Improved COBAS® TaqMan® HCV Test, Version 2.0 For Use With The High Pure System:
Enhanced Genotype Inclusivity and Performance Characteristics in a Multi-Site Study

Running title: Improved genotype inclusivity of HPS / COBAS TaqMan HCV 2.0


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ABSTRACT

We have evaluated the COBAS® TaqMan® HCV Test, v2.0 For Use With The High Pure System (HCVHPS V2), a new revised real-time RT-PCR assay developed to improve the genotype quantitation of its previous 1.0 version (HCVHPS V1). Revisions were made in the wash buffer and in the reverse-transcription temperature. Genotype inclusivity of HCVHPS V2 was evaluated at 3 different sites using HCVHPS V2, HCVHPS V1, and COBAS AMPLICOR HCV MONITOR Test, v2.0 (CAHCM). The fully automated COBAS Ampliprep/COBAS Taqman HCV test was also used in one of the participating laboratories. Mean difference in HCV RNAs between HCVHPS V2 and CAHCM and between HCVHPS V2 and HCVHPS V1 ranged, respectively, from -0.21 to 0.13 log and from 0.24 to 1.27 log, with higher than 0.5 log differences for genotypes 2, 3, 4, and 5. Using a NIBSC panel of HCV genotypes 1 through 6, the measured HCVHPS V2 values were within 0.25 log of the nominal values for all 6 genotypes. When testing serial dilutions of genotype specific HCV clinical specimens, the assay showed a limit of detection between 10 and 20 IU/mL and a 25 IU/mL to 3.91x10^8 IU/mL linear range. A 100% clinical and analytical specificity was demonstrated with 100 HCV sero-negative specimens as well as with 12 non-HCV members of Flaviviridae and 22 additional micro-organisms. These data indicate that HCVHPS V2 is a robust and accurate test for the quantitation of all six HCV genotypes and useful in monitoring viral load in all HCV clinical specimens.
INTRODUCTION

Viral load monitoring is an important part of the disease management strategies that have been developed for chronic hepatitis C. Changes in HCV RNA levels occurring during the early phase of anti-viral therapy have been derived from complex models of viral kinetics and applied to the prediction of treatment outcomes (14, 24-25). The high negative predictive value of a less than 2 logarithms decrease at week 12 of pegylated interferon / ribavirin combination therapy compared to baseline has lead to the introduction in treatment guidelines of a stopping rule which helps shortening therapy duration for non-responsive patients (2, 11). More recent studies suggest that this approach may be applied at earlier time-points during treatment and that the predictive value of viral load at week 4 may help in individualizing treatment duration (3, 7, 12-13). Research and guidelines for treatment in this field will be better supported by the development and use of more accurate quantitative assays that allow for precise measurement and monitoring of viral kinetics (8).

Real-time PCR assays have been recently introduced to offer higher sensitivity and broader dynamic ranges than end-point PCR tests (6, 15). One of these assays, the semi-automated, real-time PCR system COBAS® TaqMan® HCV Test For Use With The High Pure System (HCVHPS V1) has been limited to genotypes 1 and 6 because of an inconsistent performance with the other genotypes (20). In this respect, we have evaluated in a multi-site study an improved HCVHPS V2 by thoroughly investigating its genotype inclusivity, sensitivity, specificity, and linear range in comparison to the licensed CAHCM and HCVHPS V1.
MATERIALS AND METHODS

Samples

Clinical specimens from patients with chronic HCV infection were obtained from various vendors, Saarland University Hospital (Homburg, Germany), TriCore Reference Laboratories (Albuquerque, New Mexico, USA), and RD KK (Roche Diagnostics K.K., Tokyo, Japan). HCV genotypes and subtypes of each sample were determined either by sequencing of the 5'-UTR region or by HCV genotyping line probe/blot assays. Armored HCV RNA was designed to contain a region of the 5’-UTR of an HCV genotype 1 specimen and prepared using processes described by WalkerPeach et al. (23). Armored HCV RNA were diluted with pools of HCV-negative human EDTA-plasma or serum prior to testing. Due to the large volume required for testing in multiple replicates in multiple tests at multiple sites, some of the clinical specimens were also diluted with pools of HCV-negative human EDTA plasma or serum prior to testing. For additional studies on genotype inclusivity, a panel of all six HCV genotypes (NIBSC code 02/202) was obtained from the National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK.

