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Evaluation of the VACUTAINER PPT Plasma Preparation Tube for Use with the Bayer VERSANT Assay for Quantification of Human Immunodeficiency Virus Type 1 RNA

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Separation and storage of plasma within 2 h of phlebotomy is required for the VACUTAINER PPT Plasma Preparation Tube (PPT) versus 4 h for the predecessor VACUTAINER EDTA tube for human immunodeficiency virus type 1 (HIV-1) viral load (HIVL) testing by the VERSANT HIV-1 RNA 3.0 assay (branched DNA). The 2-h limit for PPT imposes time constraints for handling and transporting to the testing laboratory. This study compares HIVL reproducibility from matched blood in EDTA tubes and PPTs and between PPT pairs following processing within 4 h of phlebotomy, stability of plasma HIV-1 RNA at 24- and 72-h room temperature storage in the tube, and comparative labor and supply requirements. Blood from 159 patients was collected in paired tubes (EDTA/PPT or PPT/PPT): 86 paired EDTA tubes and PPTs were processed 4 h following phlebotomy and their HIVLs were compared, 42 paired PPT/PPT pairs were analyzed for intertube HIVL reproducibility, and 31 PPT/PPT pairs were analyzed for HIV-1 RNA stability by HIVL. Labor and supply requirements were compared between PPT and EDTA tubes. PPTs produce results equivalent to standard EDTA tube results when processed 4 h after phlebotomy. PPT intertube analyte results are reproducible. An average decrease of 13% and 37% in HIVL was observed in PPT plasma after 24 and 72 h of room temperature storage, respectively; thus, plasma can be stored at room temperature up to 24 h in the original tube. PPTs offer labor and supply savings over EDTA tubes.

The BD VACUTAINER PPT Plasma Preparation Tube (PPT) (Becton Dickinson and Company, Franklin Lakes, NJ) has replaced the BD VACUTAINER EDTA tube for human immunodeficiency virus type 1 (HIV-1) viral load (HIVL) testing (3, 4, 5). The PPT, unlike the EDTA tube, contains a gel plug that physically separates cells from plasma during centrifugation to facilitate easy handling, storage, and freezing. This differs from the EDTA tube. Following centrifugation, plasma from the EDTA tube must be manually transferred to a storage tube by an aseptic technique using a sterile, disposable pipette and then frozen. Compared to the PPT, the EDTA tube requires additional labor and introduces a potential source of error in specimen labeling.

The PPT has been validated for use with the Bayer VERSANT HIV-1 RNA 3.0 assay (branched DNA [bDNA]) (hereafter referred to as the HIV-1 bDNA assay; Bayer HealthCare LLC, Tarrytown, NY) but specifies a maximum 2-h holding time from phlebotomy to sample processing (3). This is in contrast to the maximum 4-h holding time validated for the EDTA tube (3). The reduced holding time for the PPT imposes stringent time limitations on transporting samples from the patient to the laboratory for sample processing and hence may restrict its use for HIV-1 bDNA assays unless alternative processing options are available. Blood collection into duplicate PPTs may be necessary for different test application requirements, and replacement of EDTA tubes with PPTs may affect work flow and supply requirements for HIVL testing.

In this report, we (i) compare plasma HIVLs on matched plasma from EDTA tubes versus PPTs processed 4 h following phlebotomy, (ii) determine the stability of HIV-1 RNA HIVL in plasma stored in PPTs at room temperature for 24 and 72 h, (iii) evaluate intertube HIVL reproducibility between matched samples collected in duplicate PPTs, and (iv) establish the comparative labor and supply requirements for the EDTA tubes versus PPT.

MATERIALS AND METHODS

Blood collection tubes. Both the EDTA tubes and PPTs generate a final K_2EDTA concentration of 1.8 mg/ml in blood when filled to the draw volume (product package insert). The PPT contains a polyester plug that physically separates cellular components from the plasma during centrifugation and maintains this separation postcentrifugation (4).

Blood collection. EDTA tubes and/or PPTs (7 ml) were collected during one phlebotomy session for each study aim at the San Francisco General Hospital Positive Health Program outpatient clinics and transported to the San Francisco General Hospital Clinical Laboratory. Tubes were centrifuged 4 h postphlebotomy at 1,100 × *g* for 10 min. Sterile plasma aliquots were prepared from each EDTA tube, transferred to screw cap polypropylene tubes, and, together with PPTs, frozen at −70°C. Plasma samples from EDTA/PPT pairs or PPT/PPT pairs were simultaneously processed and frozen.

