Comparative Stabilities of Quantitative Human Immunodeficiency Virus RNA in Plasma from Samples Collected in VACUTAINER CPT, VACUTAINER PPT, and Standard VACUTAINER Tubes

MARK HOLODNIY,^{1*} LARRY MOLE,¹ BELINDA YEN-LIEBERMAN,² DAN MARGOLIS,¹
COLLEEN STARKEY,² RICHARD CARROLL,³ TIM SPAHLINGER,⁴
JOHN TODD,⁵ AND J. BROOKS JACKSON⁴

*AIDS Research Center, Veterans Affairs Medical Center, Palo Alto,¹ and Chiron Corporation, Emeryville,⁵ California;
Department of Clinical Pathology, Section of Microbiology, Cleveland Clinic Foundation,² and University Hospitals
of Cleveland and the Institute of Pathology, Case Western Reserve University,⁴ Cleveland, Ohio;
and Syracuse Research Group, Syracuse, New York³*

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This study compared the levels of human immunodeficiency virus (HIV) virion RNA in plasma from whole blood collected in VACUTAINER CPT (cell preparation tube), VACUTAINER PPT (plasma preparation tube), VACUTAINER SST (serum separation tube), and standard VACUTAINER tubes with sodium heparin, acid citrate dextrose, sodium citrate, and potassium EDTA used as anticoagulants. Quantitative plasma HIV RNA levels were measured by branched-DNA signal amplification. Blood from all tubes was either processed within 1 to 3 h after collection or stored at room temperature or at 4°C for analysis at 6 to 8 and 30 h postdraw. Immediately separated plasma from sodium citrate CPT tubes held at 4°C maintained better stability of HIV RNA equivalents than whole blood held at room temperature or 4°C. The highest number of HIV RNA equivalents was seen with EDTA VACUTAINER tubes. HIV RNA equivalents in all types of plasma were significantly higher than in SST tubes. Although a decline in HIV RNA equivalents was seen in all collection devices after 30 h, a significantly greater decline in plasma HIV RNA equivalents occurred in acid citrate dextrose VACUTAINER tubes than in citrate CPT, PPT, and standard EDTA VACUTAINER tubes. In order to minimize the variability of quantitative HIV RNA test results, our data suggest that samples collected for a particular assay should be processed at the same time postdraw using a particular tube type throughout a given study.

Determination of human immunodeficiency virus (HIV) viral load is important for monitoring antiretroviral activity and understanding the extent of disease progression. Toward this end, virion-associated HIV RNA in plasma has been found to be a dynamic marker of HIV viral load (3). Although biologic stability (6) and significant changes after initiation or discontinuation of therapy have been described (11), little is known about the stability of cell-free HIV RNA in either whole blood or plasma separated prior to sample processing. Published reports have described variability of HIV infectious titer or nucleic acid recovery depending upon the length of elapsed time prior to sample processing (2, 8, 9) or the type of anticoagulant used in blood collection (4). Therefore, the parameters encountered in blood collection (sampling time and the type of anticoagulant) should be standardized. This is especially important given the increase in overnight shipment of clinical samples to referral laboratories for processing and the fact that a majority of clinical trials are being carried out at multiple clinical centers. To achieve this standardization, a simple blood collection and transport system is needed at clinical sites without biohazard facilities to efficiently process HIV-infected samples. However, prior to initiating the routine use of such a

system, the stability of the marker being measured needs to be established.