Test procedures

The specimens were tested by COBAS® TaqMan® HCV Test, v2.0 For Use With The High Pure System (HCVHPS V2), COBAS® TaqMan® HCV Test For Use With The High Pure System (HCVHPS V1), COBAS AmpliPrep/COBAS TaqMan HCV Test (HCMCAP), and COBAS AMPLICOR HCV MONITOR™ Test, v2.0 (CAHCM), according to instructions described in their respective package inserts. The differences between the HCVHPS V2 test and the HCVHPS V1 test are briefly discussed below.
(i) **Extractions by High Pure System.** In the HCVHPS V2 test, the Wash Buffer reagent is reconstituted with 30 mL of deionized water and 50 mL of 96 – 100% ethanol resulting in a final ethanol concentration of 48-50%. In the HCVHPS V1 test, the Wash Buffer reagent is reconstituted with 80 mL of 96 – 100% ethanol resulting in a final ethanol concentration of 77-80%.

(ii) **Amplification and detection.** The temperature for the reverse transcription step is 64°C for the HCVHPS V2 test and 59°C for the HCVHPS V1 test.

**HCV genotype inclusivity**

The accuracy of quantitation for all HCV genotypes using the HCVHPS V2 test was demonstrated by testing multiple sets of clinical specimens representing HCV genotypes 1 through 6 by three different tests (HCVHPS V2, CAHCM, and HCVHPS V1) in triplicate at three different sites: Saarland University Hospital (SUH, Homburg, Germany), TriCore Reference Laboratories (TRL, Albuquerque, New Mexico, USA), and Roche Molecular Systems (RMS, Pleasanton, California, USA). Approximately 10 clinical specimens of each of the six HCV genotypes were tested at each of the three sites with some of the clinical specimens overlapping among the three sites. All values were log_{10} transformed and each value of each specimen at each site for each test was compared to the corresponding value obtained from the other tests. The mean difference between any two tests for all specimens within each genotype at each site was then calculated. The site-to-site difference for the subset of specimens shared by more than one site was also calculated for the HCVHPS V2 test.

At one of the sites (RMS), an additional set of 114 clinical specimens of all HCV genotypes were tested by four different tests: HCVHPS V2, HCVHPS V1, CAHCM, and HCMCAP. Furthermore, a
panel of all six HCV genotypes from NIBSC (code 02/202) was also evaluated using the HCVHPS V2 test.

Additional studies were performed at the fourth site, RDKK (Roche Diagnostics, K.K., Tokyo, Japan) using 30 genotyped HCV serum specimens. The specimens were tested in triplicate by each of the three tests: HCVHPS V2, CAHCM, and HCVHPS V1.

**Limit of detection (Sensitivity) for all HCV genotypes**

To establish that the HCVHPS V2 test exhibited similar Limit of Detection (LOD) for all HCV genotypes, the HCV Secondary Standard (Lot 0002, Genotype 1a, 20,900 IU/mL) and available characterized HCV clinical specimens representing genotypes (2a, 2b, 3, 4, 5, and 6) were utilized. Serial dilutions to final concentrations of 30, 20, 10, 5, and 2.5 IU/mL were made in HCV-negative human EDTA plasma and serum. The concentrations of the source materials for the dilutions of the HCV genotypes 2a, 2b, 3, 4, 5, and 6 were determined by the CAHCM test. The HCV Secondary Standard was prepared using a genotype 1a HCV clinical specimen calibrated to the First WHO International Standard for HCV RNA NAT assays (NIBSC code 96/790) (10, 18). For each of the two different lots of the HCVHPS V2 kit, at least 3 independent dilution series were tested for each specimen matrix and each genotype. A minimum number of 12 replicates per concentration were tested with each HCVHPS V2 kit lot for a total of 24 replicates per concentration. For each genotype, the lowest tested concentration that yielded a minimum of 95% positive results as well as the calculated concentration for 95% positive results by PROBIT analysis was determined.

