JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1996, p. 3016–3022 Vol. 34, No. 12
0095-1137/96/$04.00+0

Multicenter Comparison of Three Commercial Methods for Quantification of Human Immunodeficiency Virus Type 1 RNA in Plasma

ROB SCHUURMAN,[1]* DIANE DESCAMPS,[2] GERRIT JAN WEVERLING,[3] STEVE KAYE,[4] JOLANDA TIJNAGEL,[1] IAN WILLIAMS,[5] REMKO VAN LEEUWEN,[3] RICHARD TEDDER,[4] CHARLES A. B. BOUCHER,[1] FRANCOISE BRUN-VEZINET,[2] AND CLIVE LOVEDAY[6]

Eijkman Winkler Institute for Medical Microbiology, Department of Virology, Utrecht University, Utrecht,[1] and National AIDS Therapy Evaluation Centre, Academic Medical Centre, University of Amsterdam, Amsterdam,[3] The Netherlands; Department of Virology, l'Hôpital Bichat Claude Bernard, Paris, France[2]; and Department of Virology[4] and Department of Sexually Transmitted Diseases,[5] University College London Medical School, and Department of Retrovirology, Royal Free Hospital,[6] London, United Kingdom

Received 6 May 1996/Returned for modification 9 July 1996/Accepted 28 August 1996

Three procedures for the quantification of human immunodeficiency virus type 1 (HIV-1) RNA from plasma were compared at three laboratories. The comparison involved the Quantiplex branched DNA assay (version 1.0) by Chiron Diagnostics, the NASBA-QT assay by Organon Teknika, and the Amplicor Monitor assay by Roche Molecular Systems. The laboratories performed each of the three assays with the same sets of reconstructed HIV-1-infected human plasma samples, cross-sectionally collected clinical plasma samples and longitudinally collected plasma samples from patients starting zidovudine therapy. Analysis of the reconstruction panel results for interlaboratory variation demonstrated that no laboratory differences in results were detected for any of the assays. A comparison of the reproducibilities of duplicate samples analyzed by batch and in separate assay runs demonstrated that the reproducibilities of the test results were similar within one assay and appeared to be independent of the HIV-1 concentration. The best reproducibility was obtained with the Quantiplex assay, but all three assays demonstrated equal reliability, which was independent of batched or unbatched analysis of replicate samples. Differences in the absolute concentrations calculated were observed for the assays, in particular in the analysis of reconstructed samples. In all assays, similar changes in plasma HIV-1 RNA concentrations were determined for longitudinally collected clinical samples.

To date a number of immunological and virological markers of human immunodeficiency virus type 1 (HIV-1) infection, such as the $CD4^+$ lymphocyte count, p24 antigenemia, and quantitative plasma and peripheral blood mononuclear cell cultures, are used to determine drug efficacy in patients treated with inhibitors of HIV replication. Most of these parameters are indirect or insensitive markers of viral replication. The CD4 lymphocyte count does not always correlate with the clinical response to antiretroviral therapy (1), and serum p24 antigen or plasma viremia by culture are not detectable in all patients. Further, quantitative plasma viremia assays are time-consuming and therefore not applicable in large clinical trials. HIV-1 RNA can be detected in plasma during all stages of the infection (19). Quantitative procedures have been developed to determine the virion-associated HIV-1 RNA concentration in the plasma of infected patients (2, 11, 16, 19, 20, 22–24). With these approaches, it has been demonstrated that significant reductions in plasma HIV-1 RNA concentrations could be determined in response to antiretroviral therapy (5, 6, 21) and that reductions in viral RNA load during antiviral therapy may be prognostic for the clinical outcome (12, 14, 15, 17, 27).

Several assays for the quantification of plasma HIV-1 RNA have been described (2, 11, 16, 19, 20, 22–24), and some have currently been transferred into commercial kits (16, 23, 24). These products are based on either signal amplification (23) or template amplification (16, 24).

We performed a comparative study to investigate the performance of these assays and to study their potential application in the evaluation of antiviral drug efficacy in the DELTA trial, a large clinical study comparing zidovudine monotherapy against combination therapy with either didanosine or zalcitabine in HIV-1-infected patients. The HIV-1 branched DNA assay (version 1.0; Chiron Diagnostics, Emeryville, Calif.), the HIV-1 NASBA-QT assay (Organon Teknika, Turnhout, Belgium), and the HIV-1 Amplicor Monitor assay (Roche Molecular Systems, Branchburg, N.J.) were compared at three virological sites with the use of reconstructed HIV-1-infected plasma, 21 cross-sectionally collected clinical samples obtained from HIV-1-infected individuals, and longitudinally collected samples obtained from four patients before and during treatment with zidovudine.

MATERIALS AND METHODS

Quantitative HIV-1 RNA assays and participating laboratories. Three commercial assays for HIV-1 plasma viral load determination were evaluated: the Quantiplex branched DNA assay (version 1.0; Chiron Diagnostic Laboratories) (23), the HIV-1 NASBA QT assay (Organon Teknika) (24), and HIV Monitor RNA-PCR assay (Roche Molecular Systems) (16). Three virology laboratories participating in the DELTA clinical trial took part in this comparison: Hôpital Bichat Claude Bernard, Paris, France; University College London Medical School, London, United Kingdom; and the Academic Medical Centre, Amsterdam, The Netherlands. For each participating laboratory, the assays were established by the companies and laboratory personnel were trained. Each laboratory performed the three assays with both reconstructed HIV-1-infected plasma sam-

* Corresponding author. Mailing address: Department of Virology, Eykman Winkler Institute for Clinical Microbiology, Utrecht University Hospital, Heidelberglaan 100, 3508 CX Utrecht, The Netherlands. Phone: 31-30-2506526. Fax: 31-30-2541770. Electronic mail address: r.schuurman@lab.azu.nl.

ples and clinical samples. The kit lots used at each site were identical, and all samples were analyzed under code.

