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# Performance of the Abbott RealTime and Roche Cobas TaqMan Hepatitis C Virus (HCV) Assays for Quantification of HCV Genotypes

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**We evaluated the Abbott RealTime (ART) and Roche Cobas TaqMan Hepatitis C virus (HCV) viral load assays for quantification of HCV genotypes in patient specimens. The ART HCV assay was a more sensitive and precise tool for accurate HCV viral load quantification across the HCV genotypes tested, especially genotype 1b.**

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 μl was required for ART, compared to 850 μl for CAP-CTM. Sample preparation for the ART assay was performed using the Abbott m2000sp instrument. HCV genotypes were identified using the Versant Inno-LIPA 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 $\log_{10}$ 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 $\log_{10}$ 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 $\pm$ 0.03 $\log_{10}$ IU/ml with the ART assay; mean $\pm$ 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 $\log_{10}$ IU/ml) was detectable in 12 out of 12 replicates and quantitated 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 $\log_{10}$ IU/ml; linear $R^2$, 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 $\log_{10}$ IU/ml, respectively, at 6.72 $\log_{10}$ IU/ml (0.9% and 1.8% CV) and 0.08 and 0.07 $\log_{10}$ IU/ml, respectively, at 2.79 $\log_{10}$ 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

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**TABLE 1** Sensitivity, linearity, and precision results for the ART assay

| Specimen source | Nominal VL ($log_{10}$ IU/ml)[a] | No. tested | No. detectable | Mean (SD) VL ($log_{10}$ IU/ml) | CV (%) |
|---|---|---|---|---|---|
| Panel[b] | 0 | 3 | 0 | Target not detected | |
| | 1.04 | 12 | 10 | <1.08[c] | |
| | 1.52 | 12 | 12 | 1.26 (0.17)[d] | 13.9 |
| | 2.20 | 12 | 12 | 1.63 (0.11) | 6.7 |
| | 2.79 | 9 | 9 | 2.21 (0.08) | 3.8 |
| | 3.67 | 9 | 9 | 3.23 (0.13) | 4.0 |
| | 4.71 | 9 | 9 | 4.29 (0.05) | 1.1 |
| | 5.28 | 9 | 9 | 4.81 (0.07) | 1.4 |
| | 5.72 | 9 | 9 | 5.29 (0.07) | 1.4 |
| | 6.73 | 9 | 9 | 6.38 (0.09) | 1.5 |
| Patient[e] | 0.89 | 5 | 5 | <1.08[c] | |
| | 1.89 | 5 | 5 | 1.66 (0.1) | 5.9 |
| | 2.89 | 5 | 5 | 2.65 (0.08) | 2.9 |
| | 3.89 | 5 | 5 | 3.68 (0.04) | 1.2 |
| | 4.89 | 5 | 5 | 4.68 (0.11) | 2.3 |
| | 5.89 | 5 | 5 | 5.72 (0.09) | 1.5 |
| | 6.89 | 3 | 3 | 6.61 (0.005) | 0.1 |
| | 7.89 | 5 | 5 | 7.89 (0.03) | 0.4 |

[a] For the panel specimens, the assay-specific nominal VL (provided by the manufacturer) was used; for the patient dilution series, the reported nominal viral load is the mean of experimental values for undiluted replicates.
[b] Specimens from the SeraCare panel (see Materials and Methods); nominal VL values are based on the manufacturer's results with the ART assay.
[c] All results were above the limit of detection but below the limit of quantitation.
[d] Mean of 7 results above the limit of quantitation.
[e] Serial 10-fold dilutions of a patient specimen with a high viral load; the nominal VL was calculated by averaging all VL results ($n = 5$) from the undiluted specimen (7.89 $log_{10}$ IU/ml) and adjusting for the dilution factor.

specimens. One specimen was excluded because only the ART assay result was above the linear reportable range (9.0 $log_{10}$ IU/ml; CAP-CTM result, 7.8 $log_{10}$ 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 $log_{10}$ 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; $n = 29$; $t$ 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

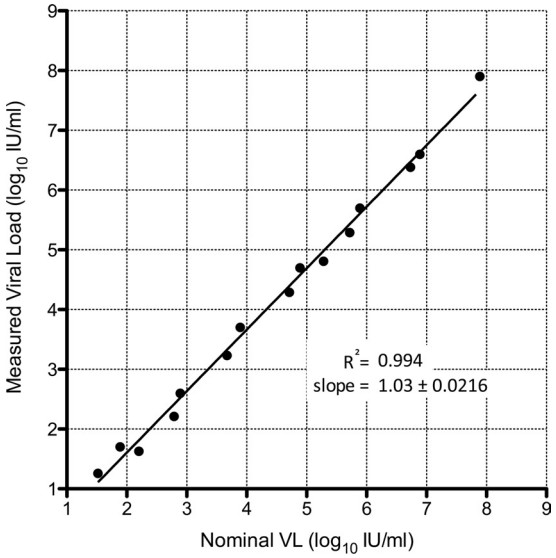


**FIG 1** Linearity of HCV viral load determinations. Results from the SeraCare panel and high-viral load patient sample dilution panel are combined. Results below the limit of quantitation but above the limit of detection were excluded.

