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Quantification of Human Immunodeficiency Virus Type 1 RNA from Dried Plasma Spots Collected on Filter Paper

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To assess dried plasma spots (DPSs) as a source of material for virus quantification, human immunodeficiency virus type 1 (HIV-1) RNA levels were quantified in matched DPS and liquid plasma samples from 73 infected patients, including 5 neonates and 4 adult patients with acute HIV-1 infection. Quantifications were performed by commercially available assays (NASBA [nucleic acid sequence-based amplification] or Amplicor, or both). There was a strong correlation between HIV-1 RNA levels in plasma and DPSs. More importantly, there was no decline in HIV-1 RNA levels in DPSs stored for as long as 2 weeks at 20°C. Similarly, storage of DPSs for 3 days at 37°C resulted in no decrease in viral RNA levels. For patients with primary infection, the DPS method allowed for the measurement of RNA levels in plasma during the initial spike in the level of viremia and in the subsequent period of suppressed viral replication. DPS quantification was equally informative in the neonatal setting, with all five newborns showing HIV-1 RNA loads of greater than 4.991 $\log_{10}$ copies/ml. We conclude that the viral RNA levels in DPSs are equivalent to those measured in fresh-frozen plasma. The ease and economy of DPS sampling, the minute volumes required, and the unexpected stability of dried RNA suggest that the use of DPSs will be particularly valuable for small-volume neonatal samples and large, population-based studies in which cold storage and transportation present special problems, as is often the case in developing countries. The ability to measure viral changes during primary infection suggests that the method will be useful for assessing vaccine efficacy in large field trials.

The ability to accurately measure viral RNA in the plasma (11, 31, 32) and intracellular (12, 24, 34, 35) compartments of human immunodeficiency virus type 1 (HIV-1)-infected persons has led to a dramatic improvement in our understanding of the natural history of HIV-1 infection and AIDS. A number of recent studies have convincingly demonstrated that high-level viral replication occurs at all stages of disease (16, 40) and that HIV-1 RNA levels in plasma are predictive of disease outcome (18, 23) and response to therapy (20, 21). These findings, combined with the introduction of potent new antiviral agents, have stimulated a growing interest in viral load monitoring.

Notwithstanding these tremendous advances, a number of issues still need to be resolved if HIV-1 RNA quantification is to be used as a routine marker of disease progression and therapeutic efficacy. Many of these issues relate to the limited stability of HIV-1 RNA in liquid plasma and the need for expedient collection, processing, and transport of samples on dry ice or liquid nitrogen. Although the logistics of specimen handling are being addressed in developed countries (27), few studies are examining the technical challenges in developing countries, where immediate sample processing and refrigeration are often unavailable. Given that the developing world bears a disproportionate share of the total number of people with AIDS (8, 41) and that clinical trials are planned for many of these countries, there is a need to develop simple and improved sampling methods that can be widely applied under difficult field conditions.

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Dried specimens collected on filter paper have the potential to facilitate large field studies by simplifying all aspects of sample collection, storage, and shipment (13, 17, 22). Since first introduced for the detection of HIV-1 DNA in 1991 (2), PCR-based dried blood spot methods have proven particularly effective for the early diagnosis of neonatal infection in developing and developed countries (4, 9, 10, 29) and for monitoring the emergence of drug resistance mutations (7), characterizing the genotype of transmitted virus (7), and tracking the global spread of HIV-1 subtypes (5). We describe here the extension of filter paper-based methods to the quantification of viral RNA in dried plasma spots (DPSs). The DPS method is reproducible and cost-effective and has broad implications for population-based research, surveillance, and monitoring of therapy.

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MATERIALS AND METHODS

Description of patients. The adult study population consisted of 53 patients enrolled in natural history and viral burden studies at Saint Paul's Hospital, Vancouver, British Columbia, Canada, and the Southern Alberta HIV Clinic at Foothill Hospital in Calgary, Alberta, Canada. Four of these patients, who had documented evidence of acute primary infection, as determined by clinical presentation (fever, rash, lymphadenopathy, diarrhea, headache, thrush) and laboratory confirmation (evidence of new infection by antibody reactivity, a positive PCR result, or the presence of p24 antigenemia), were monitored serially from the time of initial acute infection through the first few months of their clinical course. The infection in the remaining patients covered the spectrum of HIV-1 disease, ranging from early-stage asymptomatic infection to AIDS. $CD4^+$ counts ranged from 69 to 962 cells/mm^3. A second set of 72 samples came from 5 neonates and 10 young children enrolled in HIV-1 perinatal transmission studies

at the British Columbia Children's Hospital. These children were followed prospectively and were diagnosed with HIV-1 infection when two or more specimens tested culture, PCR, and (or) p24 antigen positive. The $CD4^+$ counts for the five newborns (ages, 8 days to 1.5 months) ranged from 1,800 to 3,690 cells/mm^3. All projects were approved by the Human Subjects Review Board of each participating center, and informed consent was obtained.

