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Performance of Five Different Assays for the Quantification of Viral Load in Persons Infected With Various Subtypes of HIV-1

*Philippe Bürgisser, †Pietro Vernazza, ‡Markus Flepp, §Jürg Böni, §Zuzana Tomasik, §Urs Hummel, ‖Giuseppe Pantaleo, Jörg Schüpbach, and the Swiss HIV Cohort Study

**Division of Immunology and Allergy, Centre Hospitalier Universitaire Vaudois, Lausanne; †Department of Medicine, Kantonsspital, St. Gallen; ‡Division of Infectious Diseases and Hospital Hygiene, Universitätsspital, Zurich; §Swiss National Center for Retroviruses, University of Zurich, Zurich; and ‖Laboratory of AIDS Immunopathology, Division of Infectious Diseases, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland*

Summary: Five methods for the assessment of plasma viral load (VL) were evaluated in 103 seropositive patients infected with various subtypes of HIV-1. The methods included three RNA-based assays (Amplicor Monitor 1.5, Quantiplex version 2.0, NucliSens), one ultrasensitive reverse transcriptase (PERT) assay and one "boosted" p24 antigen (Ag) enzyme immunoassay (EIA). Subtyping was based on sequencing in *env*. The sensitivities were, in decreasing order, Amplicor > PERT > p24 Ag > NucliSens > Quantiplex. The low sensitivity of NucliSens was related to the missing of several non-B (A, E, F, G) or recombinant strains, whereas that of Quantiplex did not depend on subtype. In the 1 group O sample and 4 group M samples, only PERT assay or p24Ag EIA produced a positive result. In the quantitative range, correlation was best between Amplicor and Quantiplex ($r = 0.8848$), fair between Amplicor and NucliSens ($r = 0.7064$) or PERT assay ($r = 0.7266$), lowest between Amplicor and p24Ag EIA ($r = 0.3989$). Amplicor underestimated VL in 1 subtype E sample. Thus, Amplicor performed best in terms of sensitivity (compared with all other assays) and accuracy (compared with NucliSens, PERT assay, and p24Ag) for non-B subtypes in group M samples. PERT assay appears useful for VL assessment in infections by group O or other highly divergent viruses. **Key Words:** HIV-1 subtypes—Viral load—Amplicor—Quantiplex—NucliSens—PERT assay—p24 antigen.

Accurate viral load (VL) determination is of prime importance in the management of HIV infection. Even so, amplification techniques used to measure VL in plasma, at least in their original version, often missed HIV-1 RNA or underestimated its concentration in patients infected with non-B subtypes of HIV-1 (1). This has been the case with version 1.0 of the Amplicor HIV-1 Monitor assay (Roche Diagnostics, Rotkreuz, Switzerland) for subtypes A, E, F, G and H (2–5) and with the NASBA HIV-1 RNA QT assay (Organon Teknika, Turnhout, Belgium) for subtypes A, E, G, and H (5–7). Even a branched-DNA technique, Quantiplex HIV-1 RNA version 2.0 (Chiron Diagnostics AG, Dietlikon, Switzerland), long thought to correctly quantify RNA from all subtypes of group M owing to the use of >40 probes spread over 2590 nucleotides of the conserved *pol* gene (8), has recently been shown to have missed RNA of a multirecombinant strain (9). Here, we have evaluated the performance of newer versions of the HIV-1 RNA target or signal amplification techniques mentioned earlier, as well as the performance of an experimental ultrasensitive reverse transcriptase (RT) assay and that of a "boosted" p24 antigen (Ag) enzyme immu-

Address correspondence and reprint requests to P. Bürgisser, Division of Immunology and Allergy, CHUV, BH 18, CH-1011 Lausanne, Switzerland; email: Philippe.Burgisser@chuv.hospvd.ch.

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noassay (EIA), in 103 study subjects suspected to be infected with non-B subtypes of HIV-1.

MATERIALS AND METHODS

Patients and Samples

In this study, 103 HIV-1–seropositive subjects presumed to have acquired HIV infection in areas of the world (sub-Saharan Africa or Thailand) where non-B subtypes predominate were recruited from the Swiss HIV Cohort Study centers. This selection included both untreated ($n = 31$) and treated ($n = 72$) patients.

