Cell-Free Plasma Human Immunodeficiency Virus Type 1 Titer Assessed by Culture and Immunocapture-Reverse Transcription-Polymerase Chain Reaction

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The relationship between plasma human immunodeficiency virus type 1 (HIV-1) infectious titer, determined by quantitative fivefold end-point dilution culture, and the detection of genomic HIV-1 RNA by immunocapture-cDNA-polymerase chain reaction was determined. The optimal plasma specimen collection and storage conditions for the use of such virologic markers for clinical trials were also determined. The variabilities in the measurement of infectious HIV_{LAI} titer associated with intra- and interdonor peripheral blood mononuclear cells were 1.2 and 0.86 log₁₀ 50% tissue culture infective doses (TCID₅₀)/ml (95% confidence interval range), respectively. Plasma HIV-1 titers did not change significantly after storing whole blood for 6 h either at 4°C or ambient temperature or plasma for a median of 267 days (range, 259 to 482) at -70°C. The detection of genomic HIV-1 RNA encapsulated in viral particles was very consistent, reproducible, and unaffected by either heparin or acid citrate or by multiple freeze-thawing. The HIV-1 RNA titers also appeared to generally correlate with the biologic titer obtained by the microculture assay. The consistency in infectious HIV-1 titer was evaluated by using 27 unfrozen plasma specimens collected from five subjects over 1 to 9 days. The median change in HIV-1 titer relative to baseline was -0.5 log₁₀ TCID₅₀/ml (interquartile range, -1.03 to 0.175 log₁₀). In contrast, no significant change in HIV-1 RNA for the same frozen plasma specimens was noted. As such, immunocapture-cDNA-polymerase chain reaction may be a useful measure of plasma viremia for studying the natural history of HIV disease and assessing response to therapy.

A change in plasma human immunodeficiency virus type 1 (HIV-1) viremia can be used as a direct virologic marker of disease progression and to assess response to antiretroviral therapy. Several investigators have measured cell-free plasma-associated HIV-1 and defined the important relationship between increasing virus load and disease progression. Plasma viremia has been assessed by using dilutional end-point culture-based assays to define the infectious titer and polymerase chain reaction (PCR) amplification of HIV cDNA to define the copy number of both cell-associated and cell-free HIV-1 RNA (1, 3, 4, 8, 11, 13, 16, 19). A decrease in either the infectious or RNA titers of HIV-1, or both, is a direct virologic measure of antiretroviral therapy (8, 9, 16). Therefore, knowledge of the stability of cell-free virions in plasma and the consistency of virion titers over time is necessary for the proper timing of collection and the methods of collection and storage of plasma specimens for antiretroviral clinical trials where plasma viremia is a virologic end point.

Lentiviruses and other enveloped retroviruses are inactivated to different extents by a variety of chemical and environmental conditions. For example, the plasma titer of HIV_{LAI} (formerly HIV-LAV) in a 50% HIV-1 antibody-negative plasma mixture decreases by less than 0.5 log₁₀ per day when left at room temperature (18). Similarly, the HIV-1 plasma titer has been shown to decrease by less than 0.5 to 1 log₁₀ in plasma stored for 3 days at ambient temperature or

4°C, respectively (15), and by 1 log₁₀ 50% tissue culture infective dose (TCID₅₀)/ml after a single freeze-thaw (11). The effect of repeated freeze-thaws and of various plasma storage conditions on the stability of both infectious virus and genomic RNA contained in viral particles is otherwise unknown.

In this study, we first assessed the variation in measuring the titer of plasma-associated HIV-1 introduced by using different donors for the peripheral blood mononuclear cells (PBMC) used in the microculture assay. We next measured the effect of storage time and temperature on infectious HIV-1 plasma titer and determined the effect of repeated freeze-thaws and of various plasma storage conditions on the stability of the genomic RNA contained in viral particles. The efficiency of detecting HIV-1 RNA was also determined in paired serum and plasma samples collected by using either heparin or acid citrate dextrose as the anticoagulant. Finally, we also determined the variation in both cell-free HIV-1 infectious and genomic RNA titers within individuals who were sampled serially over relatively short periods of time. Finally, our results help to define the conditions for collecting and storing HIV-infected plasma for clinical trials and document the relationship between infectious titer and genomic HIV-1 RNA detected by immunocapture-cDNA-PCR.

