

DETERMINATION OF THE EFFECT OF FREEZING BD VACUTAINER® PPT™ PLASMA *in situ* ON HEPATITIS C (HCV) VIRAL LOADS AS MEASURED BY THE ROCHE COBAS® TAQMAN® HCV ASR

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BACKGROUND

Previous studies in our laboratory have shown elevated viral loads in specimens collected from human immunodeficiency virus (HIV)-1 infected patient samples when they are collected and frozen *in situ* in BD Vacutainer® PPT™ (PPT) (BD, Franklin Lakes, NJ) and tested using the Roche COBAS® AMPLICOR HIV-1 Monitor® Test, v1.5. The objective of this study was to determine whether freezing PPT™ plasma *in situ* affects hepatitis C virus (HCV) viral loads as compared to plasma obtained from BD Vacutainer® K₂EDTA Plus tubes (EDTA) or aspirated from PPT™ prior to freezing when assayed in the Roche COBAS® TaqMan® ASR test.

MATERIALS AND METHODS

- Study subjects were consented HCV positive adults attending the UMDNJ-Infectious Disease Clinic, Newark, New Jersey. (IRB #0120060079).
- All subjects had HCV viral loads of less than 500,000 IU/ml at the previous testing (2-4 weeks prior to the study).
- HCV viral loads at the time of the study ranged from 146 IU/ml to 7,210,000 IU/ml.
- Venous whole blood was collected from 33 subjects into three tubes: one EDTA tube (A = EDTA) and two PPT™ tubes (B = PPT™, aspirated; C = PPT™ frozen *in situ*) according to the manufacturer's instructions.
- Specimens were processed according to the manufacturer's instructions, and plasmas were aspirated from the EDTA tube and one of the PPT™ tubes and frozen at -20°C until the time of testing. The remaining processed PPT™ tubes were frozen at -20°C until the time of testing.
- HCV RNA was extracted from thawed plasmas using the Roche MagNA Pure LC instrument and the Roche Total Nucleic Acid Isolation Kit.
- All specimens were amplified and quantitated in the Roche COBAS® TaqMan® 48 Analyzer using HCV Analyte Specific Reagents (ASR's).
- A schematic of the study protocol is depicted in Figure 1.