VACUTAINER CPT (cell preparation tube) and VACUTAINER PPT (plasma preparation tube) tubes contain an anticoagulant and a polyester gel barrier. Centrifugation of PPT tubes containing whole blood removes most cells from plasma, including platelets (5). A liquid density gradient medium in CPT tubes allows for separation of plasma and peripheral blood mononuclear cells (PBMCs) from erythrocytes, platelets, and granulocytes following a single 20-min centrifugation step. Once separated, either the plasma and mononuclear cell fractions can be removed for analysis or storage or the unopened tube can be shipped to a regional testing laboratory. We have previously shown that the use of sodium citrate CPT tubes (CPT-C tubes) resulted in enhanced stability of HIV RNA levels in plasma for up to 24 h postcollection compared with that of standard VACUTAINER acid citrate dextrose (ACD) tubes (V-ACD tubes) as measured by both reverse transcription PCR (RT-PCR) and branched-DNA (bDNA) assays (7). The present multicenter study greatly expands upon this observation by comparing the recovery and stability of HIV virion-associated RNA in plasma prepared from blood collected in three different types of commonly used VACUTAINER tubes, three different CPT tubes, a PPT tube, and an SST tube (serum separation tube). In order to statistically measure small (less than twofold) differences in plasma HIV RNA levels between the tube types (13), we chose to use the bDNA assay in the current study.

* Corresponding author. Mailing address: AIDS Research Center, Veterans Affairs Medical Center, 3801 Miranda Ave., #111-ID, Palo Alto, CA 94304. Phone: (415) 852-3408. Fax: (415) 858-3978.

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MATERIALS AND METHODS

Blood collection and specific tube processing. Informed consent was obtained from 40 HIV-seropositive subjects participating in this study at the AIDS Research Center (ARC), Veterans Affairs Medical Center, Palo Alto, Calif., and at the AIDS Clinical Trial Unit (ACTU), Case Western Reserve University, Cleveland, Ohio. Information collected for each subject included CD4⁺ cell count, current Centers for Disease Control classification (1), and antiretroviral history. Whole blood was collected by venipuncture in the following blood collection tubes: 8-ml CPT-C, sodium heparin CPT (CPT-H), and potassium EDTA CPT (CPT-E) tubes; 8-ml sodium citrate PPT tubes; 10-ml SST tubes; and 10-ml standard sodium heparin VACUTAINER (V-H), V-ACD, and standard potassium EDTA VACUTAINER (V-E) tubes (Becton Dickinson, Franklin Lakes, N.J.). CPT-H, CPT-E, and PPT tubes are currently experimental and not commercially available. The following experimental protocols were conducted at the two institutions.

Effects of storage time and temperature. An experiment was performed to determine whether initial separation of plasma versus storage as whole blood affected plasma RNA levels. In addition, the effect of storage temperature was also considered. At the Cleveland ACTU, whole blood from 10 HIV-seropositive subjects was collected in seven CPT-C tubes. Three of the CPT tubes were centrifuged in a swinging bucket rotor (GS-6; Beckman Instruments, Fullerton, Calif.) for 20 min at room temperature at $1,500 \times g$ within 2 h of blood collection. The plasma fraction was removed and then centrifuged for 10 min at $400 \times g$ to remove PBMCs. The plasma prepared from one of the tubes was frozen immediately at -70°C , and the remaining two tubes were left at 4°C . Plasma was processed from these tubes at 8 and 30 h postcollection and frozen at -70°C . The additional CPT tubes drawn at the same times were held as whole blood (no immediate centrifugation) at room temperature and 4°C , respectively. The unseparated whole blood in CPT tubes was then centrifuged (parameters described above) at 8 and 30 h postcollection, and plasma was stored at -70°C for batch bDNA analysis.

Effects of anticoagulant and collection tube type. An experiment was designed to compare the amounts of RNA signals from several tubes containing different anticoagulants at a single time point. At the Palo Alto ARC, whole blood from 10 HIV-seropositive subjects was collected in CPT-E, CPT-H, and CPT-C tubes; in V-E, V-H, and V-ACD tubes; and in SST tubes. The CPT and SST tubes were centrifuged in a swinging bucket rotor (Beckman GS-6) for 20 min at room temperature at $1,500 \times g$ within 2 h of collection. For the CPT tubes, the plasma fraction was removed and then centrifuged for 10 min at $400 \times g$ to remove PBMCs. The standard VACUTAINER tubes (V tubes) containing whole blood were centrifuged for 10 min at $400 \times g$ within 2 h of collection. The V-tube plasma fraction was then removed and clarified with an additional 30-min centrifugation at $400 \times g$ in a swinging bucket rotor (Beckman GS-6). Plasma and serum were frozen at -70°C within 2 h of collection.