MATERIALS AND METHODS

Preparation of donor PBMC for isolation of HIV. Lymphocytes were obtained from HIV antibody-negative volunteer

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donors at low risk for HIV infection. Whole blood was collected in sterile 60-ml polypropylene syringes containing preservative-free heparin (final concentration, 20 U/ml of whole blood; Sigma Chemical Co., St. Louis, Mo.) in phosphate-buffered saline (PBS). The blood was diluted 1:1 with sterile PBS and underlayered with lymphocyte separation medium (Organon Teknica, West Chester, Pa.), and the sample was centrifuged at $800 \times g$ for 20 min. The PBMC layer was removed and washed twice with PBS, and the cell pellet was resuspended in medium A (RPMI-1640, 16% fetal calf serum, penicillin [100 U/ml], streptomycin [100 µg/ml], and glutamine [0.3 mg/ml]). The mononuclear cells were counted with a hemocytometer, distributed into T-75 flasks (Corning Glass Works, Corning, N.Y.) at a final concentration of 2×10^6 cells per ml of medium A, and stimulated with phytohemagglutinin (5 µg/ml) for 3 or 4 days in a 5% carbon dioxide atmosphere at 36.5°C.

Plasma microculture method. Undiluted plasma was obtained from heparinized whole blood after low-speed centrifugation at $800 \times g$ for 20 min. The plasma was removed and either respun at the same centrifugal force or filtered through a 0.45-µm-pore-size filter (no. 25902, Corning). Either fresh plasma or plasma stored at -80°C was used in microculture. A fivefold dilution series from 1:5 to 1:625 was prepared in sterile screwcap microtubes (12 by 7.5 mm) with sealing O-rings (Sarstedt, Inc., Princeton, N.J.) by using 240-µl aliquots of undiluted plasma and 960 µl of medium B (RPMI-1640, 16% fetal calf serum, penicillin, streptomycin, 5% purified human interleukin-2 [Pharmacia, Piscataway, N.J.], and 0.5 ml of DEAE dextran [10 µg/ml; 500,000 Da; Sigma]). A 400-µl aliquot from each dilution was added to duplicate wells (24-well plate; Costar, Cambridge, Mass.) containing 2×10^6 3- to 4-day-old phytohemagglutinin-stimulated donor cells, and the volume was brought to 2.0 ml in each well with medium B. The 24-well plates were placed in low-density polyethylene bags (no. 6255-058; Nalgene, Rochester, N.Y.) and incubated at 36°C in a 5% CO_2 nonhumidified incubator. After 3 days, 1.0 ml of culture supernatant was exchanged for fresh medium B; this was repeated at day 7 but, in addition, 500×10^3 3- to 4-day-old phytohemagglutinin-stimulated PBMC was added. At day 14 of culture, a 200-µl aliquot was removed for measurement of HIV p24 antigen by a solid-phase sandwich-type enzyme immunoassay (Abbott Laboratories, North Chicago, Ill.). The culture was considered positive if the p24 antigen was >30 pg/ml. The titer of plasma-associated HIV was expressed as either the reciprocal end-point dilution or as the TCID_{50} per milliliter of plasma as calculated by the Reed and Muench method (17).

Viral capture and amplification. Before capture, clarified plasma was spun at 10,000 rpm for 10 min in a microcentrifuge to remove platelets and other potential cellular debris. Intact viral particles were captured from 50 µl of serum or plasma by using monoclonal anti-gp120 and anti-gp41 immunoglobulin G antibodies coupled to 0.1-µm-diameter latex microparticles. For the experiments described in Fig. 2 to 4, the genomic RNA was extracted from captured virions by sodium dodecyl sulfate-proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation as described previously (6). For the experiments described in Fig. 1 and 5, we used the following modified, simpler procedure. Fifty microliters of serum or plasma was diluted in 150 µl of sterile PBS and incubated for 1 h with 50 µl of microparticle suspension. The microparticles were centrifuged for 10 min at 5,000 rpm in a microcentrifuge. After the liquid phase was removed and the solid phase was washed once with 150 µl of

PBS, the RNA was extracted from virions bound to the solid phase by incubation with 30 µl of direct lysis buffer (Abbott) for 30 min at 37°C and then by heating at 95°C for 5 min.