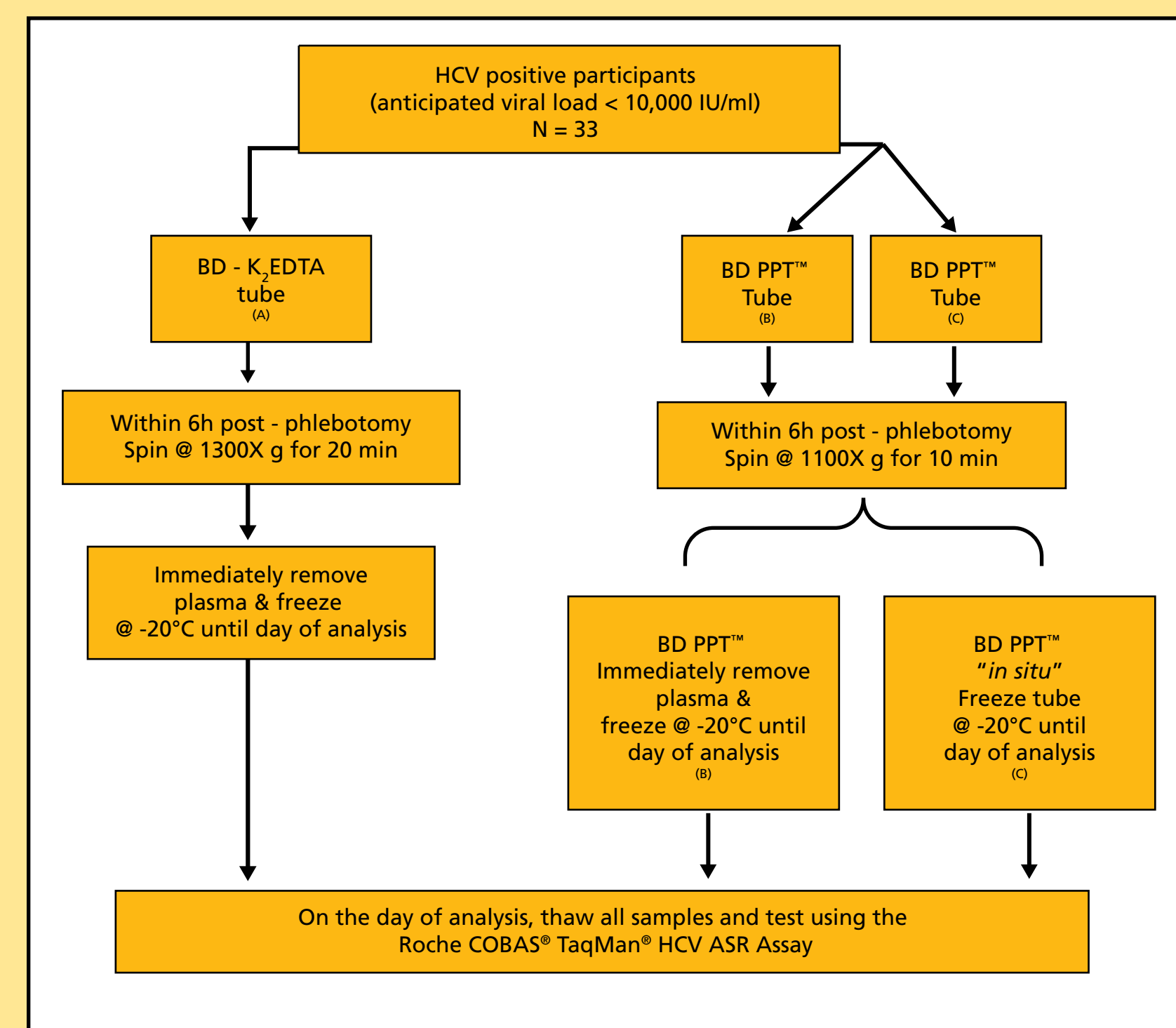


Figure 1.
Schematic diagram of the study protocol.