Effect of benchtop storage time. An experiment to further assess within-day and next-day RNA signals in immediately separated plasma from V-ACD tubes compared with SST, CPT-C, and PPT tubes was designed. At the Cleveland ACTU, whole blood from 10 HIV-seropositive subjects was collected in SST, V-ACD, PPT, and CPT-C tubes. The V-ACD, CPT, PPT, and SST tubes were centrifuged in a swinging bucket rotor (Beckman GS-6) for 20 min at room temperature at $1,500 \times g$ within 2 h of collection. For the CPT tubes, the plasma fraction was removed and then centrifuged for 10 min at $400 \times g$ to remove PBMCs. An aliquot of plasma from the V-ACD tubes and an aliquot of serum from the SST tubes were processed and frozen at -70°C within 2 h of collection. The remaining plasma was removed from the V-ACD blood collection tube and transferred to a separate plastic tube. The PPT and CPT-C tubes were either processed within 2 h of collection (plasma frozen at -70°C) or held at room temperature as clarified plasma and as a plasma-PBMC suspension, respectively. At 6 and 30 h postcollection, aliquots of plasma from V-ACD, PPT, and CPT-C tubes were processed and then frozen at -70°C .

Effects of anticoagulant, collection tube type, and benchtop storage time. An experiment was designed to compare next-day recovery of plasma RNA equivalents from all of the collection devices. At the Palo Alto ARC, whole blood from an additional 10 HIV-seropositive subjects was collected in duplicate CPT-C, CPT-H, and CPT-E tubes; in V-ACD, V-H, and V-E tubes; and in sodium citrate PPT tubes. All CPT and PPT tubes were centrifuged in a swinging bucket rotor (Beckman GS-6) for 20 min at room temperature at $1,500 \times g$ within 2 h of collection. For the CPT tubes, the plasma fraction was removed at either 2 or 30 h (stored at room temperature) and then centrifuged for 10 min at $400 \times g$ to remove PBMCs. One set of V tubes containing whole blood was centrifuged for 10 min at $400 \times g$ within 2 h of collection. The V-tube plasma fraction was then removed and clarified with an additional 30-min centrifugation at $400 \times g$ in a swinging bucket rotor (Beckman GS-6). An aliquot of each plasma type was frozen at -70°C within 2 h of collection. An additional V tube of each type containing whole blood was stored at room temperature for 30 h. The V tubes

containing whole blood were processed as described above to remove residual leukocytes and platelets with a 30-min centrifugation at $400 \times g$. Plasma from the CPT, PPT, and V tubes was then frozen at -70°C .

In addition to a 2-h collection and processing time, which could be considered ideal, 6- to 8- and 30-h postcollection times were chosen for these experiments to best simulate sample processing time during the day of collection and after overnight shipment. All serum and plasma samples were then batch assayed for virion-associated HIV RNA within 2 weeks of collection.

HIV-1 RNA quantification by the bDNA assay. HIV type 1 (HIV-1) RNA was quantified by bDNA signal amplification-based hybridization (7, 10, 13, 14) using the Quantiplex HIV RNA assay kit according to the instructions of the manufacturer (Chiron Corporation, Emeryville, Calif.). Briefly, virus was concentrated from duplicate 1-ml plasma or serum specimens by centrifugation in a benchtop microcentrifuge (centrifuge model 17RS, rotor 3753; Heraeus Sepratech, South Plainfield, N.J.) at $23,500 \times g$ for 1 h at 4°C . The virus pellet was extracted in a buffer containing proteinase K, lithium lauryl sulfate, and target probes complementary to the HIV-1 *pol* gene and then transferred to microwells coated with a capture probe. The RNA-probe complex was captured on the surface of the microwell during an overnight incubation via hybridization of the solid-phase capture probe with a subset of the target probes. The wells were washed and the bDNA amplifier molecule was hybridized to the immobilized target-probe complex, and then multiple alkaline phosphatase-labeled probes were hybridized to each bDNA molecule. The complex was then incubated with a chemiluminescent substrate (dioxetane), and the light emission was measured in a luminometer (Chiron Corporation). The light emission was proportional to the amount of labeled probe bound to captured target RNA. The quantity of RNA in a specimen, expressed as HIV RNA equivalents per milliliter, was determined from a standard curve with a dynamic range of 1×10^4 to 1.6×10^6 HIV RNA eq/ml of plasma.

