

# Multicenter Evaluation of the VERSANT<sup>®</sup> HCV RNA 3.0 Assay [bDNA] with the VERSANT<sup>™</sup> 440 Molecular System

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## Abstract

**Background and Aim:** The VERSANT HCV RNA 3.0 Assay [bDNA] (HCV 3.0) is a signal amplification nucleic acid probe assay for the direct quantification of human HCV RNA in serum or plasma using the System 340 bDNA Analyzer (S340). The VERSANT 440 Molecular System (V440) is an upgraded system (not yet commercially available in the U.S.) designed to more fully automate the VERSANT bDNA assay steps. This study examined the analytical sensitivity, specificity, precision, and linearity of HCV 3.0 using the V440. Results obtained on clinical specimens were compared for correlation between V440 and S340.

**Methods:** Three clinical testing laboratories participated in this study using multiple HCV 3.0 kit lots and two operators at each testing site. An 8-member dilution panel of HCV RNA standards (SeraCare Diagnostics, West Bridgewater, MA, and ProMedDx LLC, Norton, MA) ranging in concentration from 523 to 7,360,623 IU/mL in plasma was tested in 108, 180, or 216 replicates for each standard to determine the lower limit of detection (LoD), lower and upper limits of quantification (LLoQ and ULoQ), linearity, and reproducibility. Analytical specificity was derived from testing 302 anti-HCV antibody-negative serum specimens collected from healthy blood donors. Matched serum, EDTA- and ACD-anticoagulated plasma specimens from 40 HCV-infected and 10 HCV-seronegative patients were tested to evaluate effects of the EDTA and ACD anticoagulants on quantification of HCV RNA. Serum or plasma specimens from 172 patients with chronic HCV infection were tested using a single HCV 3.0 kit lot on both V440 and S340 for correlation of results. These specimens included the following numbers of HCV genotypes: 116 G1, 13 G2, 26 G3, 9 G4, 7 G5, and 5 G6.

**Results:** For HCV 3.0 used with V440, both the LoD (95% detection rate) and LLoQ (<35% CV and within  $\pm 0.1$  log IU/mL from linearized expected titer) were 1,215 IU/mL (95% CI, 1,124 to 1,313 IU/mL). The detection cutoff (95% negative rate) and ULoQ were 615 IU/mL and 7,692,308 IU/mL, respectively, the same as for HCV 3.0 with S340. Within the V440 quantification range, inter-assay %CV ranged from 11.5% to 38.6% with excellent linear correlation ( $r = 0.999$ ) between expected and observed mean log IU/mL titers of the HCV RNA standards. Differences between linearized mean and observed mean titer results varied from -0.06 to 0.04 log IU/mL. Analytical specificity was 99.3%. Mean differences of 0.04 log IU/mL and -0.01 log IU/mL were obtained for EDTA plasma vs. serum and for ACD plasma vs. serum, respectively, with >95% of the titer differences falling within  $\pm 0.5$  log IU/mL. HCV 3.0 quantification results (ranging from 662 to 7,180,286 IU/mL) for 164 of the 172 clinical serum or plasma specimens showed good correlation ( $R^2 = 0.990$ , Deming regression) between V440 and S340, with a mean difference of -0.01 log IU/mL (range, -0.30 to 0.38) and a range of -0.20 to 0.18 log IU/mL for 95% of the titer differences.

**Conclusions:** HCV 3.0 used with V440 is a reliable and accurate quantitative assay with analytical sensitivity, specificity, reproducibility, and range of quantification equivalent to those of HCV 3.0 with S340 for the measurement of HCV RNA levels in serum or plasma. V440 has the advantage of automated bDNA processing for high testing throughput when compared to S340.

## Introduction

VERSANT<sup>™</sup> 440 Molecular System (V440; Siemens Medical Solutions Diagnostics, Tarrytown, NY) is a walk-away, automated diagnostic instrument designed for reagent addition, incubation, washing, plate-sealing, agitation, and luminescence reading processes of the VERSANT branched-DNA (bDNA) assays. This integrated system allows the operator to prepare and place assay reagents and clinical serum or plasma specimens on board the instrument at testing set-up, and the instrument will automatically complete the bDNA assay steps. The V440 provides on-board temperature control of the assay reagents, automated preparation and delivery of all assay reagents to the microtiter well plates, while processing 12 to 168 samples per assay run. A touch screen computer user interface and bi-directional interface between the V440 and laboratory information system also improve laboratory workflow and efficiency (Figure 1).



Figure 1: VERSANT<sup>™</sup> 440 Molecular System (CE-Marked in Europe; not yet commercially available in U.S.)