Committee on Human Research. This study was performed in accordance with the guidelines of the Committee on Human Research of the University of California, San Francisco. To preserve patient confidentiality, specimens were unlinked to identifier information, including date, patient identifier, patient name, and health center identifier.

Comparison of HIVL values between matched patient plasma samples from paired EDTA tubes and PPTs. One EDTA tube and one PPT were collected from each of 86 patients and processed as described in this report.

Stability of plasma HIV-1 RNA in PPTs held at room temperature for 24 and

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72 h following centrifugation. Two PPTs were collected from each of 31 patients and processed as described in this report. A 1-ml aliquot of plasma was removed at baseline ($t = 0$) from one PPT in each pair by use of a sterile technique and stored immediately at −70°C. The remaining plasma was kept at room temperature (20°C to 23°C) in the original PPT. At 24 and 72 h postcentrifugation, the PPT was vortexed and 1-ml aliquots were removed from each pair and frozen at −70°C. Of 31 PPT pairs, 12 did not contain sufficient plasma for a 72-h aliquot.

Evaluation of intertube reproducibility by HIVL between matched samples collected in duplicate PPTs. Two PPTs were collected from each of 42 patients and processed as described in this report.

Comparison of labor and supplies for EDTA tubes versus PPT. Labor (hands-on time) and supplies required to process one EDTA tube were compared to those required to process one PPT. Time included application of a preprinted patient label and placement and removal of the tube from the centrifuge. Additional time for one EDTA tube included application of a preprinted patient label to a screw cap collection tube and transfer of plasma from the EDTA tube to a 5-ml polypropylene screw-cap tube. Supplies included all disposables used to isolate and store the plasma.

Quantitative HIVL measurements. Plasma-derived HIV-1 RNA was quantified using HIV-1 bDNA assays according to the manufacturer's instructions. All paired plasma samples (EDTA/PPT and PPT/PPT) as well as each plasma sample series for the HIV-1 RNA time course stability were tested in batch in the same run to control for experiment variability.

Statistical analyses. The HIVLs were analyzed on the log scale and evaluated by comparing the mean log quantification results for the reference and test conditions. Only values above the assay cutoff (50 copies/ml) were used in the calculations. Comparative HIVLs were considered equivalent when the 90% confidence interval of the log difference was within 0.16 $\log_{10}$ copies/ml. A difference of ± 0.16 $\log_{10}$ would result in a shift of up to 5% in percent concordance between two viral load measurements. Percent concordance is defined as the percentage of comparative HIVLs that are not statistically different. Consistency of log differences across the dynamic range of the assays was assessed using the Bland-Altman procedure (1). For each comparative HIVL assay the difference between the log quantification and the average log quantification was calculated. Log differences were plotted against their respective log means, and a linear regression line was calculated for these points. A slope significantly different from zero would indicate a proportional bias in log differences across the quantification range. The 95% confidence limits for individual log differences were drawn on the plot along with a horizontal reference line at the mean log difference.

Percent coefficient of variation was calculated for intertube HIVL variability between matched samples collected in duplicate PPTs.

RESULTS

Comparison of HIVL values between matched patient plasma samples from paired EDTA tubes and PPTs. HIVLs were determined in plasma from 86 patient plasma samples. Plasma HIVL from EDTA tubes ranged as follows: 30 samples at <50 copies/ml, 14 samples at 51 to 1,000 copies/ml, 15 samples at 1,001 to 10,000 copies/ml, 17 samples at 10,001 to 100,000 copies/ml, and 10 samples at >100,000 copies/ml. Plasma HIVL from matched PPTs ranged as follows: 31 samples at <50 copies/ml, 14 samples at 51 to 1,000 copies/ml, 15 samples at 1,001 to 10,000 copies/ml, 19 samples at 10,001 to 100,000 copies/ml, and 7 samples at >100,000 copies/ml. One plasma EDTA tube plasma sample with detectable viral load (65 copies/ml) was <50 copies/ml in the matched plasma PPT sample. The EDTA results were on the average 0.094 $\log_{10}$ higher than PPT results. The differences in results between the two were considered equivalent, as the 90% confidence interval of the mean $\log_{10}$ difference of 0.094 was within the specification of ± 0.16 $\log_{10}$ copies/ml. There was no significant difference from a slope of 0 for viral load values across the range analyzed (Fig. 1).