RESULTS

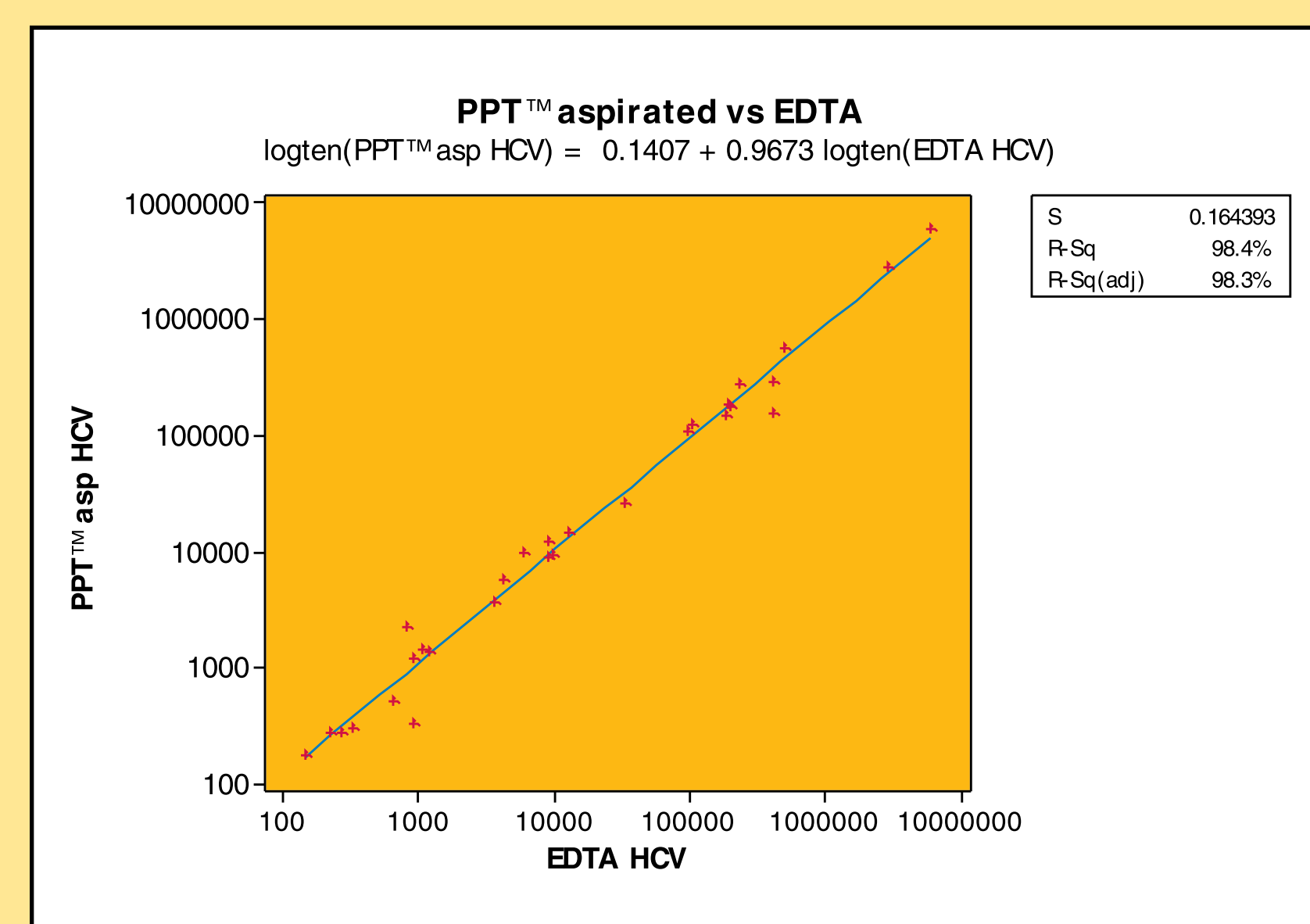


Figure 2.
Correlation between PPT™ frozen *in situ* and EDTA plasma

Static	IU/mL units			Log ₁₀ IU/mL units		
	EDTA	PPT asp	PPT <i>in situ</i>	EDTA	PPT asp	PPT <i>in situ</i>
HCV	Mean	392005	371502	4.143	4.148	4.141
	StDev	1210555	1176198	1.311	1.278	1.287
	Median	9093.33	9566.67	3.959	3.981	3.998
	Minimum	146.333	173.333	2.165	2.239	2.212
	Maximum	6020000	5856667	6.780	6.768	6.858
	N	29	29	29	29	29

Table 1.
Comparison of viral loads in IU/ml and Log₁₀ IU/mL in EDTA, aspirated PPT™ and PPT™ frozen *in situ* plasmas for 29 HCV infected subjects.