Study samples. Whole blood, collected in tubes containing EDTA or acid-citrate-dextrose, was centrifuged at 1,200 × *g* for 10 min. The clarified plasma was removed, aliquoted into sterile 1.5-ml microcentrifuge tubes (0.5 ml/tube), and stored at −70°C prior to evaluation. To prepare dried plasma spots, measured aliquots (50 μl) of thawed plasma representing specimens with low, intermediate, and high viral copy numbers were applied to individual circles of filter paper (no. 903; Schleicher & Schuell) by using a micropipettor and plugged tips (2, 15). With the exception of pediatric samples, at least four collection cards containing five 50-μl spots were prepared from each plasma sample. The filters were left to dry for 3 h in a laminar flow hood prior to testing at time zero and prior to storage under different environmental conditions. To test the effect of low-humidity storage, air-dried DPSs were placed in regular bond paper envelopes, which were then inserted into an outer PuroLetter envelope containing desiccant. The PuroLetter was sealed and stored either at 4°C in a refrigerator or at ambient temperatures of 20 to 22°C for periods of 7 to 16 days. To simulate more extreme conditions of temperature and humidity, dried (or partially dried) DPSs were again placed in bond envelopes and sealed in a PuroLetter envelope in the presence or absence of desiccant. The entire package was heated in a 37°C incubator containing trays of water to maintain a high relative humidity.

Measurement of HIV-1 RNA viral burden. The levels of HIV-1 RNA in dried plasma and freshly thawed liquid plasma were quantified by using the Amplicor HIV Monitor kit (Roche Molecular Systems, Inc., Somerville, N.J.) or the NASBA (nucleic acid sequence-based amplification) HIV-1 RNA QT System (Organon Teknika, Durham, N.C.), or both, according to the manufacturers' instructions, with modifications made to the RNA extraction protocol to accommodate smaller sample volumes.

For the Amplicor assay, each DPS was placed in a microcentrifuge tube and was reconstituted to 200 μl with sterile water. The RNA was extracted by heating the reconstituted specimen for 30 min at 65°C (with shaking) in 600 μl of Working Lysis Reagent (Amplicor HIV Monitor kit; Roche Molecular Systems, Inc.) containing guanidine isothiocyanate and a known amount of HIV Quantitation Standard (QS RNA) (33). To compensate for the smaller 50-μl volume of DPS specimens and to maintain the same relative ratio of QS RNA to specimen RNA, the amount of QS RNA added to the Working Lysis Reagent was reduced from 100 to 25 μl. After extraction, the RNA was precipitated with 1.0 ml of isopropanol at room temperature, washed in 70% ethanol, air dried for 20 min, and resuspended in 100 μl of water treated with diethyl pyrocarbonate.

In initial experiments, to serve as reference standards, RNA was extracted from 200-μl aliquots of the remaining (unspotted) plasma, and the RNA was amplified in parallel, exactly as specified in the Amplicor HIV Monitor protocol (33). In later studies, to approximate more closely the DPS method, 50-μl aliquots of cryopreserved plasma were diluted to 200 μl with water and processed as additional reference standards. When starting with 50 μl of plasma, the reagent and diluent volumes used during RNA processing were identical to those used for extraction of RNA from DPSs. Equivalent amounts (50 μl) of RNA extracted from DPSs and plasma, amplified by HIV-1 reverse transcription PCR, were quantified on microwell plates coated with a bovine serum albumin-conjugated SK102 oligonucleotide probe. Calculation of HIV-1 RNA copy number was performed according to the manufacturer's instructions. The Amplicor assay is reported to have a quantitation limit of 2.699 $\log_{10}$ HIV-1 genome equivalents per ml of plasma (on the basis of a plasma input volume of 200 μl) and to be linear at levels as high as 6.000 $\log_{10}$ RNA copies/ml of plasma. When the sample volumes are reduced to 50 μl, this translates into a quantification limit of 6.301 $\log_{10}$ copies of viral RNA/ml of plasma.

