Hepatitis C virus (HCV) infection is associated with significant liver-related morbidity and mortality around the world. The World Health Organization estimates that 3% of the global population is infected with HCV and that there are approximately 170 million people at risk of developing cirrhosis or hepatocarcinoma (23, 24). Treatment of HCV infection typically consists of pegylated interferon plus ribavirin or pegylated interferon, ribavirin, and a direct-acting antiviral (DAA) protease inhibitor (triple therapy) for non-genotype 1 and genotype 1, respectively (11, 12, 13).

Depending on the particular treatment regimen and the genotype of HCV, treatment success as measured by failure to detect viral replication 24 weeks after cessation of treatment can be achieved in 50 to 80% of patients (12).

Measurement of HCV viral load (VL) for the different HCV genotypes is crucial to clinical management of HCV-infected patients, both treated and not, for disease staging, decisions regarding treatment initiation, and individualization of treatment strategy (i.e., dosage and duration based on response kinetics) (1, 6, 7, 13). Furthermore, with the advent of DAAs, VL monitoring may help prevent protease inhibitor resistance development by allowing for switching or stopping therapy if VL does not decrease or returns while on treatment.

There are currently several commercially available HCV VL assays; real-time PCR assays are generally preferred because of their wide dynamic ranges and good sensitivities (1, 3). Two commercial real-time PCR platforms are available, the Cobas AmpliPrep/Cobas TaqMan HCV assay version 1.0 (CAP-CTM; Roche Molecular Systems, Pleasanton, CA) and the Abbott RealTime HCV assay (ART; Abbott Molecular, Des Plaines, IL). We characterized the performance of the ART assay and compared results obtained with both assays using clinical specimens of diverse HCV genotypes in a university hospital central testing laboratory.

HCV VL was determined with the ART and the CAP-CTM as per the manufacturers’ recommendations. A serum specimen volume of 500 l was required for ART, compared to 850 l for CAP-CTM. Sample preparation for the ART assay was performed using the Abbott m2000sp instrument. HCV genotypes were identified using the Versant Inno-LI PA HCV Genotype 2.0 assay (Siemens Healthcare Diagnostics, Deerfield, IL); specimens with indeterminate results were tested with the RealTime Genotyping II RUO assay (Abbott Molecular, Des Plaines, IL) or by direct sequencing and phylogenetic analysis of NS5B (performed by Siemens Clinical Laboratories, Berkeley, CA). Statistical tests were performed using Prism 5.0 (GraphPad Software, La Jolla, CA).

To evaluate the sensitivity, linearity, and intra- and interrun precision of the ART HCV assay, 2 panels spanning a wide range in VLs were used. First, a prepared dilution panel (9 different concentrations, from 1.04 to 6.73 log10 IU/ml, HCV genotype 1) was purchased from a commercial source (HCV RNA linearity panel PHW804; SeraCare Life Sciences, Milford, MA). The panel was tested in triplicate over 3 to 4 days by two operators (9 to 12 replicates in total). Second, to expand the range of VLs tested at the high end, an HCV genotype 1A patient specimen was used to prepare 8 serial 10-fold dilutions (0.89 to 7.89 log10 IU/ml), each of which was tested 3 to 5 times over 5 days. The nominal VL for this dilution panel was calculated by averaging all the VL results from the undiluted specimen (7.89 ± 0.03 log10 IU/ml with the ART assay; mean ± standard deviation [SD]) and adjusting for the dilution factor. The results are summarized in Table 1. At 11 IU/ml, 10/12 replicates were detectable but below the limit of quantification (LOQ). The sample with a nominal VL of 33 IU/ml (1.52 log10 IU/ml) was detectable in 12 out of 12 replicates and quantified in 7 of these. At 78 IU/ml and above, all replicates were above the LOQ. Based on probit analysis, the limit of detection (LOD; defined as the lowest concentration of HCV RNA in which 95% of replicates were positive) in our laboratory was calculated to be 12 IU/ml.

The measured VL was highly correlated with the expected (nominal) VL over the range tested (1.52 to 7.89 log10 IU/ml; linear R2, 0.99; slope, 1.03) (Fig. 1). Overall, assay precision was excellent, with coefficient of variation (CV) values between 0.4 and 13.9% (Table 1). The mean intrarun and interrun precision SDs were 0.05 and 0.09 log10 IU/ml, respectively, at 6.72 log10 IU/ml (0.9% and 1.8% CV) and 0.08 and 0.07 log10 IU/ml, respectively, at 2.79 log10 IU/ml (4.8% and 4.1% CV).

