Comparison of an Amplified Enzyme-Linked Immunosorbent Assay with Procedures Based on Molecular Biology for Assessing Human Immunodeficiency Virus Type 1 Viral Load

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The sensitivity of the enzyme-linked amplified sorbent test (ELAST) was compared with those of other classic enzyme-linked immunosorbent assays (ELISAs), with or without previous acidic immunocomplex dissociation (ICD), in a series of samples at different stages of human immunodeficiency virus type 1 (HIV-1) infection. The limit of viral detection of ELAST was assessed with fresh HIV-1 preparations quantified by reverse transcription-PCR and with the P24 antigen (Ag) Sanofi Pasteur Calibrator containing lyophilized virus. The P24 Ag detection capacity of ELAST was compared with that of NASBA in samples obtained from infected subjects with less than 250 CD4\(^+\) cells. The results of the present study show that ELAST was the most sensitive method for detecting P24 Ag compared to classic ELISA and ICD plus ELISA. ELAST was able to detect 0.5 pg of P24 Ag per ml in a whole virus preparation and the equivalent of 330 to 1,000 RNA copies/ml of HIV. The rate of detection of P24 Ag was always higher in subjects with low levels of anti-P24 antibodies. The number of positive results was dramatically enhanced (from 37% to 94% for subjects with <250 CD4\(^+\) cells) when the incubation period was prolonged from 1 to 16 h. In a third series of 84 samples (<250 CD4\(^+\) cells) tested in parallel, NASBA yielded 83% of the positive results and ELAST yielded 79%. Considering the high sensitivity, low cost, simplicity of equipment (only a plate reader), and possibility for full automation, ELAST appears to be a promising new tool for measuring viral load, especially in areas with few resources, in which the procedures based on molecular biology techniques may be difficult to install.

Several surrogate markers have been used to monitor disease progression in human immunodeficiency virus (HIV)-infected individuals (23, 42). These markers include CD4 count, serum P24 antigen (Ag) levels with or without immune complex dissociation (ICD) before ELISA, quantitative culture of peripheral blood mononuclear cells (PBMCs) or plasma, DNA levels in infected circulating cells, and RNA levels in plasma. A number of studies have shown that in HIV-infected persons, an increased viral burden is associated with the degree of immune deficiency and enhanced risk of perinatal transmission of the virus to the child (19, 38). Moreover, the correlation between the disease stage, CD4\(^+\) cell number, and HIV-1 provirus and virus levels has frequently been reported (19, 23, 25, 38, 42, 47). Methods that are used for quantification of circulating HIV-1 include in vitro culture (cell or plasma) (15, 40), PCR of serial dilutions of PBMCs (1), reverse transcription-PCR (RT-PCR) of serial dilutions of plasma or serum (21, 51), and amplification techniques with no need for serial dilutions (49). Overall, the results of these approaches indicate that there is a significant association between levels of viral activity and disease progression. The measure of circulating HIV load allows for the monitoring of treatments (3, 31) and permits a better understanding of the natural history of the infection (11, 34).

HIV p24\(^{Ag}\) (P24 Ag) has been widely used as a marker of virus replication. However, only a small fraction of HIV-infected individuals have measurable circulating P24 Ag levels when measured by classical immunocapture techniques (enzyme-linked immunosorbent assay [ELISA]) (14, 33). The P24 Ag level has been shown in different studies to correlate with disease progression (8, 16, 23, 32, 36), and it was further suggested that P24 Ag may be used as a criterion for entry into clinical trials and subsequently for monitoring antiviral therapy (24, 29, 43). Treatment of serum samples with acid (26, 28) or base (41) can dissociate P24 Ag complexed with antibodies and increase the rate of positivity (ICD) (31, 35, 39). However, it is no longer accepted that P24 Ag ELISAs, even after ICD, meet the goals of sensitivity required for reliable surrogate markers (12). Thus, the quantification of the HIV viral load based on molecular biology techniques (MBTs) (RT-PCR, NASBA [Organon Teknika], and branched DNA) has become the yardstick for the follow-up of HIV infection. Nevertheless, one of the major concerns which limits the widespread use of MBTs is intra- and interlaboratory variability (technical problems, nondetectable genotypes, etc.). Moreover, the cost of setting up the required facilities to avoid contamination, the price of materials and reactants, and the time required for comprehensive training of staff in MBTs, etc., curtail even further the utilization of MBTs, especially in areas with reduced resources. Therefore, there is a crying need to evaluate and validate affordable techniques to assess HIV viral load.