Synthesis of cDNA and amplification with the SK38 and SK39 primers were performed either in separate steps as previously described (Fig. 2 to 4) (6) or in a one-step fashion (Fig. 1 and 5) as follows. Duplicate 15-µl aliquots of the RNA extract were each mixed with 85 µl of a solution containing 2.5 U of avian myeloblastosis virus reverse transcriptase (GIBCO-BRL, Gaithersburg, Md.), 0.2 µg each of SK38 and SK39 primers, 0.235 mM each dATP, dTTP, dCTP, and dGTP (Pharmacia), 11.7 mM Tris base (pH 8.3), 2.06 mM MgCl_2 , 58.82 mM KCl, 10 U of RNasin (Promega, Madison, Wis.), and 1.0 U of *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, Conn.). After being incubated for 30 min at 42°C to allow for reverse transcription, the mixture was heated at 95°C for 3 min and then cycled 35 times for 1 min at 94°C and 2 min at 56°C. Detection was accomplished by liquid hybridization with a ^{32}P end-labeled SK19 probe and then by polyacrylamide gel electrophoresis and autoradiographic exposure for 1 to 4 hours with an intensifying screen at -70°C . All extractions and amplifications were done in duplicate. Previous results comparing amplification with and without reverse transcription demonstrated that this method detected only genomic RNA (6).

RESULTS

Reproducibility and stability of plasma HIV titer. The inter- and intradonor PBMC variability on plasma culture titer was assessed by titrating a unique, aliquoted, frozen (-70°C) HIV_{LAI} stock by using 3- to 4-day-old phytohemagglutinin-stimulated PBMC from 24 different HIV-seronegative donors and from five repeat donors, respectively. The mean titer measured by using PBMC from 24 different donors was $5.91 \log_{10} \text{TCID}_{50}/\text{ml}$, with a 95% confidence interval ranging from 5.48 to $6.34 \log_{10} \text{TCID}_{50}/\text{ml}$. The mean intradonor variability between paired HIV_{LAI} titrations by using PBMC from the five repeat donor pairs was $0.169 \log_{10} \text{TCID}_{50}/\text{ml}$, with a confidence interval ranging from -0.415 to $0.753 \log_{10} \text{TCID}_{50}/\text{ml}$ of plasma. Therefore, both the inter- and intradonor variabilities of plasma titer were similar, 0.86 and $1.2 \log_{10} \text{TCID}_{50}/\text{ml}$, respectively, or about $1 \log_{10} \text{TCID}_{50}/\text{ml}$. As a result, a unique PBMC donor need not be used for measuring potential plasma titer changes associated with experimental conditions.

The stability of infectious HIV in plasma was determined by collecting heparinized whole blood from six HIV-infected subjects with low-level plasma HIV viremia titers ranging from 5 to 125 reciprocal infectious units as determined by using our fivefold dilutional end-point infectivity assay. Aliquots of the blood specimens were held at ambient temperature and at 4°C for 2 to 23 h, after which plasma was separated and frozen at -70°C for batched fivefold ($0.7 \log_{10}$) serial end-point quantitative culture. As shown in Table 1, a 0.5-log_{10} difference in infectious titer was frequently observed between determinations. However, no specific pattern was associated with the variations observed. For example, patient B had a 0.5-log_{10} decrease after 6 and 18 h at 4°C but more than a 1-log_{10} increase after 18 h at ambient temperature. Therefore, aside from assay variability, no significant impact on plasma titer due to storage of blood for 1 day at ambient temperature or at 4°C was observed.

For six subjects, plasma HIV infectious titers were also measured after a single freeze-thaw before and after storage of the plasma at -70°C for 258 to 482 days. As shown in

TABLE 1. Stability of plasma HIV-1 titer in whole blood stored at ambient temperature or 4°C

Patient	Temp ^a	Titer ^b after storage time of:			
		2 h	4 h	6 h	18 to 23 h
A	A	125	25	125	5
	4°C	25	5	125	125
B	A	125	125	25	3,125
	4°C	5	25	0	0
C	A	125	5	625	5
	4°C	25	25	25	25
D	A	25	25	25	125
	4°C	125	625	125	25
E	A	5	ND ^c	5	5
	4°C	5	25	25	5
F	A	5	25	5	0
	4°C	25	125	5	3,125

^a A, ambient temperature of ~20 to 21°C.^b Titer as reciprocal of duplicate fivefold end-point dilution series.^c ND, not determined.

Table 2, the titer of three specimens decreased by 0.5 log₁₀, one was unchanged, and two increased by 0.5 log₁₀. Therefore, freezing plasma for at least 1 year at -70°C did not significantly change the infectious titer of HIV-1.