Preparation of a reconstruction panel of HIV-1-infected human plasma samples. Human EDTA-plasma samples from eight HIV-1-negative healthy blood donors were obtained from the Dutch Red Cross Bloodbank (Amsterdam, The Netherlands). In order to test each of the individual plasmas for the absence of nonspecific inhibitory factors, a sample of each donation was spiked with a particle-counted HIV-1 HXB3 stock virus (13) up to 4×10^5 HIV-1 RNA molecules per ml. The samples were subsequently analyzed in duplicate by each method, and the mean results were calculated. A donation was excluded from the preparation of the reconstructed HIV-1-infected plasma panel when an assay result was more than twofold different from the assay mean. Eight donations were finally pooled and used for the preparation of a reconstructed HIV-1-infected EDTA-plasma panel. The master sample consisted of 10 ml of pooled plasma spiked with electron microscopically particle-counted HIV-1 stock virus (HIV-1 HXB3 [13]) at a concentration of 5×10^8 HIV-1 particles per ml, equal to 10^9 HIV-1 RNA genomes per ml. This master dilution was subsequently divided into equal volumes to prepare two HIV-1 concentration series (panels A and B). Each panel consisted of twofold dilutions of the virus in pooled human EDTA-plasma at concentrations of 10^8 to 50 HIV-1 RNA molecules per ml. Each dilution step resulted in 25 ml of reconstructed HIV-1-infected plasma, which was subsequently aliquoted into appropriate volumes for the respective tests, and stored at −70°C until used.

For HIV-1 RNA quantification experiments, duplicate samples of all odd-numbered dilutions of panel A and all even-numbered dilutions of panel B were used. Merging the dilutions from panels A and B that were analyzed consequently resulted in a series of samples with twofold differences in the HIV-1 RNA concentrations between subsequent dilutions.

Intra-assay variation between duplicates analyzed in separate assay runs was compared with intra-assay variation of samples analyzed in the same assay run by analyzing the duplicates of set A in separate runs and analyzing the duplicates of set B in the same assay run.

Clinical samples. To compare the HIV-1 RNA quantitative assays of clinical samples, human EDTA-plasma specimens were collected from 21 HIV-1-infected patients: 10 asymptomatic patients (Centers for Disease Control [CDC] defined criterion A1 or A2) and 11 symptomatic patients (CDC B3 or C3) (3). Patients were either antiretroviral therapy naive ($n = 3$) or on stable therapy for at least 3 months ($n = 18$). All samples were aliquoted in assay-specific volumes and stored at −70°C until used. Duplicates were analyzed in each laboratory by the three HIV-1 RNA quantification methods.

To compare methods for their use in evaluating drug efficacy, longitudinal samples were obtained from four drug-naive patients starting zidovudine treatment. EDTA-plasma was collected at −2, 0, 1, 2, 3, 4 and 6 weeks and stored at −70°C. Because of the limited availability of plasma, single measurements of the plasma HIV-1 RNA load were made.

HIV-1 RNA quantification. Each HIV-1 RNA quantification assay was performed according to the protocol of the manufacturer. Two milliliters of plasma was needed for a single result for the Quantiplex assay, and 200 and 100 μl of plasma were needed for the Monitor and NASBA-QT assays, respectively. All NASBA determinations were performed with 10-fold-diluted calibrators. Results were considered valid or invalid as defined by the individual manufacturer's criteria. The dynamic range of the Quantiplex assay predefined by the manufacturer was between 10^4 and 10^6 HIV-1 RNA copies per ml; the NASBA QT assay had a lower threshold of 10^3 HIV-1 RNA copies per ml, with no predefined upper limit. No dynamic range limitations were predefined for the HIV-1 Monitor assay, but the lower limit for detection was considered 200 HIV-1 RNA molecules per ml.

Data collection and analysis of the results. The raw experimental data were translated into HIV-1 RNA concentrations by using the computer software supplied with each assay. The results, on diskette and as hard copy printout, were sent to a central data collection site, where they were checked, decoded, and read into a database. All results were $\log_{10}$ transformed prior to further statistical analysis.

The quantitative range of an assay was defined as the range within which at least five of six replicates in the reconstruction panel generated a result within the dynamic range for an assay.

To study reproducibility in relationship to plasma viral load, the means and standard deviations were calculated for all replicates for which the results were within the quantitative range of an assay. In addition, inter- and intra-assay variation and interlaboratory variation were calculated by using analysis of variance. Intraclass correlation coefficients of reliability (ICC) and corresponding lower confidence intervals were calculated for all concentrations in the quantitative ranges of the assays (8). The ICC of a result represented the ratio of the standard deviation (SD) to the SD plus a standard error [SD/(SD + e)]. An ICC value of 1 represented perfect reproducibility, whereas an ICC value of 0 represented nonreproducibility. Linearity of results was calculated by linear regression analysis. An input-versus-output curve was considered linear when the regression coefficient (b) was $0.9 < b < 1.1$ and the correlation coefficient (r^2) was ≥0.99.

Parallel line analysis was performed to investigate whether the experimental output curves paralleled the theoretical curve in the quantitative range of an assay (7). Subsequently, the potency of an assay was calculated to relate the output concentrations generated by each assay to the amount of virus in the particle-counted stock virus used for the panel preparation and to relate the numbers calculated by one assay to those calculated for the others (7).

RESULTS

Three commercial assays for plasma HIV-1 RNA load determination were evaluated with respect to their quantitative range, intra- and interassay variation, and interlaboratory variation. The study involved the quantitation of plasma HIV-1 RNA in 177 reconstructed HIV-1-infected EDTA-plasma samples and in 173 clinical EDTA-plasma samples.