**Linear range**

Two linearity panels consisting of 15 members of Armored HCV RNA (genotype 1) in EDTA plasma or serum were tested using three unique lot combinations of COBAS® TaqMan® HCV Test,
v2.0 and High Pure System Viral Nucleic Acid kits. The 15 members of the panels covered a range from 7.83 HCV RNA IU/mL to 7.83x10^8 HCV RNA IU/mL based on the source material whose concentration was determined by comparison to the HCV Secondary Standard (Lot GTR015), prepared using a Genotype 1a HCV clinical specimen calibrated to the Second WHO International Standard for HCV RNA NAT assays (NIBSC code 96/798) (10, 19). Twelve replicates per level for each of the three lot combinations were tested for a total of 36 replicates per level. Linear range was determined by following the CLSI EP6-A guideline using a maximum allowable bias of 0.2 log_{10} (22).

**Precision**

The total imprecision of the HCVHPS V2 test for EDTA plasma specimens was assessed by testing each of 6 different concentrations of a genotype 1 specimen in EDTA plasma over 48 times in 24 runs using 2 lots of COBAS® TaqMan® HCV Test, v2.0 kits, 3 analysts, and 2 COBAS® TaqMan® 48 Analyzer instruments. The total imprecision of the HCVHPS V2 test for serum specimens was assessed by testing each of 7 different concentrations of a genotype 1 specimen in serum over 72 times in 36 runs using 2 lots of COBAS® TaqMan® HCV Test, v2.0 kits, 3 analysts, and 3 COBAS® TaqMan® 48 Analyzer instruments. Lot-to-lot, instrument-to-instrument, operator-to-operator, between-run, within-run, and total imprecision were evaluated using a nested ANOVA of log_{10} transformed HCV RNA values. The %CV was calculated using the formula, % CV = SQRT [(10^{SD x SD x ln 10} -1)] x 100, where SQRT, SD, and ln10 represent square root, standard deviation of log_{10} transformed values, and the natural log of 10, respectively (4).

**Specificity**
The clinical specificity of the HCVHPS V2 test was determined by testing 50 HCV sero-negative EDTA plasma and 50 HCV sero-negative serum specimens with 2 different COBAS® TaqMan® HCV Test, v2.0 kit lots. The analytical specificity was evaluated by adding micro-organisms into HCV-negative human EDTA plasma and serum. Twelve non-HCV members of Flaviviridae (West Nile Virus, St. Louis Encephalitis Virus, Dengue Virus Types 1 through 4, Yellow Fever Virus, Zika Virus, Banzi Virus, Ilheus Virus, Murray Valley Virus, and Hepatitis G Virus) and twenty-two additional micro-organisms (Staphylococcus aureus and epidermis, Epstein Barr Virus, Propionibacterium acnes, Adenovirus Human Type 3, Cytomegalovirus Davis and Towne, Herpes Simplex Virus type 1 MacIntyre and type 2G, Hepatitis A Virus, Hepatitis B Virus genotypes A and B, Human Papilloma Virus 11, 18, and 6B, Varicella Zoster, Candida albicans, HTLV I/II, Influenza B, Mycobacterium avium, and HIV) were tested for possible cross-reactivity using the HCVHPS V2 test.