Detection and stability of HIV RNA in various media. Calibrated plasma controls, consisting of end-point dilutions of virions added to seronegative plasma, and RNA controls, consisting of end-point dilutions of purified total RNA extracted from H9-IIIB-infected cells, were analyzed in parallel with each experiment (Fig. 1). A 10-fold change in plasma load or RNA level was easily and reproducibly detected by immunocapture-cDNA-PCR.

Paired serum and plasma samples were obtained from seven HIV-1-seropositive individuals and stored at ambient temperature, and HIV RNA was isolated by viral capture within 24 h of collection. Six subjects had an AIDS-defining illness and one had AIDS-related-complex symptoms. The percentages of CD4⁺ lymphocytes ranged from 6 to 0%. As shown in Fig. 2 for three of these subjects, the signal obtained after immunocapture and reverse transcription-PCR was consistently and significantly higher in plasma than in serum. Thus, plasma was preferred over serum for detecting HIV RNA by immunocapture-cDNA-PCR.

The stability of virion-associated HIV-1 RNA was evaluated for two subjects, J and K, having 19 and 10% CD4⁺ cells, respectively. Paired citrated or heparinized plasma specimens were obtained, and the specimens were stored for 24 h at ambient temperature. HIV RNA was extracted from each fresh sample and also after freezing at -70°C and thawing to room temperature for 2 to 4 h one, three, five,

FIG. 1. Procedural controls for detection of plasma HIV-1 RNA by immunocapture-cDNA-PCR. Plasma controls A, B, and C represent the three lowest positive 10-fold dilutions (10^{-3} , 10^{-4} , and 10^{-5} , respectively) of a virus preparation added to seronegative plasma. RNA controls D and E represent the two lowest positive 10-fold dilutions (10^{-6} and 10^{-7} , respectively) of total cell-associated HIV RNA purified from an H9-IIIB-infected cell line. The DNA controls F and G correspond to approximately 500 and 50 copies of HIV-1, respectively, isolated from the 8E5 cell line (a gift of B. Polsky, Sloan Kettering Memorial Institute, New York, N.Y.). N, negative controls.

seven, and nine times. Specimens were also tested after storage for 2 and 6 days at ambient temperature. Purified RNAs were kept in ethanol at -70°C until completion of the extractions and then resuspended in sterile water, reverse transcribed, and amplified as described previously (6). As shown for subject J in Fig. 3A, the signals obtained after each freeze-thaw step were not significantly different from those obtained with fresh plasma. Also, samples stored for up to 6 days at ambient temperature gave signals similar to those obtained from fresh plasma (Fig. 3B). Lastly, there was no significant difference between plasma collected in the presence of heparin or in the presence of citrate. A two- to threefold variation of signal intensity was sometimes observed between duplicate amplifications of the same sample (i.e., freeze-thaw 3), and, occasionally, duplicate reverse transcription-PCR produced significantly discrepant results. These findings suggest that the reverse transcription step is inherently variable and stress the importance of duplicate testing for cDNA-PCR assays. Nevertheless, these results indicate that several cycles of freeze-thawing, prolonged storage at ambient temperature, and the nature of the anticoagulant had no significant effect on the detection of HIV-1 RNA by immunocapture-cDNA-PCR. Similar results were obtained with samples from subject K (data not shown).

To demonstrate that no significant change in viral load was associated with these experimental conditions, we determined the titers of HIV-1 RNA by immunocapture-cDNA-PCR after serial dilution of the plasma. As shown in Fig. 4, the HIV-1 RNA titer measured by end-point titration for the same two patients, J and K, was 10^{-1} and 10^0 , respectively. In contrast to the minor differences in signals observed between freeze-thaws, a 1-log₁₀ decrease in plasma viral load

TABLE 2. Stability of plasma HIV-1 titer at 70°C

Patient	Storage time (days)	Baseline titer ^a	Storage titer ^b
A	267	25	125
B	259	25	25
F	267	25	5
G	258	125	625
H	482	125	25
I	356	625	125

^a Baseline titer was determined from frozen plasma.^b Titer as reciprocal of duplicate fivefold end-point dilution series.

FIG. 2. Detection of HIV-1 RNA in paired serum (S) and plasma (P) samples from three individuals.

FIG. 3. Effect of multiple freeze-thaws (A) and storage at ambient temperature (B) on the detection of HIV-1 RNA by viral capture in plasma from subject J. (A) The detection of HIV-1 RNA was performed on plasma collected in the presence of heparin (H) or citrate (C) before freezing and after one, three, five, seven, and nine cycles of freeze-thaw. (B) An experiment parallel to that described for panel A was carried out on the same sample after 0, 2, and 6 days at ambient temperature.

was associated with a consistent and significant reduction in PCR signal. Therefore, the RNA viral load was not significantly reduced under the experimental and clinical conditions described above.

