Evaluation of the VACUTAINER® Brand PPT™ Tubes for HCV Viral Load Testing

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We investigated the performance of a new collection tube for the preparation of EDTA anti-coagulated plasma for viral load testing. HCV RNA copy numbers were measured with a commercially available assay (the Amplicor HCV Monitor™ test kit). Blood specimens were collected from HCV-seropositive individuals in the VACUTAINER® Brand Plasma Preparation Tube™ and a control, non-gel separator VACUTAINER® Brand PLUS K_2EDTA tube. We found that viral loads from plasma prepared in the PPT™ tube were equivalent to those in plasmas prepared in conventional EDTA tubes. Additionally, in contrast to the EDTA tube, the PPT™ tube provided a closed system for the collection, preparation and transport of a plasma specimen with no plasma transfer or re-labeling steps.

Hepatitis C virus (HCV) RNA level as measured by gene or signal amplification techniques has been used to detect early and ongoing HCV infection, assess the efficacy of specific treatment (1), and predict clinical outcome in treated patients (2).

HCV viral loads are significantly affected by several factors including the conditions of sample collection and processing. Manzin, et al. found a sample-to-sample variability in the loss of detectable HCV RNA in serum compared with that in plasma from the same patient (3). Others have reported a loss of HCV viral copy number attributable to increased time to centrifugation of whole blood, (4) or whether the test sample was serum rather than plasma (5,6). It is therefore highly important to minimize pre-analytical variables caused by differences in sample handling and treatment.

Since previous studies have shown that HCV RNA levels are initially higher and more stable in plasma than in serum (5), and that EDTA is preferred over heparin as an anti-coagulant for samples used in PCR-based HIV viral load assays (7), Becton Dickinson VACUTAINER Systems has recently developed a plastic evacuated tube with EDTA anti-coagulant for the collection of venous blood which upon centrifugation separates undiluted plasma for use in molecular diagnostic test methods. The tube contains 9 mg of dried K_2EDTA, yielding a ratio of 1.8 mg/mL of blood when the evacuated tube is filled correctly to its 5 mL draw volume. The tube also contains a material that upon correct centrifugation (1,100 x g for 10 minutes, in a swing-out bucket rotor) forms a barrier between the plasma and most of the cellular elements, allowing for transportation of the sample without first removing the plasma into secondary tube(s).

The purpose of this study was to compare HCV viral loads obtained from plasma produced in this new gel-separator K_2EDTA plasma preparation tube, VACUTAINER® Brand PPT™ (PPT), to those obtained from plasma produced in a non-gel separator VACUTAINER® PLUS™ K_2EDTA tube (EDTA).

MATERIALS AND METHODS

Patient population. Venous blood samples were drawn from sixty-five (65) HCV-positive patients. Thirty (30) subjects were adults attending an HCV-positive out-patient clinic in Sacramento, California (Site 1), and thirty-five (35) patients were adults attending the University of Michigan Medical Center outpatient clinic in Ann Arbor, Michigan(Site 2).

Testing Site	R	Regression Line Slope	P Value	EDTA Tubes Log_{10}(mean Viral Load)	PPT Tubes Log_{10}(mean Viral Load)
Site 1	**0.9708**	**0.9994**	**0.9606**	**5.8204**	**5.8184**
Site 2	**0.9967**	**0.9872**	**0.8886**	**5.3503**	**5.3551**

Table 1. ANOVA and least squares mean analysis of log_{10} viral load results for PPT vs. EDTA plasmas. EDTA and PPT plasma samples collected from 65 HCV positive subjects: 30 paired plasma samples from Site 1 and 35 paired plasma samples from Site 2. EDTA plasma aliquots and paired, spun PPT tubes were frozen, stored, and shipped at –70°C to the testing laboratory where they were thawed on the day of testing (Site 1) or frozen and stored at –70°C until the day of testing (Site 2).

All subjects had been previously identified by immunoassay and/or PCR as HCV positive. Patient identities, clinical status, and antiviral treatment regimen were blinded to study monitors. Patient informed consent was obtained in accordance with policies of the sponsoring site's Institutional Review Board (IRB). Viral load results obtained from plasma specimens produced in the new tube were not made available to physicians for patient management.

Sample collection and processing. Two tubes for the preparation of plasma were drawn from each patient: one PPT tube and one EDTA tube. Tubes containing whole blood were transported from the phlebotomy laboratory to the processing laboratory at ambient temperature. All whole blood samples collected in the EDTA and PPT tubes were centrifuged in a swing-out bucket rotor at 1100 x g for 10 minutes within two hours of collection. Plasma from EDTA tubes was withdrawn from the tubes immediately after centrifugation, placed in secondary tubes, mixed and aliquoted (approx. 500 uL/ aliquot). Plasma in spun PPT tubes was stored *in situ*.

