)	612 (1.0) (1,913)	0.32 — (1.0)	0.55 — (1.7)	1,404 — (1.8)	780 — (1.0)	810 — (1.0)
2	1.62 × 10 ⁷ (823) (1.25 × 10 ⁷)	19,680 (1.0) (15,138)	1.3 — (1.0)	26 — (20)	3,120 — (2.7)	804 — (1.0)	804 — (1.0)

^aSerum from an HIV-1 seropositive subject was diluted 2×10^3 -fold with buffer and 10 μ l of the diluted serum was incubated with 100 fmol each of 2,4-dinitrophenyl-bovine serum albumin-recombinant RT (rRT) and rRT β -D-galactosidase conjugate in the presence of 50 μ g of inactive β -D-galactosidase and 10 μ l of nonspecific rabbit serum in a total volume of 150 μ l for 3.5 to 4 hr and with two colored polystyrene beads (3.2 mm in diameter) coated with affinity-purified (anti-2,4-dinitrophenyl group) IgG for 1 hr. The buffer used was 10 mmol/L sodium phosphate buffer, pH 7.0, containing 0.4 mol/L NaCl, 1 mmol/L $MgCl_2$, 1 g/L bovine serum albumin and 1 g/L NaN_3 . After washing, the colored polystyrene beads were incubated with 150 μ l of 1 mmol/L ϵ N-2,4-dinitrophenyl-L-lysine for 0.5 hr. The eluate was incubated with two white polystyrene beads coated with affinity-purified (anti-human IgG γ -chain) IgG for 1 hr (Contact between colored and white beads—No). Alternatively, the colored polystyrene beads were incubated with the white polystyrene beads in 150 μ l of 1 mmol/L ϵ N-2,4-dinitrophenyl-L-lysine for 1 hr (Contact between colored and white beads—Yes). The signals in the absence of antibodies to RT and the signal for RT- β -D-galactosidase conjugate added are the fluorescence intensities for β -D-galactosidase activity assayed for 20 hr, and the signals in the presence of antibodies to RT is the fluorescence intensities for β -D-galactosidase activity assayed for 1 hr.

^bValues in parentheses are the ratios of signals to those for β -D-galactosidase activity bound to the colored polystyrene beads before transfer of the immune complex and the white polystyrene beads after transfer of the immune complex without contact between the colored and white polystyrene beads.

tested. With less polymerized conjugate, the signal in the presence of antibody IgG to RT was reduced 1.8-fold by the immune complex transfer, but the signal in its absence was reduced 1,913-fold by the immune complex transfer process. As a result, the detection limit of antibody IgG to RT was lowered 1,000-fold by the immune complex transfer. This was 1×10^4 -fold more sensitive than Western blotting for p66 band when β -D-galactosidase activity was assayed for 1 hr and was 1×10^5 -fold more sensitive when assayed for 20 hr. (RT consists of two subunits: p51 and p66). With highly polymerized conjugate, the signal in the presence of antibody IgG to RT was reduced 2.7-fold by the immune complex transfer, but the signal in its absence was reduced 15,138-fold. As a result, the detection limit of antibody IgG to RT was lowered no less than 5,600-fold by the immune complex transfer. This was 1×10^4 -fold more sensitive than Western blotting for p66 band when β -D-galactosidase activity was assayed for 1 hr and was 1×10^5 -fold more sensitive when assayed for 20 hr.

In general, effect of the immune complex transfer tends to be greater in cases where the signal for β -D-galactosidase activity bound to the colored polystyrene beads; the first solid phase is higher.

FACTORS LIMITING THE POTENTIAL OF THE IMMUNE COMPLEX TRANSFER ENZYME IMMUNOASSAY TO IMPROVE THE SENSITIVITY

In the immune complex transfer enzyme immunoassays described above, the colored polystyrene beads were incubated with ϵ N-2,4-dinitrophenyl-L-lysine for 0.5 hr to elute the immune complexes, and the eluates were incubated with the white polystyrene beads for 1 hr to transfer the immune complexes. Alternatively, the colored polystyrene beads can be incubated with the white polystyrene beads in the presence of ϵ N-2,4-dinitrophenyl-L-lysine for 1 hr. The immune complexes tended to be transferred more efficiently within a shorter period of time (Tables 1 and 2). However, the nonspecific signals were enhanced by the direct contact between the colored and white polystyrene beads during the incubation (Tables 2 and 3). Notably, when highly polymerized rRT- β -D-galactosidase conjugate was used, the nonspecific signal was enhanced 20-fold by the direct contact between the colored and white polystyrene beads.

Although human serum samples were not added in the immune complex transfer enzyme immunoassays described above, human serum samples are used for their practical uses. When serum samples from HIV-1 seronegative subjects, which contained neither HIV-1 p24 antigen nor antibody IgGs to p17 and RT of HIV-1, were added, the nonspecific signals were enhanced to various degrees, limiting the sensitivity of the immune complex transfer enzyme immunoassays. In the immune complex transfer enzyme immunoassay for HIV-1 p24 antigen, the detection limit of p24 antigen by 1 hr assay of β -D-galactosidase assay using 10 μ l of serum samples was

10 amol or 0.24 pg/ml of serum and was 40 to 80-fold lower than those obtained with currently available commercial kits using 100 to 200 μ l of serum samples (10 to 20 pg/ml of serum). However, the highest signal in the presence of 10 μ l of serum samples from HIV-1 seronegative subjects by 20 hr assay of β -D-galactosidase activity was slightly lower than that corresponding to 0.05 amol of HIV-1 p24 antigen in the absence of human serum samples. Therefore, 20 hr assay of β -D-galactosidase activity only slightly lowered the detection limit of HIV-1 p24 antigen in the presence of human serum samples as compared with 1 hr assay, although did lower it approximately 10-fold to 10 zmol in the absence of human serum samples. Similarly, the nonspecific signals in the immune complex transfer enzyme immunoassays for antibody IgGs to p17 and RT were also enhanced to various extents by the presence of human serum from HIV-1 seronegative subjects, hampering the improvement of the sensitivity by 20 hr assay of β -D-galactosidase activity. The detection limits of antibody IgGs to p17 and RT by 1 hr assay of β -D-galactosidase using 10 μ l of serum samples were 1×10^4 to 3×10^4 -fold lower than those by Western blotting for p17 and p66 bands.

The adverse effect of serum samples from HIV-1 seronegative subjects in the immune complex transfer enzyme immunoassay for HIV-1 p24 antigen might be due to the presence in those serum samples of protein(s) reactive with anti-p24 antibodies used and/or the presence in the preparations of anti-p24 antibodies used of protein(s) other than anti-p24 antibodies. The adverse effect in the immune complex transfer enzyme immunoassay for antibody IgGs to p17 and RT might be due to the presence of proteins other than rp17 and rRT in the preparations of rp17 and rRT used and/or the presence of antibody IgG(s) reactive with rp17 and rRT in serum samples from HIV-1 seronegative subjects.

RAPID IMMUNE COMPLEX TRANSFER ENZYME IMMUNOASSAY

In the above immune complex transfer enzyme immunoassays, p24 antigen and antibody IgGs to p17 and RT antigens were reacted with the two conjugates for 0.5 to 4 hr to form the immune complexes, and the immune complexes were trapped for 1 hr and were transferred for 1 to 1.5 hr (18). The period of time used for the immuno-reactions involved was 2.5 to 6.5 hr. This has been shortened to 15 to 30 min without lowering the sensitivity, which will be described in detail elsewhere.

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