Amplification of signals of bound proteins with a catalyzed-reporter deposition technique (enzyme-linked amplified sorbent test [ELAST]) (DuPont de Nemours-REN, Paris, France) enhances levels of detection of P24 Ag (9). Schüpbach et al. (45) recently showed that disruption of immunocomplexes by heating 1/6-diluted plasma prior to ELAST detected HIV expression as sensitively as RT-PCR.

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The aim of this work is to compare the results obtained with ELAST with those of other ELISAs used currently to detect P24 Ag in a series of human samples at different stages of HIV-1 infection. Sensitivity was gauged with (i) a fresh HIV-1 preparation quantified after serial dilution by RT-PCR and NASBA and (ii) the P24 Ag Sanofi Pasteur Calibrator (lyophilized virus recognized as standard by the French health authorities). Finally, in a different series of blind samples (plasma obtained from HIV-infected people), ELAST was compared to NASBA.

MATERIALS AND METHODS

Samples. In all of the studies presented here, the tests were performed with blind samples. The first two series of sera (classic ELISA versus ELAST) were obtained from different subjects living in France, in which the dosage of P24 Ag was prescribed before MBTs were available for routine HIV viral load determinations. The sera were kept at −20°C for more than 12 months. The clinical status and immunological status of these subjects were monitored in public hospitals. Since genotyping or subtyping is not performed during routine follow-up, no data on the HIV-1 subtypes were available (in France, clade B is largely in the majority).

To compare NASBA with ELAST, tests were performed with plasma samples derived from blood anticoagulated with sodium citrate. The plasma samples for NASBA were prepared within a maximum of 3 h after blood collection, by centrifugation of the capped tubes. They were then immediately stored at −80°C. NASBA ELAST was performed under routine conditions.

P24 standard. The standard (Sanofi-Diagnostics Pasteur) approved by the Agence du Medicament (France)—containing lyophilized HIV-1 (BRU strain) grown in CEM cells, purified by ultracentrifugation, inactivated and diluted in human serum, and negative for hepatitis B surface Ag, hepatitis C viral antibodies, and HIV-1 and HIV-2 antibodies—was used for comparative tests of sensitivity. For each experiment, calibration plots were plotted with dilutions of the standard in a pool of HIV-1- and HIV-2-negative sera, ranging between 0.1 (100 fg) and 100 ng.

Testing for P24 Ag. Samples were tested by the immunocapture ELISA (HIV-p24 Core Profile ELISA; Du Pont de Nemours) according to the manufacturer’s directions. Ag-antibody complexes (immunocomplexes) were disrupted (ICD) by using low-pH solutions (1 volume of sample plus 1 volume of glycine reagent with 1 volume of Tris for neutralization).

ELAST. Circulating P24 Ag was detected with the immunocapture ELISA (HIV-p24 Core Profile ELISA; Du Pont de Nemours) followed by amplification of the bound antigen (5, 7). For running the tests, 20 µl of 5% Triton, 100 µl of samples or controls, and 100 µl of a glycine solution (pH 2) were incubated for 90 min at 37°C for ICD. Neutralization was performed with 100 µl of Tris buffer for 10 min at room temperature. (Only in a small number of tests [<5%] did we experience difficulties in obtaining a neutralization. When that happened, we strongly suggested ICD.) It was always possible to perform the tests on the volume outside the gel (>150 µl in all cases).

One hundred fifty microliters was then transferred to the ELISA plate (HIV-1 p24 Core Profile Add Kit; Du Pont) and incubated for 1 h or overnight (16 h) at room temperature. (After each of the incubations during ELAST, a specific washing program was applied. Plates were washed with 300 µl of DuPont’s washing solution nine times.) After washing, 100 µl of detector antibody was added and washed. After the third wash, 100 µl of biotinyl-tyramide (BT) was added (100 µl of BT concentrate in 10 ml of BT diluent at a 1:2 ratio). HRP converts phenol derivatives to free radicals and catalyzes the activation of the phenolic groups of BT, resulting in covalent binding to protein-blocking molecules on the solid phase. Plates were incubated for 30 min at room temperature and washed, and 100 µl of HRP-ELAST solution was added (20 µl of HRP-ELAST in 10 ml of ELAST diluent for HRP). After incubation (30 min at room temperature) and washing, 100 µl of OPD was added, and the reaction was stopped after 15 min (100 µl of HCl [1 N]). Optical densities (ODs) were determined at 490 or 620 nm, and concentrations were calculated from the calibration curves plotted from the standard at concentrations between 0.10 and 64 pg/ml.