For NASBA, which is based on isothermal amplification with a three-component enzyme system (38, 39), each DPS was eluted directly into 0.9 ml of lysis buffer containing guanidine thiocyanate, Triton X-100, and a known amount of undiluted calibrators (or diluted calibrators, depending on the expected HIV-1 copy number). After a 1-h incubation at room temperature with intermittent vortexing, the tubes were centrifuged briefly (at 11,000 × *g* for 10 s), and the supernatant, which contained the released nucleic acid and calibrators, was transferred to a fresh tube. Silicon dioxide was added as a solid-phase capture system, and the bound nucleic acids (and calibrators) were washed, eluted, amplified, and detected as specified for the NASBA HIV-1 RNA QT chemiluminescence assay. As with the Amplicor assay, NASBA has a reported quantitation limit of 2.600 $\log_{10}$ HIV-1 RNA copies/ml of plasma when used with diluted calibrators and plasma input volumes of 200 μl. This corresponds to a sensitivity of 3.301 $\log_{10}$ HIV-1 RNA copies for 50-μl input volumes. As shown in this study, the NASBA assay continues to be linear above 6.301 $\log_{10}$ copies of HIV-1 RNA/ml of plasma (see Fig. 1).

Statistical analyses. For convenience of analysis and to evaluate correlations between different environmental conditions, all viral RNA values are reported as $\log_{10}$ transformed copy numbers of HIV-1 RNA per milliliter of plasma (or dried plasma equivalent), with samples containing amounts of RNA below the limit of detection being assigned a value of 3.000 $\log_{10}$ copies. The viral load patterns in patients with primary HIV-1 infection were computed by linear regression and were expressed as $\log_{10}$ copies per milliliter as a function of time (in days). Statistical analyses were performed by using StatView (Abacus Concepts, Inc., Berkeley, Calif.).

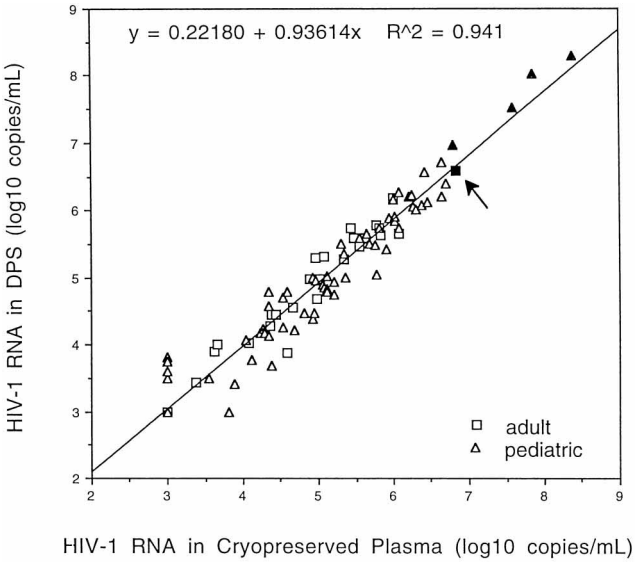

FIG. 1. Regression analysis of viral RNA levels in paired liquid plasma and DPS specimens. Linear regression analysis of $\log_{10}$ transformed HIV-1 *gag* RNA levels for the 97 matched plasma-DPS pairs analyzed by NASBA revealed a slope of 0.9361, with a squared correlation coefficient (R^2) of 0.941. These values were calculated by the formula y = intercept + slope (x) for the curve fit. Specimens from adult patients are represented as squares; those from pediatric patients are represented as triangles. The arrow and closed square represent the specimen from the patient who was seronegative for HIV-1 but who was a rapid progressor from primary infection to death.

RESULTS

Viral RNA quantification across the spectrum of HIV-1 disease. In initial pilot studies, viral load determinations were performed in duplicate with a coded panel of 110 matched DPS and cryopreserved, liquid plasma samples collected from 47 patients at different stages of HIV-1 disease, including acute primary infection (n = 4), chronic persistent infection (n = 23), and AIDS (n = 5), as well as patients with early perinatal infection (n = 15). Eleven of the paired DPS-plasma samples were quantified by both the Amplicor and NASBA assays, while the remaining pairs were analyzed by the Amplicor assay (13 sets) or the NASBA assay (86 sets) only. Linear regression analysis of log-transformed RNA measurements for these paired specimens revealed a strong correlation between HIV-1 RNA levels in liquid plasma and DPSs and between results of the Amplicor and NASBA assays. Data for the 97 pairs tested by the NASBA assay are presented in Fig. 1. Regression analysis revealed a slope of 0.9361 with a squared correlation coefficient of 0.941. The mean ± standard deviation HIV-1 RNA levels in liquid plasma and DPS for these pairs were 5.042 ± 1.199 and 4.965 ± 1.158 $\log_{10}$ copies/ml, respectively. This 0.077-$\log_{10}$ difference between the levels in plasma and those in DPSs was not statistically significant by the Wilcoxon signed rank test. The median difference between duplicate samples measured in the same assay was 0.120 $\log_{10}$ copies/ml for liquid plasma and 0.158 $\log_{10}$ copies/ml for DPSs. Similar results were obtained for 24 matched plasma-DPS pairs tested by the Amplicor assay (data not shown).