Interference

Twenty-three different drug compounds (Tenofovir disoproxil fumarate, Enfuvirtide, Nevirapine, Efavirenz, Lamivudine, Zidovudine, Zalcitabine, Stavudine, Ribavirin, Abacavir sulfate, Didanosine, PEG interferon alpha 2a, Interferon alpha 2b, Interferon alpha 2a, Indinavir sulfate, Ritonavir, Nelfinavir sesylate, Saquinavir, Amprenavir, Lopinavir/Ritonavir, Paroxetine, Fluoxetine, and Sertraline) at concentrations of 3 times their respective Cmax values (peak plasma concentrations) in EDTA plasma and serum were tested by the HCVHPS V2 test. An HCV specimen of approximately $2 \times 10^4$ IU/mL with and without the spiked drug was examined by the HCVHPS V2 test in triplicate. The difference in mean log HCV RNA (IU/mL) values between the drug-spiked and the control (no
drug) specimens was calculated. A mean difference within 0.3 $\log_{10}$ was considered as no interference for the drug tested.
RESULTS

HCV genotype inclusivity

The results from HCV genotype inclusivity studies performed with HCVHPS V2, HCVHPS V1, and CAHCM at three sites (TRL, SUH, and RMS) are shown in Table 1. All three sites generated results with similar mean differences in log HCV RNA (IU/mL) when comparing HCVHPS V2 to HCVHPS V1 or HCVHPS V2 to CAHCM for all genotypes except the result for genotype 2 at SUH for the comparison between HCVHPS V2 and HCVHPS V1. Mean differences in log HCV RNA (IU/mL) between the HCVHPS V2 test and the HCVHPS V1 test for all 3 sites combined were 0.24, 0.56, 1.27, 1.05, 0.73, and 0.25 log_{10} for genotypes 1 through 6, respectively. These results showed greatly enhanced genotype inclusivity of the HCVHPS V2 test in comparison to the HCVHPS V1 test for genotypes 2, 3, 4, and 5. For the comparison of the HCVHPS V2 test to the CAHCM test, mean differences in log HCV RNA (IU/mL) between the two tests for all 3 sites combined were much smaller at -0.21, -0.11, 0.13, -0.02, -0.13, and -0.16 log_{10} for genotypes 1 through 6, respectively. The results demonstrated that the genotype inclusivity of the HCVHPS V2 test is very similar to that of the CAHCM test for all six genotypes.

When analyzing differences among the three sites for the subset of shared specimens tested by the HCVHPS V2 test at more than one site, we observed mean differences in log HCV RNA (IU/mL) between any 2 sites of 0.10 log_{10} or less for all specimens and 0.21 log_{10} or less for each of the six HCV genotypes (data not shown).

The variable differences between the HCVHPS V2 test and the HCVHPS V1 test among the 3 sites for specimens of HCV genotype 2, possibly due to site-specific subtype distribution, was examined at a fourth site, RDKK (data not shown). Mean differences in log HCV RNA (IU/mL) between the
HCVHPS V2 test and the HCVHPS V1 test were -0.03, 0.67, and -0.09 log_{10} for genotypes 1b, 2a, and 2b, respectively. For the comparison of the HCVHPS V2 test to the CAHCM test, mean differences in log HCV RNA (IU/mL) between the two tests were -0.12, -0.34, and 0.14 log_{10} for genotypes 1b, 2a, and 2b, respectively. The results at RDKK demonstrated greatly enhanced genotype inclusivity of the HCVHPS V2 test for subtype 2a in comparison to the HCVHPS V1 test and are consistent with the above possibility for variable composition of subtypes for the genotype 2 specimens tested at the three sites.

In order to further demonstrate the equivalent quantitation of HCV genotypes by the HCVHPS V2 test, two more studies were carried out at one of the sites (RMS). In the first study, 114 clinical specimens of various HCV genotypes were examined by four different tests: HCVHPS V2, HCVHPS V1, CAHCM, and HCMCAP. The results shown in Table 2 demonstrated that the mean log HCV RNA (IU/mL) for genotypes 2a, 3, 4, and 5 by the HCVHPS V2 test are 0.48 to 0.90 log_{10} higher than those by the HCVHPS V1 test. In addition, the mean log HCV RNA (IU/mL) by the HCVHPS V2 were similar to those of the CAHCM test and the HCMCAP test for all HCV genotypes. Also in this series, genotype 2a, although dramatically improved over the HCVHPS V1 test, was somewhat less efficiently quantified than the other genotypes. Quantification of this genotype was within 0.5 log_{10} of the HCMCAP test. In the second study, the panel of HCV genotypes 1 through 6 from NIBSC (code 02/202) was tested in singlicate using the HCVHPS V2 test. As shown in Table 3, all 6 of the HCV genotype specimens in the NIBSC panel yielded values that were within 0.25 log of the expected nominal value.

