DETERMINATION OF THE EFFECT OF FREEZING BD VACUTAINER® PPT™ PLASMA in situ ON HEPATITIS B (HBV) VIRAL LOADS USING THE ROCHE COBAS® TAQMAN® HBV RUO ASSAY

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BACKGROUND
The BD Vacutainer® PPT™ Plasma Preparation Tube (PPT) is an evacuated blood collection tube which upon centrifugation yields undiluted plasma for use in molecular diagnostic test methods. This study was initiated to determine if freezing PPT plasma in situ affects hepatitis B virus (HBV) viral loads as compared to plasma obtained from BD Vacutainer® K+EDTA Plus Tubes (EDTA) or plasma aspirated from PPT prior to freezing.

MATERIALS AND METHODS
• Study subjects were consented HBV positive adults attending the UMDNJ-Infectious Disease Clinic, Newark, New Jersey.
• All subjects had HBV viral loads of less than 500,000 IU/ml at the previous testing (2-4 weeks prior to the study).
• HBV viral loads at the time of the study ranged from 9 IU/ml to 1,780,000 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.
• HBV DNA was extracted from thawed plasmas using the High Pure System Viral Nucleic Acid Kit (Roche Molecular Systems).
• All specimens were amplified and quantitated in the Roche COBAS® TaqMan® 48 Analyzer using the COBAS® TaqMan® HBV research use only (RUO) assay.
• A schematic of the study protocol is depicted in Figure 1.

RESULTS

• Of the 33 patients in the study, 26 subjects had detectable viral loads of > 100 IU/mL in all three tubes collected.
• The HBV viral load in the 78 specimens (three tubes/per subject) ranged from 9 IU/ml to 1,780,000 IU/ml.
• The median viral load in the EDTA tube was 78,288 IU/ml as compared to 78,590 IU/ml in the PPT aspirated plasma and 76,622 IU/ml in the PPT frozen in situ plasma.
• The correlation coefficients between the three different specimens gave coefficients between 0.969 and 0.991.
• 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 HBV 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 of this study, we have determined that freezing plasma in PPT does not affect HBV viral loads.

SUMMARY
• Plasma frozen in situ in PPTs can be used with the Roche COBAS® TaqMan® HBV RUO assay.