Although the Amplicor and NASBA assays gave comparable results for plasma and DPS samples with viral loads of between

3.301 and 6.301 $\log_{10}$ HIV-1 RNA copies per ml of plasma, there were some discrepancies at the upper and lower limits of quantification. Of particular note were samples from five patients who had viral burdens of $>5.27 \times 10^6$ (6.72 $\log_{10}$) RNA copies/ml of plasma. At these high levels, the Amplicor assay showed a plateau in viral load, making it necessary to dilute the plasma specimen prior to RNA extraction and analysis. NASBA, on the other hand, continued to be linear at levels as high as 8.38 $\log_{10}$ RNA copies/ml. The NASBA results were highly reproducible for both cryopreserved plasma and dried plasma spots and in assays performed with diluted and undiluted calibrators. One of the five patients (Fig. 1) had been studied previously and had been found to be a seronegative rapid progressor who advanced from primary HIV-1 infection through AIDS with multiple opportunistic infections to death within 10 months (25). This patient had viral RNA levels in excess of 6.000 $\log_{10}$ throughout the course of his disease (see Fig. 3D). The four remaining patients with high viral loads (6.785 to 8.380 $\log_{10}$ RNA copies/ml) (Fig. 1) were newborns tested in the first 39 days of life. Two of the newborns were retested at subsequent time points: one at 3 months and the other at 4 and 33 months. There was little change in viral copy number over time, with both infants showing prolonged, elevated HIV-1 RNA levels in excess of 6.690 copies/ml. Although plasma RNA levels of 7.505 $\log_{10}$ have previously been reported in newborns, the detection of levels as high as 8.380 $\log_{10}$ was unexpected and warrants further investigation. In our study, the mean ± standard deviation levels for all five neonates tested in the first 6 weeks of life were 7.195 ± 1.177 for liquid plasma and 7.147 ± 1.308 for DPSs. One sample fell below the limits of detection by the NASBA assay. This sample had an Amplicor viral burden of 3.46 $\log_{10}$ for liquid plasma and 3.33 $\log_{10}$ for dried plasma.

Effect of DPS storage on HIV-1 RNA quantification. To examine the effects of cold storage and mild climatic conditions on HIV-1 RNA stability, replicate sets of DPSs prepared from a cross-section of infected patients were enclosed in PuroLetter envelopes and placed in a refrigerator at 4°C, or, alternatively, they were left on top of a bench in an air-conditioned laboratory at 20 to 22°C for periods of 7 to 16 days. The replicates, stored under different environmental conditions, were then tested for HIV-1 RNA in the same Amplicor assay by using matched liquid plasma as the reference standard. As indicated in Fig. 2, storage of DPSs for up to 16 days at 4°C (Fig. 2A) or 20°C (Fig. 2B) had no deleterious effect on our ability to quantify viral RNA. When compared to liquid plasma, linear regression analysis gave a slope of 1.097 and a squared correlation coefficient of 0.952 for refrigerated DPS and a slope of 0.979 and a squared correlation coefficient of 0.939 for DPSs stored at room temperature. The mean ± standard deviation RNA levels were 5.039 ± 0.827 $\log_{10}$ copies/ml for plasma and 4.976 ± 0.930 and 4.959 ± 0.835 $\log_{10}$ copies/ml for DPSs stored at 4 and 20°C, respectively. Again, the −0.063 and −0.080 log differences were not statistically significant by the Wilcoxon signed rank test.

