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PERFORMANCE OF THE ABBOTT \textit{m}2000_{\text{SP}}/\textit{m}2000_{\text{RT}} REAL-TIME POLYMERASE CHAIN REACTION ASSAY FOR HEPATITIS C VIRUS RNA QUANTIFICATION

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\textbf{Running Title}: \textit{m}2000_{\text{SP}}/\textit{m}2000_{\text{RT}} FOR HCV RNA QUANTIFICATION

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ABSTRACT

Quantification of hepatitis C virus (HCV) RNA is essential for the everyday management of chronic hepatitis C therapy. “Real-time” polymerase chain reaction (PCR) techniques are potentially more sensitive than classical PCR techniques, are not prone to carryover contamination, and have a consistently wider dynamic range of quantification. Thus they are rapidly replacing other technologies for routine quantification of HCV RNA. We extensively evaluated the intrinsic characteristics and clinical performance of m2000_{SP}/m2000_{RT}, the Abbott real-time PCR platform for HCV RNA quantification. The study shows that m2000_{SP}/m2000_{RT} is sensitive, specific, precise and reproducible, and has a broad dynamic range of quantification. When comparing HCV RNA levels measured in the same individuals with m2000_{SP}/m2000_{RT} and the third-generation branched DNA assay, a trend towards a modest overestimation of HCV RNA levels was observed in m2000_{SP}/m2000_{RT} in all genotypes except genotype 5. The differences were however unlikely to have any impact in clinical practice. In conclusion, our study shows that the Abbott m2000 real-time PCR system for HCV RNA quantification is sensitive, specific, precise and reproducible and that its broad dynamic range of quantification is well suited to HCV RNA monitoring in the clinical setting.
INTRODUCTION

Monitoring of hepatitis C virus (HCV) RNA levels is essential for the management of chronic hepatitis C therapy with the combination of pegylated interferon alpha and ribavirin. Indeed, the rapid virologic response (undetectable HCV RNA at week 4 of therapy) and the early virologic response (more than 2 Log_{10} drop or undetectable HCV RNA at week 12 of therapy) are strong predictors of the likelihood of a sustained viral eradication and are used to tailor treatment duration and improve cure rates (2, 6, 7, 10, 14, 16, 23, 26, 27). HCV RNA quantification will remain of major importance in the management of therapy with the new HCV drugs in development to assess the virologic response and to detect virologic breakthroughs related to viral resistance early enough to alter therapy and rescue the antiviral efficacy (20).

Ideally, HCV RNA quantification assays should be sensitive, specific, accurate, precise, and reproducible. They should have a broad range of linear quantification that fully covers the HCV RNA levels observed in clinical practice in both untreated and treated patients, and quantification should be independent of the HCV genotype. Real-time polymerase chain reaction (PCR) techniques currently are replacing classical PCR methods and the branched DNA technology which did not fulfill all of these criteria. Real-time PCR methods are sensitive, with lower limits of detection/quantification of the order of 10-15 HCV RNA international units (IU)/mL, and are not prone to carryover contamination. They benefit from a broad dynamic
range of quantification of 7 to 8 $\log_{10}$ that covers most of the HCV RNA levels encountered in clinical practice.

In this study, we investigated the intrinsic and clinical performances of the recently developed Abbott $m2000$ real-time PCR system for HCV RNA quantification, which uses the automated extractor $m2000_{SP}$ and the $m2000_{RT}$ device for automated real-time PCR amplification and detection of PCR products ($m2000_{SP}/m2000_{RT}$, Abbott Diagnostic, Chicago, Illinois).

**MATERIALS AND METHODS**

**Materials**

**Standards.** A standard panel (OptiQuant™ HCV RNA, AcroMetrix, Benicia, California) containing different concentrations of HCV RNA from a single source, i.e. a hepatitis C infected individual with HCV genotype 1b (15), was used to study the analytical performance of the assay. The 7 panel members are NAP-000, NAP-HCV5E1, NAP-HCV5E2, NAP-HCV5E3, NAP-HCV5E4, NAP-HCV5E5, NAP-HBV5E6, which contain no HCV RNA, $5 \times 10^1$ IU/mL ($1.7 \log_{10}$ IU/mL), $5 \times 10^2$ IU/mL ($2.7 \log_{10}$ IU/mL), $5 \times 10^3$ IU/mL ($3.7 \log_{10}$ IU/mL), $5 \times 10^4$ IU/mL ($4.7 \log_{10}$ IU/mL), $5 \times 10^5$ IU/mL ($5.7 \log_{10}$ IU/mL) and $5 \times 10^6$ IU/mL ($6.7 \log_{10}$ IU/mL), respectively.