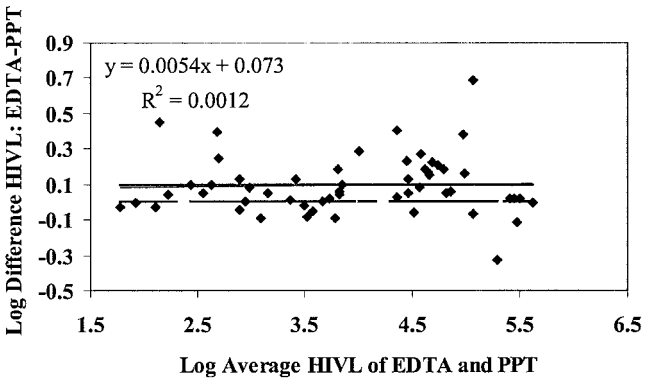

FIG. 1. Comparison of HIVL values for blood collected in PPT versus EDTA tubes. One EDTA tube and one PPT were collected from each of 86 patients, and plasma was isolated and tested for HIVLs using the HIV-1 bDNA assay.

Stability of plasma HIV-1 RNA in PPTs held at room temperature for 24 and 72 h following centrifugation. The HIVL was evaluated in plasma collected in PPTs from 31 patients following centrifugation at 4 h post blood collection. Plasma HIVLs at $t = 0$ h included: 8 samples at 51 to 1,000 copies/ml, 11 samples at 1,001 to 10,000 copies/ml, 8 samples at 10,001 to 100,000 copies/ml, and 4 samples at >100,001 copies/ml. At 24 h there was a log difference of −0.06 $\log_{10}$ (95% confidence interval, −0.11, −0.01), or a 13% reduction in mean quantification, from baseline values. The HIVLs were considered equivalent to baseline, as the 90% confidence interval for the log difference was within ± 0.16 $\log_{10}$. At 72 h, there was a log difference of −0.20 $\log_{10}$ (95% confidence interval, −0.26, −0.14) or a 37% reduction in mean quantification from baseline. The HIVLs at 72 h were not considered to be equivalent to baseline (Table 1).

Evaluation of intertube reproducibility by HIVL between matched samples collected in duplicate PPTs. Matched plasma from duplicate PPTs was evaluated from 42 patients. The plasma HIVLs from PPT 1 were as follows: 16 samples at <50 copies/ml, 5 samples at 51 to 1,000 copies/ml, 9 samples at 1,001 to 10,000 copies/ml, 8 samples at 10,001 to 100,000 copies/ml, and 4 samples at >100,001 copies/ml. The plasma HIVLs from PPT 2 were as follows: 16 samples at <50 copies/ml, 6 samples at 51 to 1,000 copies/ml, 8 samples at 1,001 to 10,000 copies/ml, 7 samples at 10,001 to 100,000 copies/ml, and 5 samples at >100,001 copies/ml. Plasma HIVLs for both paired tubes from all samples were equivalent, with a mean log difference of 0.0016 and a coefficient of variation of 17.6% (Fig. 2). This is comparable to the mean within-run percent coefficient of variation reported for the product (3).

TABLE 1. Stability of PPT plasma held at room temperature (24 and 72 hours) postcentrifugation

Time (h)	HIVL (log mean)	90% Confidence interval		Log difference with baseline	90% Confidence interval difference ($\log_{10}$)	
0	3.69	3.65	3.72			
24	3.63	3.59	3.66	−0.06	−0.11	−0.01
72	3.49	3.44	3.54	−0.20	−0.26	−0.14

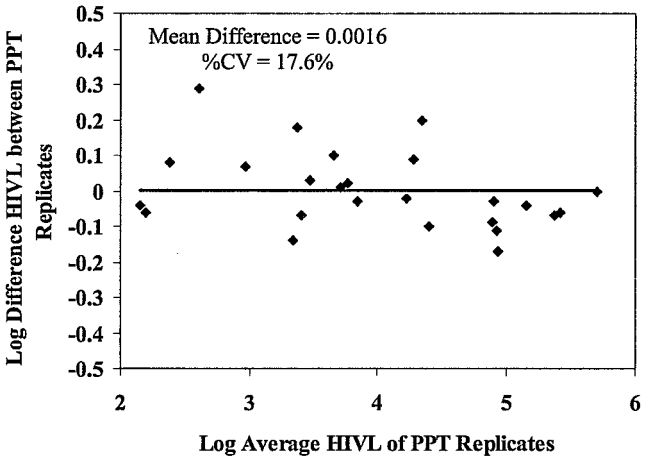

FIG. 2. Comparison of HIVL values for blood collected in duplicate PPT. Two PPTs were collected from each of 42 patients, and plasma was isolated and tested for HIVLs using a HIV-1 bDNA assay.