RESULTS

Table 1 shows the results obtained with the first series of 279 samples. Classic ELISA was able to detect only 8 out of 114 samples positive for the P24 Ag when the sera were obtained from persons with less than 250 CD4+ cells. When acidic ICD was performed before ELISA, 26 samples became positive. The amplification of the signals with no prior ICD (classic ELISA plus amplification) showed 38 positive results, and ICD plus amplification (ELAST) showed 42 positive results. Even after dilution (for ICD, sera were diluted 1/3 with the acidic and the neutralizing solutions), ELAST showed the highest rates of positivity for all of the samples independently of their CD4+ cell count ranking. In samples obtained from subjects with more than 300 CD4+ cells—in which classic ELISA did not detect any positivity—a dramatic increase in sensitivity was obtained with ELAST, with 11 positive samples out of 30 (37%). For sera obtained from subjects with CD4+ cell counts between 250 and 500, ELAST was also the most sensitive method, with positive results for 33% of the samples. Global analysis of the results of the first series of 279 HIV+ sera (in which at least 40% were obtained from subjects in an advanced stage of infection), indicates that only 13 (4.2%) samples were positive by classic ELISA. After ICD, 22% of the samples became positive. When amplification was performed after

| CD4+ cell count | No. (%) of samples with positivity* | Total no. of samples tested (n = 279) |
|----------------|-----------------------------------|-------------------------------------|
| <250          | 8 (7)                             | 38 (37)                             |
| 250–500       | 5 (4.9)                           | 22 (23.5)                           |
| >500          | 0 (0)                             | 11 (13.3)                           |
| NA            | 0 (0)                             | 14 (42.4)                           |

*Classic, detection of the P24 Ag by ELISA (immunocapture); Classic + Ampl, amplification of the signal after classic ELISA with no previous ICD.

**ELAST was performed after a 1-h incubation.

**NA, cell count not available.
ELISA, but with no previous ICD, 24% of all the samples were positive. ICD previous to ELISA and BT amplification (ELAST) increased the rates of detection to 36%.

Table 2 shows the results of the first series of 279 samples stratified into two groups: positive and negative anti-P24 antibodies. The levels of Ag detection for all methods (classic ELISA, ICD plus ELISA, classic ELISA plus amplification, and ELAST) were significantly higher in samples with no anti-P24 antibodies. ELAST showed the highest level of antigen detection (19% of the positive samples and 85.7% of the negative samples). ICD with no amplification was less sensitive (10% and 55.7%, respectively). Classic ELISA did not detect the P24 Ag in any of the samples obtained from subjects with detectable levels of anti-P24 antibodies.

Acidic ICD enhanced the detection levels for P24 Ag from 0 to 10% in the group of anti-P24 detectable levels of anti-P24 antibodies. The coupling of ICD with amplification (ELAST) in the anti-P24 group enhanced detection levels to 19.6%.

In a second series of 100 samples (CD4+ count of <250), ELAST was performed after 1 or 16 h of incubation. P24 Ag was positive for 37% of the samples after 1 h of incubation, but the rate dramatically increased to 94% when the incubation period was prolonged up to 16 h. Out of 100 samples tested, 62 samples were negative and 1 was borderline (sample ODs ≤ 10%, which was the cutoff value) when the Ag immunocapture on the microplates was performed according to the manufacturer's indications (1 h). When the incubation was prolonged up to 16 h, only five samples were negative and one sample was borderline. Forty samples from HIV-seronegative subjects were also run in triplicate, and they were all negative for ELAST performed after 1 or 16 h of incubation.

For ELAST (16 h), reproducibility was 100% for negative samples and 100% for all of the samples containing the equivalent of >1 pg/ml. For 15 samples (0.25 to 1 pg/ml) tested repeatedly (four times), reproducibility was 95% and 57 determinations showed the same values (picograms per milliliter). Intralaboratory variability for retesting of the samples with reactants from different lots (picograms per milliliter) was always <20%. With different lots of reactants retested at different dates, linearity ranged between 0.5 and 16 pg/ml.