Quantitative agreement between the ART and CAP-CTM assays was assessed retrospectively using 253 deidentified remnant patient specimens (203 positive and 50 negative) submitted to the Ohio State University Medical Center Clinical Microbiology Laboratory for HCV VL testing. Genotypes 1, 2, 3, 4, and 6 were included. Paired results were obtained from 201 of the positive
One specimen was excluded because only the ART assay result was above the linear reportable range (9.0 log₁₀ IU/ml; CAP-CTM result, 7.8 log₁₀ IU/ml), and another was excluded because the CAP-CTM assay result was below the LOQ (43 IU/ml; ART result, 34 IU/ml).

All 50 HCV-negative specimens had undetectable VLs by both assays. The mean difference (CAP-CTM minus ART) between results from the positive specimens was 0.38 log₁₀ RNA IU/ml (95% confidence interval, −0.17 to 0.94) (Fig. 2). Linear regression analysis of log-transformed VL results yielded an $R^2$ value of 0.986 (data not shown). Mean differences by genotype or subtype were all equal to or below 0.5; the largest difference was observed for subtype 1b (mean difference, 0.5; $t$ test $P = 0.011$ versus non-1b), and the smallest difference was observed for genotype 4 (mean difference, 0.14; $n = 6$; $t$ test $P = 0.035$ versus non-4) (Table 2). The proportion of specimens with a VL difference greater than 0.5 was highest for subtype 1b (52%), whereas the proportion outside the 95% confidence interval was lowest for...
TABLE 2 Viral load differences between the CAP-CTM and ART assays according to genotype and subtype

<table>
<thead>
<tr>
<th>Genotype(a)</th>
<th>n</th>
<th>Mean (SD) VL difference (log(_10) IU/ml, CAP-CTM minus ART)</th>
<th>% with difference &gt;0.5 log</th>
<th>% with difference &gt;95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>103</td>
<td>0.42 (0.22)</td>
<td>31</td>
<td>2</td>
</tr>
<tr>
<td>1a</td>
<td>70</td>
<td>0.38 (0.23)</td>
<td>23</td>
<td>3</td>
</tr>
<tr>
<td>1b</td>
<td>29</td>
<td>0.50 (0.19)</td>
<td>52</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>0.38 (0.46)</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>2a</td>
<td>23</td>
<td>0.36 (0.48)</td>
<td>22</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>33</td>
<td>0.32 (0.20)</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>3a</td>
<td>31</td>
<td>0.33 (0.20)</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.14 (0.47)</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>0.29 (0.08)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ND(d)</td>
<td>29</td>
<td>0.38 (0.30)</td>
<td>31</td>
<td>7</td>
</tr>
<tr>
<td>All</td>
<td>201</td>
<td>0.38 (0.29)</td>
<td>27</td>
<td>4</td>
</tr>
</tbody>
</table>

\(a\) Genotypes 1, 2, and 3 include subsets, listed separately by subtype (1a and 1b, 2b, and 3a).

\(b\) P = 0.011 versus non-1b.

\(c\) P = 0.035 versus non-4.

\(d\) ND, not determined.

Since our study was completed, a new version (2.0) of the CAP-CTM assay has been described, in order to remedy a problem with underestimation of the VL for certain HCV genotypes (8, 21); however, the CAP-CTM version 2.0 assay is not yet FDA approved, and so laboratories in the United States are still using version 1.0. The linearity, precision, and sensitivity portion of this study was performed only with the ART assay, making direct comparisons to the CAP-CTM assay difficult. Nonetheless, overall our results are consistent with those from other studies that indicate improved accuracy across genotypes with the ART assay, combined with excellent performance characteristics in our hospital laboratory setting. Additionally, the ART assay is currently the only assay with automated sample processing, with an LOD and LOQ of 12 IU/ml, which meets the requirement for monitoring HCV VL in patients undergoing triple therapy with DAA.

ACKNOWLEDGMENTS

We are grateful to Abbott Molecular for study support and to Neil Parkin (Data First Consulting, Inc.) for assistance with data analysis.

REFERENCES