Comparison of plasma culture and RNA detection. We determined the variability in the plasma HIV titer of five HIV-viremic subjects ($CD4^+$ count, <50 cells per μ l) who were receiving zidovudine therapy. Heparinized whole blood was collected serially over two sampling intervals of from 2 to 9 days duration but separated by 40 to 60 days. All of the cultures were performed in real time on unfrozen plasma specimens. For cultures of plasma drawn on the same day, the same phytohemagglutinin-stimulated donor cells were used, but phytohemagglutinin-stimulated PBMC from different donors were used for cultures set up on different days. All specimens from each donor were assayed together for plasma RNA after freezing of the samples at

-70°C . For 27 specimens, the median change relative to the baseline HIV titer for each of two sampling intervals was $-0.5 \log_{10}$ TCID₅₀/ml; the interquartile range was from -1.025 to $0.175 \log_{10}$ TCID₅₀/ml, which was comparable to the interassay variation described earlier. Thus, a change in titer should be equal to or greater than $1.2 \log_{10}$ TCID₅₀/ml to be considered biologically significant. Four samples collected from two individuals showed such significant variations in titer.

The levels of HIV-1 RNA were determined on frozen samples from four of those five subjects. Interestingly, no significant changes were observed between sequential samples from each subject, whereas culture titers frequently fluctuated by $1 \log_{10}$ TCID₅₀/ml or more (Fig. 5). The HIV-1 RNA levels decreased significantly upon 10-fold dilutions but remained equivalent for samples from each subject (data not shown). In general, the HIV-1 RNA signals became undetectable when specimens were diluted by 2 to 4 $\log_{10}$.

DISCUSSION

Our study provides new insight into the stability and variability of plasma-associated HIV-1 under the specimen collection and storage conditions used by the National Institute for Allergy and Infectious Disease AIDS Clinical Trial Group protocols. An understanding of the stability and variability of the HIV-1 titer in blood plasma is important for both the proper collection and storage of plasma specimens and for the design of antiretroviral clinical trials where a change in plasma HIV-1 titer is used as a virologic measure of therapeutic efficacy.

The infectivity of plasma collected from HIV-1-seropositive individuals did not change significantly when whole blood was kept for up to 6 h at room temperature or after the plasma had been frozen for at least 1 year at -70°C . The

FIG. 4. End-point titration of plasma HIV-1 RNA in two samples. Specimens from subject J and K were serially diluted 10-fold in plasma obtained from a seronegative subject, and HIV-1 RNA was detected by immunocapture-cDNA-PCR.

FIG. 5. Consistency in plasma HIV-1 infectious titer ($\log_{10}$ TCID₅₀ per milliliter) and genomic RNA detection. The infectious titer is indicated by different symbols for each of the five subjects (A to E), and the RNA autoradiogram signal corresponding to each cultured sample (indicated as 1 to 7) is represented at the top of each panel (RNA).

differences observed probably reflected the variability of the culture method itself rather than true biological changes in the HIV-1 titer. Therefore, accurate results can be obtained from plasma culture if the plasma is separated on the day of collection and either cultured fresh or frozen at -70°C for culturing later.

The results of the inter- and intradonor variability studies suggested that a change in plasma titer greater than $1.2 \log_{10}$ TCID₅₀ is required to show a true biological difference in

infectious titer, whereas smaller variations probably correspond to assay variability. Repeat short-term determinations of plasma titers in five subjects also showed that the microculture plasma HIV assay used by us had an inherent mean variability of approximately $0.5 \log_{10}$ infectious units. Therefore, on the basis of a fivefold dilution ($0.7 \log_{10}$) protocol, significant changes in HIV-1 titer must equal or exceed two well dilution steps.

HIV-1-specific RNA has been successfully detected by

direct extraction of serum and plasma (7, 9). The disadvantage of this approach, however, is that some anticoagulants, such as heparin, have a high affinity for nucleic acids and can strongly inhibit the amplification (10). In addition, other substances present in the serum or plasma may also remain associated with the nucleic acids during the extraction and inhibit enzymatic activities during the reverse transcription and/or the amplification. Immunocapture or centrifugation, followed by washing, circumvent these potential problems by selectively removing only HIV virions from plasma before extraction or amplification of the viral RNA (6, 16). Our results showed that the material of choice for viral capture cDNA-PCR is plasma rather than serum. The lower cDNA-PCR signal obtained in serum than in plasma may be due to the trapping of viral particles in the fibrin network during blood clotting. For plasma samples, HIV-1 RNA was detected equally well if specimens had been collected in the presence of either citrate or heparin.