Reconstruction experiments. (i) Individual plasma donations. Initially, HIV-1 RNA quantification experiments with eight individual EDTA-plasma donations spiked with HIV-1 HXB3 virus at 4×10^5 virus genomes per ml were performed. All results were within a twofold difference from the mean assay result for all three assays, and therefore all donations were included in the preparation of the reconstructed HIV-1-infected EDTA-plasma panels.

(ii) Reconstruction panels. The results were first analyzed for the presence of interlaboratory variation. No significant differences in results between laboratories were determined by the three HIV-1 RNA quantification methods (analysis of variance, $P > 0.9$). Therefore, the results for replicates analyzed at different laboratories were pooled for further analysis.

For each assay, reproducibility was determined within the range of HIV-1 RNA concentrations for which quantitative results were obtained, i.e., the ranges within which at least five of six replicates (each dilution analyzed in duplicate in each of the three countries) generated quantitative results. The highest level of reproducibility was obtained for the Quantiplex assay (Fig. 1); the SD for all dilutions ranged between 0.05 and 0.12 $\log_{10}$ units. The NASBA and Monitor assays gave generally higher SD values, ranging from 0.09 to 0.49 and from 0.06 to 0.45 $\log_{10}$ units, respectively. An SD value of >0.35 was observed once for NASBA at the last concentration of the quantitative range and on one occasion for the Monitor assay.

The reliability of duplicate results obtained for samples in reconstruction panel A or B demonstrated the highest ICC value (0.99; confidence interval [CI] of ±0.01) for panel B samples analyzed by the Quantiplex assay; otherwise, the ICC values calculated for panel A or B samples were >0.97 for each of the assays (CI of ±0.01 or 0.02). A higher CI (±0.03) was observed only for panel B results obtained by NASBA.

Analysis of the pooled results of panel A and B samples showed no differences in reliability between panel A and panel B results, indicating that the reliability of duplicates analyzed in separate runs was not different from the reliability of duplicates analyzed in the same run. Therefore, the results for panels A and B were pooled in all further analyses. Pooling the data gave a mean SD of 0.08 $\log_{10}$ unit by the Quantiplex assay, and mean SDs for the NASBA and Monitor assay of 0.17 and 0.18 $\log_{10}$ units, respectively.

The quantitative ranges for the respective assays (Fig. 2) were based on the mean output concentrations calculated for each dilution. The Quantiplex assay gave quantitative results between determined HIV-1 RNA concentrations of 4.29 and 6.10 $\log_{10}$ molecules per ml, with a linear relationship between input and output concentrations being observed throughout this range (regression coefficient (b) of 1.016; r^2 of 1.00). The NASBA assay gave quantitative results between determined HIV-1 RNA concentrations of 3.47 to 7.14 $\log_{10}$ molecules per ml. The highest degree of linearity (b of 0.912; r^2 of 0.99) between input and output concentrations was obtained between HIV-1 RNA concentrations of $\log_{10}$ 4.23 and 6.30. The

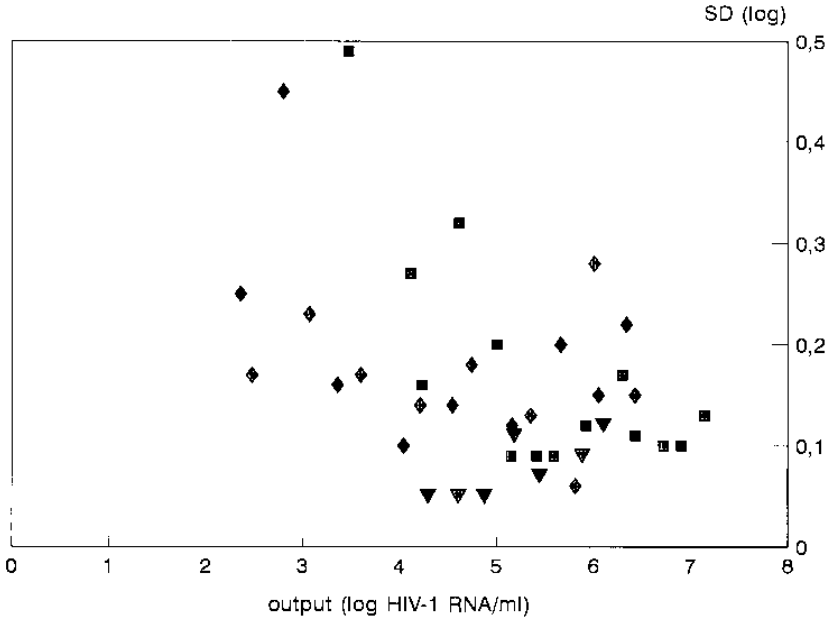

FIG. 1. SD expressed as $\log_{10}$ values, calculated with the results for replicates of both reconstruction panels A (stippled symbols) and B (closed symbols) in the quantitative range of an assay. Each point is the mean for at least five replicates. For panel A, the samples were analyzed in separate runs; for panel B, the samples were analyzed in the same assay run. ▽, Quantiplex; □, NASBA; ◇, Monitor.

difference from the theoretical input concentration increased outside this range (Fig. 2). The Monitor assay, which like NASBA has no predefined upper assay limit, gave quantitative results between determined HIV-1 RNA concentrations of 2.36 and 6.43 $\log_{10}$ molecules per ml. Linearity between input and output HIV-1 RNA concentrations was observed between output concentrations of 2.48 and 6.05 $\log_{10}$ molecules per ml (b of 0.927; r^2 of 1.00).

The determined (output) HIV-1 RNA concentrations (Fig. 2) were all lower than the estimated input concentration, but the input/output ratio was different for every assay (analysis of variance, P of <0.01). Potency calculations with the results in the quantitative range shared by the assays demonstrated that in general the Quantiplex assay results were 1.00 $\log_{10}$ unit lower, Monitor assay results were 0.92 $\log_{10}$ unit lower, and NASBA assay results were 0.55 $\log_{10}$ unit lower than the input concentration.

