

Evaluation of Specimen Handling Conditions in BD Vacutainer® Plasma Preparation Tube HIV-1 Viral Load as Measured by the Abbott RealTime HIV-1 Assay

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INTRODUCTION

A number of studies have been conducted to determine the effect of specimen handling parameters on the measurement of HIV viral load. Results of some studies indicated that freezing of plasma *in situ* in the BD Vacutainer® Plasma Preparation Tube (PPT) resulted in an elevated HIV-1 viral load when quantified in the Roche COBAS Amplicor HIV-1 Monitor® assay. An *investigation revealed that the elevated viral loads in specimens that were frozen in PPT (PPT *in situ*) were possibly due to the presence of proviral DNA released from cells associated with the gel barrier indicating that the assay does not discriminate between amplification of viral RNA and proviral DNA. The objectives of this study were to:

1. Evaluate the effect of freezing plasma *in situ* in PPT on the accuracy of HIV-1 viral load results using the Abbott RealTime HIV-1 assay, *and*
2. Evaluate the effect of whole blood storage in the PPT for six hours at room temperature prior to centrifugation (PPT6H) instead of two hours as specified in the PPT product insert (PPT control).

* H Wan, P Belem, A Seth and H Fernandes. Co-Amplification of HIV Proviral DNA and Viral RNA in the Roche Cobas Amplicor™ HIV-1 Monitor Assay. Poster presented at the **Clinical Virology Symposium 2006**.

MATERIALS AND METHODS

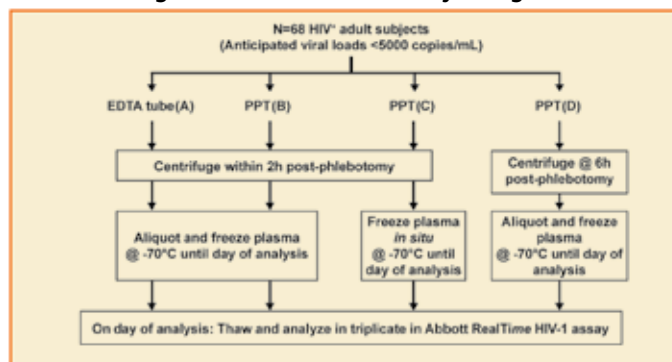
STUDY DESIGN

A total of 68 consented adult HIV+ subjects whose viral loads in previous tests were less than 5000 copies/mL participated in the study. (Figure 1)

- One 10 mL, BD Vacutainer® K₂EDTA Tube (EDTA), and three 8.5 mL PPTs were collected from each subject.
- The EDTA tube and two PPTs were centrifuged within two hours of collection, and the third PPT was centrifuged six hours after collection.
- For each subject, plasma from the EDTA tube and two PPTs was aliquoted and frozen at -70°C.
- Plasma from the third PPT was frozen *in situ* at -70°C until the day of analysis.
- On the day of analysis, all specimens were thawed and tested in triplicate for viral load with the Abbott * assay.

* The average of three samples was used as the viral load count (VLC) for that tube. For statistical purposes, a value of 0 was assigned to samples where HIV was “undetected.”

Figure 1: Schematic of Study Design



Blood Collection Tubes

Tube ID	Tube Description	Draw Volume
A	BD Vacutainer® K ₂ EDTA Tube 16 x 100 mm, K ₂ EDTA, Ref. #366643	10 mL
B,C,D	BD Vacutainer® Plasma Preparation Tube (PPT) 16 x 100 mm, K ₂ EDTA, Ref. #362799	8.5 mL

Acceptance Criteria

- The mean tube bias between the test tube (PPT) and the EDTA tube must be within +/- 0.3 on the Log₁₀ scale with 95% confidence using the average values of triplicate measurements for each tube.
- No more than one subject with undetectable viral load in the EDTA tube will show viral loads ≥ 160 copies/mL in the test tubes (PPTs), provided that at least 17 subjects are tested undetectable.

RESULTS

Results from 64 of the 68 subjects could be statistically evaluated. Twenty-nine (29) subjects had average Log_{10} VLCs of >40 copies/mL (LOQ) in all tubes, while there were 35 subjects for which target was either “undetected” or below the LOQ in all or some of the tubes regardless of handling condition. For the 29 subjects with VLCs $>LOQ$, summary data can be found in **Table 1**.

