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

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

*ND=Not Detected

RESULTS

Figure 2: Correlation plot of Log_{10} HIV-1 viral load in PPT 2H (B) to EDTA

![Figure 2](image1)

Intercept = 0.213
Slope = 0.947
R squared = 92.1%

Figure 3: Correlation plot of Log_{10} HIV-1 viral load in PPT 6H (C) to EDTA

![Figure 3](image2)

Intercept = 0.259
Slope = 0.92
R squared = 92.6%
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
1. 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.

2. Figures 2–6 show that $R^2$ 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 $R^2$ values for comparisons of Tubes E and F to Tube A were 87.2% and 88.6% respectively.

3. 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 no longer than 6h at ambient temperature.
- Storage of plasma 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.

**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.

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