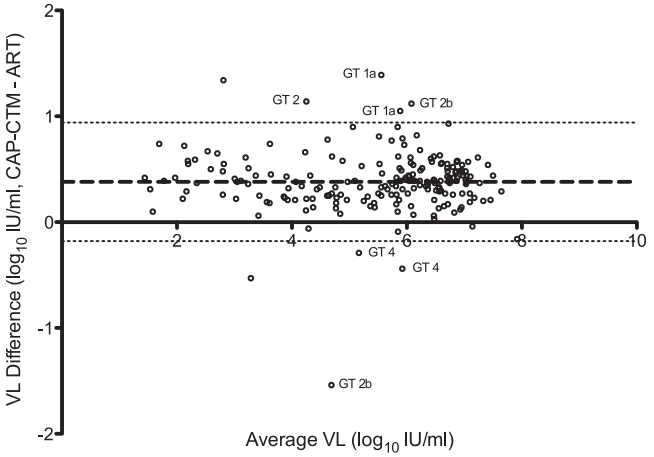


**FIG 2** Bland-Altman plot of differences in viral loads between CAP-CTM and ART assays versus the means of the 2 results. Genotypes of outliers are indicated except for those with indeterminate genotypes. The bold dotted line indicates the mean VL difference, and the lighter dotted lines indicate the 95% confidence limits.

**TABLE 2** Viral load differences between the CAP-CTM and ART assays according to genotype and subtype

| Genotype[a] | *n* | Mean (SD) VL difference ($log_{10}$ IU/ml), CAP-CTM minus ART | % with difference >0.5 log | % with difference >95% CI |
|---|---|---|---|---|
| 1 | 103 | 0.42 (0.22) | 31 | 2 |
| 1a | 70 | 0.38 (0.23) | 23 | 3 |
| 1b | 29 | 0.50 (0.19)[b] | 52 | 0 |
| 2 | 27 | 0.38 (0.46) | 22 | 11 |
| 2b | 23 | 0.36 (0.48) | 22 | 9 |
| 3 | 33 | 0.32 (0.20) | 15 | 0 |
| 3a | 31 | 0.33 (0.20) | 16 | 0 |
| 4 | 6 | 0.14 (0.47)[c] | 33 | 33 |
| 6 | 3 | 0.29 (0.08) | 0 | 0 |
| ND[d] | 29 | 0.38 (0.30) | 31 | 7 |
| All | 201 | 0.38 (0.29) | 27 | 4 |

[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.

this subtype and genotype 3 (0%) and highest for genotype 4 (33%) (Table 2). For subtype 1b, this indicates a consistent but relatively small difference between results that is likely to be related to the subtype. Individual specimens with difference values outside the 95% confidence interval belonged to subtypes 1a (1.38 and 1.06), 2b (1.12 and −1.54), and genotype 4 (−0.29 and −0.44), and there were 2 specimens whose genotype could not be determined) (Fig. 2). However, for subtypes 1a and 2b, the mean difference in VL was not significantly different from non-1a or non-2b specimens, respectively, indicating that the underlying reason for the large discrepancy in VL results for these specimens was not related to subtype.

The observed precision, linearity, and sensitivity of the ART assay reported here are similar to results from other laboratories and testing environments (2, 5, 15, 17–19, 22). In addition, the negative bias of results from the CAP-CTM assay compared to those from the ART assay that we described is consistent with most (14–16), although not all (2, 17), published studies. The basis for this difference in absolute VL is unknown but reinforces recommendations to use a single assay that can accurately quantify HCV genotypes when comparisons between measurements are clinically important, such as longitudinal sampling over time for individual patients.

Previous studies have noted a bias in VL results from specific genotypes or subtypes between assays (4, 10, 17, 20, 22). Our observations of a tendency for the ART assay to give higher results with genotype 4 and lower VL results with subtype 1b, compared to the CAP-CTM assay, are similar to those reported by others (9, 11, 20, 22). However, in our study as well as others, individual outliers of many genotypes have been observed, and generalization to all specimens of any particular subtype or genotype is not warranted. This is especially true since the numbers of specimens tested from genotypes 4 and 6 were low; the differences we observed require confirmation in larger studies. In most cases, underestimation of VL is probably a result of sequence polymorphism in one of the primer or probe target regions (4, 10), which may be more common in some genotypes but may be expected to occur in any specimen.

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.