**Clinical specimens.** Serum samples were obtained from patients followed in the Department of Hepatology and Gastroenterology of Henri Mondor Hospital. Group A included 202 HCV-seronegative individuals, i.e. subjects without total anti-
HCV antibodies in a third-generation enzyme immunoassay (Vitros ECi, Ortho-Clinical Diagnostics, Raritan, New Jersey). Group B included 141 patients with chronic HCV infection, characterized by the presence of both total anti-HCV antibodies and HCV RNA. The HCV genotype was determined in all cases by means of direct sequence analysis of a portion of the non structural 5B (NS5B) gene that encodes the RNA-dependent RNA polymerase followed by phylogenetic analysis, as recently described (3). Group B included 55 patients infected with HCV genotype 1, 21 with genotype 2, 29 with genotype 3, 24 with genotype 4, 9 with genotype 5 and 3 with genotype 6. Serum was separated from whole blood by centrifugation, placed into sterile tubes and frozen at -70°C until use in this study. Group C included 10 patients infected with HCV genotype 1, 7 with genotype 2, 8 with genotype 3, 9 with genotype 4 and 1 with genotype 5 whose sera had been tested for HCV RNA with the Cobas Ampliprep®/Cobas TaqMan® (CAP/CTM) real-time PCR assay (Roche Molecular Systems, Pleasanton, California).

Assessment of \( m_{2000}^{SP}/m_{2000}^{RT} \) performance.

Analytical sensitivity. In order to evaluate the analytical sensitivity of the \( m_{2000}^{SP}/m_{2000}^{RT} \) assay relative to the manufacturer’s stated lower limit of detection (12 IU/mL), serial 1/2 dilutions of the NAP-HCV5E4 standard down to 6.25 IU/mL were tested 20 times.

Specificity. The specificity of the \( m_{2000}^{SP}/m_{2000}^{RT} \) assay was assessed by testing the 202 HCV-seronegative clinical specimens from group A.

Linearity, accuracy and influence of the HCV genotype. The linearity of quantification in \( m_{2000}^{SP}/m_{2000}^{RT} \) was assessed by testing the seven samples of the standard panel OptiQuant™ HCV RNA, which contain up to \( 5 \times 10^6 \) IU/ml (6.7
Log\textsubscript{10} IU/ml). Each panel member dilution was tested six times in the same experiment with both \textit{m2000\textsubscript{SP}/m2000\textsubscript{RT}} and three times in the same experiment with the third-generation bDNA-based assay, Versant\textsuperscript{®} HCV RNA 3.0 Assay. The average measured values were compared with the expected HCV RNA levels. In addition, serial one-fifth dilutions down to signal extinction were tested in 10 genotype 1, 10 genotype 2, 9 genotype 3, 10 genotype 4, 4 genotype 5 and 3 genotype 6 samples from group B. The dilutions were made with the Nucleic Acid Test (NAT) dilution matrix (AcroMetrix), a defibrinated, delipidized normal human plasma.

We also compared the results of \textit{m2000\textsubscript{SP}/m2000\textsubscript{RT}} with those obtained for the same samples with the third-generation bDNA-based assay in the 141 samples from group B. The bDNA assay can be confidently used as a comparator since it has been shown to be precise and accurate and to equally quantify HCV genotypes 1 to 6, due to the use of a set of 6 capture and 17 extender oligonucleotide probes spanning the full-length 5' non-coding and the 5' third of the core coding region for hybridization of the HCV genome (1, 8, 9, 12, 13, 17-19, 24).

Finally, HCV RNA levels obtained with \textit{m2000\textsubscript{SP}/m2000\textsubscript{RT}} were compared with those obtained in the same samples with the CAP/CTM assay and the third-generation bDNA-based assay in the 35 HCV-positive samples from group C.

\textit{Precision and reproducibility}. In order to assess precision (or intra-assay reproducibility), each member of the OptiQuant\textsuperscript{™} HCV RNA panel was tested six times. In order to assess inter-assay reproducibility, the low positive control (LPC) and the high positive control (HPC) provided in the kits were tested 38 times in the corresponding runs on different days.

