

Evaluation of the Effect of Specimen Handling Conditions in BD Vacutainer® PPT on the Stability of HIV-1 Viral Load using Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test

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INTRODUCTION

Accurate quantification of HIV-1 RNA is essential to the management of HIV-1 infected patients. The aim of this study was to validate the performance of the BD Vacutainer® Plasma Preparation Tube (PPT™) with the Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test and evaluate the effect of the following parameters on HIV-1 viral load:

- 1) Storage of whole blood in PPT for six hours prior to centrifugation.
- 2) Storage of plasma *in situ* in the PPT at room temperature (RT) or 4°C for up to 5 days.
- 3) Agitation (to simulate transport) of the tube followed by a re-centrifugation.

MATERIALS AND METHODS

Study Design: See Figure 1.

Patient Population:

Blood was collected from 55 adult, consented HIV+ subjects with previous viral load (VL) test results of <1000 copies/mL.

Collection Tubes:

- Tube A: BD Vacutainer® K₂EDTA Tube, Ref.#367861
- Tubes B-G: BD Vacutainer® Plasma Preparation Tube (PPT™), Ref. #362788

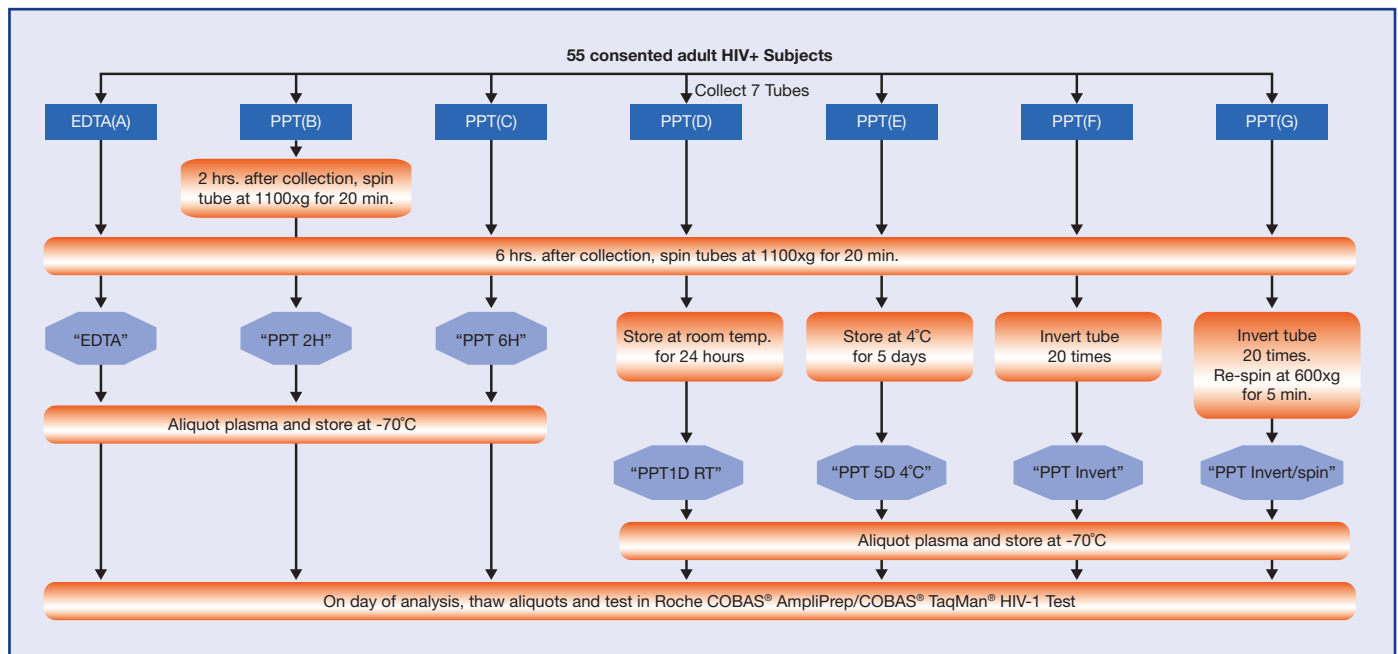
Assay:

Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test (CAP/CTM)

Statistical Analysis:

- Cochran's Q test to compare the proportion of subjects with undetectable viral load count (ND) and results under the limit of detection (LOD) of <48 copies/mL between tubes.
- McNemar's Test for paired samples.
- Analysis of concordance of dichotomized CAP/CTM HIV-1 Test, v1.0 results (<50 copies/mL, ≥50 copies/mL) using 2x2 tables for comparison between results from all PPT tube handling conditions to EDTA results.

