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Comparison of HIV-1 Viral Load between Abbott m2000 and Roche COBAS TaqMan Methods

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Abstract

Accurate quantification of HIV-1 viral load (VL) is crucial for disease monitoring and management. This study was designed to compare HIV-1 VL determination between two of the major commercial real-time PCR-based methods, i.e., the Roche's COBAS AmpliPrep/COBAS TaqMan HIV-1 Test and the Abbott Real Time HIV-1 Assay. Out of 308 paired plasma tested, 85.1% (262/308) of test results were concordant with 173 samples were quantifiable for VL and 89 were "Not Detected" (ND). There was a strong overall correlation of the quantifiable VL between the two methods (R^2=0.952). Comparison of the mean VL with differences of the two methods using the Bland Altman plot showed rough symmetric distribution of the differences, indicating neither method is better than the other for measuring VL. However, a relative high 14.9% (46/308) of discordant results was found between the two methods. $\chi 2$ test of those discordant results indicated a significant difference ($\chi 2$= 96.37; p = <0.001). Of the 104 ND Roche samples, 15 (14.4%) were detected by Abbott method; of the 120 ND Abbott samples, 31 (25.8%) were detected by Roche method. Differences in gene target, test sensitivity, input volume and their abilities to detect different HIV-1 subtypes could potentially explain some of the discordance.

Keywords: HIV-1 viral Load; Quantification; Real-time PCR; Abbott m2000; Roche COBAS TaqMan

Introduction

Accurate quantification of HIV-1 Viral Load (VL) in plasma compartment is crucial for disease monitoring and management (Braun et al., 2007; Ciotti et al., 2008). This method has now become a standard method for monitoring HIV-infected patients on antiretroviral therapy in the United States. This trend has lead to the development and approval of a number of diagnostic assays (For reviews of these assays, see Constantine and Zhao, 2005). There are currently two major commercial and FDA-approved real-time PCR-based methods, i.e., the Cobas AmpliPrep/Cobas TaqMan HIV-1 test (the Roche method; Roche Molecular Systems, Inc., Branchburg, NJ) and the Abbott Real Time HIV-1 assay (the Abbott method; Abbott Molecular Inc., Des Plaines, IL). These two assays share three common features 1) Additional reduction in the lower limits of detection (LOD) of HIV-1 RNA from the earlier version; 2) New primers and probes designs for recognition of the different viral subtypes and circulating recombinant forms (CRFs); and 3) Reduction in hand-on time by configuring the test near to full automation. Table 1 summarizes the assay characteristics of these two assays.

Another common feature of these two assays is the use of real-time PCR as their underlying technology for the measurement of HIV-1 viral load. Real-time PCR, also known as the TaqMan technology or 5'exonuclease assay, quantifies PCR products cycle-by-cycle ("real-time") as they accumulate (Holland et al., 1991). This gene-target based amplification method is based on the determination of the threshold cycle (*CT*) when the amplified product is detected for the first time and the PCR is still in its exponential phase (Ciotti et al., 2008; Gibson et al., 1996; Gordillo et al., 2005; Scott et al., 2009). Different from the conventional PCR, an internal probe is added to the detection process, which is an oligo nucleotide with both a fluorescent reporter and a fluorescent quencher dye attached. If a target sequence is present, the probe anneals between the forward and reverse primers and is then digested by the 5' nuclease activity of the DNA polymerase as PCR proceeds. Digestion of the probe DNA separates the reporter dye from the quencher dye, making the reporter dye signal detectable. Detection of the resulting fluorescence collectively provides an immediate real-time quantification of the PCR process.

The Roche's COBAS AmpliPrep/COBAS TaqMan HIV-1 test (hereafter as the Roche method) uses the COBAS® AmpliPrep Instrument for automated specimen processing, which is configured to either one of the following automated amplification and detection systems: the COBAS TaqMan analyzer in a docked or undocked configuration and the COBAS TaqMan 48 analyzer in an undocked configuration (Roche, 2007; Schumacher et al., 2007; Sloma et al., 2009; Wolff and Gerritzen, 2007). The COBAS AmpliPrep can process up to 72 samples in each run. The assay can also quantify HIV-1 RNA over the linear dynamic range of 48 - 10,000,000 copies/mL (Roche, 2007). Using the TaqMan analyzer, the sample extraction volume protocol is 0.85mL and this requires sample input volume between 1.0 and 1.05mL (Sloma et al., 2009).