Plasma, prepared from ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood and frozen at −80°C within 6 hours of collection, was used for HIV-1 RNA determination by three techniques and for the PERT assay. A second tube of EDTA-anticoagulated blood was used to separate peripheral blood mononuclear cells (PBMC, for subtyping) from plasma (for p24 Ag determination) by centrifugation on a Ficoll-Hypaque density gradient within 24 hours of collection.

HIV-1 RNA Assays

HIV-1 RNA levels were determined by reverse transcription–polymerase chain reaction (RT-PCR, Amplicor HIV-1 Monitor version 1.5, Roche Diagnostics) (3,10), branched DNA (bDNA) signal amplification assay (Quantiplex HIV-1 RNA version 2.0, Chiron Diagnostics AG) (8), and transcription-based RNA amplification (NucliSens HIV-1 RNA QT, Organon Teknika AG, Pfäffikon, Switzerland) (11). In the conditions used, the lower detection level as stated by the manufacturer ranged generally between 100 and 400 copies/ml (Amplicor and NucliSens), whereas that of Quantiplex was constant at 500 copies/ml.

A few samples with undetectable RNA by the standard Amplicor assay were reanalyzed using a supersensitive version of the assay (version 1.5) and 1.0 ml of plasma (12). The lower detection limit was ~10 copies/ml.

Ultrasensitive Reverse Transcriptase Assay

The product-enhanced reverse transcriptase (PERT) assay was performed as previously described (13,14), with some modifications. Briefly, thawed plasma was diluted fivefold with phosphate-buffered saline (PBS), filtered through 0.2-μm filters after which 1 ml was used for virus sedimentation with 20,000 ×*g* at 4°C for 60 minutes in a microfuge. The pellet was resuspended in 50 μl PBS. The RT reaction was carried out in duplicate with 10 μl resuspended virus in a total reaction volume of 30 μl at 37°C for 90 minutes. After heat inactivation of the enzyme, template RNA was degraded by RNase A digestion (400 ng) at 45°C for 45 minutes. Subsequent amplification for 40 cycles in a total reaction volume of 55 μl was performed on a ABI7700 Sequence Detector (Perkin Elmer Biosystems, Norwalk, CT, U.S.A.) using a FAM-labeled TaqMan probe (FAM-5′-CGAGACGCTAC-CATGGCTATCGCT-3′-TAMRA). For quantitation of RT, a dilution series of recombinant HIV-1 RT (Roche Molecular Biochemicals, Rotkreuz, Switzerland) was used to generate an external standard curve. Anti-RT antibody-dependent inhibition of the assay was assessed by testing each sample a second time in the presence of 50 μU/ml of recombinant HIV-1 RT. Inhibition was calculated as follows:

$$\% \text{ inhibition} = 100 \times \left(1 - \frac{\text{activity}_{(\text{sample + RT})} - \text{activity}_{(\text{sample})}}{\text{activity}_{(\text{PBS + RT})} - \text{activity}_{(\text{PBS})}}\right)$$

Activities were corrected for inhibition if they were at least four times higher than that of the PBS control and inhibition did not exceed 95%. Otherwise, specimens were scored as failures. The cutoff of this modified assay for positivity was 22 nU/ml of recombinant HIV-1 RT.

"Boosted" p24 Antigen (p24 Ag) Enzyme Immunoassay

Immune complexes were first dissociated by heat denaturation. Next, p24 Ag was quantified using an HIV-1 p24 EIA and a tyramide-based signal amplification technique as described by Nadal et al (15). Briefly, 100 μl plasma was diluted with 500 μl 0.5% Triton X-100 in 1.5-ml Eppendorf tubes, denatured by heating at 100°C for 5 minutes on a Techne (Cambridge, U.K.) dry heat block and tested in duplicate with the NEN/DuPont HIV-1 Core Profile enzyme-linked immunosorbent assay (ELISA) in combination with the ELAST ELISA amplification system (both purchased from NEN Life Science Products, Geneva, Switzerland). The cutoff level for positivity in diagnostic testing was determined for each assay plate by calculating the mean absorbance of 8 HIV-1–negative controls run on the same plate plus five standard deviations (SD). For quantification, a cutoff corresponding to the mean plus three SD was used. Absorbance was read using a Dynatech MR5000 ELISA reader (Microtech Produkte, Embrach, Switzerland). Antigen was quantified with a kinetic analysis using the Quanti-Kin Detection System (DL3, Diagnostica Ligure srl, Genoa, Italy). This allowed quantification in a range from ~500 to 6,250,000 fg/ml with a single sample dilution.