The VERSANT<sup>®</sup> HCV RNA 3.0 Assay (HCV 3.0) is an in vitro diagnostic signal amplification nucleic acid probe assay approved by the FDA for the quantification of hepatitis C virus (HCV) RNA in serum or plasma using the semi-automated System 340 bDNA Analyzer (S340). This assay has been validated previously to quantify HCV RNA over the range of 615 to 7,692,308 IU/mL in clinical serum or plasma specimens containing HCV genotypes 1 to 6.

This study was conducted to 1) determine the accuracy, linearity, reproducibility, analytical sensitivity, and specificity of HCV 3.0 using the V440, 2) evaluate the effects of EDTA and ACD anticoagulants on quantification of HCV RNA by HCV 3.0 using the V440, and 3) compare quantitative results obtained by HCV 3.0 using the V440 to those obtained by the same assay using the S340 for a set of clinical serum and plasma specimens.

## Materials and Methods

1. V440 performance characteristics - accuracy, linearity, reproducibility, analytical sensitivity, and specificity

- A panel of 8 HCV standards (QC1 to QC8) ranging from 523 to 7,360,623 IU/mL were prepared from serial dilution of recombinant HCV bacteriophage or whole HCV particles (SeraCare Diagnostics, West Bridgewater, MA, and ProMedDx LLC, Norton, MA) in HCV RNA-negative plasma. Each panel member was assigned an HCV RNA target concentration by Siemens Medical Solutions Diagnostics (SMSD) after testing of replicates on multiple assay plates by multiple operators. For accuracy, linearity, reproducibility, and analytical sensitivity, 2 operators at each of the 3 participating clinical testing laboratories performed the testing using 3 different HCV 3.0 kit lots. Each of the 8 panel members were tested in replicates of 6, 10, or 12 on each bDNA assay plate for a total of 108 (QC1 to QC4), 180 (QC5 and QC6), or 216 (QC7 and QC8) replicates, respectively.

Detection cutoff (DC) = The HCV RNA level below which 95% of the negative specimens quantify.

Limit of detection (LoD) = The lowest HCV RNA concentration that yields an assay result at or above the DC for 95% of the time.

Lower limit of quantification (LLoQ) = The lowest quantifiable HCV RNA level that shows <35% CV and within  $\pm 0.1$  log IU/mL from the linearized expected titer.

Upper limit of quantification (ULoQ) = The highest quantifiable HCV RNA level that shows <35% CV and within  $\pm 0.1$  log IU/mL from the linearized expected titer.

- Analytical specificity was determined only at one testing site (SMSD Clinical Laboratory) using 2 different HCV 3.0 kit lots in testing 302 unique serum specimens collected from healthy blood donors who were negative for anti-HCV, anti-HIV-1/2, and HBsAg.

2. Effects of EDTA and ACD anticoagulants

Matched serum, EDTA-, and ACD-anticoagulated plasma specimens from 40 HCV-infected and 10 HCV-seronegative patients were tested at SMSD Clinical Laboratory using one HCV 3.0 kit lot. The HCV-infected subjects were known to have HCV RNA levels >962 but <7,692,308 IU/mL prior to specimen collection. Matched serum and plasma specimens from each subject were tested in the same assay run for quantitative comparison.

3. Correlation between V440 and S340 in clinical specimens

Serum or EDTA-anticoagulated plasma specimens from 172 patients with chronic HCV infection were divided across and tested by the 3 testing sites all using a single HCV 3.0 kit lot on the V440. Quantification results on these specimens using the same HCV 3.0 kit lot on the S340 were obtained only at one testing site (SMSD Clinical Laboratory). These specimens contained HCV RNA levels spanning the range of HCV 3.0 and included the following numbers of HCV genotypes: 116 GT1, 13 GT2, 26 GT3, 9 GT4, 7 GT5, and 5 GT6.

## Results

1. V440 performance characteristics - accuracy, linearity, reproducibility, analytical sensitivity, and specificity

- DC and ULoQ were 615 IU/mL and 7,692,308 IU/mL, respectively, for HCV 3.0 using V440, same as those using the S340.
- Both the LoD and LLoQ were determined to be 1,215 IU/mL (95% CI, 1,124 to 1,313 IU/mL) (Table 1).
- The maximum difference between corresponding assigned titer and mean observed titer was 0.08 log IU/mL (QC7), while that between corresponding mean linearized titer and mean observed titer was -0.06 log IU/mL (QC5).
- Deming regression analysis showed good linearity ( $r = 0.999$ ) and correlation ( $R^2 = 0.999$ ) between the assigned titers and mean observed titers across the quantification range of HCV 3.0 using V440 (Figure 2).
- Within the quantification range of HCV 3.0 using V440, inter-assay %CV ranged from 11.5% to 38.6% (Table 1).
- Of the 302 unique serum or plasma specimens collected from healthy blood donors, 300 showed HCV RNA titers of <615 IU/mL, resulting in an analytical specificity of 99.3% (lower 95% confidence limit of 97.9%).