The Abbott Real Time HIV-1 assay (here after as the Abbott method) is carried out on an integrated *m*2000sp and *m*2000rt instrument system (Marshall et al., 2007), which consists of two separate instruments, i.e., the *m*2000sp that carry out automated extraction, purification and preparation of HIV-1 RNA, and the *m*2000rt that amplifies, detects and measures the HIV-1 RNA VL (Abbott, 2007b; Sloma et al., 2009; Wolff and Gerritzen, 2007). Four

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Name	Extraction system	Amplification system	HIV-1 target region	Amplification and detection method	Assay software	Sample input volume (mL)	Linear dynamic range
Abbott RealTi*m*e HIV-1 assay	Automated *m*2000sp	*m*2000rt	*Pol* IN	Real-time PCR , fluorescent detection	Open mode 0.6mL RNA HIV-1 assay version 2	0.6	40-1×10^7 copies/mL
Cobas AmpliPrep- Cobas TaqMan HIV-1 assay	Automated COBAS AmpliPrep	COBAS TaqMan	*gag*	Real-time PCR, fluorescent detection	AMPLILINK 3.1.2	0.85	48-1×10^7 copies/mL

Reference: (Abbott, May 2007a; Abbott, June 2008; Abbott, May 2007b; Gueudin et al., 2007; Roche, May 2007; Scott et al., 2009)

Table 1: Summary of Assay characteristics.

different sample extraction volumes (0.2 mL, 0.5 mL, 0.6 mL, and 1.0 mL) are used with the Abbott method with sample input volume ranging from 0.7 to 1.8 mL (Sloma et al., 2009). The Abbott method uses real-time platforms with fluorescence detection systems (Abbott, 2007b; Scott et al., 2009). It runs 96 samples per run per day with more if done overnight. The instrument can detect HIV-1 RNA with a linear dynamic range from 40 – 10,000,000 copies/mL.

Even though both the Roche and Abbott methods use the real-time PCR technology, the gene targets are different. The Roche method detects the HIV-1 *gag* gene whereas the Abbott method targets the *IN* gene. In addition, their principles in designing primers and probes are also different. While the Roche method uses the standard TaqMan technology, a different and partially double stranded probe is introduced to the Abbott method (Finan and Zhao, 2007; Johanson et al., 2001). Specifically, instead of labeling the probe with both the reporter and quencher molecules on a single strand of oligonucleotide, the reporter molecule is labeled at the 5' end of the gene-specific probe; the quencher molecule is labeled to an oligonucleotide complementary to the 5' end of the probe. In this configuration, signal is not created by probe hydrolysis but by separation of the probe from quencher oligonucleotide. The rationale is that the uncoupling of the probe hydrolysis from polymerase extension will give rise to clean background and hybridization of the longer probe oligonucleotide to the gene target at relative low temperature (56°C) will yield tolerance to mismatches thus allowing less stringent hybridization and broad coverage for the detection of HIV-1 subtypes (Swanson et al., 2006).

Consequently, even though both methods are based on the same principle, due to the differences in their proprietary primer and probe designs, compatibility of these two methods has not been well documented. The objective of this study was to compare results of HIV-1 VL quantification between the two methods.

Materials and Methods

Study population and sample collection

All patient samples were collected at the University of Maryland Medical Center (UMMC) in Baltimore, Maryland. This study was approved by the Institutional Research Board under the protocol number of H-30472. All blood samples were collected in EDTA tubes. The plasma samples were first used for routine HIV-1 VL clinical testing using the Roche method. VL of the same plasma samples was re-measured using the Abbott m2000 in the same day without freeze/thaw. The Abbott *m*2000 0.6 mL protocol with a LOD of 40 copies/mL was chosen because of its volume requirement and sensitivity is comparable to the Roche method (LOD=48 copies/mL).

Sample extraction

The AMPLILINK 3.1.2 software was used on the COBAS AmpliPrep instrument for an automated sample extraction. The instrument processed 850µL of plasma. HIV viral particles were first lysed and an HIV-1 Quantitation Standard (QS) Armored RNA molecule was added to each sample, which was followed by a series of processes which involves incubation, introduction of magnetic particles, washing to remove unbound particles and elution of nucleic acid (NA) at elevated temperatures. The processed specimen containing the released HIV-1 RNA and HIV-1 QS RNA were then added to the amplification mixture and transferred to the COBAS TaqMan Analyze (Roche, 2007).

The Version 2 of the *m*2000 open mode 0.6 mL RNA HIV-1 assay protocol was used for sample extraction. This automated system uses magnetic microparticles-based reagents for the purification of nucleic acid from the samples. The general principle for NA extraction is similar to that of the Roche method, i.e., following lyses of the sample, the NA was fixed to magnetic beads and unbounded beads removed by a series of wash steps. Finally, the NA was released using an elution buffer. An internal control was added into the samples preparation procedure. The extracted NA was mixed with the amplification reagents before amplification with the *m*2000rt (Abbott, 2007a; Abbott, 2008). Manufacturer's instructions were strictly followed in both extraction processes.