Clinical samples. (i) Cross-sectionally collected plasma samples. To analyze the accuracy and reproducibility of the assays by using clinical material as opposed to reconstructed

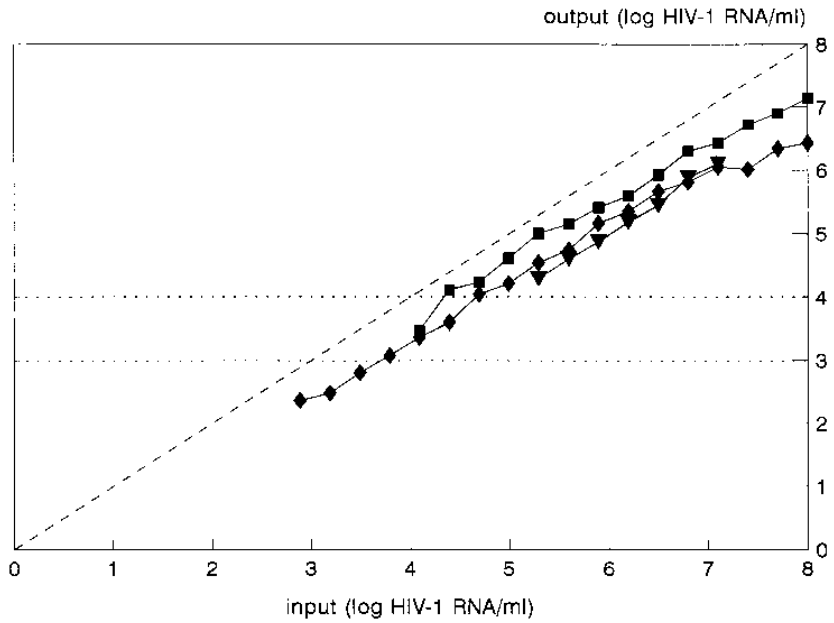

FIG. 2. Graphical representation of mean determined (output) HIV-1 RNA concentrations versus calculated (input) concentrations in the quantitative ranges of the individual assays. The diagonal dashed line represents complete homology between input and output results. Each point is the mean of at least five replicates. Horizontal dotted lines indicate the predefined lower detection limits for the Quantiplex (10^4 molecules per ml) and NASBA (10^3 molecules per ml). ▽, Quantiplex □, NASBA; ◇, Monitor.

samples, we analyzed a panel of 21 plasma samples cross-sectionally collected from patients. All samples were analyzed in duplicate at each laboratory.

The individual patient's results for these experiments are depicted in Fig. 3. Interlaboratory differences were analyzed by using the results for nine patients harboring HIV-1 RNA at concentrations in the shared dynamic range of the assays. Statistically significant interlaboratory differences were observed (analysis of variance, P of <0.01). However, the highest observed mean interlaboratory difference was 0.18 $\log_{10}$ unit and was therefore considered to be of no clinical significance. The statistical difference between laboratories was not taken into account in further analyses.

Patients with a quantitative result in at least five of six replicate determinations were used to calculate the mean SD of an assay. This analysis demonstrated that the SD for the assays were comparable to those calculated with the reconstruction panel: 0.09, 0.19, and 0.17 $\log_{10}$ units for Quantiplex, NASBA, and Monitor, respectively.

In general the determined HIV RNA concentrations for the symptomatic patients were 0.3 $\log_{10}$ unit higher than those for the asymptomatic patients, independent of the assay used. In all samples, the determined amount of HIV-1 RNA was $<10^6$ molecules per ml, indicating that none of the results exceeded the preset upper limit of the Quantiplex assay. Of all cross-sectional samples analyzed by the Quantiplex assay, 30.8% were below the threshold of the assay, compared with 13.9 and 1.6% of all samples by NASBA and Monitor assays, respectively.

The HIV-1 RNA loads determined with NASBA technology for asymptomatic patient G and symptomatic patients L and N resulted in significantly lower viral RNA concentrations than those determined with both of the other quantitative strategies. For the other patients, except symptomatic patient T and asymptomatic patient I for whom the virus load could be quantified only by the Monitor assay, the results for one assay overlapped those for one or both of the others.

For 9 of 21 patients the determined plasma HIV-1 RNA concentrations of all replicate samples were within the dynamic ranges of the respective assays. These nine patients' results were used to calculate the mean HIV-1 RNA concentrations for each assay and demonstrated no significant differences between assay results ($P > 0.05$). This analysis was repeated for the 17 patients' samples for which all replicates were within the dynamic ranges of both the NASBA and Monitor assays. Differences in HIV-1 plasma RNA concentrations determined by both assays were not statistically significant ($P > 0.05$).

(ii) Longitudinally collected plasma samples. To compare the ability of the assays to detect changes in viral load during therapy, longitudinal samples from four patients before and during zidovudine treatment were analyzed single fold by each of the laboratories. Monitor and NASBA assay results were generated by all three laboratories, whereas Quantiplex results from one laboratory failed (Fig. 4). All patients had high plasma virus loads at baseline, ranging from 4.6 to 6.0 $\log_{10}$ HIV-1 RNA molecules per ml determined by the Quantiplex assay and from 4.6 to 6.9 and 4.2 to 5.8 $\log_{10}$ HIV-1 RNA molecules per ml by the NASBA and Monitor assays, respectively. All four patients showed a maximal HIV-1 RNA load decline between 1 and 4 weeks, which increased in three of four patients by 3 to 6 weeks. For one patient, the HIV-1 RNA concentration fell below the cutoff for the Quantiplex assay at week 1. None of the results fell below the cutoffs for the NASBA and Monitor assays at any time point. The patterns of changes in viral load in these patients were indistinguishable by each assay used (Fig. 3), but the number of patients was too small to perform further statistical analysis.