Subtyping

DNA was extracted from peripheral blood mononuclear cells (PBMC) and a 0.7-kb amplicon including regions V3-V5 of gp120 *env* was produced by nested PCR using primers ED5/ED12 and ES7/ES8 (16). This amplicon was sequenced and subtyped as described (17). Possible virus recombinants were detected by the HIV-1 subtyping tool of the National Center for Biotechnology Information (NCBI, Bethesda, MD, U.S.A). PBMC from 1 asymptomatic Cameroonian patient, with a blood $CD4^+$ T-cell level of 327 cells/μl, was negative for proviral DNA by PCR for subtyping. This patient was suspected to have been infected with a subtype O strain on the basis of serology (HIV-1 Western blot, faint gp160 and gp41 *env* bands but strong *gag* [p24, p55] and *pol* [p32, p51, p66] bands; indeterminate HIV-2 Western blot with *gag* and *pol*, but no *env* bands) and absence of detectable plasma HIV-1 RNA (Amplicor). On coculture of her PBMC, high PERT values but no HIV-1 RNA were measured in the supernatant. The RU5 region of the viral RNA was amplified from culture supernatant using a procedure developed for the identification of unknown retroviruses and the product was identified as representing subtype O by sequencing (18).

RESULTS

Polymerase chain reaction for subtyping, performed in 100 study subjects, was successful in 92. Of these, 34

were found to be infected with subtype A, 16 with B, 12 with C, 7 with D, 10 with E, 1 with F, and 1 with G. In the remaining 11, the amplified *env* sequence showed evidence for intersubtype recombination A/C/A ($n = 4$), and 1 case each of A/E/A, C/A, B/F, G/E/G, H/A/H, H/C/H, and predominantly F). In addition, 1 patient was found to be infected with a strain belonging to group O as detailed in Materials and Methods.

The sensitivity of the assays is shown in Table 1. Only those specimens ($n = 83$) that could be tested with all five assays were considered; of the remaining 20 samples, 11 failed in the PERT assay and 9 had insufficient volume. VL was undetectable by any method in 28 specimens, as a result of efficient antiretroviral treatment. Results with the remaining samples demonstrated considerable differences in the sensitivity of the five assays (Table 1). Stratifying the results according to treatment status produced little change in the relative sensitivity of the assays (Table 1). No HIV RNA was detectable in the group O sample by any of the three methods, but a positive signal was measured by both PERT assay and p24 Ag EIA (Fig. 1).

Samples positive by Amplicor but negative by Quantiplex all contained <8000 copies/ml according to Amplicor, and included 4 with >400 copies/ml, which belonged to subtype B (Fig. 1*A*). In contrast, several samples positive by Amplicor but negative by NucliSens contained high VL according to Amplicor (Fig. 1*B*). All NucliSens-negative samples with >400 copies/ml (Amplicor) and known sequence contained viruses that belonged to non-B subtypes (A, E, F, G) or were recombinant in *env* (A/C/A, B/F, G/E/G) (Fig. 1*B*). Samples missed by the PERT assay all contained <4000 copies/ml (Amplicor) and included 2 with subtype B strains (Fig. 1*C*). These 2 subtype B strains were detected by p24 Ag EIA. Specimens missed by the p24 Ag EIA all contained <40,000 copies/ml (Amplicor), with only non-B subtypes represented in the range between 1000 and 40,000 copies/ml (Fig. 1*D*).

Among 14 samples with low copy levels (<400 copies/ml) in the Amplicor assay, a few were reactive in either the PERT assay or the p24 Ag EIA (Fig. 1*C* and 1*D*). In addition, 2 group M specimens (1 subtype A, 1 with an undetermined subtype in a Thai patient) were reactive in the PERT assay but tested negative by all other techniques (Fig. 1*C*). Similarly, 3 group M specimens (2 subtype A, 1 H/A/H) were reactive in the p24 Ag EIA only (Fig. 1*D*). These 5 samples were retested using a supersensitive version of Amplicor 1.5. The results were as follows: 61 copies/ml, and RNA present but in concentrations <10 copies/ml in the 2 PERT-positive samples, respectively; 35 copies/ml, no RNA detectable, and 26 copies/ml in the 3 p24 Ag–positive samples, respectively.