\textbf{HCV RNA quantification}.  

m2000SP/m2000RT. HCV RNA was extracted from 500 µl of serum in the automated extractor m2000SP, according to the manufacturer’s instructions. The m2000RT device was then used for automated real-time PCR amplification and detection of PCR products according to the manufacturer’s instructions. HCV RNA levels were expressed in IU/mL.

bDNA. In the Versant HCV RNA 3.0 Assay, HCV RNA was recovered from 50 µl of serum and quantified in the semi-automated System 340® bDNA analyzer (Siemens Medical Solutions Diagnostics, Tarrytown, New York), according to the manufacturer’s instructions. HCV RNA levels were expressed in IU/mL.

CAP/CTM. HCV RNA was extracted from 850 µl of serum in the automated Cobas AmpliPrep® extractor, according to the manufacturer’s instructions. The Cobas TaqMan 48® Analyzer was used for automated real-time PCR amplification and detection of PCR products. Generated data were analyzed with AmpliLink® software. HCV RNA levels were expressed in IU/ml.

Statistical analysis. Descriptive statistics are shown as the mean ± standard deviation (SD) or medians and interquartile ranges as appropriate. Comparisons between groups were made using the Kruskall-Wallis test or Mann-Whitney test. The relationship between quantitative variables was studied by means of regression analysis. p values <0.05 were considered significant.

RESULTS

Intrinsic performance of the m2000SP/m2000RT assay
**Analytical sensitivity.** Serial 1/2 dilutions of a standard containing 50 IU/mL HCV RNA down to 6.25 IU/mL were tested 20 times in different experiments with $m_{2000sp}/m_{2000RT}$. HCV RNA was detected in 20/20, 20/20, 13/20 and 13/20 replicate tests of samples containing 50 IU/mL (1.7 $\log_{10}$ IU/mL), 25 IU/mL (1.4 $\log_{10}$ IU/mL), 12.5 IU/mL (1.1 $\log_{10}$ IU/mL) and 6.25 IU/mL (0.8 $\log_{10}$ IU/mL), respectively. This result was in keeping with the manufacturer’s stated lower limit of detection of the assay of 12 IU/mL.

**Specificity.** The specificity of $m_{2000sp}/m_{2000RT}$ was assessed by testing 202 samples from anti-HCV seronegative patients (group A). No sample tested positive above the lower limit of detection (LLOD) of 12 IU/mL (specificity: 100%, 95% confidence interval: 99-100%).

**Precision and reproducibility.** Precision (intra-assay reproducibility) was assessed by testing the seven members of the OptiQuant™ HCV RNA standard, that contain 0, 1.7, 2.7, 3.7, 4.7, 5.7 and 6.7 $\log_{10}$ IU/mL, respectively, six times in the same experiment. As shown in Table 1, the coefficients of variation varied from 0.76% to 7.68%. Inter-assay variability was assessed by testing both the high positive control (HPC) and the low positive control (LPC), extracted like clinical samples, 38 times in different experiments. The coefficients of variation were of 1.98% and 3.91%, respectively (Table 1).

**Accuracy, linear quantification and influence of the HCV genotype**
Linear quantification of standard panel dilutions. The OptiQuant™ HCV RNA genotype 1b standard panel, containing 5 × 10^1 IU/mL (1.7 Log_{10} IU/mL) to 5 × 10^6 IU/mL (6.7 Log_{10} IU/mL), was used to assess the linearity of HCV RNA quantification in the m2000SP/m2000RT assay. The panel was tested six times and three times in the same experiments with m2000SP/m2000RT and bDNA, respectively. As shown in Figure 1A, a significant relationship was found between the average measured and the expected HCV RNA levels (r = 0.9980, p < 0.0001). The difference between the average measured and the expected HCV RNA levels varied from 0.23 to 0.57 Log_{10} IU/mL. A significant relationship was also found between the average measured and the expected HCV RNA levels in the bDNA assay (r = 0.9997, p < 0.0001; Figure 1B). The difference between the average measured and the expected HCV RNA level varied from 0.21 to 0.33 Log_{10} IU/mL. The HCV RNA level of three standards, NAP-000, NAP-HCV5E1 and NAP-HCV5E2, was below the LOD of the bDNA assay (615 IU/mL, i.e. 2.79 Log_{10} IU/mL).