Figure 1: Study Design



RESULTS

Table 1: Available Results per Tube Type and Handling Condition

Tube/Condition	Total Count	*ND Count	Data Not Available	<48 Copies/mL	>48 Copies/mL
EDTA (A)	55	13	1	11	30
PPT 2H (B)	55	14	1	9	31
PPT 6H (C)	55	18	0	9	28
PPT 1D RT (D)	55	18	0	5	32
PPT 5D 4°C (E)	55	14	0	13	28
PPT invert (F)	55	4	1	6	44
PPT invert/spin (G)	55	15	2	6	32

*ND=Not Detected

RESULTS

Figure 2: Correlation plot of Log₁₀ HIV-1 viral load in PPT 2H (B) to EDTA

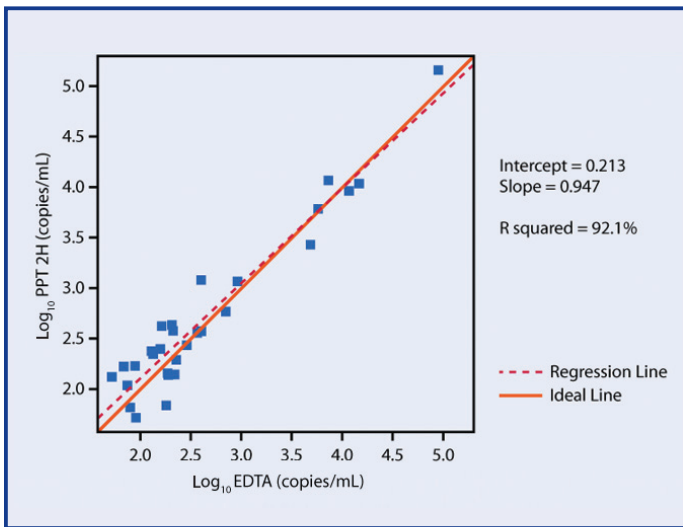