Limit of detection (Sensitivity) for all HCV genotypes
To verify that the HCVHPS V2 test had a similar LOD for all HCV genotypes, limiting dilution experiments were performed. For each genotype, the lowest concentration required to yield positive results in at least 95% of replicates as well as the concentration for 95% positive results by PROBIT analysis are shown in Table 4. The LOD values for all HCV genotypes for both specimen matrices were between 10 and 20 HCV RNA IU/mL based on the observed lowest level to yield at least a 95% positivity rate. PROBIT analysis of this data showed similar LOD values that ranged from 6.3 (Genotype 3 in EDTA plasma) to 19.4 (Genotype 2a in serum) HCV RNA IU/mL. No trend was observed for consistently lower or higher quantitation for a given specimen matrix.

**Linear range**

The analysis of linearity study results showed that the linear range for EDTA plasma matrix for the HCVHPS V2 test was from 23.5 HCV RNA IU/mL to $3.91 \times 10^8$ HCV RNA IU/mL (Figure 1a). The largest difference between the linear fit and the third order polynomial fit, for all levels within the linear range, was $0.16 \log_{10}$. This value was within the pre-set $0.2 \log_{10}$ allowable bias (data not shown). For serum matrix, the linear range was from 15.7 HCV RNA IU/mL to $3.91 \times 10^8$ HCV RNA IU/mL (Figure 1b) with the largest difference of $0.20 \log_{10}$ between the linear fit and the third order polynomial fit (data not shown).

**Precision**

Tables 5 and 6 show the %CV of each component of the variance analysis and the total %CV for HCV genotype 1 for in EDTA plasma and serum, respectively. For EDTA plasma, the total %CV values for the 6 levels ranged from 32.2% to 35.8% and were fairly constant. For serum, the total %CV values for the 7 levels ranged from 25.1% to 57.5%. The total %CV values for serum matrix were dependent on the concentrations of the levels tested. For the five levels with
concentrations ranging from 13.1 to $1.17 \times 10^6$ HCV RNA IU/mL, the total %CV values were less than 33%. For the lowest two concentrations tested (65.6 HCV RNA IU/mL and 32.8 HCV RNA IU/mL), the total %CV was equal to or greater than 49%.

In general, for both EDTA plasma and serum samples, relatively low level or no variance was attributable to “Lot-to-Lot” or “Instrument-to-Instrument” components. For most of the samples tested, each of the rest of the components of the variance examined (“Operator-to-Operator”, “Between-Run” and “Within-Run”) showed approximately equal contributions to the total variance. An exception was for the low concentration samples for which the contributions by “Within-Run” and “Between-Run” were much greater than those by the rest of the components.

Specificity

All of the 200 test results by the HCVHPS V2 test for the 50 sero-negative EDTA plasma specimens and the 50 sero-negative serum specimens using two different kit lots were negative to yield 100% clinical specificity (data not shown). None of the 12 non-HCV members of the *Flaviviridae* or 22 additional micro-organisms tested were positive for HCV RNA by the HCVHPS V2 test (data not shown). Based on this data, the analytical specificity would also be 100%.