Quantification of HCV RNA in clinical samples containing HCV genotypes 1 to 6. One hundred and forty-one samples from patients with chronic hepatitis C infected with HCV genotypes 1 to 6 (group B, see Methods section) were tested with both m2000SP/m2000RT and the third-generation bDNA assay. All of these samples fell within the dynamic range of quantification of both assays. As shown in Figure 2, there was a significant relationship between the HCV RNA levels obtained in the same samples with m2000SP/m2000RT and bDNA, for genotype 1 to 5 (only 3 genotype 6 samples have been tested). The regression lines were slightly above the expected equality line, as a result of higher values obtained with m2000SP/m2000RT.
than with bDNA in the same sample in the majority of cases (131 out of 141 clinical specimens tested) (Figure 2).

Figure 3A shows a Bland-Altman analysis of HCV RNA levels measured in the 141 samples from group B in both $m2000_{SP}/m2000_{RT}$ and bDNA. The figure plots the difference between the two measured values ($m2000_{SP}/m2000_{RT}$ minus bDNA) as a function of the mean of both measurements. A moderate overestimation of HCV RNA levels by $m2000_{SP}/m2000_{RT}$ as compared to bDNA was observed in 131 of the 141 samples (92.9%) containing all HCV genotypes (median $m2000_{SP}/m2000_{RT}$ minus bDNA difference: 0.30 Log$_{10}$ IU/mL). Six samples (one genotype 1, two genotype 2, two genotype 4 and one genotype 6) had an $m2000_{SP}/m2000_{RT}$ minus bDNA difference below -1.96 times the mean difference, whereas 8 samples (all of genotype 1) had a $m2000_{SP}/m2000_{RT}$ minus bDNA difference above +1.96 times the mean difference. The individual differences between $m2000_{SP}/m2000_{RT}$ and bDNA were however always below 1.0 Log$_{10}$ IU/mL in these samples. Box plots of individual differences between the two methods are shown for each genotype in Figure 3B. They confirm the global, very moderate overestimation of HCV RNA levels in $m2000_{SP}/m2000_{RT}$ compared to bDNA, independently of the HCV genotype. The median differences were 0.22 Log$_{10}$ IU/mL for genotype 1, 0.33 Log$_{10}$ IU/mL for genotype 2, 0.31 Log$_{10}$ IU/mL for genotype 3, 0.32 Log$_{10}$ IU/mL for genotype 4, and -0.08 Log$_{10}$ IU/mL for genotype 5 ($p = 0.013$). HCV genotype 6 is not shown on Figure 3B because only 3 samples have been tested.

Linear quantification of serial dilutions of HCV-infected sera. Serial one-fifth dilutions down to signal extinction were tested in 10 genotype 1, 10 genotype 2, 9 genotype 3, 10 genotype 4, 4 genotype 5 and 3 genotype 6 samples from group B.
Serial dilution quantification was linear in all cases, whatever the HCV genotype, with Pearson’s coefficient ranging from 0.9940 to 0.9996 for HCV genotype 1, 0.9930 to 0.9996 for HCV genotype 2, 0.9971 to 0.9996 for HCV genotype 3, 0.9812 to 0.9997 for HCV genotype 4, 0.9938 to 0.9989 for HCV genotype 5 and 0.9975 to 0.9983 for HCV genotype 6. The expected difference between two successive 1/5 dilutions is 0.70 Log_{10} IU/mL. The mean±SD differences between the undiluted sample and the first 1/5 dilution, and between each dilution and the subsequent one were 0.81±0.12, 0.76±0.12, 0.74±0.08, 0.77±0.13, 0.76±0.11, and 0.75±0.11, respectively (not significantly different).

Comparison with CAP/CTM

Sera from 35 patients with chronic HCV infection of different genotypes (group C) were tested with the m2000SP/m2000RT assay, the CAP/CTM assay and the third-generation bDNA-based assay. Figure 4A shows the better correlation between m2000SP/m2000RT and bDNA than between CAP/CTM and bDNA. The greatest differences between CAP/CTM and bDNA were seen for HCV genotypes 2 and 4 samples, as already reported (4). Figure 4B shows the relationship between HCV RNA levels measured in the same samples by m2000SP/m2000RT and CAP/CTM.