Figure 3: Correlation plot of Log₁₀ HIV-1 viral load in PPT 6H (C) to EDTA

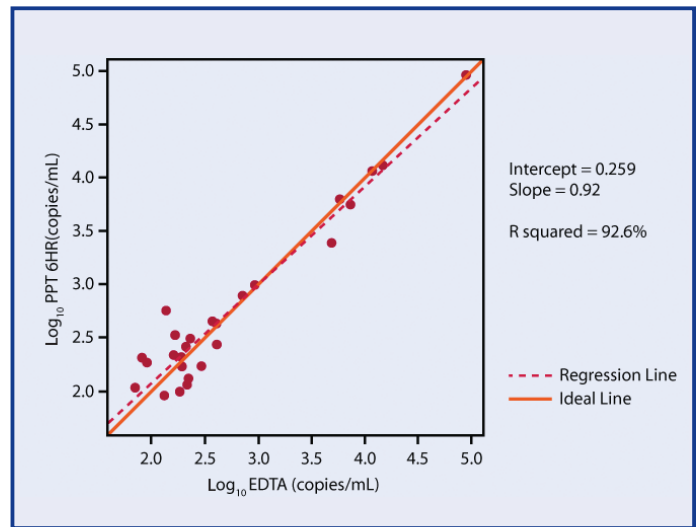


Figure 4: Correlation plot of Log_{10} HIV-1 viral load in PPT 1D RT (D) to EDTA

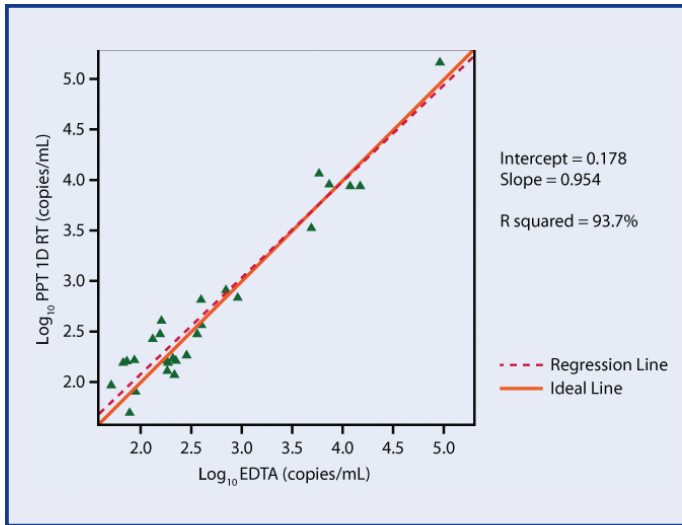


Figure 5: Correlation plot of Log_{10} HIV-1 viral load in PPT 5D 4°C (E) to EDTA

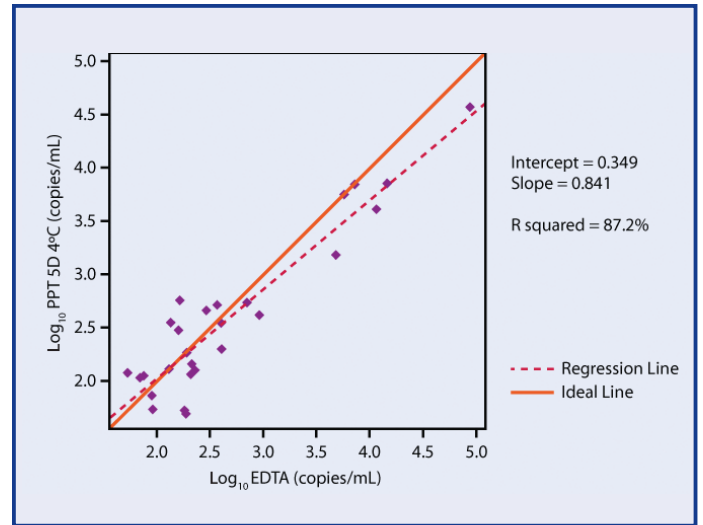


Figure 6: Correlation plot of Log_{10} HIV-1 viral load in PPT invert (F) to EDTA

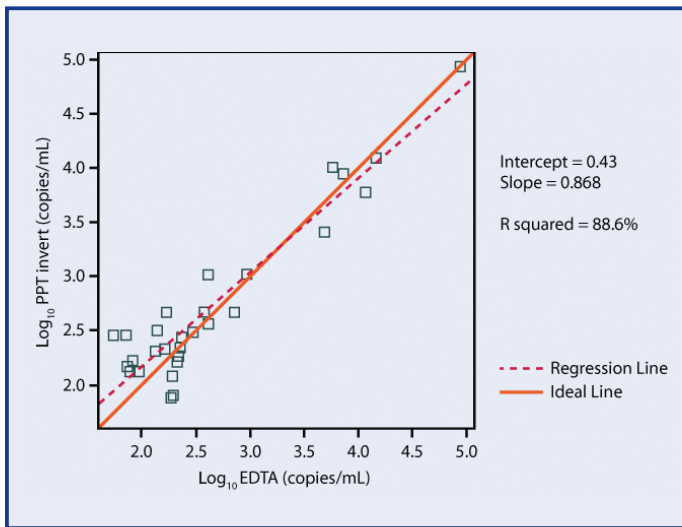


Figure 7: Correlation plot of Log_{10} HIV-1 viral load in PPT invert/spin (G) to EDTA