At Site 1, for each patient, one spun PPT tube and plasma aliquots from one EDTA tube were frozen, stored at –70°C and shipped on dry ice to Laboratory Corporation of America. Samples were stored at –70°C for up to one week before testing. On the day of testing, paired PPT/EDTA samples were thawed at ambient temperature and tested using the Roche AMPLICOR HCV-1 MONITOR™ Test.

At Site 2, for each patient, one spun PPT tube and plasma aliquots from one EDTA tube were frozen, stored at –70°C up to two weeks until the day of testing. On the day of testing, paired PPT/EDTA samples were thawed at ambient temperature and tested using the Roche AMPLICOR HCV-1 MONITOR™ Test. Different lots of reagents were used at the two testing sites.

RESULTS

An analysis of variance, ANOVA, was performed to compare viral load results between the PPT tube and the EDTA control using a log transformation of the Roche Amplicor™ Monitor assay results. The ANOVA was performed using a complete block design, with subjects as blocks and the two tubes as treatments. Residual plots indicated that a logarithmic transformation was necessary to stabilize the variance across the treatments for viral counts.

In addition to the ANOVA, correlation plots of evaluation *versus* control tubes and corresponding regression analyses were performed for all (log_{10}(viral count)) subjects. Correlation coefficients (Pearson's R values) and regression line slopes and intercepts were calculated.

Table 1 shows the results of ANOVA and least squared means analysis for PPT vs. EDTA HCV RNA results for Site 1 and Site 2. In particular, note that the p-values, given in the column labeled "P value" for the effect of tube type on viral load results are 0.9606 and 0.8886 respectively, both of which are greater than 0.05. Therefore, the differences in log_{10} viral count between the EDTA and PPT tubes are not significant at the 0.05 level.

The correlation plots are given in Figures 1 and 2. The correlation estimates are very high for both sites: R = 0.9708 and 0.9967 for Site 1 and Site 2 respectively.

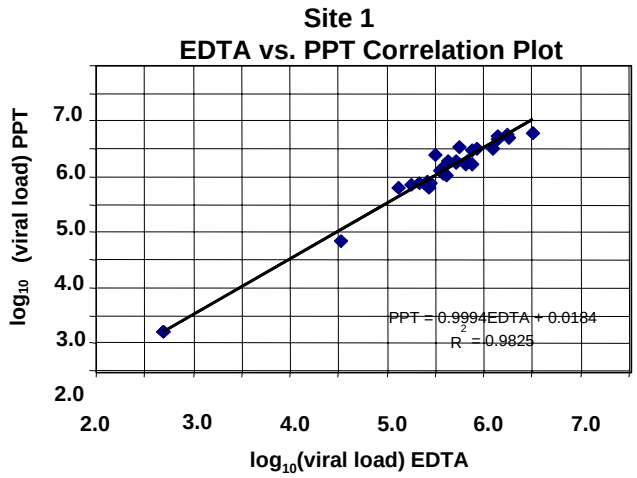

FIG. 1: Log_{10} HCV RNA concentrations for 30 subject plasmas collected in PPT tubes plotted against the log_{10} HCV RNA concentrations for the matching EDTA tube plasmas (Site 1). Spun PPT tubes and plasma aliquots from EDTA tubes were frozen, shipped and stored at –70°C until the day of testing.

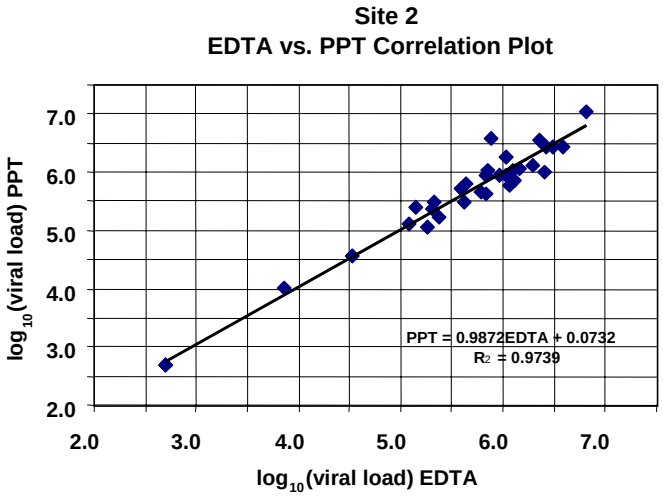

FIG. 2: Log_{10} HCV RNA concentrations for 35 subject plasmas collected in PPT tubes plotted against the log_{10} HCV RNA concentrations for the matching EDTA tube plasmas (Site 2). Spun PPT tubes and plasma aliquots from EDTA tubes were stored frozen at –70°C until the day of testing.