DISCUSSION

Our assessment of the intrinsic performance of the m2000SP/m2000RT platform for HCV RNA quantification shows an excellent specificity, precision and reproducibility of the technique, in keeping with previous reports (11, 22). The
manufacturer's stated lower limit of detection of the assay is 12 IU/ml, as confirmed in the present study.

In clinical samples from patients with chronic hepatitis C infected with the 6 HCV genotypes, we observed a significant relationship between the HCV RNA levels measured in the same samples with \( m^{2000_{SP}}/m^{2000_{RT}} \) and the third-generation bDNA assay. The use of the third-generation bDNA assay as the comparator was justified by the fact that this assay is accurate and reproducible, quantifies HCV RNA independently of the HCV genotype and has been well calibrated to the World Health Organization (WHO), as confirmed in this study (1, 8, 9, 12, 13, 24, 25). The correlation was found for all HCV genotypes (it was not significant for genotype 6 but only 3 samples could be tested) and the median individual differences between the HCV RNA levels measured in the two assays were below ±0.5 Log\(_{10}\) IU/mL (i.e. a 3-fold difference) in all but 10 clinical samples.

The HCV RNA quantification panel used in this study was calibrated with the WHO international HCV RNA standard by means of the second-generation bDNA assay (15, 21). A minor underestimation of HCV RNA levels was observed with both \( m^{2000_{SP}}/m^{2000_{RT}} \) and the third-generation bDNA assay relative to the expected HCV RNA values, in spite of a linear relationship. When comparing HCV RNA levels measured in the same individuals with both techniques, we observed a trend towards a modest overestimation of HCV RNA levels in \( m^{2000_{SP}}/m^{2000_{RT}} \) compared to the third-generation bDNA in all genotypes except genotype 5. The differences were however unlikely to have any impact in the clinical use of the \( m^{2000_{SP}}/m^{2000_{RT}} \) platform. Importantly, no sample was substantially underquantified in the \( m^{2000_{SP}}/m^{2000_{RT}} \) real-time PCR assay relative to bDNA. In contrast, we have shown that another real-time PCR platform for HCV RNA quantification, CAP/CTM,
underestimates HCV RNA levels in approximately 30% of HCV genotype 4 and 15% of HCV genotype 2 infections as a probable result of mispairing of the PCR primers and/or Taqman probe (4). We recently reported two patients infected with HCV genotype 4 with undetectable HCV RNA in CAP/CTM and high viral levels in \textit{m2000SP/m2000RT} and bDNA (5). When testing the same patients' samples with \textit{m2000SP/m2000RT}, CAP/CTM and bDNA in the present study, we observed a better accuracy of \textit{m2000SP/m2000RT} than CAP/CTM for HCV RNA quantification, especially in HCV genotypes 2 and 4 samples.

We tested serial dilutions of clinical samples of the different genotypes. Quantification was linear in all cases, whatever the HCV genotype. This is unlike the CAP/CTM assay in which we found that HCV RNA levels were overestimated in undiluted clinical samples, but not when the same samples were tested after dilution, a phenomenon observed with all six HCV genotypes (4). These findings explain the greater overestimation of HCV RNA levels relative to bDNA in CAP/CTM than in \textit{m2000SP/m2000RT}. The molecular basis for the HCV RNA overestimation by CAP/CTM in undiluted samples is unknown. We hypothesized a biochemical interaction with a blood component during one of the reaction steps, an interaction that vanishes when the concentration of this putative component is reduced in diluted samples (4). The results of the present study suggest that this putative interaction does not occur during the \textit{m2000SP/m2000RT} reaction.

In conclusion, our study shows that the Abbott \textit{m2000} real-time PCR system for HCV RNA quantification (\textit{m2000SP/m2000RT}) is sensitive, specific, precise and reproducible and that its broad dynamic range of quantification is well suited to HCV RNA monitoring in the clinical setting. This is of particular importance in the current clinical context. Indeed, novel therapeutic strategies that will include HCV protease
and polymerase inhibitors will require sensitive and accurate assays with a broad dynamic range of quantification to monitor the virological responses and tailor treatment in order to prevent resistance.