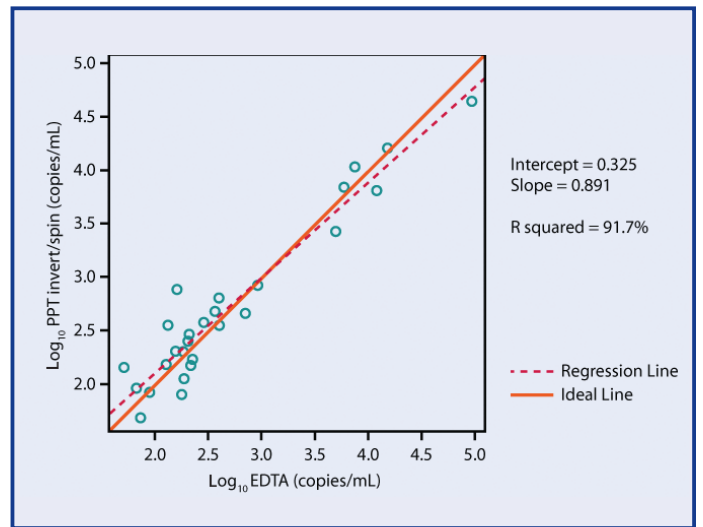


Table 2: Summary of Results per PPT Tube Handling Condition vs. EDTA

Eval. Tube	Total Subjects	Both Eval. and EDTA <50cp/mL (Concordant)	Eval. <50cp/mL EDTA ≥50cp/mL (Discordant)	Eval. >50cp/mL EDTA <50cp/mL (Discordant)	Both Eval. and EDTA ≥50cp/mL (Concordant)	Overall Agreement ¹ vs. EDTA	P-value for McNemar's Test ²
PPT 2H (B)	53	22	1	1	29	96.2%	1.0000
PPT 6H (C)	54	23	4	1	26	90.7%	0.1797
PPT 1D RT (D)	54	20	3	4	27	87.0%	0.7055
PPT 5D 4°C (E)	54	24	4	0	26	92.6%	0.0455
PPT invert (F)	53	10	0	14	29	73.6%	0.0002
PPT invert/spin (G)	52	19	2	4	27	88.5%	0.4142

¹ Percent overall agreement = Number of subjects with concordant results/Total number of subjects x 100.

² P-value is for McNemar's Test for paired samples with two binary outcomes (<50 copies/mL; >50 copies/mL).

A P-value of <0.05 may indicate a statistically significant difference between the evaluation and control tubes being compared.

No adjustment for multiple comparisons was performed.

SUMMARY OF RESULTS

- Table 1** shows that the number of specimens with quantifiable HIV-1 VL in Tubes A, B, C, D, E, and G were comparable (28–32). The tube that was inverted to simulate transportation had more specimens (44) with quantifiable VL.
- Figures 2–6** show that R² values in correlation plots of evaluation tubes A, B, C, D, and G for comparisons to EDTA control (Tube A) ranged from 91.7% to 93.7% while R² values for comparisons of Tubes E and F to Tube A were 87.2% and 88.6% respectively.
- Table 2** shows that the PPT invert (Tube F) was significantly different to the EDTA control (Tube A). The PPT 5D at 4°C (Tube E) may also differ from the EDTA control. The p-value for McNemar's Test was <0.05 for both comparisons, though no adjustment for multiple comparisons was made. The overall agreement, however, for Tube E was 92.6% while that for Tube F was 73.6% indicating that inverting the tube without re-centrifugation may have a greater impact on VL results <50 copies/mL as compared to EDTA than storing the plasma for 5D at 4°C.

SUMMARY

This study shows that with the Roche TaqMan HIV-1 viral load assay (CAP/CTM), HIV VL overall agreement with EDTA is unaffected by:

- Storage of whole blood in PPT for 6h at room temperature before centrifugation.
- Storage of plasma overnight *in situ* in PPT at room temperature.
- Storage in PPT at 4°C for up to 5 days.

Additionally:

- As compared to EDTA plasma, VLs are increased in a significant number of samples in PPT near or below the LOQ when PPT was inverted and not re-centrifuged.

CONCLUSIONS

We conclude that the BD Vacutainer® PPT™ is equivalent to EDTA plasma for HIV-1 viral load as measured by the Roche TaqMan HIV-1 viral load assay (CAP/CTM) if:

- Whole blood is stored in PPT for no longer than 6h at ambient temperature.
- Plasma is stored in PPT for no longer than 1 day at ambient temperature or 5 days at 4°C.
- PPT is re-centrifuged at 600 x g for 5 minutes in the receiving laboratory prior to aliquoting, testing, or further storage.

Acknowledgements: We would like to thank Karen Byron and Valentin Parvu (BD) and John Duncan (Roche Molecular Systems) for statistical analyses.