Statistical analysis. All analyses were carried out by using the nonparametric, two-sided Wilcoxon signed rank test. The quantitative virologic markers to be measured were assumed to neither change over time nor differ between the types of collection tubes used. Significance was defined as a P of <0.05 .

RESULTS

Characteristics for the 40 HIV-infected subjects involved in this study are as follows. The average CD4⁺ lymphocyte count $\pm$ standard deviation was 123 ± 121 cells per mm^3 . Twelve of the subjects had not received any Food and Drug Administration-approved or investigational antiretroviral therapy in the previous 3 months. Twenty-eight subjects were currently on an antiretroviral regimen. Twenty of the 40 subjects had symptomatic disease (Centers for Disease Control classes B2 through C3) (1).

Effects of storage time and temperature. Blood was collected in CPT tubes and held at either 4°C or room temperature for up to 30 h. The mean RNA levels obtained for 7 of 10 evaluable subjects who had $>10^4$ HIV RNA equivalents per ml of plasma are presented in Table 1. The mean CD4⁺ count for this group was 165 cells per mm^3 , with a range of 30 to 500/ mm^3 . All seven were currently receiving antiretroviral therapy. Centrifuging the CPT tubes within 2 h of collection resulted in plasma that yielded stable RNA quantitation for up to 30 h postcollection, even when the plasma was left in the tubes held at 4°C . In contrast, not centrifuging the tubes (i.e., leaving the blood whole) resulted in plasma with diminished RNA levels compared with those of the centrifuged plasma. The worst conditions were obtained by storing the uncentrifuged tubes at room temperature; for instance, relative to RNA recovery with plasma prepared by centrifuging CPT tubes within 2 h of collection, leaving whole blood in the CPT tubes at room temperature for 8 and 30 h resulted in recoveries of RNA that were 58 and 44% ($P = 0.016$ and 0.018), respectively. Mean RNA levels from whole blood held at 4°C for 8 and 30 h declined to 82 and 56% of the 2-h levels, respectively ($P = 0.018$). Mean RNA levels were significantly higher at the 8-h sampling time for whole blood held at 4°C than for whole blood held at room temperature ($P = 0.028$). RNA levels at the 30-h sampling time were also higher when the samples were held as whole blood at 4°C than when they were kept at room temperature, and they almost reached significance ($P =$

TABLE 1. Effects of processing time and temperature on HIV RNA levels in plasma from sodium citrate CPT tubes

Sampling time (h) ^a	HIV RNA level (% of 2-h value; <i>P</i>) under the indicated conditions ^b		
	Spun CPT, 4°C	Unspun CPT, 4°C	Unspun CPT, 22°C
2	293 ± 139		
8	299 ± 163 (102; NS)	241 ± 127 (82; 0.018)	169 ± 81 (58; 0.016)
30	299 ± 158 (102; NS)	164 ± 80 (56; 0.018)	128 ± 57 (44; 0.018)

^a Time that plasma was separated from unspun and spun CPT tube samples and frozen at -70°C. *n* = 7 subjects.

^b RNA values are expressed as 10³ RNA equivalents per milliliter of plasma (means ± standard errors of the means [SEM]). Comparisons of signal decay between hours are expressed as percentages of the 2-h values. NS, not significant.