Spearman correlation coefficients between VL values in the quantitation range of each method are depicted in Figure 1. The correlation was best between Quantiplex and Amplicor ($r = 0.8848$; $p < .0001$). However, VL values measured by Quantiplex were on the average 0.75 $\log_{10}$ lower than that measured by Amplicor. The correlation was fair between NucliSens ($r = 0.7064$; $p < .0001$) and Amplicor, as well as between PERT assay ($r = 0.7266$; $p < .0001$) and Amplicor. It was lowest between p24 Ag EIA and Amplicor ($r = 0.3989$; $p = .024$).

In the Quantiplex versus Amplicor plot (Fig. 1*A*), 1

TABLE 1. *Sensitivity of five different assays in the detection of HIV-1 in the plasma of 83 patients*

Subtype	Total no. of samples[a]	No. of samples testing positive				
		Amplicor 1.5	Quantiplex 2.0	NucliSens	PERT	Boosted p24 Ag EIA
A	25	19	10	10	16	15
B	15	9	2	7	5	5
C	8	6	3	3	4	3
D	3	2	1	2	1	0
E	10	6	3	4	5	3
F	1	1	0	0	1	0
G	1	1	1	0	1	1
Recombinant	10	6	5	3	6	7
O	1	0	0	0	1	1
Unknown	9	5	1	1	3	3
	83	55 (66.3%)	26 (31.3%)	30 (36.1%)	43 (51.8%)	38 (45.8%)
Not treated ($n = 25$)[b]		84.0%	56.0%	60.0%	84.0%	68.0%
Treated ($n = 58$)[b]		56.9%	20.7%	25.9%	37.9%	36.2%

[a] Includes only samples for which results by all five methods are available. Very similar results were obtained when samples with missing data were included (Amplicor (Roche), 66.3%; Quantiplex (Chiron), 33.7%; NucliSens (Organon Teknika), 37.5%; PERT assay, 50.0%; p24 Ag EIA, 43.1%).
[b] Antiretroviral treatment.