ACKNOWLEDGMENTS

The \( m^{2000}_{SP}/m^{2000}_{RT} \) kits used in this study have been kindly provided by Abbott Molecular Diagnostics (Rungis, France). This work is part of the activity of the VIRGIL European Network of Excellence on Antiviral Drug Resistance supported by a grant (LSHM-CT-2004-503359) from the Priority 1 "Life Sciences, Genomics and Biotechnology for Health" programme in the 6\(^{th}\) Framework Programme of the European Union. The authors are grateful to Françoise Darthuy and Guillaume Dameron for helpful technical assistance.
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FIGURE LEGENDS

Figure 1. HCV RNA quantification of a commercial standard panel containing $5 \times 10^1$ ($1.7 \text{ Log}_{10}$) to $5 \times 10^6$ ($6.7 \text{ Log}_{10}$) HCV RNA IU/mL (OptiQuant™ HCV RNA, AcroMetrix, Benicia, California) by $m_{2000_{SP}}/m_{2000_{RT}}$ (A) and bDNA (B). The average measured results are shown as a function of the expected results. The dashed line is the equality line.

Figure 2. Correlation between HCV RNA levels measured in $m_{2000_{SP}}/m_{2000_{RT}}$ and bDNA in the same samples from group B, consisting of 141 clinical samples containing HCV genotype 1 (Figure 2A), 2 (2B), 3 (2C), 4 (2D), 5 (2E) and 6 (2F).

Figure 3. (A) Bland-Altman plot analysis of HCV RNA levels measured in both $m_{2000_{SP}}/m_{2000_{RT}}$ and bDNA in the 141 samples from group B. The difference between HCV RNA levels measured in $m_{2000_{SP}}/m_{2000_{RT}}$ and bDNA, respectively, is represented as a function of the mean of both values. Different genotypes are represented by different colors. The gray area corresponds to the mean difference ± 1.96 standard deviation. (B) Distribution of the differences between HCV RNA levels measured in $m_{2000_{SP}}/m_{2000_{RT}}$ and bDNA in the same samples according to the HCV genotype (1 to 5). The results are presented as box-plots, where the horizontal line represents the median value, the gray box the 50th percentiles and the upper and lower bars the 95th percentiles.

Figure 4. Correlation between HCV RNA levels measured: (A) in CAP/CTM and bDNA (left) and in $m_{2000_{SP}}/m_{2000_{RT}}$ and bDNA (right), and (B) in CAP/CTM and
$m_{2000_{SP}}/m_{2000_{RT}}$ in the same samples from group C, consisting of 35 clinical samples containing HCV genotypes 1 to 5.
Table 1. Intra-assay (precision) and inter-assay reproducibility of $m2000_{SP}/m2000_{RT}$ HCV real-time PCR assay. For intra-assay reproducibility, the seven members of the standard panel (NAP-000 to NAP-HCV5E6) containing 0 to $5 \times 10^6$ IU/ml, i.e. 6.70 Log$_{10}$ IU/mL, respectively, have been tested six times in the same experiment. For inter-assay reproducibility, the assay low positive control (LPC) and high positive control (HPC) have been tested 38 times in different experiments.

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<th>No. of determinations</th>
<th>Mean (SD) measured HCV RNA (Log$_{10}$ IU/ml)</th>
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Figure 1
HCV RNA level in Versant HCV 3.0 Assay (Log10 IU/mL)

Genotype 1 (n = 55)

Genotype 2 (n = 21)

Genotype 3 (n = 29)

Genotype 4 (n = 24)

Genotype 5 (n = 9)

Genotype 6 (n = 3)

Figure 2

$r = 0.9911; p < 0.0001$

$r = 0.9736; p < 0.0001$

$r = 0.9784; p < 0.0001$

$r = 0.9802; p < 0.0001$

$r = 0.9781; p < 0.0001$

$r = 0.9911; p < 0.0001$

$r = 0.9292; p = 0.23$
Mean of HCV RNA levels measured in m2000SP/m2000RT and Versant HCV 3.0 Assay (in Log10 IU/mL)

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<th>Mean</th>
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Difference between HCV RNA levels measured in m2000SP/m2000RT and in Versant HCV 3.0 Assay (in Log10 IU/mL)

+1.96 mean difference

-1.96 mean difference

A Figure 3A
Figure 3B

B

Difference between HCV RNA levels measured in m2000_sp/m2000_RT and in bDNA (in Log10 UI/ml)
Figure 4

Genotype 1 (n=10)
Genotype 2 (n=7)
Genotype 3 (n=8)
Genotype 4 (n=9)
Genotype 5 (n=1)