0.07). These results demonstrate that if plasma cannot be prepared immediately, for maximum RNA recovery, CPT tubes should be centrifuged soon after specimen collection and then stored refrigerated at 4°C.

Effects of anticoagulant and collection tube type. Comparative quantification of HIV RNA in plasma specimens from 10 subjects as measured in eight different collection tubes is presented in Table 2. The mean CD4⁺ count for this group was 249 cells per mm³, with a range of 35 to 550 cells per mm³. Seven of the 10 subjects were currently receiving antiretroviral therapy. Plasma samples were prepared within 2 h of collection and stored at -70°C. The mean RNA copy number was significantly higher in the CPT-C tubes than in the CPT-H and V-H tubes (*P* = 0.0048 and 0.009, respectively) and significantly higher in plasma from V-E tubes than from V-ACD and V-H tubes (*P* = 0.049 and 0.018, respectively). Levels in plasma from V-E tubes were comparable to those for CPT-E tubes. RNA levels in serum were significantly lower than those in any kind of plasma (*P* = 0.0009). Thus, in this experiment, the greatest recovery of HIV RNA was obtained when blood was collected in V-E or CPT-E tubes.

Effect of benchtop storage time. The stability of HIV RNA in plasma that had been removed within 2 h from cells and left at room temperature for 6 and 30 h was assessed next. Six of 10 subjects had HIV RNA equivalents in plasma of >10⁴/ml and therefore could be included for analysis. The mean CD4⁺ count for this group was 160 cells per mm³, with a range of 4 to 520 cells per mm³. Five of the six subjects were currently receiving antiretroviral therapy. As presented in Table 3, the 2-h levels in serum were significantly lower than the levels in plasma. The mean levels of RNA in plasma decreased after 30 h of storage at room temperature in comparison with levels in plasma that was frozen within 2 h. RNA levels were least stable in plasma prepared from V-ACD tubes; after 6 and 30 h of storage, 87% (*P* = 0.11) and 60% (*P* = 0.0039) of the RNA was recovered, respectively, compared with that recovered from plasma frozen within 2 h of collection. RNA levels in plasma obtained from the other tubes (CPT-C and PPT) also declined with time; however, 100 and 95% of the original

values were maintained after 6 h (*P* = 0.20) and 86 and 86% were maintained after 30 h, respectively (*P* = 0.15 and 0.16, respectively). After storage at room temperature for 30 h, there were no differences in RNA levels between plasma from PPT and CPT-C tubes; however, RNA levels were lower in plasma from V-ACD tubes (*P* < 0.05).

Effects of anticoagulant, collection tube type, and benchtop storage time. Testing of the effect of storing whole blood in the collection device for up to 30 h on RNA levels was next expanded to include seven types of tubes. The results for 10 additional subjects are presented in Table 4. The mean CD4⁺ count for this group was 118 cells per mm³, with a range of 7 to 495 cells per mm³. Five of the 10 subjects were currently receiving antiretroviral therapy. By 30 h, the mean RNA levels in plasma had decreased irrespective of the collection device. The smallest decrease occurred in plasma from V-E tubes (13%). This decrease was not statistically significant. The mean RNA level declined by 27 to 28% in plasma from CPT-C and CPT-H tubes (*P* = 0.036 and 0.046, respectively). The mean RNA levels in plasma from V-H and V-ACD tubes declined by 34 and 33%, respectively (*P* = 0.009 and 0.05, respectively). Mean RNA levels in PPT tube plasma declined by 33% (*P* = 0.0069). After 30 h of storage, the largest amount of HIV RNA was found in plasma prepared from V-E tubes, and this amount was statistically significant (*P* = 0.009) compared with those for all other tubes.