Figures 3 and 4 show confidence intervals based on the least squares means. Log_{10} of mean viral loads for Site 1 plasmas collected in EDTA and PPT tubes were 5.8204 and 5.8184 respectively. For plasmas collected at Site 2, the log_{10} of mean viral loads were 5.3503 and 5.3551 for EDTA and PPT tubes respectively. The overlapping of the two intervals (PPT and EDTA) for both plasma treatment types for samples collected at two different sites graphically demonstrates that the the log_{10} viral count between the two tube types were statistically equivalent.

DISCUSSION

In this study, a new plasma preparation tube for viral load testing was evaluated and compared to a conventional EDTA tube. Results of this study indicate that viral load measurements obtained from plasma collected in the VACUTAINER® Brand PPT™ tube are substantially equivalent to results obtained with plasma collected in a non-gel separator EDTA tube for HCV-positive plasmas. Furthermore, blood can be collected**,** processed into plasma, stored and shipped in the PPT™ tube which provides a closed system for

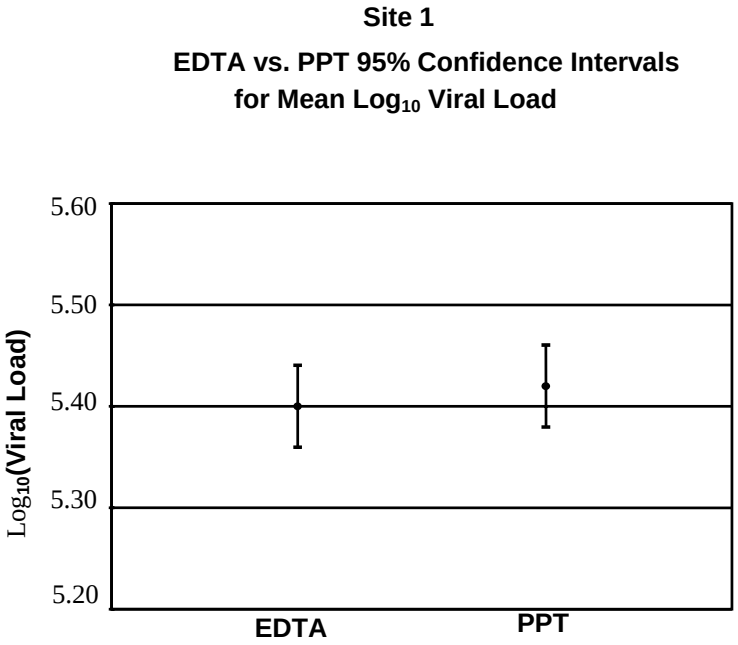

Figure 3: Pairwise 95% confidence intervals for mean $\log_{10}$ viral count in 30 HCV positive plasma samples from EDTA and PPT tubes (Site 1)

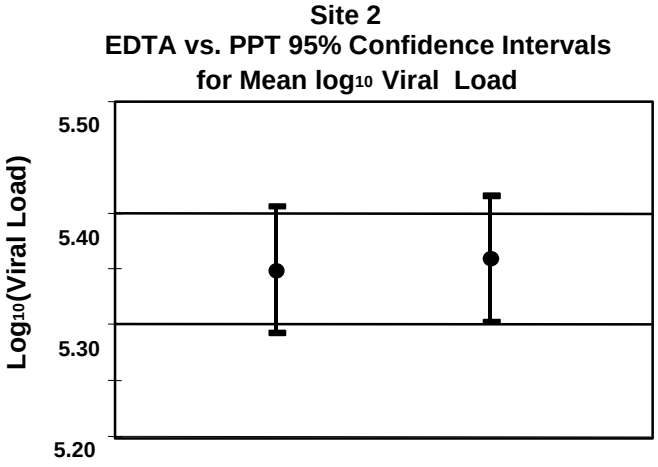

Figure 4: Pairwise 95% confidence intervals for mean $\log_{10}$ viral count in 35 HCV positive plasma samples from EDTA and PPT tubes (Site 2).

sample collection and transport. These are important advantages over conventional non-gel separator tubes, the plasma from which must be separated and transferred to a secondary tube(s) prior to analysis. The PPT™ tube minimizes operator exposure to infected samples and reduces the chance of sample identification error inherent in transfer of plasma to secondary tubes.

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