We next examined the stability of HIV-1 RNA under environmental conditions resembling those found in more tropical climates. Four replicate sets of eight DPSs were placed at room temperature (20 to 22°C) (one set) or at 37°C with desiccant (two sets) (Fig. 2C) and without desiccant (one set). One of the sets with desiccant was only partially dried (Fig. 2D) to simulate conditions of high relative humidity, in which case the filters would never completely dry. Storage for up to 3 days at 37°C caused no significant reduction in the viral RNA load compared with the viral RNA levels measured in the cryopreserved, liquid samples. The mean ± standard deviation RNA levels in cryopreserved plasma, DPSs stored at 20°C, and DPSs stored at 37°C with desiccant, wet with desiccant, and without desiccant were 4.697 ± 0.745, 4.666 ± 0.847, 4.691 ± 0.800, 4.624 ± 0.663, and 4.642 ± 0.739 $\log_{10}$ copies/ml. The log differences between frozen plasma and DPSs stored under the various conditions, −0.031, −0.006, −0.073, and −0.055, respectively, were not significantly different.

Variations in HIV-1 RNA levels during acute primary infection. The final set of experiments was designed to examine the in vivo dynamics of viral load changes in four patients with acute HIV-1 infection. As indicated in Fig. 3, equivalent information was obtained with DPSs and liquid samples. The first three patients (patients A to C) displayed a 2- to 3-log decline in viral RNA levels within the first 50 days of presentation with acute retroviral infection. In samples from patient A, high levels of viral RNA in plasma (6.018 to 6.333 $\log_{10}$ copies/ml) were detected at least 2 weeks prior to seroconversion. This was followed by a precipitous decline and stabilization of the patient's viral load at levels ranging from 3.988 to 4.506 $\log_{10}$ copies of HIV-1 RNA per ml of liquid or dried plasma. The stabilization occurred approximately 26 days after the initiation of symptoms and was associated with a relatively high $CD4^+$ count (>580 cells/μl) that was maintained beyond the first year (some $CD4^+$ data not shown). In sharp contrast, patient D showed only a modest suppression of HIV-1 RNA levels in the interval following the acute phase of infection. This patient, who was first sampled 95 days after the initiation of symptoms showed a plateau of high-level viremia (5.70 to 6.300 $\log_{10}$ copies/ml) that persisted until day 236, the last time point at which a sample was obtained. As described previously (25), patient D failed to seroconvert, had low $CD4^+$ counts (100 to 180 cells/μl), and progressed from primary infection to death in less than 1 year. The high viral loads observed for this patient are consistent with the patient's rapid clinical outcome. Overall, the mean ± standard deviation RNA levels for liquid and DPS samples from patients with primary infection were 4.904 ± 0.759 and 4.793 ± 0.949 $\log_{10}$ copies/ml, respectively, for patient A; 4.686 ± 0.712 and 4.677 ± 0.739 $\log_{10}$ copies/ml, respectively, for patient B; 4.816 ± 0.952 and 4.873 ± 0.917 $\log_{10}$ copies/ml, respectively, for patient C; and 5.872 ± 0.248 and 5.95 ± 0.300 $\log_{10}$ copies/ml, respectively, for patient D. Taken together, these longitudinal data showing typical changes in primary viral load patterns provide strong support that quantification of RNA levels in DPSs is biologically relevant and equivalent to conventional RNA quantification with cryopreserved plasma.

DISCUSSION

This study indicates that regardless of the test kit selected, Roche Amplicor or Organon Teknika NASBA, the testing of DPSs collected on filter paper is an accurate and reliable method for the quantification of viral RNA. In all studies (>668 tests performed with specimens from 73 patients with a wide range of $CD4^+$ cell counts), analysis of paired DPSs and fresh-frozen plasma revealed a close correlation between HIV-1 RNA levels in dried and liquid plasma over a dynamic range of RNA measurements (~5 logs; 3.207 to 8.380 $\log_{10}$ copies/ml) with replicate samples tested by different technologists and assay formats. The mean log differences in viral RNA levels for all assays ranged from 0.006 to 0.090 $\log_{10}$, with the variation for DPS samples being comparable to those observed for cryopreserved plasma samples. These findings indicate that HIV-1 RNA elutes completely from the filter paper and that there is no measurable loss of nucleic acid during extraction. The sensitivity limits of quantification with DPSs was in the