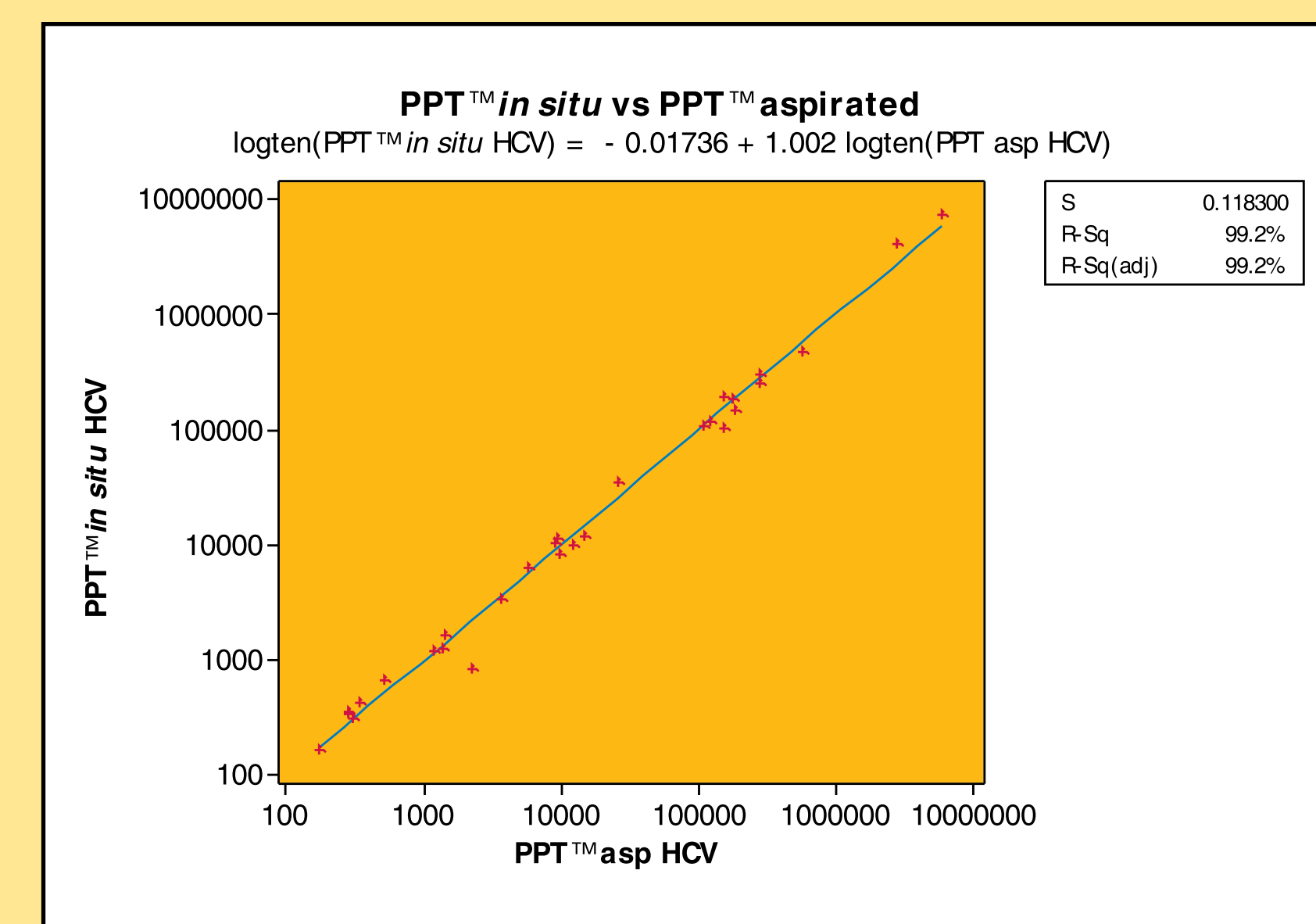


Figure 3.
Correlation between PPT™ frozen *in situ* and PPT™ aspirated plasma.