Interference

All of the drug-spiked samples yielded results that were within 0.2 log$_{10}$ of the results for their respective controls. Therefore, none of the 23 different drugs (that are commonly prescribed to HCV patients) tested by the HCVHPS V2 test were shown to interfere with the quantitation of HCV RNA (data not shown).
DISCUSSION

In recent years quantitative HCV RNA tests have been developed to address the increasing demand for sensitive and accurate assays that are useful for monitoring and predicting treatment outcomes in chronic hepatitis C (1, 16). In the present study we evaluated the HCVHPS V2 test, a real-time PCR test that has been developed to overcome certain genotype specific limitations shown by its previous version, the HCVHPS V1 test. Because of significant under-estimation of genotypes 2 through 5, the HCVHPS V1 test has been restricted to the quantitation of genotypes 1 and 6 only. Changes in the ethanol concentration of the wash buffer in sample preparation and the temperature of the reverse transcription step in RT-PCR were then introduced in the new version HCVHPS V2. The performance of this HCVHPS V2 test was then evaluated utilizing all HCV genotypes. Indeed, our results show that the HCVHPS V2 test provides high sensitivity and accurate quantitation for all six HCV genotypes when compared to the CAHCM test. In all participating laboratories, using a variety of clinical specimens and standards, the mean difference between the HCVHPS V2 test and the CAHCM test was within ±0.3 log_{10}. As expected, the mean log HCV RNA (IU/mL) by the HCVHPS V2 test were significantly greater for genotypes 2 through 5 than those by the HCVHPS V1. Furthermore, the mean differences in log HCV RNA (IU/mL) values for each of the six genotypes when comparing HCVHPS V2 to HCVHPS V1 or HCVHPS V2 to CAHCM were very similar for all three laboratories with a sole exception of the result for genotype 2 at one of the three laboratories. Additional experiments performed on subtype-specific clinical specimens showed that the difference between the two HCVHPS test versions is higher for genotype 2a than for 2b, suggesting a variable subtype composition for genotype 2 samples at the four study sites. Furthermore, the HCVHPS V2 test gave very similar results for all HCV genotypes when compared
to a fully automated HCMCAP test (5, 20-21). These experimental results support that the decrease in the ethanol concentration of the wash buffer and the increased temperature of the reverse transcription step significantly improved quantitation across HCV genotypes 2 to 5.

The remainder of the experiments presented in this study address the performance of the improved version of the assay. The linear range of the HCVHPS V2 assay is as would be expected based on the previous version of the assay. In both specimen matrices, EDTA plasma or serum, the assay was shown to have a broad linear range from 25 IU/mL to $3.91 \times 10^8$ IU/mL. Extensive precision analysis, including lot-to-lot, operator-to-operator, within-run, and between-run variability, showed the assay to be robust and reproducible for both EDTA plasma and serum samples. In general, between-run and within-run variances and, to a lesser extent, the operator-to-operator variance contribute the most to the total variance seen with the HCVHPS V2 test. The higher variability observed at the lower limit of the dynamic range in serum is to be expected because of analyte distribution in the sample matrix at the 10 to 50 IU/ml level (16). This variation at the lower dilutions was not observed in the EDTA plasma sample analysis.

The analysis of the Limit of Detection was performed for all HCV genotypes in both EDTA plasma and serum specimen matrices. The results of this analysis show that the LOD values of the HCVHPS V2 based on Probit analysis are between 6 to 17 HCV RNA IU/mL for EDTA plasma and 11 to 19 HCV RNA IU/mL for serum. The LOD for each genotype is similar and there is no apparent bias for any genotype.

The HCVHPS V2 test appeared to be highly specific. No cross-reactivity was observed when tested with closely related Flaviviruses or with other unrelated viruses, bacteria, or fungal agents that could be present in serum or plasma were tested in the assay. In addition, the HCVHPS V2 test was not
affected by 23 different drugs that may be administered to patients with chronic hepatitis C, often presenting with concurrent diseases or co-infections (9, 17).