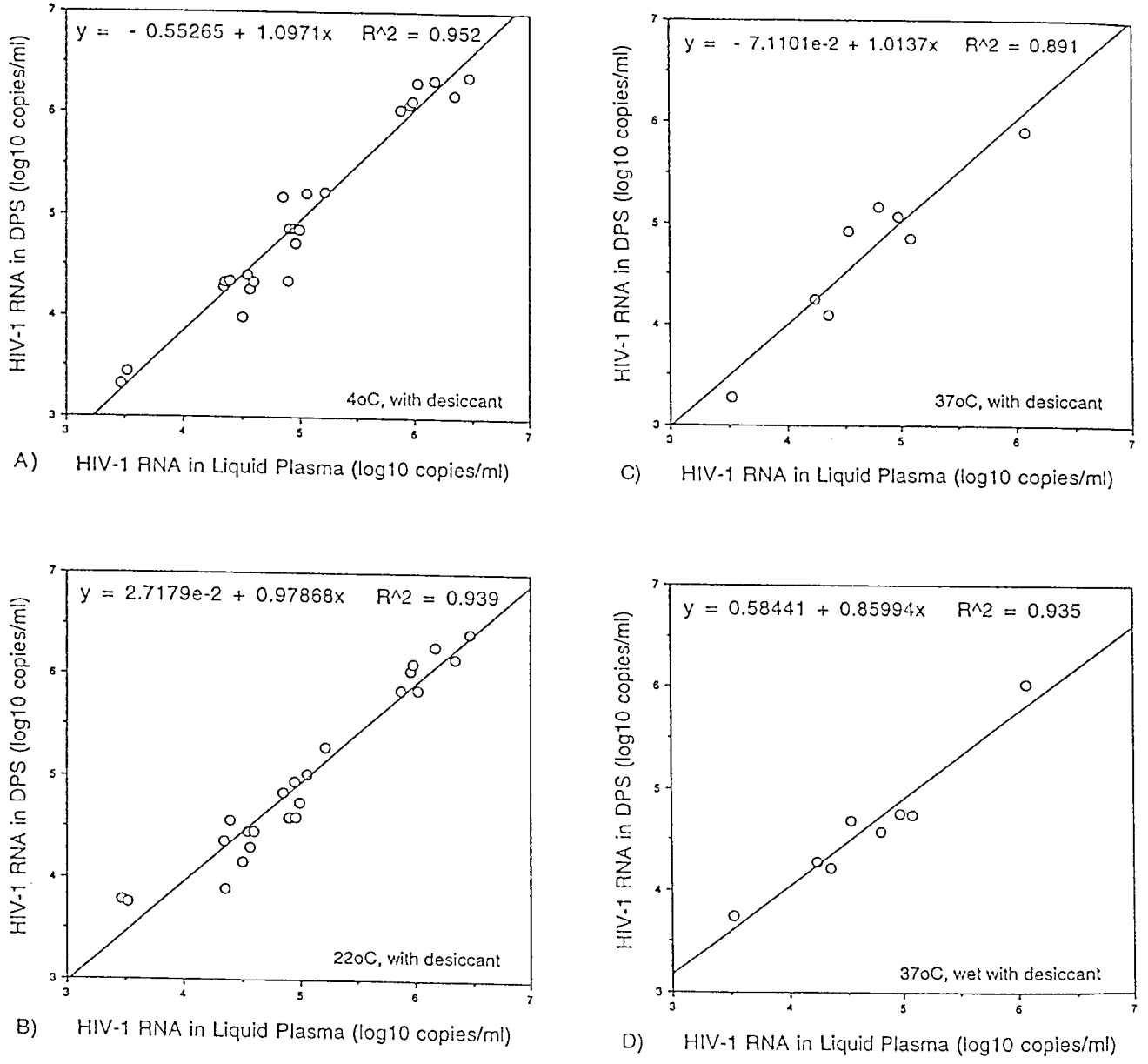

FIG. 2. Stability of HIV-1 RNA detection in DPSs as determined with the Amplicor HIV-1 Monitor kit. Replicate sets of DPSs were stored under a variety of different environmental conditions and were analyzed in parallel with an aliquot of the same plasma stored under optimal conditions (fresh frozen at −80°C). Linear regression analysis of $\log_{10}$ transformed HIV-1 *gag* RNA levels is presented for matched sets of cryopreserved plasma and DPSs. The formula for the curve fit is given by y = intercept + slope (x) along with the square of the correlation coefficient (R^2). Storage conditions for dried (A, B, and C) and partially dried (D) plasma spots are shown: 16 days at 4°C (A), 16 days at 22° (B); 3 days at 37°C, high humidity (C); 3 days at 37°C, high humidity (D).

range of ~3.000 $\log_{10}$ HIV-1 RNA copies/ml, which is the expected value for 50-μl sample volumes.