DISCUSSION

Overall, the support in the literature for the stability of HIV viral load markers (especially HIV RNA in plasma) with respect to sampling processing is minimal. This lack of controlled data has left it up to the investigator to define the acceptable parameters for specimen collection and storage. We chose to study the effect of storing whole blood versus plasma and to compare sample storage at room temperature and 4°C in an attempt to mimic the conditions encountered in a multicenter clinical trial. Repeated sample mixing and the temperature changes observed in transport could not be standardized for the purpose of this study and were not evaluated for their effect on viral marker stability.

We have attempted to identify the blood collection tubes, storage conditions, and processing times that result in optimal stability of plasma HIV RNA levels, using the bDNA assay. Thus, the information presented here for HIV RNA stability in plasma does not necessarily reflect RNA stability determined by using RT-PCR or nucleic acid sequence-based amplification (NASBA). In our investigation, we evaluated new collection devices, namely, the VACUTAINER CPT and PPT tubes, as well as V tubes. The data presented here demonstrate that HIV virion RNA in plasma, as measured by a DNA signal amplification assay, can be detected in most types of collection tubes. However, significant quantitative differences in RNA

TABLE 2. Effect of collection tube on plasma HIV RNA levels when plasma was separated and frozen within 2 h of collection^a

Tube type	10 ³ RNA eq/ml of plasma (mean ± SEM)
V-ACD	127 ± 32
V-H	106 ± 24
V-E	147 ± 37
CPT-C	127 ± 31
CPT-E	140 ± 36
CPT-H	89 ± 21
SST	55 ± 15

^a *n* = 10 subjects.

TABLE 3. Stability of HIV RNA levels in plasma stored at room temperature

Sampling time (h) ^a	HIV RNA level (% of 2-h value; <i>P</i>) with the following tube type ^b :			
	V-ACD	PPT	CPT-C	SST
2	74 ± 23	77 ± 22	62 ± 20	39 ± 9
6–8	64 ± 22 (87; NS)	73 ± 25 (95; NS)	62 ± 22 (100; NS)	
30	44 ± 13 (60; 0.0038)	67 ± 21 (86; NS)	54 ± 17 (86; NS)	

^a Time that plasma was removed from the collection device and frozen at -70°C. *n* = 6 subjects, except for SST, for which *n* = 5 subjects.

^b RNA values are expressed 10³ RNA equivalents per milliliter of plasma (means ± SEM). Comparisons of signal decay between hours, within tubes, are expressed as percentages of the 2-h values. NS, not significant.

levels were evident in plasma obtained from the different blood collection tubes, and serum was clearly inferior to any kind of anticoagulated plasma. Plasma containing heparin produced lower RNA levels than other anticoagulants in both V and CPT tubes. The reason for this decline is not clear. PCR technology has been shown to be inhibited by the presence of heparin (4). However, the bDNA assay utilizes signal amplification, not PCR-based template amplification. We have noted that equal recoveries can be obtained when cell-free HIV is added to paired seronegative plasma containing EDTA and heparin (data not shown). Thus, heparin does not appear to interfere with the bDNA quantification. V-E tubes produced a higher RNA level than CPT-E or other anticoagulant-containing tubes. This may in part be related to an anticoagulant dilution phenomenon. V-E tubes contain only 50 µl of liquid EDTA as an anticoagulant (or may be internally spray coated), whereas V-ACD tubes contain 1.5 ml and CPT tubes contain 1 ml of anticoagulant. In addition, plasma from CPT tubes (but not from PPT tubes) contains 0.8 ml of Ficoll-Hypaque after centrifugation. Thus, a plasma sample, and hence RNA copy number, could be diluted by as much as 20% in these tubes compared with V-E tubes. Another possibility for differences in plasma RNA quantification may be related to virion absorption to platelets in "cell-free" plasma. We have found that <5% of RNA signal is platelet associated (5). This is consistent with previously published case studies (12). Plasma from PPT and CPT tubes contains essentially no platelets because of the efficient removal of platelets via centrifugation through the gel barrier. Variable levels of platelets can remain in plasma in V tubes. Thus, a small contribution to RNA signal could potentially be made by residual platelets.