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## REFERENCES

1. **Al Olaby RR, Azzazy HM.** 2011. Hepatitis C virus RNA assays: current and emerging technologies and their clinical applications. Expert Rev. Mol. Diagn. **11**:53–64.
2. **Caliendo AM, et al.** 2006. Multilaboratory comparison of hepatitis C virus viral load assays. J. Clin. Microbiol. **44**:1726–1732.
3. **Chevaliez S.** 2011. Virological tools to diagnose and monitor hepatitis C virus infection. Clin. Microbiol. Infect. **17**:116–121.
4. **Chevaliez S, Bouvier-Alias M, Brillet R, Pawlotsky JM.** 2007. Overestimation and underestimation of hepatitis C virus RNA levels in a widely used real-time polymerase chain reaction-based method. Hepatology **46**: 22–31.
5. **Chevaliez S, Bouvier-Alias M, Pawlotsky JM.** 2009. Performance of the Abbott real-time PCR assay using m2000sp and m2000rt for hepatitis C virus RNA quantification. J. Clin. Microbiol. **47**:1726–1732.
6. **Chevaliez S, Pawlotsky JM.** 2006. Hepatitis C virus serologic and virologic tests and clinical diagnosis of HCV-related liver disease. Int. J. Med. Sci. **3**:35–40.
7. **Chevaliez S, Pawlotsky JM.** 2007. Hepatitis C virus: virology, diagnosis and management of antiviral therapy. World J. Gastroenterol. **13**:2461–2466.
8. **Colucci G, et al.** 2007. Improved COBAS TaqMan hepatitis C virus test (version 2.0) for use with the High Pure system: enhanced genotype inclusivity and performance characteristics in a multisite study. J. Clin. Microbiol. **45**:3595–3600.
9. **Elkady A, et al.** 2010. Performance of two real-time RT-PCR assays for quantitation of hepatitis C virus RNA: evaluation of HCV genotypes 1-4. J. Med. Virol. **82**:1878–1888.
10. **El Sharkawi FZ, Chudy M, Hanschmann KM, Kress J, Nubling CM.** 2008. Consistency of quantitation of HCV genotype 4 from Egypt across three HCV-RNA amplification assays. J. Med. Virol. **80**:2086–2091.
11. **European Association for the Study of the Liver.** 2011. EASL clinical practice guidelines: management of hepatitis C virus infection. J. Hepatol **55**:245–264.
12. **Ghany MG, Nelson DR, Strader DB, Thomas DL, Seeff LB.** 2011. An update on treatment of genotype 1 chronic hepatitis C virus infection: 2011 practice guideline by the American Association for the Study of Liver Diseases. Hepatology **54**:1433–1444.
13. **Ghany MG, Strader DB, Thomas DL, Seeff LB.** 2009. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology **49**:1335–1374.
14. **Halfon P, Bourliere M, Penaranda G, Khiri H, Ouzan D.** 2006. Real-time PCR assays for hepatitis C virus (HCV) RNA quantitation are adequate for clinical management of patients with chronic HCV infection. J. Clin. Microbiol. **44**:2507–2511.
15. **Michelin BD, Muller Z, Stelzl E, Marth E, Kessler HH.** 2007. Evaluation of the Abbott RealTime HCV assay for quantitative detection of hepatitis C virus RNA. J. Clin. Virol. **38**:96–100.

16. **Pyne MT, Konnick EQ, Phansalkar A, Hillyard DR.** 2009. Evaluation of the Abbott investigational use only RealTime hepatitis C virus (HCV) assay and comparison to the Roche TaqMan HCV analyte-specific reagent assay. J. Clin. Microbiol. **47**:2872–2878.
17. **Sabato MF, Shiffman ML, Langley MR, Wilkinson DS, Ferreira-Gonzalez A.** 2007. Comparison of performance characteristics of three real-time reverse transcription-PCR test systems for detection and quantification of hepatitis C virus. J. Clin. Microbiol. **45**:2529–2536.
18. **Schutten M, Fries E, Burghoorn-Maas C, Niesters HG.** 2007. Evaluation of the analytical performance of the new Abbott RealTime RT-PCRs for the quantitative detection of HCV and HIV-1 RNA. J. Clin. Virol. **40**:99–104.
19. **Schutten M, et al.** 2007. Multicenter evaluation of the new Abbott RealTime assays for quantitative detection of human immunodeficiency virus type 1 and hepatitis C virus RNA. J. Clin. Microbiol. **45**:1712–1717.
20. **Tuaillon E, et al.** 2007. Impact of hepatitis C virus (HCV) genotypes on quantification of HCV RNA in serum by COBAS AmpliPrep/COBAS TaqMan HCV test, Abbott HCV RealTime assay and Versant HCV RNA assay. J. Clin. Microbiol. **45**:3077–3081.
21. **Vermehren J, et al.** 2011. Development of a second version of the Cobas AmpliPrep/Cobas TaqMan hepatitis C virus quantitative test with improved genotype inclusivity. J. Clin. Microbiol. **49**:3309–3315.
22. **Vermehren J, et al.** 2008. Differences between two real-time PCR-based hepatitis C virus (HCV) assays (RealTime HCV and Cobas AmpliPrep/Cobas TaqMan) and one signal amplification assay (Versant HCV RNA 3.0) for RNA detection and quantification. J. Clin. Microbiol. **46**:3880–3891.
23. **World Health Organization.** 1999. Global surveillance and control of hepatitis C. Report of a WHO consultation organized in collaboration with the Viral Hepatitis Prevention Board, Antwerp, Belgium. J. Viral Hepat. **6**:35–47.
24. **World Health Organization.** 2002. Hepatitis C. World Health Organization, Geneva, Switzerland. http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index.html. Accessed 30 September 2011.