Table 1: Accuracy, linearity, and reproducibility of HCV 3.0 using V440 for HCV standards

HCV Std	No. of replicates	Assigned titer (IU/mL)	Mean linearized titer (IU/mL)	Mean obs titer (IU/mL)	Titer difference (Log IU/mL)		% Detected	Total % CV
					AT vs MOT	MLT vs MOT		
QC1	108	7,360,623	7,042,173	6,941,514	0.03	0.01	100.0	11.5
QC2	108	836,512	757,984	709,430	0.07	0.03	100.0	16.4
QC3	108	73,606	70,422	71,443	0.01	-0.01	100.0	14.4
QC4	108	83,651	75,798	84,094	0.00	-0.05	100.0	17.9
QC5	180	8,365	7,580	8,702	-0.02	-0.06	100.0	21.3
QC6	180	2,091	1,895	1,780	0.07	0.03	100.0	24.2
LoD*	-	1,215	1,104	-	-	-	95.0	32.8
QC7	216	1,042	948	873	0.08	0.04	88.4	34.9
DC*	-	615	558	-	-	-	32.9	38.6
QC8	215 <sup>§</sup>	523	474	472	0.04	0.00	15.8	39.8

\* Results were interpolated for the limit of detection (LoD) and detection cutoff (DC).

<sup>§</sup> V440 did not yield result for a replicate of QC8 due to excessive reagent volume detected in reaction well.

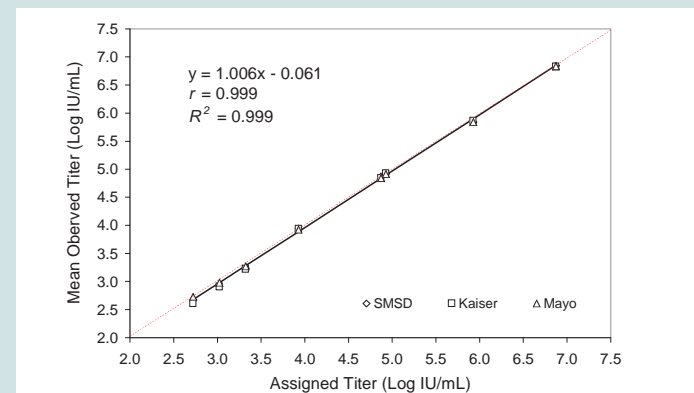


Figure 2: Correlation between mean observed and assigned (expected) titers of HCV standards

2. Effects of EDTA and ACD anticoagulants on HCV 3.0 with V440

- Of the 40 matched sets of serum, EDTA-, and ACD-anticoagulated plasma specimens containing HCV RNA, 1 serum specimen had insufficient volume for testing and results from the matched specimen results were excluded for analysis.
- Deming regression analysis (Figure 3) of quantification titers between serum and EDTA plasma showed good linearity ( $r = 0.996$ ) and correlation ( $R^2 = 0.992$ ), with mean titer difference of 0.04 log IU/mL (range, -0.16 to 0.50 log IU/mL).
- Quantification titers in serum also showed good linearity ( $r = 0.997$ ) and correlation ( $R^2 = 0.993$ ) with those in ACD plasma (Figure 4), with mean titer difference of -0.01 log IU/mL (range, -0.28 to 0.29 log IU/mL).
- All 10 matched sets of HCV RNA-negative serum, EDTA-, and ACD-anticoagulated plasma specimens showed quantification results that were below the detection cutoff value (615 IU/mL) of HCV 3.0 using V440 (i.e., 100% agreement).

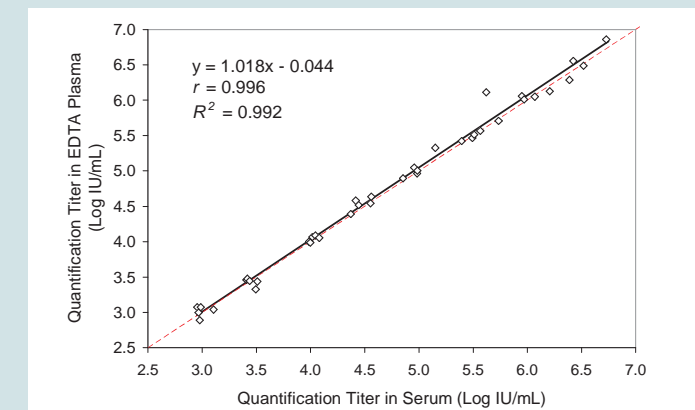


Figure 3: Correlation of HCV RNA quantification titers between EDTA-anticoagulated plasma and serum specimens

