DETERMINATION OF THE EFFECT OF FREEZING BD VACUTAINER® PPT™ PLASMA in situ ON HEPATITIS C (HCV) VIRAL LOADS AS MEASURED BY THE ROCHE COBAS® TAUqMAN® 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® AMPLIFICOR 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® K™_EDTA Plus tubes (EDTA) or aspirated from PPT™ prior to freezing when assayed in the Roche COBAS® TagMan® ASR test.

MATERIALS AND METHODS

• Study subjects were consenting 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 BD - K™_EDTA (EDTA) or BD PPT™ tubes (B = PPT, aspirated from BD Vacutainer) tubes 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 (ASRs).
• A schematic of the study protocol is depicted in Figure 1.

RESULTS

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

CONCLUSIONS

• Of the 33 patients in the study, 29 subjects had detectable VL's of > 10,000 IU/ml in all three tubes collected.
• The HCV viral load in the 87 specimens (three tubes/subject) ranged from 146 IU/ml to 7,210,000 IU/ml.
• The median viral load in the K™_EDTA 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 EDTA tubes.

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

<table>
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<th>HCV</th>
<th>EDTA</th>
<th>PPT aspirated</th>
<th>Log10_EDTA</th>
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<td>IU/ml</td>
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</table>

Figure 1. Schematic diagram of the study protocol.

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

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

Figure 4. Correlation between PPT™ aspirated and EDTA plasma.