VL determination (Amplification and Detection)

HIV-1 RNA amplification and detection was first performed with the automated COBAS TaqMan Analyzer. Following reverse transcription, the RNA: cDNA hybrid was amplified several times using primers that target the specific target sequence. Real-time PCR was used for detection. Dual-labeled fluorescent probes for HIV-1 and HIV-1 QS-specific oligonucleotides were used, each labeled with different reporter dyes and a quencher dye. The amount of HIV-1 RNA in the sample was quantified by measuring the fluorescence of the HIV-1 probe. The fluorescence activity was converted into VL yields by the analyzer (Roche, 2007).

The Abbott *m*2000rt performed the automated amplification and detection process. Following reverse transcription, the cDNA was amplified several times annealed to an HIV-1 and IC primers. Detection by real-time technology used two probes of different lengths: a longer fragment bounded to a fluorescent marker and complementary to the target sequence and a shorter fragment bounded to a quencher molecule. The fluorescence activity of the HIV-1 probe correlated to the amount of HIV-1 target sequence in the sample. The fluorescence counts were converted into VL measure by the analyzer (Abbott, 2007a; Abbott, 2008). Manufacturer's instructions were strictly followed.

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Statistical analysis

All VL values were first transformed into the $\log_{10}$ format. Pearson correlation test was used to measure the overall correlation of the VL between the two methods. The Bland-Altman analysis was used for the analysis of the concordance of the two methods (Bland and Altman, 1999). The $\chi 2$ analysis was used to compare the discordant results. Statistical significance was set at $p < 0.05$.

Results

A total of 308 paired plasma samples were tested in the same day by both methods (Table 2). Among them, 173 samples were quantifiable for VL and 89 samples were "Not Detected" (*ND*) by both methods. The Abbott method positively identified 188 samples out of 308 with the detectability of 61.0%; similarly, the Roche method detected 204/308 with 66.2% detectability. Among the detected samples, there was a strong overall correlation between the two methods based on the linear regression analysis (Figure 1; R^2=0.952; p<0.0001). Consistently, comparison of the distribution and median values of VL between these two methods also showed similar median VL values but a slight wider distribution by the Roche method than the Abbott method was seen (Figure 2). Specifically, a $\log_{10}$ median value ± standard deviation (SD) of 3.25 ± 1.27 copies/mL and 3.35 ± 1.29 copies/mL were observed for the Abbott method and the Roche method, respectively. The difference between these two methods was Log_{10} 0.10 ± 0.29 copies/mL with 93.7 % of the samples differing by less than 0.5 log, which is typically considered as normal assay variation.

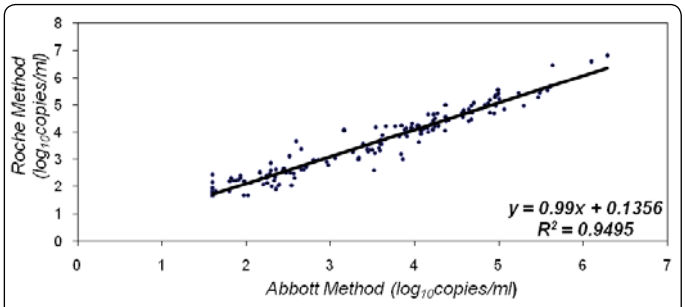

Figure 1: Regression analysis of the quantitative results of the plasma HIV-1 RNA VL measured by the Roche method (y-axis) and the Abbott method (x-axis). Each data point represents one of the paired 173 quantifiable plasma samples. The best fit for the regression analysis is indicated by the solid line. The equation of the fitted line and the Pearson coefficient of determination are presented on the plot.

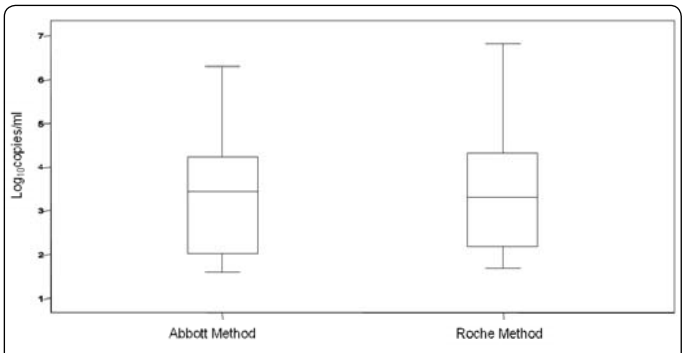

Figure 2: Box and Whisker plot for the quantifiable plasma samples. The plot to the left represents the distribution of the VL from the Abbott method assay and the plot to the right represents the distribution of VL from the Roche assay. The boxes present the distribution of the middle 50% of the data (median value) and the remaining 50% are represented by the upper and lower whiskers (vertical lines). The solid line in the box indicates the median value and the ends of the whiskers indicate the minimum and maximum values.