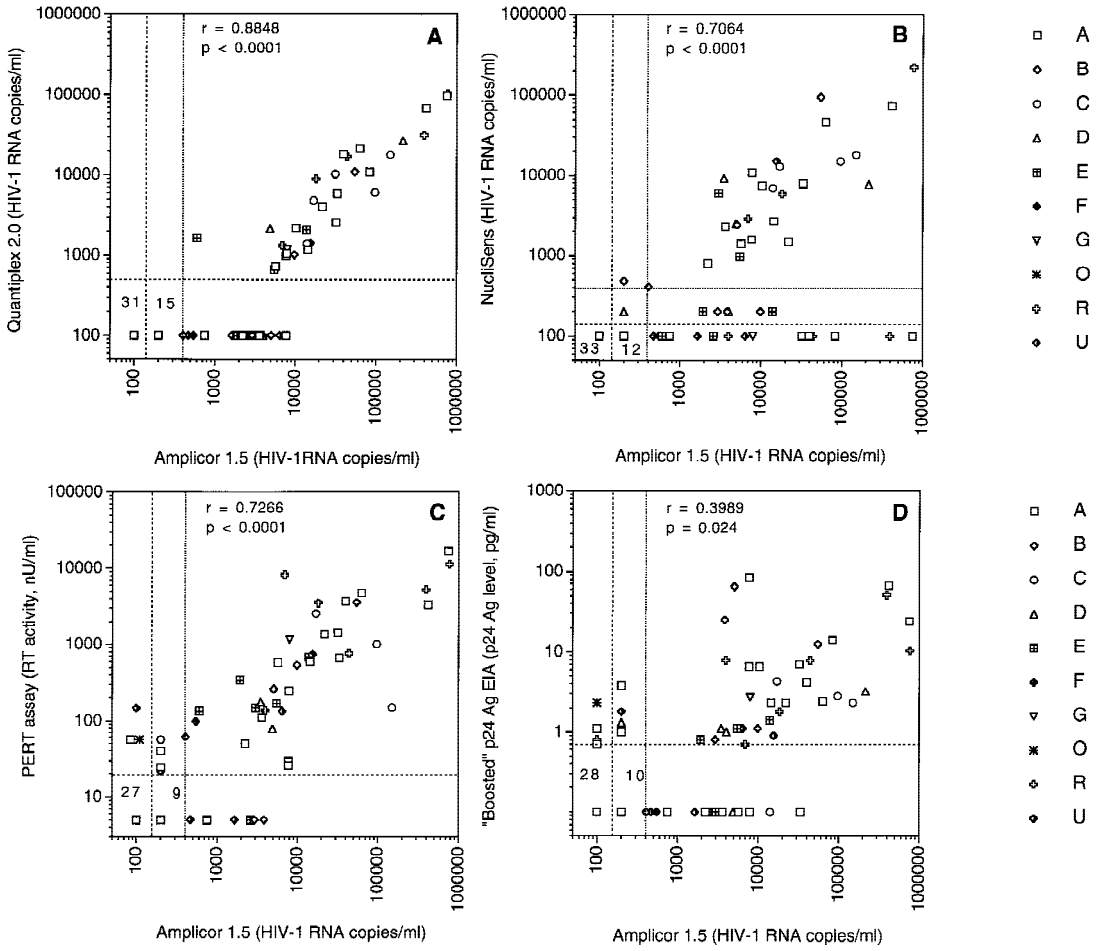

FIG. 1. Comparison between viral load (VL) measurement methods by scatter plotting, using all available results for 103 patients. (**A**) Quantiplex 2.0 assay versus Amplicor 1.5 assay. (**B**) NucliSens assay versus Amplicor 1.5 assay. (**C**) Product-enhanced reverse transcriptase (PERT) assay versus Amplicor 1.5 assay. (**D**) p24 Antigen (Ag) enzyme immunoassay (EIA) versus Amplicor 1.5 assay. An arbitrary value of 100 copies/ml (RNA assays), 5 nU/ml (PERT assay), or 0.05 pg/ml (p24 Ag EIA) has been attributed to all samples with VL below the lower detection level (*dashed line*, number of samples indicated in the corresponding quadrant). An arbitrary value of 200 copies/ml (Amplicor and NucliSens assays) has been attributed to all samples with detectable RNA but VL below the lower quantitation limit (*dotted line*, number of samples indicated in the corresponding quadrant). r, Spearman correlation coefficients between values in the quantitative range; R, recombinant subtype; U, subtype unknown.

specimen subtyped as E was obviously distinct, that is, VL as determined by Amplicor was 1.20 $\log_{10}$ lower than that expected from the linear regression curve, taking VL by Quantiplex as a reference. This sample was positive in the PERT assay (133 nU/ml), but was not detected by NucliSens and the Ag p24 EIA.

DISCUSSION

Of 103 HIV-1–seropositive study subjects investigated here, 72 were receiving antiretroviral therapy. This explains the relatively low percentage of specimens with detectable viral RNA. Amplicor version 1.5, equipped with the new *gag* primers SK145 and SKCC1B, proved to be the most sensitive assay in this study in which samples containing subtypes A, C, E, and recombinant viruses predominated. This differs from findings obtained using previous versions (1.0, 1.0+) of Amplicor (2–4,10).

In contrast, Quantiplex (version 2.0) and NucliSens had the lowest sensitivity, but for different reasons. With Quantiplex, the failure was not related to subtype but reflected the low intrinsic (i.e., irrespective of the number of copies indicated by the manufacturer) sensitivity of the assay. This conclusion is supported by a similar observation we made in a previous unpublished comparison of Quantiplex version 2.0 and Amplicor version

1.0+, using samples from subjects likely to be infected with subtype B. Indeed, of 88 samples with VL levels ranging from 400 to 6000 copies/ml according to Amplicor, 62 were found to have <500 copies/ml according to Quantiplex (data not shown). It should be mentioned here that a new 10-times-more-sensitive version (3.0) of the Quantiplex assay has become available since completion of this study. Results obtained using this new version have been reported to match closely those obtained using Amplicor (19). With NucliSens, low sensitivity resulted predominantly from the lack of detection of RNA in several samples containing subtype A, E (actually a recombinant subtype with A sequence in the targeted *gag* region [20]), and G viruses, in accordance with previous reports (5,21). Interestingly, HIV RNA detection and quantification were achieved in other samples also containing subtype A and E viruses, probably as the result of critical sequence variation in the NucliSens primer binding sites within a given subtype (22).

A close correlation existed between VL values by Quantiplex and those by Amplicor, even though the former were 5.6 times lower than the latter. Again, this appeared not to be related to HIV-1 subtypes, but mainly to the different calibration of the two kits (23,24). Indeed, in a preliminary comparison using 55 samples likely to contain subtype B strains, we observed that Quantiplex 2.0 produced VL values 3.0 times lower than that obtained using Amplicor 1.5 (data not shown). Such discrepancies, also reported by other investigators (6,24–26) emphasize the need to always use the same assay for the follow-up of a given patient. Of all samples in the quantifiable range by both Amplicor and Quantiplex ($n = 30$), only 1 subtype E specimen, also quantifiable by PERT but not by NucliSens, showed evidence of VL underestimation by Amplicor 1.5. This finding stresses that underestimation of VL with the latest version of Amplicor in samples containing non-B viruses exists (26), even though it seems to be rare (10).