Variation in RNA quantification was also assessed longitudinally for a small group of patients with primary HIV-1 infection. The patterns of viral RNA clearance, as measured by quantification of RNA in DPSs, paralleled those observed for fresh-frozen plasma. Three of four patients (patients A, B, and C) showed a dramatic 2- to 3-log reduction in plasma virion RNA levels to levels approaching 4.000 $\log_{10}$ copies/ml. Clearance of this magnitude, during the earliest stages of infection, has been associated with a favorable prognosis compared to that for patients who failed to adequately suppress their viremia in the interval immediately following the acute phase of primary infection (19, 23). The rapid disease course for patient D and the persistent high-level viremia, as measured by RNA quantification methods with both DPS and conventional samples, supports the interpretation that failure to effectively clear virus in the primary stage of infection is associated with rapid clinical progression. These serial studies emphasize the clinical importance of viral load monitoring and provide compelling evidence that quantification of RNA levels in DPSs and liquid plasma samples provides biologically important information.

Our investigations also demonstrate the feasibility of using DPSs for the early detection and quantification of HIV-1 RNA in infected neonates. As in the studies with adults, there was a strong correlation between the results obtained by testing

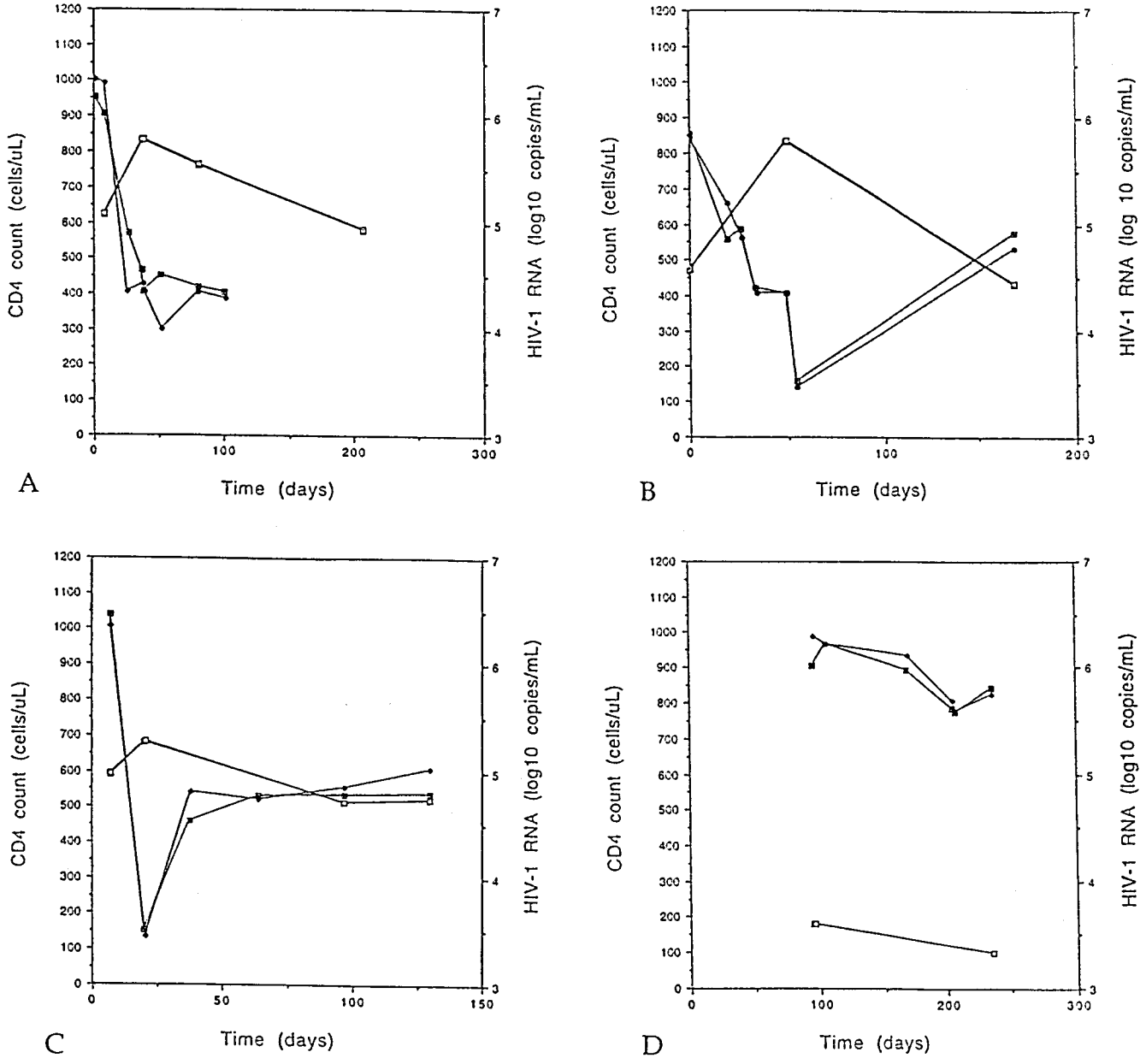

FIG. 3. Viral RNA load dynamics in four patients with primary HIV-1 infection. HIV-1 *gag* RNA levels, determined from both DPS (circles) and fresh-frozen plasma (closed squares), and $CD4^+$ cell counts (open squares) against time are presented for each patient. Time refers to days from the time of onset of clinical symptoms.

DPSs and liquid plasma, with high levels of HIV-1 RNA being measured in all five infants tested during the first 6 weeks of life. In two infants sampled longitudinally, HIV-1 RNA levels remained elevated at 3 and 33 months. Although these findings need to be confirmed in studies with larger numbers of neonates, they are consistent with the findings of two other studies showing persistent high-level viremia throughout the first year of life (30, 37). The detection of viral burdens that are six times higher (up to 8.288 $\log_{10}$) than previously reported (7.505 $\log_{10}$) may be a result of the smaller (50-μl) plasma input volumes used in our study. With samples with high copy numbers and large (200-μl) input volumes, it is conceivable that the amplification reactions may become saturated. This interpretation is supported by results indicating that the dilution of samples from neonates can improve linearity, especially in the Amplicor assay, which tends to plateau at high copy numbers. Alternatively, since only a small number of neonates have been studied to date, it is conceivable that the full range of values for neonates has not yet been determined.

