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Successful use of Plasma Preparation Tubes™(PPTs) in the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test

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Abstract

Background—Since switching to the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v. 1.0 from the Amplicor HIV-1 Monitor Test, v. 1.5, an increase in detectable viral load results was noted. We were concerned that this was due to the use of Plasma Preparation Tubes (PPT) in this test.

Objective—To assess the impact of different pre-analytical processing conditions on HIV-1 viral load results on the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test.

Study design—Sixty-three HIV-infected patients were consented and had 3 PPTs and 1 K₂EDTA drawn for HIV-1 viral load testing. Three methods of PPT processing were compared against the referent K₂EDTA tube which was spun at 1100 × *g* for 20 min, poured off and frozen; PPT1 was refrigerated with an additional centrifugation prior to testing, PPT2 was processed similarly to EDTA, and PPT3 was centrifuged, frozen and centrifuged again prior to testing.

Results—PPT1 and PPT3 yielded results that were most similar to the referent EDTA processing, with a concordance correlation coefficient (CCC) of 0.80 and 0.85, compared to PPT2 with CCC of 0.37. Both PPT1 and PPT3 involved additional centrifugation prior to testing. In 26 patients with residual samples from the PPT2 processing, 9 (34.6%) were found to have the presence of proviral DNA, which likely contributed to the elevated HIV-1 RNA viral loads in these individuals.

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Competing interests

Reagents for the study were provided by Roche Diagnostics.

AMC and CEH are members of the Roche Diagnostics Scientific Advisory Board. The other authors have no conflict of interest.

Ethical approval

Emory Institutional Review Board approval was obtained for this study (#44146).

Conclusion—PPTs can be used in the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test with an additional centrifugation in order to avoid misleading elevated HIV-1 RNA viral loads that may change patient management.

Keywords

HIV-1; Viral load; COBAS Amplicor/COBAS Ampliprep; Plasma Preparation Tubes

1. Background

After replacement of the Amplicor HIV-1 Monitor Test, v. 1.5 (Roche Diagnostics, Indianapolis, IN), with the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v. 1.0 (Roche Diagnostics), our institution noted an increase in detectable HIV-1 RNA viral loads. Plasma Preparation Tubes (PPT™; Becton Dickinson, Franklin Lakes, NJ) frozen *in situ* have been associated with artificially increased HIV-1 RNA viral loads.¹⁻⁴ Since PPTs are not validated for use in the TaqMan test, our laboratory performed a validation study to use PPT instead of K₂EDTA (EDTA; Becton Dickinson, Franklin Lakes, NJ) for specimen collection. Given that the literature had implicated that freezing PPTs *in situ* resulted in elevated HIV-1 viral load levels,^{2,5} our validated protocol required pouring off the plasma prior to freezing. Despite these precautions, the number of patients who had low level viral loads after changing to the TaqMan test increased significantly.

Cellular material retained during separation of plasma from whole blood can falsely elevate the HIV-1 RNA viral load, due to co-amplification of integrated HIV-1 proviral DNA.⁶ Residual platelets with adherent HIV-1 virions may also falsely increase viral load values.^{7,8} Elevated viral load values cause great concern to practitioners and patients and the detection of low levels of HIV-1 RNA (<2000 copies/ml) in patients with previously undetectable viral loads may result in unnecessary HIV-1 genotypic resistance testing⁹ and changes in the antiretroviral regimen. Our objective was to assess the impact of different pre-analytical processing conditions on HIV-1 viral load results using the TaqMan test.

2. Study design

2.1. Selection of study individuals

This study was approved by the Emory University IRB. Patients who were HIV-1 infected and had venous blood specimen collection as part of their routine surveillance starting May 1st, 2010 and ending October 30th, 2010 were eligible. Individuals with unquantifiable HIV-1 viral load levels on their previous visit were approached, and 63 individuals consented to participate in the study.

2.2. Pre-analytical processing conditions

Four tubes (1 EDTA and 3 PPTs) were obtained from consenting patients and processed as described in Fig. 1. EDTA and PPT2 were spun for 20 min per manufacturer's instructions for EDTA. PPT1 and PPT3 were spun for 10 min per manufacturer's instructions for PPT. PPT1 specimens were tested with other clinical specimens received that day, and the research specimens (EDTA, PPT2, and PPT3) were stored as indicated and tested together on a separate run. All specimens were tested using the Taqman test as per manufacturer's protocol.

2.3. Amplification of HIV-1 proviral DNA

Proviral DNA was detected from plasma specimens using a qualitative nested PCR assay as described elsewhere.⁶ Amplicons were run on a gel and the results were arbitrarily scored

from 1+ → 4+ (low → high), based on densitometric analysis. Historical data showed no evidence of inhibition of amplification.

2.4. Statistical analysis

Viral load values were $\log_{10}$ transformed prior to statistical analysis. For undetectable viral loads, a value of 20 was assigned; for viral loads <48 copies/ml (<1.68 $\log_{10}$ copies/ml), a value of 30 was assigned.

To assess agreement between each PPT method and EDTA, the concordance correlation coefficient (CCC) was calculated.¹⁰ Similar to the Pearson correlation coefficient, the range of CCC is between -1 and 1, inclusive. Calculations of CCC and Pearson r (and their 95% confidence intervals) were performed using SAS[®] version 9.3 (SAS Institute Inc., Cary, NC).