The genomic HIV-1 RNA encapsulated in viral particles is not measurably degraded upon repeated freeze-thawing of plasma or after several days of storage at room temperature. Similar results have been recently reported on the stability of hepatitis C virus RNA in plasma (20). We also found similar results empirically by using a variety of well-defined panels and positive controls. For example, the calibrated semiquantitative plasma controls we used for this study were established more than 2 years earlier and have always given very consistent results (data not shown). Although we did not precisely determine the maximum number of freeze-thaws that can be tolerated by a given specimen without detectable loss of HIV-1 RNA signal, our results consistently showed that after five cycles of freeze-thawing or 3 days at ambient temperature, there was no significant decline in plasma-associated HIV-1 RNA. We have analyzed seroconversion samples that had been kept for 3 to 5 years at -20°C as well as heparinized plasma samples kept for 1 to 3 years at -70°C and showed that there was very little effect, if any, associated with storage of plasma at subzero temperatures on the detection of HIV-1 RNA (2, 5, 6).

Our finding that several cycles of freeze-thaw have no adverse effect on the detectability of HIV-1 RNA in plasma have important implications for monitoring antiviral therapy. For example, specimens could be tested in real time and additional corresponding aliquots could be frozen for later use. Therapeutic efficacy measured during a clinical trial could be subsequently confirmed by batch testing of frozen specimens. Also, the ability to retest the same sample several times would obviate the need to aliquot multiple specific vials for the sole purpose of measuring viral load by the immunocapture method.

In the five subjects from whom serial plasma titers were determined over several days, plasma culture was significantly more variable than HIV-1 RNA detection by immunocapture-cDNA amplification. Four observations of two patients showed variations in titer that exceeded $1.2 \log_{10}$, which could represent biologic changes in infectious HIV-1 titer specific to those subjects. The HIV-1 RNA signals, however, were almost identical for all samples collected from each individual. This may indicate that an occasional variation greater than $1.2 \log_{10}$ in titer does not necessarily reflect a corresponding change in HIV-1 RNA load. It emphasizes the need for measuring a sustained reduction in titer equal to or greater than $1.2 \log_{10}$ on at least two or more consecutive samples to attribute a change in titer to a specific therapeutic or biologic effect.

There are several possible explanations for the discor-

dance between the variation in infectious plasma HIV titer and the lack of variation in HIV RNA. The inherent variation in the plasma microculture infectivity assay may result from the many determinants that are necessary for establishing in vitro infectivity of the plasma-associated virions. For example, neutralizing antibody, viral envelope shedding (14), genetically defective virus (12), and deterioration of virions on transport from the clinic to the laboratory may interfere with the reproducibility of our measurement of infectious titer. Clearly, the determinants for detection of infectious virus outnumber those for detecting HIV genome alone. Of note, the HIV-1 RNA signal was approximately equivalent to the infectious titer in that it represented about $3 \log_{10}$ of infectious virus equivalent. As such, it is unlikely that the number of cell-free plasma-associated infectious HIV virions differed greatly from the number that was detected by immunocapture-cDNA-PCR. This may indicate that the level of infectious virus may fluctuate within a less-variable pool of total plasma-associated HIV RNA. The measurement of virion-associated HIV-1 RNA may be better than infectious titer for showing relative changes in viral load during therapy because the measurement of HIV-1 RNA does not require duplicate specimens, which may be necessary to optimally assess the inherent variability of infectious titer. Further studies are required to more precisely quantitate the RNA copy number in relation to the infectious plasma HIV titer.

In summary, we have shown the following: (i) the titer of infectious cell-free plasma-associated HIV is stable under the routine conditions of specimen collection and storage used by the AIDS Clinical Trial Group virology laboratories; (ii) the inherent variation in the PBMC-based assay for cell-free HIV is approximately $1.2 \log_{10}$; and (iii) the measurement of HIV-1 RNA by immunocapture-cDNA-PCR is subject to less variation than is infectious titer, the signal obtained is higher in plasma than in serum, and the signal is not affected by anticoagulant and is stable to multiple freeze-thaws. As such, immunocapture of cell-free HIV virions, followed by reverse transcription and amplification, appears to be a promising method for measuring the antiviral response to anti-HIV therapy.

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