In a preliminary study, we compared detection limits for classic ELISA, ELAST with 1 h of incubation, and ELAST with 16 h of incubation. According to the curve performed with the standard, they were, respectively, 20, 2, and 0.25 pg/ml. In the present study, Table 3 shows the results obtained with a supernatant of a fresh HIV-1 preparation serially diluted. This preparation was titrated by RT-PCR and NASBA, and the results with the aliquot used as a standard were, respectively, 1,000 and 800 RNA copies/ml. Classic ELISA was able to detect the equivalent of 20,000 viral RNA copies. ELAST was able to detect the equivalent of 2,500 viral RNA copies with an incubation time of 1 h. When the incubation was prolonged for 16 h, ELAST detected the equivalent of 1,000 viral RNA copies. These results were reproducible in four different tests with determinations performed each time in triplicate. Since before antigen capture, all of the samples were diluted 1/3 for acidic ICD, the signal detection limit of ELAST (16-h incubation) may be adjusted down to the equivalent of at least 1,000 viral RNA copies/ml.

Table 4 shows the results obtained with a third series of 84 samples (plasma) obtained from persons with less than 250 CD4+ cells (range, 50 to 250), tested in parallel with NASBA and ELAST. From these 84 samples, 10 were negative by the two techniques. NASBA yielded 83% positive results, and ELAST yielded 79% positive results. Four samples were found positive by ELAST and negative by NASBA. (Three of these four samples showed weak ODs, corresponding to concentrations below 1 pg/ml according to the standard curve.) Eight samples were positive by NASBA but negative by ELAST, and one sample (ELAST negative, repeated three times) had more than 40,000 copies of HIV RNA according to NASBA. Two samples containing between 800 and 2,000 RNA copies were not detected by ELAST. Six samples out of eight with viral load values (NASBA) of <2 × 10^3 RNA copies/ml were detected by ELAST (results ranged between 0.1 and 4 pg/ml). ELAST rates of positivity were 85% in the NASBA category ≥2 × 10^3 and <40 × 10^3 RNA copies/ml (44 samples) and 95% when viral load values ranged between >40 × 10^3 and <200 × 10^3 RNA copies/ml (20 samples). All of the samples with values of >2 × 10^3 RNA copies/ml were detected by ELAST.

The plasma samples tested in the third series of comparative studies were frozen at −20°C and thawed for retesting (triplicate) 1 month later. Intra- and intertest variabilities for ELAST (16 h) were <20% (picograms per milliliter).

### Table 2. Positive P24 antigenemia in samples obtained from HIV-infected patients according to presence or absence of anti-P24 antibodies

| Antibody status | No. (%) of samples with positivity | Total no. of samples tested |
|----------------|-----------------------------------|----------------------------|
| Anti-P24+      | 0 (0)                             | 209                        |
| Anti-P24−      | 13 (18.5)                         | 70                         |
| Total          | 13 (4.6)                          | 279                        |

* Determined according to Abbot's anti-P24 antibody kit.

### Table 3. Comparison of viral detection capacities of classic ELISA and ELAST by using a supernatant of fresh HIV-1 quantified by RT-PCR

| Method           | 250   | 125   | 62    | 31    | 16    | 8     | 4     | 2     | 1     | 0.5   | 0.25  | 0.12  | 0.10  | 0.050 |
|------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Classic ELISA    | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| ELAST 1-h incubation | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |
| ELAST 16-h incubation | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     | +     |

* Borderline, sample ODs = OD cutoff values −10%.
TABLE 4. Comparison between NASBA and ELAST with samples obtained from subjects with <250 CD4+ cells/ml

| ELAST resulta | NASBA resultb | No. of samples |
|---------------|---------------|---------------|
|               | Negative      | Positive (1,000 RNA copies/1,000 µl) |               |
|               | >0.8–<2       | >2–<40        | >40–200       | >200–<500     |
| Negative      | 10            | 2             | 5             | 1             | 0             |
| Positive (pg/ml) | 0.1–1       | 3             | 4             | 11            | 8             | 2             |
|               | 1.0–4         | 1             | 2             | 6             | 4             | 2             |
|               | 4.0–16        | 0             | 0             | 10            | 2             | 1             |
|               | >16           | 0             | 0             | 2             | 5             | 5             |

a ELAST results after a 16-h incubation.

b Note that there were 10 samples ELAST negative and NASBA negative, 8 samples ELAST negative and NASBA positive, 4 samples ELAST positive and NASBA negative, and 62 samples ELAST positive and NASBA positive.

DISCUSSION

No laboratory test (whether virological or immunological) is able today to fulfill the expected requirements of correlation with progression, change if the patient improves or worsens, variation with successful treatments, direct association with HIV natural history, intra- and interlaboratory reproducibility, facilities for setup and routine running (i.e., not requiring sophisticated structures or larger surfaces), comparable cost to other laboratory tests, and affordability to the largest number of infected people around the world.