DISCUSSION

The quantification of plasma HIV-1 RNA in HIV-1-infected individuals is an important tool with which to study the natural history of the virus infection and the role of viral load in disease progression and transmission (4, 12, 15, 17, 19). Moreover, quantitative plasma HIV-1 RNA determinations generate important information on the relative efficacy of inhibitors of viral replication. Detailed analysis of viral load changes after treatment of patients with strong inhibitors of HIV-1 replication has also generated new insights into the dynamics of virus replication (10, 18, 21, 25).

Initially, most of these investigations were performed with in-house PCR-based HIV-1 RNA quantification assays (2, 11, 19, 20, 22). In general, these methods were laborious and lacked proper quality assurance and control. At present, the use of commercial assays to determine HIV RNA load, particularly in clinical trials of patients receiving antiretroviral therapy, is extensive. Several of these trials are performed as multicenter studies, with laboratory tests performed at the local sites.

To control and assure that high-quality results are generated by different laboratories, there is a need for standardized reference panels. Moreover, these panels can be used to compare the performance of available assays, thereby addressing issues such as inter- and intra-assay variation as well as variation between different production lots of any particular assay (interkit variation) (26).

The present study was designed to address intra- and inter-assay variation and to study interlaboratory variation. This is the first multicenter study in which the performances of three commercial assays for plasma HIV-1 RNA quantification with an identical set of reconstructed and clinical HIV-1-infected plasma samples have been compared. It should be emphasized that the comparison involved only HIV-1 subtype B viruses. It cannot be excluded that the evaluated assays express differences in sensitivity to detect and quantify HIV-1 clades other than subtype B.

For each of the evaluated assays, statistical analysis of the results generated with the reconstructed samples demonstrated no significant differences between results generated at different laboratory sites. The observed interlaboratory difference with the cross-sectional samples was small and not clinically relevant but reached significance as a result of the high reproducibility of the assays. It can be concluded that these assays are appropriate for application in multicenter studies and that there is no apparent need for centralized analysis of samples. It should be emphasized that in this study all technicians were well trained in performing the assays. It cannot be excluded that the experience of the technician who performs an assay may influence the quality of the results (26).

The results obtained with the reconstructed plasma samples demonstrate that for each of the assays, the reproducibility of duplicate analyses performed in batch (same assay run) was similar to the reproducibility of duplicates analyzed in separate runs (different assay runs) (Fig. 1). Moreover, within one assay, reproducibilities were generally independent of the HIV RNA concentration. These results imply that under ideal laboratory circumstances there is no need for batchwise analysis of clinical samples. Results from the reconstructed plasma panel and the clinical samples demonstrated that the Quantiplex assay expressed the smallest mean SD (<0.10 $\log_{10}$ units) and that