Taken together these data indicate that the HCVHPS V2 test has significantly improved the quantitation of genotypes 2 through 5 over the previous version. In addition, the changes in the extraction and thermal cycling parameters have not compromised the performance characteristics of the assay. The data demonstrate that the HCVHPS V2 test has a broad linear range, good precision through the linear range, a low limit of detection across all HCV genotypes, an excellent specificity for HCV, and lack of interference by drugs administered to HCV infected patients. We conclude that the HCVHPS V2 test can be used reliably to detect and quantitate viral load in HCV infected patients.
REFERENCES


**TABLES AND FIGURES LEGENDS**

**Table 1.** Genotype Inclusivity: Mean Differences in log HCV RNA (IU/mL) for Each Genotype at Three Different Sites (TRL, SUH, and RMS) Among the Three Tests - HCVHPS V2, HCVHPS V1, and CAHCM.

**Table 2.** Genotype Inclusivity: Mean Differences in log HCV RNA (IU/mL) for Each Genotype at RMS Among the Four Assays – HCVHPS V2, HCVHPS V1, CAHCM, and HCMCAP.

**Table 3.** Genotype Inclusivity: Difference in log HCV RNA (IU/mL) Between the Observed and Expected Values for HCV Genotypes 1 through 6 from the NIBSC HCV Genotype Panel (02/202).

**Table 4.** Limit of Detection: HCV Genotype Detection for HCVHPS V2.

**Table 5.** Precision of the COBAS® TaqMan® HCV Test, v2.0 in EDTA Plasma.

**Table 6.** Precision of the COBAS® TaqMan® HCV Test, v2.0 in Serum.

**Figure 1:** Linear Range of the COBAS® TaqMan® HCV Test, v2.0 in EDTA Plasma* (a) and Serum* (b).
1TRL, SUH, and RMS denote TriCore Reference Laboratories, Saarland University Hospital, and Roche Molecular Systems, respectively. 2The numbers inside the parentheses denote the number of clinical specimens tested.

Table 1

<table>
<thead>
<tr>
<th>Site</th>
<th>Methods compared</th>
<th>Differences in log values</th>
<th>All Specimens</th>
</tr>
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<td></td>
<td>Gt 1</td>
<td>Gt 2</td>
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<tr>
<td>TRL</td>
<td>HCVHPS V2 - HCVHPS V1</td>
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<td>HCVHPS V2 - HCVHPS V1</td>
<td>0.30 (10)</td>
<td>0.88 (10)</td>
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<td>HCVHPS V2 - HCVHPS V1</td>
<td>0.23 (7)</td>
<td>0.43 (9)</td>
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<td>0.24 (25)</td>
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<td>-0.21 (25)</td>
<td>-0.11 (28)</td>
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1The numbers inside the parentheses denote the number of clinical specimens tested.

Table 2.
Each panel member was tested in singlicate.

### Table 3

<table>
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<th>HCV Genotype</th>
<th>log HCV RNA (IU/mL)</th>
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<td>6</td>
<td>3.00</td>
<td>3.15</td>
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\(^1\) Each panel member was tested in singlicate

### Table 4

<table>
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<tr>
<th>Genotype</th>
<th>Actually Observed Lowest Level With At Least 95% Positive Results (IU/mL)</th>
<th>Concentration for 95% Positive Results by PROBIT Analysis (IU/mL)</th>
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<tr>
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<td>EDTA Plasma</td>
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</tr>
<tr>
<td>6</td>
<td>10</td>
<td>10</td>
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</table>

Table 3

Table 4.
The % CV values of each component of the variance analysis and of total variance are shown for each of the six levels tested.

Table 5.

<table>
<thead>
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<th>Component of Variance</th>
<th>Concentration of HCV Genotype 1 (IU/mL) in EDTA Plasma</th>
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<tbody>
<tr>
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<td>Lot-to-Lot</td>
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<td>Instrument-to-Instrument</td>
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<td>Operator-to-Operator</td>
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<td>Between-Run</td>
<td>18.6%</td>
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<tr>
<td>Within-Run</td>
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<tr>
<td>Total %CV</td>
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<tr>
<td>n</td>
<td>48</td>
</tr>
</tbody>
</table>

The % CV values of each component of the variance analysis and of total variance are shown for each of the seven levels tested.

Table 6.
Fig. 1 a.

*Error bars denote 2 standard errors of the mean values

Fig. 1 b