Mean biases of VLCs between EDTA plasma and PPTs B, C, and D were calculated for subjects with VLCs >40 copies/mL. The mean bias plot and summary data are presented in **Figure 2** and **Table 2** respectively. In these subjects, the mean biases among tubes, regardless of handling conditions, were within ± 0.3 (Log_{10}).

Regression line plots and R^2 comparing VLCs in EDTA plasma to VLCs in PPT control (B), PPT *in situ* (C) or PPT6H (D) tubes are presented in **Figures 3, 4, and 5**. The regression line plot and R^2 for the comparison of PPT control (B) to PPT 6H (D) is shown in Figure 6. All correlation coefficients (r) for all comparisons were >0.96 .

There were 15 subjects for which the target in all EDTA aliquots was “undetected.” For these subjects, the viral load in all PPT aliquots, regardless of sample handling condition, was either undetected or <40 copies/mL.

A VLC frequency for all subjects vs tube type is presented in **Figure 7**.

Table 1: Summary of data for 29 subjects with average VLCs of >40 copies/mL

	EDTA(A)	PPT(B)	PPT <i>in situ</i> (C)	PPT6H(D)
Mean	2.51	2.50	2.52	2.51
Std. Deviation	0.80	0.90	0.85	0.84
Minimum	1.22	0.80	1.11	1.32
Maximum	4.69	4.75	4.85	4.82
No Data / Total	0 / 29	1 / 29	0 / 29	0 / 29

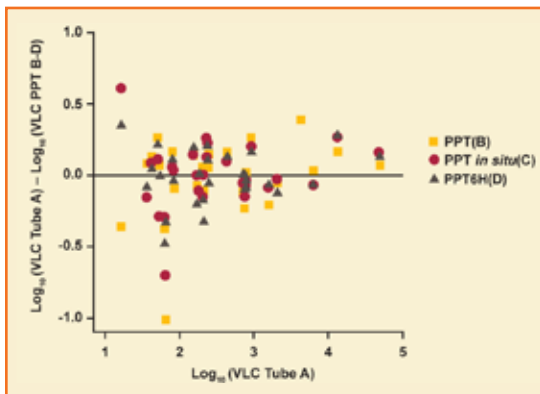
Table 2: Estimated mean bias of PPT tubes versus EDTA tube on the Log_{10} scale

Analyte	*Mean Bias Criterion	PPT(B)	PPT <i>in situ</i> (C)	PPT6H(D)
Log_{10} VLC	± 0.3	-0.026 (-0.133, 0.081)	0.005 (-0.101, 0.110)	-0.001 (-0.107, 0.104)

Numbers in () are the minimum and maximum biases respectively for that tube type in comparison to the EDTA tube (A).

*Mean tube bias criterion used in this study for tubes to be considered equivalent.

Figure 2: Difference plots of PPT (B), PPT *in situ* (C), PPT6H versus EDTA and of PPT *in situ*, PPT6H versus PPT



Graphs show more variability on the Log_{10} scale for small viral load counts. These differences are due to observations under the limit of quantification.

RESULTS

Figure 3: Log-log linear regression of PPT control(B) versus EDTA(A)

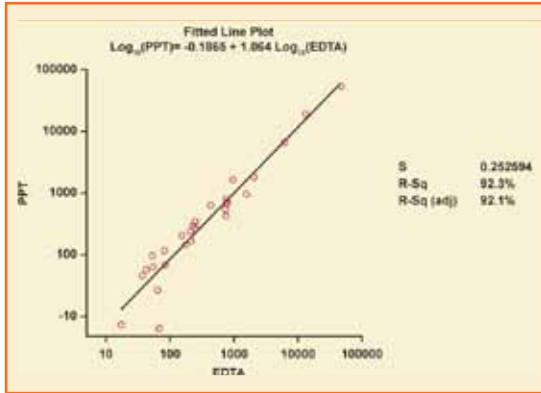


Figure 4: Log-log linear regression of PPT *in situ*(C) versus EDTA(A)