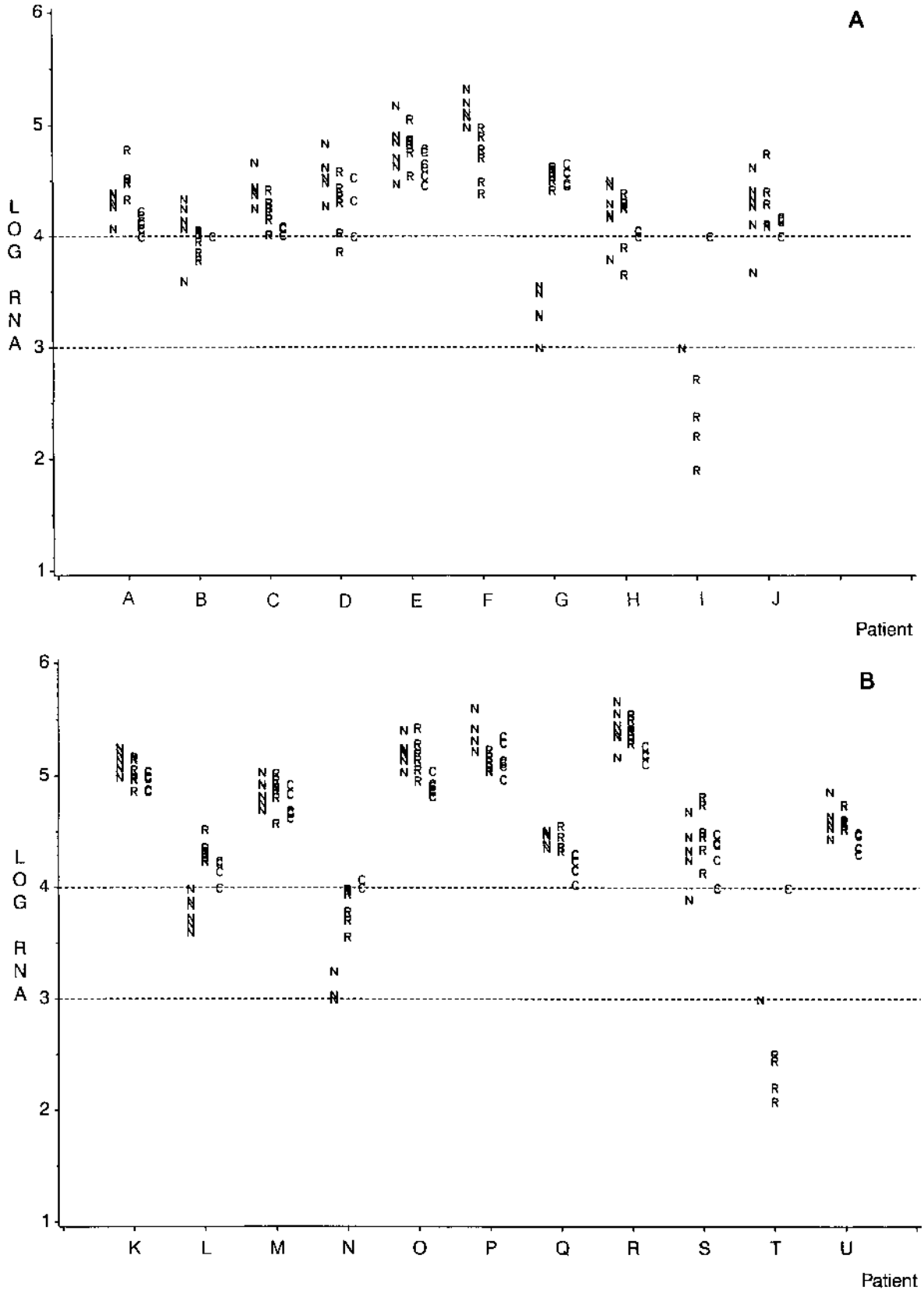

FIG. 3. Determined plasma viral RNA concentrations in cross-sectional collection of samples obtained from asymptomatic (A) or symptomatic (B) HIV-1-infected patients. Each letter represents a single observation for one assay (N, NASBA; C, Quantiplex; and R, Monitor). All samples were analyzed in duplicate at all three laboratories. The amount of viral RNA in plasma from asymptomatic patient F could not be determined by the Quantiplex assay, because of limited availability of plasma. Horizontal lines indicate the predefined lower detection limits for Quantiplex (10^4 molecules per ml) and NASBA (10^3 molecules per ml) assays.

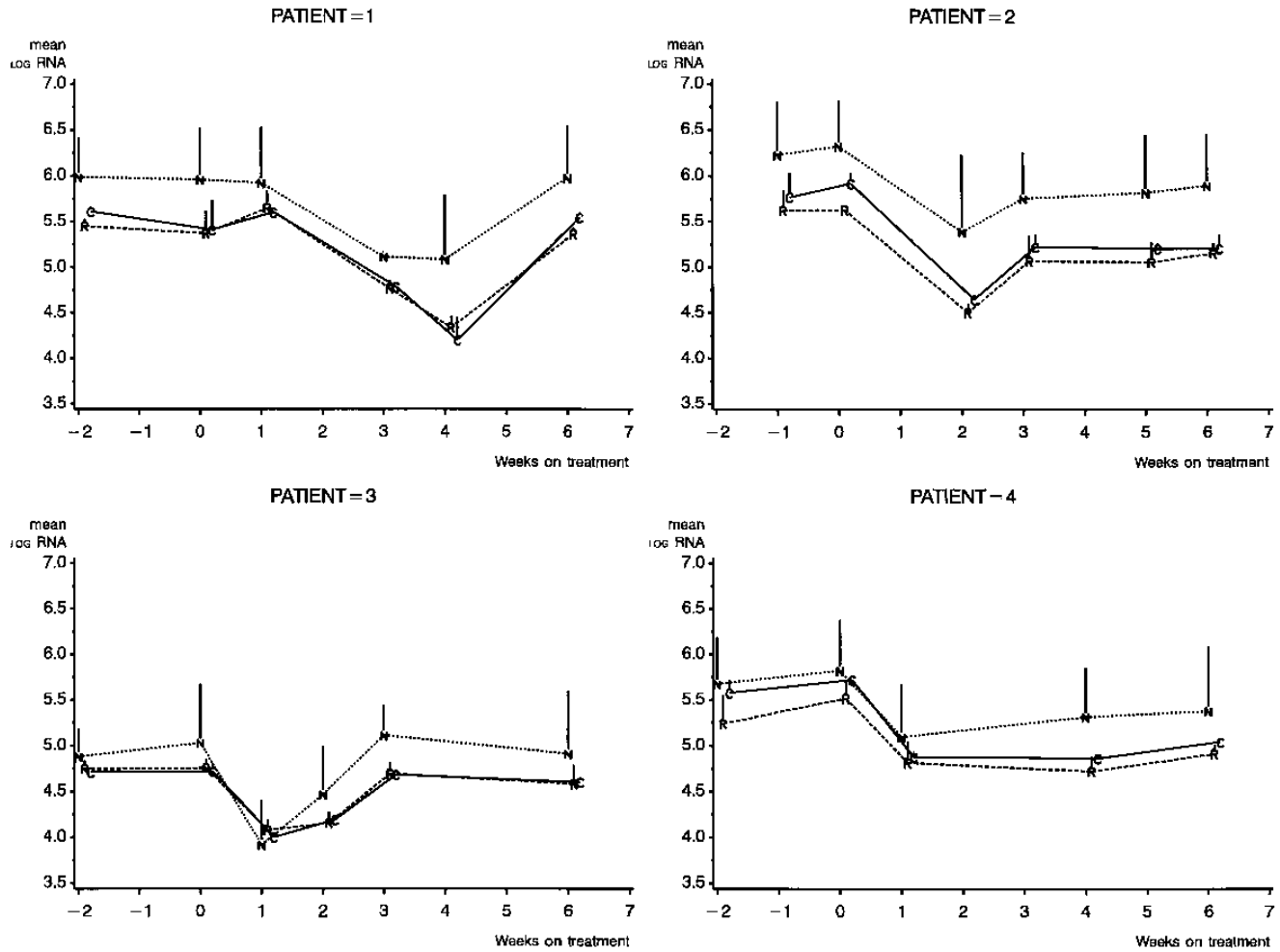

FIG. 4. Determined viral RNA concentrations in longitudinal plasma samples obtained from four HIV-1-infected patients starting zidovudine therapy. The samples were analyzed singlefold by the three assays in each laboratory. The Quantiplex results were generated by two laboratories. Vertical error bars represent +1 SD. N, NASBA; C, Quantiplex; R, Monitor.

slightly higher mean SD values were obtained with the NASBA and the Monitor assays (mean SD of <0.20 $\log_{10}$ units).