Correlation between VL values by NucliSens and those by Amplicor was not as high as that found in the Quantiplex versus Amplicor comparison. This reflected, at least in part, underestimation of VL by NucliSens in some cases of infection by subtype A or E.

The PERT assay was the second-best method as far as sensitivity is concerned. Because it does not rely on HIV RNA sequence, its performance was expected to be independent from subtype. This was indeed the case, as shown with the group O sample, in which VL was undetectable by any of three RNA-based assays. In addition, correlation between RT activities by PERT assay and RNA levels by Amplicor was fair. Thus, PERT appears to be suitable for the follow-up of patients infected with highly divergent HIV-1 strains, such as those constituting groups O or N. One weakness of the PERT assay as performed here lay in its failure to quantify RT activity in the presence of high titers of enzyme-inhibiting antibodies (11% of the samples in the present study). In addition, the PERT assay missed 6 samples containing various group M subtypes, including 2 with subtype B, that had VL >400 copies/ml according to Amplicor. This may reflect loss of viral particles from the pellet after centrifugation. There are other possible explanations, however. Several patients were receiving antiretroviral treatment. About 24% of patients with treatment failure are known to be resistant to nucleosidic RT inhibitors but not to protease inhibitors (27). In such patients, protease inhibitors may have hampered cleavage of the *gag-pol* precursor, leading to low activity of the unprocessed RT as measured by PERT assay (28). Alternatively, inhibition of the processing of the *gag* precursor may have produced viral particles resistant to Triton X-100 disruption in the assay (29). In patients treated with nonnucleoside RT inhibitors, enough drug may have remained bound to the enzyme to inhibit its activity in vitro. In addition to the group O sample, the PERT assay may have uncovered 2 cases of infection by group M strains that were negative by the standard Amplicor as well as by all other assays. However, a supersensitive version of Amplicor detected low levels of HIV RNA in those 2 samples. This may represent further cases of VL underestimation with non-B subtypes. Alternatively, the few virions in these samples may have contained unusually high levels of RT (30).

The "boosted" p24 Ag EIA was more sensitive than either Quantiplex or NucliSens and was even able to detect antigenemia in the group O sample. A low level of antigenemia was also found in 3 group M samples with otherwise undetectable VL by standard techniques. Two of them, however, contained low levels of RNA as assessed by the supersensitive version of Amplicor. This discrepancy between p24 Ag and RNA measurement may result from the fact that plasma often contains a large excess (4–60 times [sometimes more], finding based on data from Piatak et al. [31]) of nonviral p24, either free or as immune complexes, over p24 as present in the viral capsid.

Correlation between p24 antigenemia and RNA levels by Amplicor was low. This could reflect the excess of nonviral over viral p24 Ag in plasma, as mentioned earlier. However, this ratio seems to be fairly constant in individual patients, as suggested by our observation that the time course of p24 Ag and HIV RNA under antiretroviral treatment are very similar (13,15,32). In addition, p24 antigenemia may have been underestimated in some

cases of non-B subtype infection if several non-B epitopes of p24 are poorly recognized by antibodies used in the assay (33).

In conclusion, Amplicor 1.5 performed best in detecting and quantifying viral RNA in cases of infection by non-B subtypes of HIV-1 group M. There was evidence of underestimation of VL in a single case of subtype E infection. Quantiplex 2.0 had a low sensitivity irrespective of subtype, but accurately quantified RNA from all group M samples with concentrations above 8000 copies/ml (Amplicor values). In contrast, NucliSens was characterized by a low sensitivity or accuracy toward some strains of subtypes A, E, F, G, or recombinant subtypes. The PERT assay could substitute to the RNA-based quantitative assays in cases of infection by group O and other highly divergent strains. The PERT assay and the "boosted" p24 Ag EIA may occasionally demonstrate the presence of viral proteins in the plasma of patients infected by non-B subtypes, when very low levels of HIV RNA are not detected by standard assays.