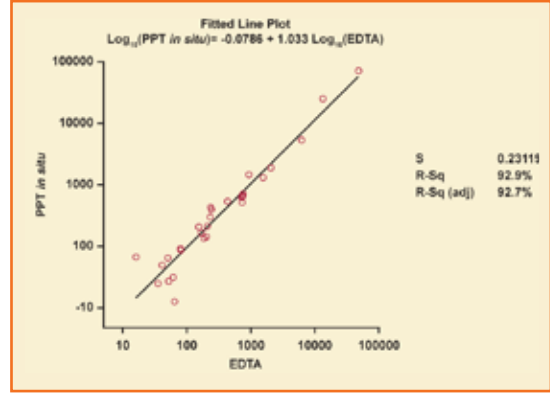


Figure 5: Log-log linear regression of PPT6H(D) versus EDTA(A)

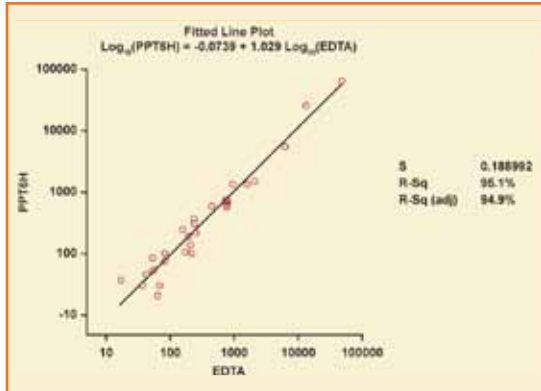


Figure 6: Log-log linear regression of PPT6H(D) versus control PPT tube(B)

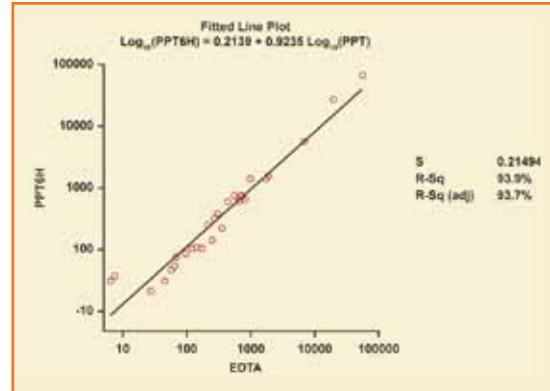
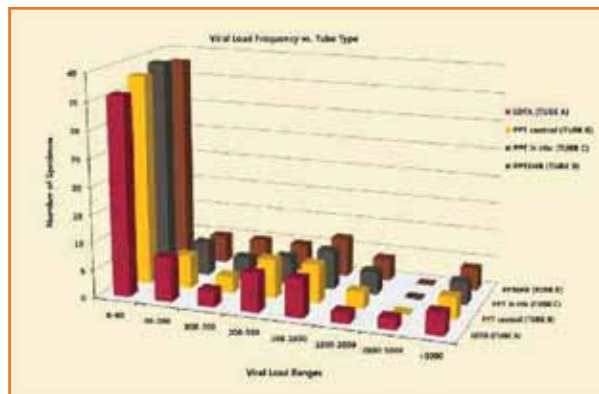


Figure 7: Distribution of VLCs vs. tube type for all 68 subjects



SUMMARY

For subjects with VLCs above the assay LOQ of 40 copies/mL, ANOVA analysis to estimate the mean bias between tubes and log-log linear regression analysis showed that all handling conditions tested were equivalent with 95% confidence and the mean bias between each PPT handling condition and the EDTA tube was shown to be within the $\pm 0.3 \text{ Log}_{10}$ VLC with 95% confidence.

For 35 subjects with VLCs <LOQ for the assay in all tubes, cross tabulation of specimens over/under the LOQ and the Cochran Q's test was performed to check if the rate of detection for these very low viral load subjects is different among the four tubes. The cross tabulation results and the McNemar's p -test yielded the same proportion of subjects over/under the LOQ in all four tubes with an overall p -value of 0.33 indicating that there was no difference in the rate of detection among the four different conditions tested.

CONCLUSIONS

We conclude that using the Abbott RealTime HIV-1 Viral Load Assay, specimens can be collected in BD Vacutainer® Plasma Preparation Tubes and:

- Centrifuged within 6 hours of phlebotomy with no effect on viral load results.
- Plasma separated in PPTs can be stored frozen *in situ* until the time of testing with no effect on viral load results.
- There is no misclassification of HIV viral load status when using PPT in combination with the Abbott RealTime HIV-1 Viral Load Assay for any of the handling conditions tested.