3. Results

Prior to switching to the Taqman test, 39.5% of the specimens collected in PPT had HIV-1 viral load values < 50 copies/ml (<1.70 $\log_{10}$ copies/ml) ($n = 1190$) using the Monitor test. After switching to the Taqman test using PPTs, only 14.4% of the specimens had viral load values below the limit of quantification of 48 copies/ml (<1.68 $\log_{10}$ copies/ml) ($n = 1999$).

Table 1 demonstrates the percent of patients with undetectable (<20 copies/ml) and/or unquantifiable (below the level of quantification; <48 copies/ml; <1.68 $\log_{10}$ copies/ml), viral load stratified by processing conditions, in comparison to those that were quantified (>48 copies/ml; >1.68 $\log_{10}$ copies/ml). For the EDTA specimen, 73.0% were either undetected or below the level of quantification compared to 79.4% for PPT1, 19.0% for PPT2 and 69.9% for PPT3. Chi-square analysis showed no difference in the values between EDTA compared to PPT1 or PPT3. There was a statistically significant difference ($p < 0.0001$) in the viral load values of EDTA compared to PPT2. The mean viral load values are listed in Table 2. The CCC for the PPT1 and PPT3 specimens compared to EDTA were very good at 0.80 and 0.85, respectively. The PPT2 specimen had poor agreement with the referent EDTA specimen with a CCC of 0.37.

Twenty-six individuals with detectable viral loads from any tube had adequate residual samples for PCR detection of proviral DNA. All of these samples had been processed for viral load testing with the PPT2 method and 2 of these individuals also had residual sample from the PPT3 method. Nine of the 26 PPT2 specimens (34.6%) had proviral DNA detected and 3 (11.5%) specimens were equivocal. Three of the nine specimens had high levels of proviral DNA (3+ → 4+) compared to 6/9 who had lower levels (1+ → 2+). Fourteen (53.8%) in the PPT2 group did not have evidence of co-amplification of proviral DNA and all of these individuals had RNA levels that were either undetectable or below the limit of quantification of the assay with the other 3 specimen types (EDTA, PPT1, and PPT3).

4. Discussion

The method of specimen collection and processing had a significant impact on the HIV-1 RNA viral load value when tested in the TaqMan test. The PPT1 and PPT3 methods of processing had statistically concordant HIV-1 viral loads as compared to the referent EDTA method, whereas the PPT2 method did not. These differences achieved statistical significance despite the relatively small sample size and resultant larger standard deviations in each group. These findings indicate that specimens that were spun, shipped, poured off from PPTs and then frozen have higher HIV-1 viral loads compared to those collected in EDTA poured off prior to shipping or those collected in PPT with a second centrifugation

prior to testing. We have demonstrated that approximately 1/3 of the patients had increased viral loads due to the co-amplification of proviral DNA, similar to Wan et al. where PPTs were frozen *in situ* and did not undergo additional centrifugation prior to testing in the Monitor test.⁶ There is evidence that transportation of PPTs between clinical sites after centrifugation and prior to pour off also resulted in elevated HIV-1 viral loads.¹¹ The results also demonstrated that if it is desirable for the laboratory to freeze the specimen, the plasma should be poured off after a centrifugation step (*i.e.* PPT3). Our laboratory instead chose to refrigerate for ease of processing, which clearly is equivalent to the referent EDTA and similar in concordance to PPT3 method.

Since the proviral DNA in most specimens was low (1+ → 2+), it is unclear if the increased HIV RNA viral load in the 9/26 patients was due exclusively to proviral DNA. Given that 2/3 of the patients did not have proviral DNA detected, it is likely that the increased viral load values were due to a combination of proviral DNA and platelet-associated RNA, as it has been shown that plasma collected in PPTs is rich in platelets.¹²

In conclusion, when using the COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, we found that an additional centrifugation¹³ is critical if the plasma has been collected in PPTs. There was high agreement in HIV-1 viral load between EDTA and whether the specimen was refrigerated (PPT1) or frozen (PPT3).

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Fig. 1. Flow chart diagram of specimen processing. EDTA, PPT1 and 3 were processed as per manufacturer's protocol. They were subsequently spun prior to testing to remove any debris and/or cellular material. PPT2 was processed similar to EDTA for comparison purposes.

Table 1

Comparison of the percentage of undetectable and <48 copies/ml between the three tube processing methods with EDTA.

Conditions <i>n</i> = 63	% Not detected	% <48 copies/ml	% >48 copies/ml	<i>p</i> -Value compared to EDTA
EDTA (−20 °C)	58.7	14.3	27.0	–
PPT1 (4 °C)	61.9	17.5	20.6	0.6750
PPT2 (−20 °C)	12.7	6.3	81.0	<0.0001
PPT3 (−20 °C)	52.4	17.5	30.1	0.7635

Table 2

Comparison of the viral loads of the three tube processing methods with EDTA.

Conditions <i>N</i> = 63	Mean log ₁₀ copies/ml (SD)	Pearson correlation coefficient (95% CI)	Concordance correlation coefficient (95% CI)
EDTA (−20 °C)	1.61 (0.53)	–	–
PPT1 (4 °C)	1.52 (0.41)	0.85 (0.76, 0.91)	0.80 (0.71, 0.87)
PPT2 (−20 °C)	2.19 (0.55)	0.59 (0.40, 0.73)	0.37 (0.22, 0.51)
PPT3 (−20 °C)	1.62 (0.50)	0.85 (0.76, 0.91)	0.85 (0.76, 0.91)