HIV-1 quantification by the MBTs RT-PCR, NASBA, and branched DNA (20, 37, 46) is gaining more and more ground in determining therapeutic performance without having to consider survival as the endpoint. However, because they are expensive and technically complex, MBTs are routinely used only in a few specialized centers (13, 50). In light of the above criteria, the search for reliable, inexpensive, easy-to-perform tests to determine HIV viral load has been a major concern for virologists.

The results of the present study indicate that ELAST (ICD plus ELISA coupled with the BT amplification system) is the most sensitive method for detecting P24 Ag, compared to classic ELISA, ICD plus ELISA, and amplification with no previous ICD.

Schüpbach et al. (45) reported in a previous study that the rate of positivity was enhanced from 27 to 53% when sera were treated with acids for ICD and to 68% when the immunocomplexes were disrupted by heating the samples (1/6 diluted) for 5 min at 100°C.

In the present study, acid ICD enhanced the detection levels for P24 Ag from 0 to 10% in the group of anti-P24+ subjects. Even with no prior ICD, BT amplification also enhanced the level of detection up to 11.5%. The coupling of ICD with amplification (ELAST) in the anti-P24+ group enhanced detection levels to 19.6%.

Schüpbach et al. (45) indicated that overall rates of detection for samples (diluted 1/6) obtained from HIV-infected people were 68% for sera and 78% for plasma. In the present study, the detection rate is almost identical to that obtained by Schüpbach et al. (45) for sera not treated for Ag-antibody dissociation. Nevertheless, the rates of positivity obtained by Schüpbach et al. were higher, both when immunocomplexes were dissociated by acids and when they were dissociated by heat. Positive results for sera in our study versus that of Schüpbach's with sera and versus that of Schüpbach's with plasma for CD4+ cell counts of 200 to 500 are 33, 49.4, and 63%, respectively. The rates for the group with a CD4+ cell count < 250 are 37, 61, and 74%, respectively. Since for the group of samples with CD4+ counts > 500, the results were, respectively, 37 and 5.6%, we cannot explain the differences observed in the first two groups just by the long storage of our series of samples at −20°C.

In the present study, the levels of P24 Ag detection for all methods (ELISA, ICD plus ELISA, classic ELISA plus amplification, and ELAST) were significantly higher in samples with no anti-P24 antibodies. When comparing the results obtained with the samples for each technique, for anti-P24 positive versus anti-P24 negative samples, ELAST showed the highest level of antigen detection (16.2% of the positive samples and 85.7% of the negative samples). ICD with no amplification was less sensitive (9.6% and 55.7%, respectively). Moreover, the lack of detection of P24 Ag is not only due to the entrapment of the viral particles or proteins by the circulating antibodies, but may reflect low levels of viral replication and/or release of virions from reservoirs. Viral replication assessed by the positivity of a freely circulating viral Ag may therefore be one of the consequences of a reduction of immune functions, since for all of the techniques tested here, the highest rates of P24 antigen detection were obtained in subjects with low (or undetectable) levels of anti-P24 antibodies (anti-P24+). Recently, Binley et al. reported results along the same lines and concluded that the disappearance of anti-Gag antibodies during infection is highly unlikely to be due to immune complex formation (6).

The first series of sera (Table 1), from persons with less than 250 CD4+ cells, showed a rate of positivity for ELAST never higher than 37% (anti-P24+ and anti-P24− together). This value is lower than that expected from previous reports in which the positivity rate for sera obtained from subjects with CD4+ cell counts < 200/ml was 60% (45). To enhance the level of detection, we performed kinetic studies with samples with ODs higher than the average of the negative control ODs but lower than the threshold (average negative control ODs + standard deviations). We carried out these studies with different incubation times (4, 8, 16, 24, 36, and 48 h). More than 50% of the samples became positive when the incubation period reached 16 h (plateau at 16 h). When standard (whole virus preparation) dilutions (four tests in triplicate in each experiment) were randomly tested, the P24 Ag detection limit for classic ELISA was never under 16 pg/ml. ELAST made it possible to detect 2 pg of P24 Ag per ml after 1 h of incubation and 0.5 pg/ml after 16 h (with 100% of the samples containing 0.5 pg of the whole viral preparation per ml positive in all of the experiments).