Comparative labor and supply requirements for EDTA tubes versus PPT. The EDTA tubes and PPT require approximately 20 s of hands-on time each for application of a preprinted patient laboratory label and placement and removal from the centrifuge. The EDTA tube required an additional 35 s for application of a preprinted patient laboratory label to a 5-ml screw-cap polypropylene tube, aspiration, and transfer of plasma from the EDTA tube to the labeled 5-ml screw-cap polypropylene tube by use of a disposable, sterile transfer pipette. In this approach, the PPT required approximately 20 s processing time, while EDTA tubes required approximately 55 s processing time, an increase of 175% in labor, as well as additional disposables, including a 5-ml screw-cap polypropylene tube, a sterile transfer pipette, and an additional patient label.

DISCUSSION

The EDTA tube was validated for use with the Bayer VERSANT HIV-1 bDNA 3.0 assay, which specifies a maximum 4 h of hold time between phlebotomy and centrifugation and storage (3). In this report we demonstrate the presence of equivalent HIVLs in matched plasma from PPT and EDTA tubes centrifuged and stored 4 h after phlebotomy. Following centrifugation and then storage in the PPT for 24 h at room temperature, the plasma HIVLs were 13% lower than baseline (i.e., a log difference of -0.06 $\log_{10}$) but were within the defined specification of $\pm$ 0.16 $\log_{10}$ HIV-1 copies/ml.

The 2-h holding time between phlebotomy and centrifugation was arbitrarily applied in an earlier study evaluating six plasma samples collected in PPTs (>10,000 copies of HIV-1 RNA detected by HIV-1 bDNA assay version 1.0), which demonstrated a 5% and 14% decline in plasma HIVL at 6 and 30 h of room temperature storage, respectively (5). The arbitrary 2-h holding time between phlebotomy and centrifugation for PPTs, in contrast to the established 4-h holding time for EDTA tubes, was subsequently used for the HIV-1 bDNA assay clinical trial (3) and consequently used as a validation specification of the PPT for use with HIV-1 bDNA assays. We now show that PPTs, in similarity to EDTA tubes, can be processed within 4 h following phlebotomy for HIV-1 bDNA assays.

In a recent study, handling and storage conditions were evaluated for PPTs versus EDTA tubes on 19 samples with >5,000 copies/ml (4) as measured by the AMPLICOR HIV-1 MONITOR assay (Roche Diagnostics, Branchburg, NJ). The study concluded that whole blood can be stored in PPTs for 6 h prior to centrifugation and that, following centrifugation, samples can be shipped overnight in the original PPT without any significant decline in HIVL (4). However, it is unclear whether these findings can be directly applied for testing by HIV-1 bDNA assay, as the technologies are dissimilar. Moreover, detection by AMPLICOR HIV-1 MONITOR utilizes one probe directed against a 142-base amplicon (6) while the HIV-1 bDNA assay incorporates 96 probes across the polymerase gene (2). Nevertheless, both that report (4) and our current findings reported in this paper suggest the handling conditions for PPTs are fairly similar for both assays.

This study is the first to demonstrate reproducible HIVL in patient-matched plasma derived from duplicate PPTs. This finding is significant in light of the tube-size option limitations, storage options, and plasma requirements for various tests. This is particularly important in terms of clinical trials when multiple PPTs are often obtained from the same patient for various HIV-1-based tests; the results can now assuredly be considered relative to each other.

Finally, as aliquoting is not required for isolating plasma from the PPT, it offers considerable labor savings as well as fewer disposable requirements than the EDTA tube and reduces the risk of cross contamination. These finding can be used to improve work-flow condition, reduce costs through lesser disposable requirements, and potentially reduce sample labeling errors.

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