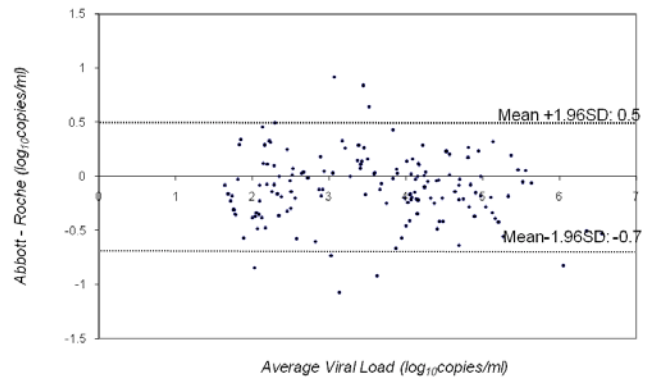

Figure 3: Bland-Altman analysis of the quantitative results. The vertical axis represenst the VL differences (the Abbott method minus the Roche method) and the horizontal axis represenst the means of VL. Each data point represents one of the paired 173 quantifiable plasma samples. The solid line indicates the mean difference between the values and the dotted lines indicates the mean ± SD 1.96 (95% limits of agreement). The number of samples within this range was 165 (95.4%).

There were a total of 46 (14.9%) discordant samples (Table 2). Of 104 *ND* Roche samples, 15 of them (14.4%) were detected by the Abbott method; similarly, of the 120 *ND* Abbott samples, 31 (25.8%) were detected by the Roche method. The χ^2 analysis of those discordant results indicated a significant difference between both methods ($\chi^2 = 96.37$, p = p <0.001).

However, comparison of the differences of VL measurements between the two methods by using the Bland Altman plot showed rough symmetric distribution of the differences and the log differences in 95.4% of the paired sample were between -Log_{10} 0.68 and Log_{10} 0.47, which are close the normal assay variation (Figure 3). Together, these data suggest that neither method is better than the other for measuring VL.

Discussion

In this study, we have compared VL quantification by the Roche and the Abbott methods side-by-side. Our data showed that these two methods are essentially indistinguishable (with a correlation coefficient of 0.952) in quantifying most of the samples tested at the University of Maryland Medical Center. However, a relative significant percentage (14.9%) of discordance was also observed between these methods (Table 2). It is unclear at the moment the source of the discrepancies. The observed differences could potentially be due to a number of intrinsic differences between these two assays, e.g., the gene target and the primer and probes designs. For examples, the Abbott method has been shown to have better detectability of non-B subtypes (Geelen et al., 2003; Swanson et al., 2005) (Gueudin et al., 2007). Thus some of the discordant samples could potentially be due to the HIV-1 subtype differences. One way to further evaluate the cause of the observed discrepancies is to determine the HIV-1 subtypes by DNA sequencing. However, this factor should not be the major contributing factor because a recent surveillance study at the University of Maryland Medical Center showed that about 98.2% of the patient samples examined were HIV-1 B subtypes, i.e., with the prevalence of the HIV-1 non B subtype of about 1.8% (Carr et al., 2010). Sensitivity of the two assays could also contribute to the differences observed as the Abbott method has a LOD of 40 copies/

		The Roche Method		
		Detected	Not Detected	Total
The Abbott Method	Detected	173	15	188
	Not detected	31	89	120
	Total	204	104	308

Table 2: Summary of HIV-1 VL test results.

mL while the Roche method has a LOD of 48 copies/mL. Furthermore, the input plasma volume is different between the two methods. 0.6 mL of plasma is used in the Abbott Method and 0.85 mL is input in the Roche assay. In addition, different precision of the methods could also potentially explain some of the discordant results.

It should be mentioned that the genetic diversity of HIV-1 subtypes could present a significant challenge in the accurate VL monitoring of HIV-infected patients who carry non-B subtypes (Damond et al., 2007; Gueudin et al., 2007; Scott et al., 2009; Swanson et al., 2005). Results of this study are unable to evaluate the potential differences between these two assays in this regard. Other assay performance characteristics such as analytical specificity, linearity, reproducibility, and precision were not evaluated.

In conclusion, the two methods showed strong correlation in measuring VL within their testing dynamic range. Various intrinsic factors including the gene target, LOD, input volume and different precision of the methods and variations in HIV-1 subtypes could potentially explain some of the discordant results. Future work includes increase of the testing population size, determination of the HIV-1 subtypes and DNA sequence analysis of those discrepant samples.

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