APPENDIX

The members of the Swiss HIV Cohort Study are M. Battegay (Chairman of the Scientific Board), E. Bernasconi, P. Bürgisser, M. Egger, P. Erb, W. Fierz, M. Flepp (Chairman of the Group "Clinics"), P. Francioli (President of the SHCS, Centre Hospitalier Universitaire Vaudois, CH-1011-Lausanne), H.J. Furrer, P. Grob, B. Hirschel, B. Ledergerber, L. Matter (Chairman of the Group "Laboratories"), A. Meynard, M. Opravil, F. Paccaud, G. Pantaleo, L. Perrin, W. Pichler, J.-C. Piffaretti, M. Rickenbach (Head of Coordination and Data Center), C. Rudin, P. Sudre, J. Schüpbach, A. Telenti, P. Vernazza, and R. Weber.

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REFERENCES

1. Workshop Report from the European Commission (DG XII, INCO-DC) and the Joint United Nations Programme on HIV/AIDS. HIV-1 subtypes: implications for epidemiology, pathogenicity, vaccines and diagnostics. *AIDS* 1997;11:UNAIDS17–36.
2. Dunne AL, Crowe SM. Comparison of branched DNA and reverse transcriptase polymerase chain reaction for quantifying six different HIV-1 subtypes in plasma. *AIDS* 1997;11:126–7.
3. Michael NL, Herman SA, Kwok S, et al. Development of calibrated viral load standards for group M subtypes of human immunodeficiency virus type 1 and performance of an improved AMPLICOR HIV-1 MONITOR test with isolates of diverse subtypes. *J Clin Microbiol* 1999;37:2557–63.
4. Alaeus A, Lilja E, Herman S, Spadoro J, Wang J, Albert J. Assay of plasma samples representing different HIV-1 genetic subtypes: an evaluation of new versions of the Amplicor HIV-1 monitor assay. *AIDS Res Hum Retroviruses* 1999;15:889–94.
5. Coste J, Montes B, Reynes J, et al. Comparative evaluation of three assays for the quantitation of human immunodeficiency virus type 1 RNA in plasma. *J Med Virol* 1996;50:293–302.
6. Parekh B, Phillips S, Granade TC, Baggs J, Hu DJ, Respess R. Impact of HIV type 1 subtype variation on viral RNA quantitation. *AIDS Res Hum Retroviruses* 1999;15:133–42.
7. Gobbers E, Fransen K, Oosterlaken T, et al. Reactivity and amplification efficiency of the NASBA HIV-1 RNA amplification system with regard to different HIV-1 subtypes. *J Virol Methods* 1997;66:293–301.
8. Kern D, Collins M, Fultz T, et al. An enhanced-sensitivity branched-DNA assay for quantification of human immunodeficiency virus type 1 RNA in plasma. *J Clin Microbiol* 1996;34:3196–3202.
9. Oelrichs RB, Dyer WB, Downie JC, Harvey K, Sullivan JS, Workman C. Inaccurate HIV-1 viral load quantification by three major commercially available methods. *AIDS* 1999;13:727–8.
10. Triques K, Coste J, Perret JL, et al. Efficiencies of four versions of the AMPLICOR HIV-1 MONITOR test for quantification of different subtypes of human immunodeficiency virus type 1. J Clin Microbiol 1999;37:110–16.
11. Segondy M, Ly TD, Lapeyre M, Montes B. Evaluation of the Nuclisens HIV-1 QT assay for quantitation of human immunodeficiency virus type 1 RNA levels in plasma. *J Clin Microbiol* 1998;36:3372–4.
12. Schockmel GA, Yerly S, Perrin L. Detection of low HIV-1 RNA levels in plasma. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;14:179–83.
13. Böni J, Pyra H, Schüpbach J. Sensitive detection and quantification of particle-associated reverse transcriptase in plasma of HIV-1-infected individuals by the product-enhanced reverse transcriptase (PERT) assay. *J Med Virol* 1996;49:23–8.
14. Pyra H, Böni J, Schüpbach J. Ultrasensitive retrovirus detection by a reverse transcriptase assay based on product enhancement. *Proc Natl Acad Sci USA* 1994;91:1544–48.
15. Nadal D, Böni J, Kind C, et al. Prospective evaluation of amplification-boosted ELISA for heat-denatured p24 antigen for diagnosis and monitoring of pediatric human immunodeficiency virus type 1 infection. *J Infect Dis* 1999;180:1089–95.
16. Delwart EL, Shpaer EG, Louwagie J, et al. Genetic relationships determined by a DNA heteroduplex mobility assay: analysis of HIV-1 env genes. *Science* 1993;262:1257–61.
17. Böni J, Pyra H, Gebhardt M, et al. High frequency of non-B subtypes in newly diagnosed HIV-1 infections in Switzerland. *JAIDS J Acquir Immune Defic Syndr* 1999;22:174–9.
18. Weissmahr RN, Schüpbach J, Böni J. Reverse transcriptase activity in chicken embryo fibroblast culture supernatants is associated with particles containing endogenous avian retrovirus EAV-0 RNA. *J Virol* 1997;71:3005–12.
19. Highbarger HC, Alvord WG, Jiang MK, et al. Comparison of the Quantiplex version 3.0 assay and a sensitized Amplicor Monitor assay for measurement of human immunodeficiency virus type 1 RNA levels in plasma samples. *J Clin Microbiol* 1999;37:3612–4.
20. Carr JK, Salminen MO, Koch C, et al. Full-length sequence and mosaic structure of a human immunodeficiency virus type 1 isolate from Thailand. *J Virol* 1996;70:5935–43.
21. Nkengasong JN, Kalou M, Maurice C, et al. Comparison of NucliSens and Amplicor monitor assays for quantification of human immunodeficiency virus type 1 (HIV-1) RNA in plasma of persons with HIV-1 subtype A infection in Abidjan, Cote d'Ivoire. *J Clin Microbiol* 1998;36:2495–8.
22. Vandamme AM, Schmit JC, Van Dooren S, et al. Quantification of HIV-1 RNA in plasma: comparable results with the NASBA HIV-1 RNA QT and the AMPLICOR HIV monitor test. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;13:127–39.
23. Brambilla D, Leung S, Lew J, et al. Absolute copy number and