One of the most important observations of this study, however, was the finding that dried plasma can be stored for prolonged periods of time at ambient temperatures without adversely affecting quantitative HIV-1 RNA determinations or changing the categorical result (from positive to negative). These findings add to the growing list of HIV-1 analytes (including antibodies, antigens, RNA, and proviral DNA) (1, 3, 5, 28) that are, unexpectedly, stable when dried onto a solid matrix. In the current study, no significant differences in viral

loads were detected when DPSs were stored for as long as 2 weeks at 20°C and for 3 days at 37°C and high relative humidity. Although these conditions and time limits should be adequate for shipping DPSs from most temperate climates, studies are under way to better understand the long-term stability of viral RNA in DPSs collected under severe tropical conditions. In studies to date, HIV-1 RNA has been successfully quantified from DPSs collected in the Middle East, stored for 2 to 3 days at ambient temperatures, and then transported by hand to Canada. Even more amazingly, we have found that HIV-1 RNA is still detectable in dried (whole) blood spots collected from Southeast Asia in 1992 and inadvertently left at room temperature (~20°C) for longer than 1 year (unpublished data). As with all quantification methods, the ultimate utility of the DPS method at the global level will depend on the ability of the amplification primers to recognize and amplify different clades with equal efficiency. When combined with viral quantification, the use of specimens on filter paper to characterize HIV-1 genetic variants by direct automated sequencing (5, 36) will provide a powerful approach for the development and refinement of RNA quantification assays that are effective globally.

The ability to preserve and transport HIV-1 RNA on filter paper has far-reaching epidemiological and public health implications. Quantification of RNA levels with DPSs will be particularly valuable in the neonatal setting, in which only minute amounts of sample are available, and internationally, when cold storage and transportation present special problems. On the basis of the ease and safety of transporting filter papers, combined with the marked stability and biological equivalency of dried and liquid plasma, it is anticipated that quantification of RNA levels with DPSs will continue to be refined and evaluated as a practical and economical tool for studying the natural history of incident HIV-1 infection in adults and neonates and assessing the effects of antiviral agents, vaccines, and other interventions on the viral burden. Current studies are directed toward a more detailed investigation of DPS stability under extreme tropical conditions and at eliminating the need for an initial, on-site, low-speed centrifugation to separate the plasma. Elimination of this step, possibly by adaptation to spots of whole blood, would broaden the applicability of the method and render it suitable for use in geographic regions that lack electricity. Recent studies in our laboratory and the laboratories of Anne Comeau (8a), Jack Moye (26), and Susan Fiscus (14) suggest that accurate quantification of HIV-1 RNA in dried whole blood will also be possible by using either the NASBA (Moye and Fiscus) or Amplicor (Comeau) methods.

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