Without removal of plasma from the collection tube, all samples demonstrated a decrease in RNA levels after 30 h at room temperature. However, immediately separated plasma

from CPT and PPT tubes and whole blood from V-E tubes maintained higher HIV RNA levels longer than plasma from V-ACD and V-H tubes. Generally, there appears to be an advantage in separating plasma from whole blood within 2 h of collection to maintain RNA levels. However, a nonsignificant decrease (13%) in HIV RNA levels was noted in plasma prepared from V-E tubes even after 30 h of storage as whole blood. Similar data have been reported for infectious plasma viremia, in which 38% of positive plasma cultures were found to have a fall in infectious titer when plasma was separated from whole blood 24 h postphlebotomy (15). In contrast, plasma that was immediately separated from whole blood and stored at 4°C was found to have no significant change in tissue culture infective dose over a 48-h period (8). Coombs et al. also reported that the titer of HIV in plasma remained unchanged up to 6 h postdraw when blood samples were left whole (2). In addition, they found that HIV RNA in plasma as measured by RT-PCR after immunocapture, when plasma was separated from whole blood, remained stable for up to 6 days. The discrepancies noted with these previous reports may be due to the lack of reproducibility of the method utilized in the specific studies and hence the inability of the method to discern changes in the level of the material measured. Recent reports have demonstrated that the bDNA assay can accurately measure changes as small as 1.4-fold in plasma RNA levels (13). Although the number of subjects we analyzed in each group is small, significant differences could still be observed. Thus, we believe that the current report more accurately reflects the stability of HIV RNA in plasma.

We have previously reported on the advantage of the VACUTAINER CPT system in maintaining stability of HIV RNA in plasma as measured by RT-PCR and bDNA techniques (7). The data presented here greatly expand on the previous observations because the effects of different anticoagulants, collection tube types, and storage temperatures were studied. Our data support the fact that most blood collection tubes can be used to sample HIV RNA in plasma by using bDNA technology. However, the preferable blood collection system is the V-E tube when maximal RNA signal after both 2 and 30 h of storage using bDNA technology is desired. Although plasma HIV RNA levels were somewhat less, benefits of the CPT and PPT closed-tube system included limited personnel exposure to the infected blood product at the clinical site, immediate separation and decreased sample processing time at both the clinic and the reference laboratory, and a limited decline of plasma viral load markers. In addition, the CPT tube separates PBMCs and plasma from whole blood with one simple centrifugation step.

With the observed decrease in RNA signal over time, the length of time to sample processing, including storage, should be standardized. Although these detectable and potentially significant falls may be rectified by immediate separation and refrigeration of a plasma sample, this level of processing would

TABLE 4. Effects of sampling time, collection tube, and anticoagulant on plasma HIV RNA levels

Tube type	HIV RNA level (% of 2-h value; <i>P</i>) at the following sampling time ^a :	
	2 h	30 h
V-ACD	142 ± 47	91 ± 30 (64; 0.05)
V-H	101 ± 33	64 ± 31 (66; 0.009)
V-E	156 ± 52	137 ± 48 (87; NS)
PPT	136 ± 44	92 ± 31 (67; 0.0069)
CPT-C	126 ± 41	90 ± 33 (72; 0.036)
CPT-E	153 ± 52	80 ± 26 (52; 0.0069)
CPT-H	94 ± 30	60 ± 19 (73; 0.046)

^a Sampling time, time that plasma was removed from the collection device and frozen at -70°C. *n* = 10 subjects. RNA levels are expressed as 10³ RNA equivalents per milliliter of plasma (means ± SEM). Comparisons of signal decay between hours, within tubes, are expressed as percentages of the 2-h values. NS, not significant.

limit the areas where clinical blood samples could be processed postcollection. Given that one tube may produce a higher value than another for a particular assay, as with the RNA detection assay used here, it would be best to perform an entire study with a particular type of tube and a standard processing time.

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