Böni et al. (9) reported that detection rates for samples obtained from treated patients during a median observation time of 25 weeks (Indinavir at 800 mg three times daily in addition to prior antiretroviral treatment) amounted to 75.6% for amplified ELISA and to 73.6% for RT-PCR (detection limit of 0.2 pg/ml). Böni et al. indicate that individual antigen levels correlated well with RT-PCR (r = 0.714). By analyzing their results in terms of group means at different time points, the average treatment induced changes in viral RNA and was closely matched by changes in viral antigen. Van Damme et al. (48) compared the abilities to measure viral load in 107 samples from 25 subjects by RT-PCR (Monitor; Roche) and NASBA and obtained a correlation coefficient on the same order as that obtained when RT-PCR and amplified P24 Ag are compared (r = 0.688). Coste et al. (17) showed in a comparative study carried out with 60 plasma samples from HIV-infected people, a concordance of positive results of 80.4% between RT-PCR and NASBA. No correlation between NASBA
or RT-PCR and rates of P24 Ag positivity was found. This lack of correlation (r < 0.3 for P24 Ag versus NASBA or versus RT-PCR or versus branched DNA) can be explained by the fact that for P24 Ag determinations, no amplification of the signals was performed and the cutoff value was set as high as 30 pg/ml.

In the present study, NASBA and ELAST testing of plasma from persons with less than 250 CD4+ cells (range, 50 to 250) yielded, respectively, 83% (>400 copies/ml) and 79% positive results. Intriguingly, one sample that was ELAST negative (repeated three times) had more than 20,000 copies of HIV RNA according to NASBA, and one sample that was ELAST positive (4 pg/ml) was NASBA negative. This sample was obtained from an individual included in a double-masked clinical trial (zidovudine plus D4T or placebo) just a few weeks before. The half-life of the released P24 proteins may be longer than RNA release after lysis from intact virions, and we should expect that the reduction in circulating P24 antigen levels may be delayed after initiation of an efficient antiviral treatment. Böni et al. (9) reported the same type of results, indicating that in a reduced number of subjects after starting antiretroviral treatments, the decrease in P24 Ag is slower than that of viral RNA or particle-associated RT activity. In the present study, four samples (4.25%) were repeatedly positive for ELAST and repeatedly negative for NASBA. No inhibitors of NASBA were detected in the plasma (controls were included for extraction amplification and quantification procedures), and technical problems were not reported during the running of these tests in the routine laboratory. Coste et al. (17) reported that NASBA is able to detect most HIV-1 subtypes, but not subtype G. For the samples studied here, no data on HIV-1 subtypes that could explain these discrepancies are available.

Moreover, in a 4-year period, the Amplicor kit was used in a United Kingdom reference laboratory for the detection or confirmation of HIV-1 infection (4). This study showed that the Amplicor assay has a sensitivity of 93.7% for adults and children over 2 years. For specimens from young children (under 2 years of age), sensitivity was 75%. The negative results were assumed to be false negative on the basis of clinical data, serological markers (including P24 Ag), and/or results for previous follow-up specimens.

Differences between MBTs and P24 Ag were also recently reported for different HIV-1 strains: significantly lower RT-PCR (Amplicor) loads were measured for most GagA, GagB, and GagF strains, whereas NASBA detected lower copy numbers in the GagH strain, GagH/EnvG recombinant strain, and in one HIV-1 group M strain. HIV-1 group O strains were not reported by others (9). In asymptomatic subjects, frequent checking would permit early detection of viral replication and, consequently, timely treatment. Also, in treated subjects, frequent viral load evaluations may warn of possible therapeutic failure.

The problem here involves both money and facilities: only a few institutions—not to mention individuals—can afford to provide regular follow-up tests with MBTs. ELISA technologies, however, are significantly cheaper than MBTs (less than 10 times the cost for samples tested with ELAST duplicate tests versus a single test with RT-PCR, NASBA, or branched DNA). Those laboratories around the world already using the standard ELISA may easily add signal amplification techniques, providing them with the added advantage of automatic processing of large numbers of samples.

If the detection threshold of 1,000 RNA copies/ml (signal produced by the equivalent of 330 copies/ml, considering a 1/3 dilution for ICD) found in the present study is confirmed by others, ELAST (acid-ICD or heat-ICD plus antigen immunocapture plus BT amplification) may become a new tool for routine determination of viral load in cases such as (i) early seroconversions, (ii) the paucisymptomatic phase of the HIV infection, (iii) mother-child HIV-1 transmission, and (iv) whether to start or wait and add or change treatments. However, comparative kinetic studies measuring decay in RNA and in P24 Ag should be performed, because ELAST (16-h incubation) might lead to erroneous conclusions in cases in which antiretroviral therapies induce very rapid reduction in viral replication.

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