The results obtained with the reconstruction panel demonstrated that viral load determinations by NASBA resulted in higher RNA equivalents than those determined by the Quantiplex or Monitor assays. In general, NASBA results were 0.42 and 0.52 $\log_{10}$ units higher than those by the Monitor and Quantiplex assays, respectively. The observed differences between the assays with the reconstruction panel could not be confirmed with the cross-sectionally collected clinical samples. Currently, there is no clear explanation for this intriguing discrepancy, but the absence of the effect with clinical samples is reassuring.

In the plasma of asymptomatic patient G of the cross-sectional study, very similar viral RNA concentrations were detected by the Monitor and Quantiplex assay, but a >1 $\log_{10}$ unit lower concentration was detected by NASBA. A similar discrepancy might also exist for symptomatic patients L and N. Each of the quantitative assays involves different hybridization and/or amplification probes. Therefore, it cannot be excluded that the inconsistent NASBA results are due to genomic variation at the primer/probe binding site of the virus variants circulating in these two patients, thereby influencing the efficiency of the amplification and/or detection reactions. Such an effect has previously been noted for the Monitor assay with non-clade-B HIV-1 variants. In particular, experience with testing samples from patients of Afro-Caribbean origin whose plasma samples were unreactive by the Amplicor Monitor assay demonstrated that, in these cases, the variants could be detected by the NASBA assay (14a).

The results obtained with the longitudinal samples demonstrate that the patterns of changes in viral RNA load were comparable for the assays. This observation, together with the presented results on intra-assay variation and assay reproducibility, indicates that changes in plasma viral RNA levels are likely to be measurable by any of these methods. For the Quantiplex assay the reproducibility was superior to those for both of the other assays over the entire quantitative range. However, at present, the analytical sensitivity of the assay needs further improvement to enable monitoring of the very low plasma viral RNA load seen in patients treated with the potent HIV-1 replication inhibitors currently used in clinical trials. Improved analytical sensitivity has now been achieved by the very recently introduced version 2.0 of the assay (8). Though both Monitor and NASBA assay results were demonstrated to be less reproducible than the Quantiplex assay re-

sults, quantitative results were generated over a 3-$\log_{10}$-unit or greater quantitative range. In particular, the Monitor assay combined this with very high analytical sensitivity. This level of sensitivity may also be achieved by using a larger input volume (1 ml) with the NASBA assay or by using the ultrasensitive Quantiplex assay (9).

In conclusion, the choice of method for quantification may be dictated by the research question as well as by operational needs. HIV-1 RNA load has become an important parameter for the evaluation of the efficacy of antiretroviral drugs. The potential of these drugs to reduce viral load is still increasing, as is the need for viral load determinations, especially in the face of effective viral suppression. Therefore, there will be a particular need for HIV RNA quantification by assays that display great analytical sensitivity, combined with a reasonable sample volume, high sample throughput, and robust specificity.

ACKNOWLEDGMENTS

We thank all members of the DELTA Virology subcommittee and Theo Cuypers and Patrick Bossuyt for helpful discussions and support. Chiron Diagnostics, Roche Molecular Systems, and Organon Teknika are acknowledged for substantial support in training technicians, for providing all necessary equipment and reagents, and for pleasant collaborations. We acknowledge Tom de Groot and Toon van Bommel for logistical assistance and Peter Nara and Jaap Goudsmit for providing us with the HIV-1 reference virus stock.

REFERENCES

1. **Aboulker, J. P., and A. M. Swart.** 1993. Preliminary analysis of the Concorde trial. Lancet **341:**889–890. (Letter.)
2. **Bruisten, S. M., M. H. Koppelman, M. T. Roos, A. E. Loeliger, P. Reiss, C. A. Boucher, and H. G. Huisman.** 1993. Use of competitive polymerase chain reaction to determine HIV-1 levels in response to antiviral treatments. AIDS **7**(Suppl. 2)**:**S15–S20.
3. **Centers for Disease Control.** 1987. Revision of the CDC surveillance case definition for acquired immunodeficiency syndrome. Morbid. Mortal. Weekly Rep. **36:**S1–S5.
4. **Coll, O., C. A. B. Boucher, C. Fortuny, B. M. de Tejada, Y. Canet, I. Caragol, J. Tijnagel, J. M. Bertran, and T. Espanol.** Vertical HIV-1 transmission correlates with a high maternal viral load at delivery. J. Acquired Immune Defic. Syndr., in press.
5. **Danner, S. A., A. Carr, J. M. Leonard, L. Lehman, F. Cudiol, J. Gonzalez, A. Rawentos, R. Rubio, E. Bouza, V. Pintado, A. G. Acuado, G. Garcia de Lomas, R. Delgado, J. C. Borleffs, A. Hsu, J. M. Valdes, C. A. B. Boucher, and D. A. Cooper.** 1995. A short-term study of the safety, pharmacokinetics, and efficacy of ritonavir, an inhibitor of HIV-1 protease. N. Engl. J. Med. **333:**1528–1533.
6. **Eron, J. J., S. L. Benoit, J. Jemsek, R. D. MacArthur, J. Santana, J. B. Quinn, D. R. Kuritzkes, M. A. Fallon, and M. Rubin.** 1995. Treatment with lamivudine, zidovudine, or both in HIV-positive patients with 200 to 500 $CD4^+$ cells per cubic millimeter. N. Engl. J. Med. **333:**1662–1669.
7. **Finnley, D. J.** 1978. Parallel line analysis, p. 69–104. *In* Statistical methods in biological assays. Charles Griffin and Company Ltd., London.
8. **Fleiss, J. L.** 1985. The design and analysis of clinical experiments. John Wiley & Sons, New York.
9. **Fultz, T., J. Todd, S. Hamren, P. Sheridan, M. Collins, T. Yeghiazarian, D. Kern, D. Chernoff, and M. Urdea.** 1995. Quantitation of plasma HIV-1 RNA using an ultra-sensitive branched DNA (bDNA) assay, p. 93. *In* Program and Abstracts of the 2nd National Conference on Human Retroviruses.
10. **Ho, D. D., A. U. Neumann, A. S. Perelson, W. Chen, J. M. Leonard, and M. Markowitz.** 1995. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature (London) **373:**123–126.
11. **Holodniy, M., D. A. Katzenstein, S. Sengupta, A. M. Wang, C. Casipit, D. H. Schwartz, M. Konrad, E. Groves, and T. C. Merigan.** 1991. Detection and quantification of human immunodeficiency virus RNA in patient serum by use of the polymerase chain reaction. J. Infect. Dis. **163:**862–866.
12. **Katzenstein, T. L., C. Pedersen, C. Nielsen, J. D. Lundgren, P. H. Jakobsen, and J. Gerstoft.** 1996. Longitudinal serum HIV-RNA quantification: correlation to viral phenotype at seroconversion and clinical outcome. AIDS **10:**167–173.
13. **Layne, S. P., M. J. Merges, M. Dembo, J. L. Spouge, S. R. Conley, J. P. Moore, J. L. Raina, H. Renz, H. R. Gelderblom, and P. L. Nara.** 1992. Factors underlying spontaneous inactivation and susceptibility to neutralization of human immunodeficiency virus. Virology **189:**695–714.
14. **Loveday, C., and A. Hill.** 1995. Prediction of progression to AIDS with serum HIV-1 RNA and CD4 count. Lancet **345:**790–791. (Letter.)