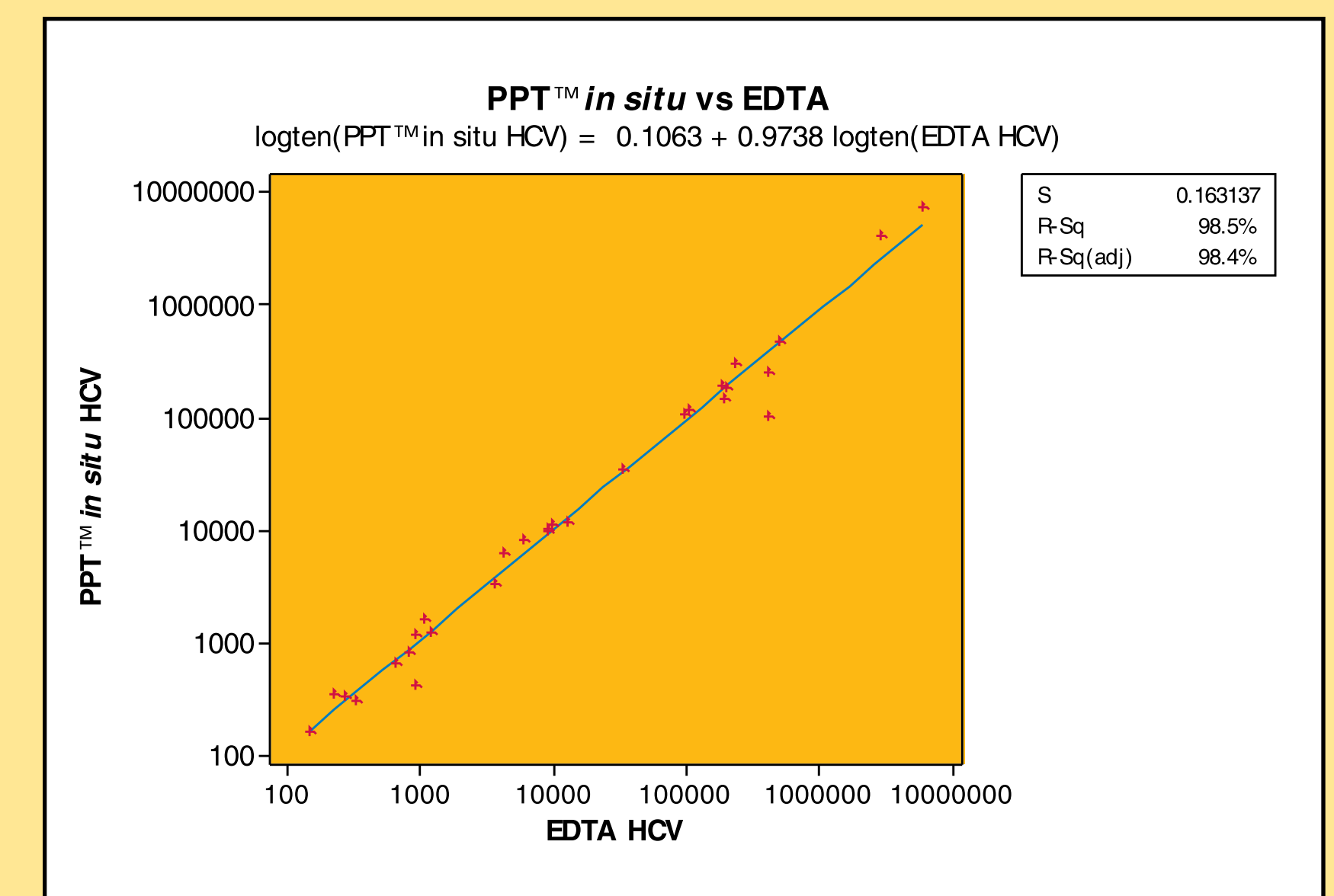


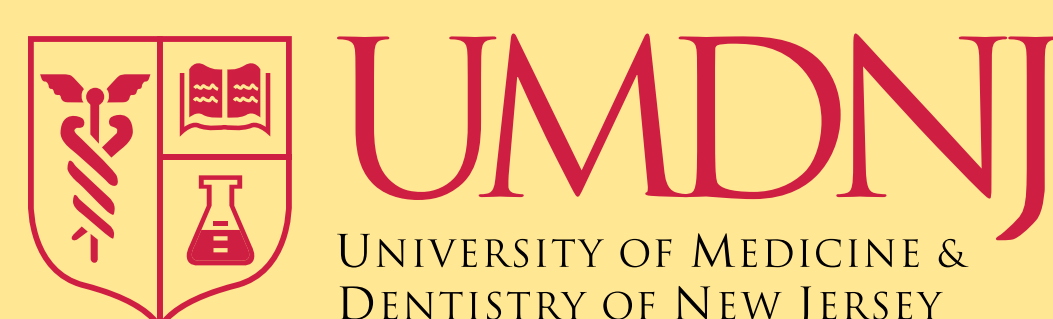
Figure 4.
Correlation between PPT™ aspirated and EDTA plasma

RESULTS

- Of the 33 patients in the study, 29 subjects had detectable VL's of > 100 IU/mL in all three tubes collected.
- The HCV viral load in the 87 specimens (three tubes/per subject) ranged from 146 IU/ml to 7,210,000 IU/ml.
- The median viral load in the K₂EDTA tube was 9,093 IU/ml as compared to 9,567 IU/ml in the PPT™ aspirated plasma and 9,950 IU/ml in the PPT™ frozen *in situ* plasma.
- The correlation coefficients between EDTA and aspirated PPT™ plasma, EDTA and *in situ* frozen PPT™ plasma, aspirated PPT™ and *in situ* frozen PPT™ plasma were 0.984, 0.985, and 0.992 respectively.
- These results indicate that there is no difference between calculated viral loads in specimens collected in PPT™ tubes (aspirated or frozen *in situ*) and EDTA tubes.

CONCLUSIONS

- This study has demonstrated that there is no significant difference in HCV viral loads between specimens frozen *in situ* in PPT™ or aspirated PPT™ plasma as compared to the plasma obtained from an EDTA tube.
- Based on the results from this study, we have determined that freezing plasma in PPT™ does not affect HCV viral loads.



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