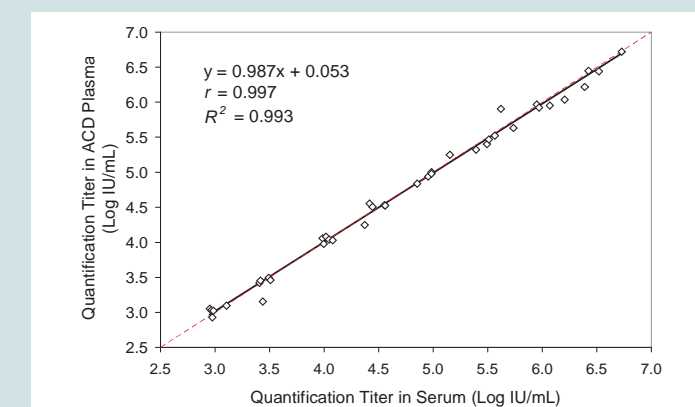


Figure 4: Correlation of HCV RNA quantification titers between ACD-anticoagulated plasma and serum specimens

3. Correlation between V440 and S340 with clinical specimens

- Of the 172 clinical serum or plasma specimens tested, 8 specimens yielded results below DC (615 IU/mL) on the S340 and could not be included for analysis.
- For the remaining 164 specimens that yielded log IU/mL titers by both V440 and S340, Deming regression analysis (Figure 5) showed good linearity ( $r = 0.995$ ) and correlation ( $R^2 = 0.990$ ) across the quantification range of both systems.
- Bland-Altman plot (Figure 6) demonstrated a mean titer difference of -0.01 log IU/mL (range, -0.30 to 0.38 log IU/mL) between V440 and S340, with 95% of the titer differences occurring within a range of -0.20 to 0.18 log IU/mL.

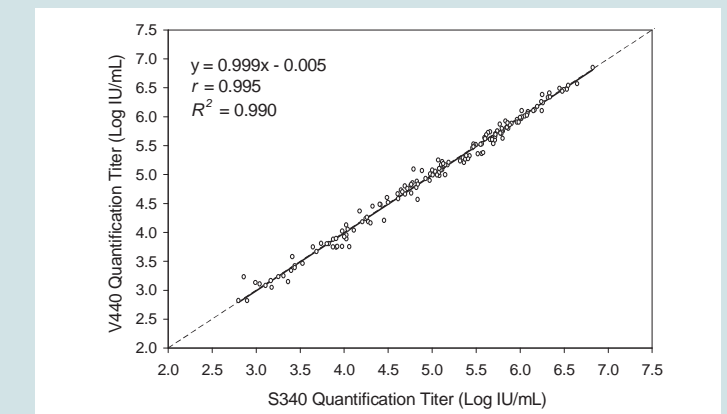


Figure 5: Correlation of quantitative HCV RNA titers in clinical specimens between V440 and S340

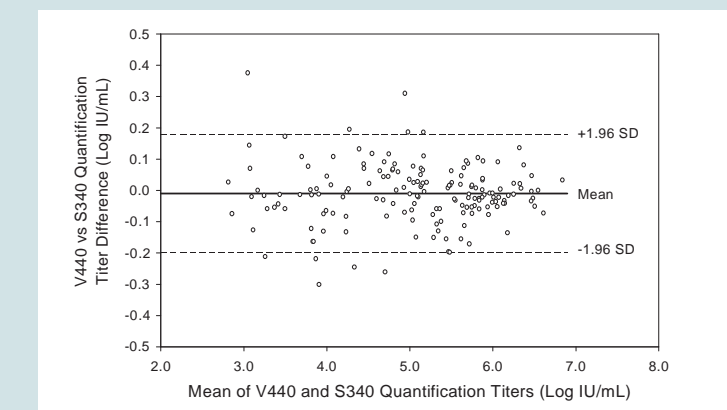


Figure 6: Bland-Altman plot of HCV RNA titer differences in clinical specimens between V440 and S340

## Conclusions

- HCV 3.0 used with V440 showed analytical sensitivity, specificity, reproducibility, and range of quantification equivalent to those of HCV 3.0 with S340 for the measurement of HCV RNA levels in serum or plasma.
- V440 has the advantages of automated bDNA processing for high testing throughput when compared to S340.