relative change in determinations of human immunodeficiency virus type 1 RNA in plasma: effect of an external standard on kit comparisons. *J Clin Microbiol* 1998;36:311–4.
24. Nolte FS, Boysza J, Thurmond C, Clark WS, Lennox JL. Clinical comparison of an enhanced-sensitivity branched-DNA assay and reverse transcription-PCR for quantitation of human immunodeficiency virus type 1 RNA in plasma. *J Clin Microbiol* 1998;36: 716–20.
25. Segondy M, Izopet J, Pellegrin I, et al. Comparison of the QUANTIPLEX HIV-1 RNA 2.0 assay with the AMPLICOR HIV-1 MONITOR 1.0 assay for quantitation of levels of human immunodeficiency virus type 1 RNA in plasma of patients receiving stavudine-didanosine combination therapy. *J Clin Microbiol* 1998; 36:3392–5.
26. Damond F, Apetrei C, Descamps D, Brun-Vézinet F, Simon F. HIV-1 subtypes and plasma RNA quantification. *AIDS* 1999;13: 286–7.
27. Cohen J. The daunting challenge of keeping HIV suppressed. *Science* 1997;277:32–3.
28. Le Grice SFJ, Mills J, Mous J. Active site mutagenesis of the AIDS virus protease and its alleviation by trans complementation. *EMBO J* 1988;7:2547–53.
29. Kaplan AH, Krogstad P, Kempf DJ, Norbeck DW, Swanstrom R. Human immunodeficiency virus type 1 virions composed of unprocessed Gag and Gag-Pol precursors are capable of reverse transcribing viral genomic RNA. *Antimicrob Agents Chemother* 1994; 38:2929–33.
30. Garcia Lerma JG, Yamamoto S, Gomez-Cano M, et al. Measurement of human immunodeficiency virus type 1 plasma virus load based on reverse transcriptase (RT) activity: evidence of variabilities in levels of virion-associated RT. *J Infect Dis* 1998;177: 1221–9.
31. Piatak M, Jr., Saag MS, Yang LC, et al. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. *Science* 1993;259:1749–54.
32. Böni J, Opravil M, Tomasik Z, et al. Simple monitoring of antiretroviral therapy with a signal-amplification-boosted HIV-1 p24 antigen assay with heat-denatured plasma. *AIDS* 1997;11:F47–52.
33. Corrigan GE, Al-Khalili L, Malmsten A, et al. Differences in reverse transcriptase activity versus p24 antigen detection in cell culture, when comparing a homogeneous group of HIV type 1 subtype B viruses with a heterogeneous group of divergent strains. *AIDS Res Hum Retroviruses* 1998;14:347–52.

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