14a. **Loveday, C., and R. Tedder.** Personal communication.

15. **Mellors, J. W., L. A. Kingsley, C. R. Rinaldo, Jr., J. A. Todd, B. S. Hoo, R. P. Kokka, and P. Gupta.** 1995. Quantitation of HIV-1 RNA in plasma predicts outcome after seroconversion. Ann. Intern. Med. **122:**573–579.
16. **Mulder, J., N. McKinney, C. Christopherson, J. Sninsky, L. Greenfield, and S. Kwok.** 1994. A rapid and simple PCR assay for quantification of HIV RNA: application to acute retroviral infection. J. Clin. Microbiol. **32:**292–300.
17. **O'Brien, W. A., P. M. Hartigan, D. Martin, J. Esinhart, A. Hill, S. Benoit, M. Rubin, C. Lahart, N. Wray, S. M. Finegold, W. L. George, G. M. Dickinson, N. Klimas, G. Diamond, S. B. Zollapazner, P. C. Jensen, C. Hawkes, C. Oster, F. Gordin, A. M. Labriola, P. Spivey, T. Matthews, K. Weinhold, G. Drusano, and M. J. Egorin.** 1996. Changes in plasma HIV-1 RNA and $CD4^+$ lymphocyte counts and the risk of progression to AIDS. N. Engl. J. Med. **334:**426–431.
18. **Perelson, A. S., A. U. Neumann, M. Markowitz, J. M. Leonard, and D. D. Ho.** 1996. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. Science **271:**1582–1586.
19. **Piatak, M., Jr., M. S. Saag, L. C. Yang, S. J. Clark, J. C. Kappes, K. C. Luk, B. H. Hahn, G. M. Shaw, and J. D. Lifson.** 1993. High levels of HIV-1 in plasma during all stages of infection determined by competitive PCR. Science **259:**1749–1754.
20. **Scadden, D. T., Z. Wang, and J. E. Groopman.** 1992. Quantitation of plasma human immunodeficiency virus type 1 RNA by competitive polymerase chain reaction. J. Infect. Dis. **165:**1119–1123.
21. **Schuurman, R., M. Nijhuis, R. van Leeuwen, P. Schipper, D. de Jong, P. Collis, S. A. Danner, J. Mulder, C. Loveday, C. Christopherson, et al.** 1995. Rapid changes in human immunodeficiency virus type 1 RNA load and appearance of drug-resistant virus populations in persons treated with lamivudine (3TC). J. Infect. Dis. **171:**1411–1419.
22. **Semple, M., C. Loveday, I. Weller, and R. Tedder.** 1991. Direct measurement of viraemia in patients infected with HIV-1 and its relationship to disease progression and zidovudine therapy. J. Med. Virol. **35:**38–45.
23. **Urdea, M. S., J. C. Wilber, T. Yeghiazarian, J. A. Todd, D. G. Kern, S. J. Fong, D. Besemer, B. Hoo, P. J. Sheridan, R. Kokka, and et al.** 1993. Direct and quantitative detection of HIV-1 RNA in human plasma with a branched DNA signal amplification assay. AIDS **7**(Suppl. 2)**:**S4–S11.
24. **van Gemen, B., T. Kievits, P. Nara, H. G. Huisman, S. Jurriaans, J. Goudsmit, and P. Lens.** 1993. Qualitative and quantitative detection of HIV-1 RNA by nucleic acid sequence-based amplification. AIDS **7**(Suppl. 2)**:**S107–110.
25. **Wei, X., S. K. Ghosh, M. E. Taylor, V. A. Johnson, E. A. Emini, P. Deutsch, J. D. Lifson, S. Bonhoeffer, M. A. Nowak, B. H. Hahn, et al.** 1995. Viral dynamics in human immunodeficiency virus type 1 infection. Nature (London) **373:**117–122.
26. **Yen-Lieberman, B., D. Brambilla, B. Jackson, J. Bremer, R. Coombs, M. Cronin, S. Herman, D. Katzenstein, S. Leung, H. J. Lin, P. Palumbo, S. Rasheed, J. Todd, M. Vahey, and P. Reichelderfer.** 1996. Evaluation of a quality assurance program for detection of human immunodeficiency virus type 1 plasma RNA by the AIDS Clinical Trials Group Virology Laboratories. J. Clin. Microbiol. **34:**2695–2701.
27. **Yerly, S., L. Kaiser, B. Mermillod, C. Baumberger, B. Hirschel, and L. Perrin.** 1995. Response of HIV RNA to didanosine as a predictive marker of survival. AIDS **9:**159–163.