

Abstract # Multi-Laboratory Comparison of Two Commercial Real-Time HCV Viral Load Assays.

U-108

A.M. Caliendo¹, S. Young², A. Gonzalez³, Y. Zhou⁴, J. Andersen⁴, A. Valsamakis⁵, G.J. Tsongalis⁶, B. Yen-Lieberman⁷, R. Pyles⁸, J.W. Bremer⁹, and N. Lurain⁹
1 Emory University School of Medicine, Atlanta, GA; 2 Tricolor Reference Laboratories, Albuquerque, NM; 3 Virginia Commonwealth University, Richmond, VA; 4 Harvard School of Public Health, Boston, MA; 5 The Johns Hopkins Medical Institutions, Baltimore, MD; 6 Dartmouth-Hitchcock Medical Center, Lebanon, NH; 7 Cleveland Clinic Foundation, Cleveland, OH; 8 University of Texas Medical Branch, Galveston, TX, 9 DAIDS Viral Quality Assessment (VQA) Laboratory, Rush University Medical Center, Chicago, IL

Emory University School of Medicine
Department of Pathology and Lab Medicine
1364 Clifton Road, H-180
Atlanta, GA 30322
Tel. 404.712.5721
Fax. 404.727.3133
Email. acalain@emory.edu

Abstract

Background: Viral load testing is the standard of care in monitoring response to HCV therapy in HCV/HIV co-infected individuals. Real-time PCR assays hold great promise for this application because they are very sensitive and have a broad linear range. However, the comparative performance of two commercial real-time viral load assays, the Abbott TaqMan ASR (Abbott) and the Roche COBAS TaqMan48 RUO (Roche) has not been evaluated. We report a multi-laboratory comparison on an evaluation of these two assays, to determine the limit of detection, linear range, reproducibility, and agreement. **Methods:** Plasma was obtained from an HCV infected individual (genotype 1b) and the viral load was determined using the Roche COBAS Amplifier HCV Monitor v2.0 test. This stock material was diluted in normal plasma to concentrations of 1 to 7 log₁₀ IU/ml. Four panels, each with 7 replicates of each concentration were tested in 3 (Abbott) or 4 (Roche) labs. Data were log₁₀ transformed prior to analysis.

Results: Both assays detected all 28 replicates with a concentration of 1 log₁₀ IU/ml and were linear to 7 log₁₀ IU/ml. Overall the Roche assay was more reproducible than the Abbott assay, and both assays were less reproducible at lower concentrations of virus. For the Roche assay, the SD was 0.18 and 0.09 log₁₀ for replicates of 2 and 5.5 log₁₀ IU/ml, respectively. While the SD for the Abbott assay was 0.42 and 0.26 log₁₀ for replicates of 2 and 5.5 log₁₀ IU/ml, respectively. For the Roche assay, the within-lab SD was below 0.15 log₁₀ for all 4 labs at viral load viral values of greater than or equal to 2.5 log₁₀ IU/ml. For the Abbott assay, the within-lab SD ranged from 0.17 to 0.43 log₁₀ at viral load values greater than 2.5 log₁₀ IU/ml; for 3 of the 4 labs the SD was less than or equal to 0.15 log₁₀ for viral load values greater than or equal to 5.5 log₁₀ IU/ml. In general, the viral load values obtained with the Roche assay were 0.2 to 0.7 log₁₀ greater than those obtained with the Abbott assay.

Conclusions: The Roche assay can reliably detect 5-fold changes in viral load for values greater than or equal to 2.5 log₁₀ IU/ml, while this level of reliability is reached with the Abbott assay at values greater than 5.5 log₁₀ IU/ml. Overall the sensitivity and linear range (at least 6 log₁₀) of these real-time assays enables them to be used for both HCV diagnostics as well as therapeutic monitoring, which is not possible with the current, most commonly used quantitative HCV assays.

Introduction

Viral load testing is the standard of care for monitoring response to HCV therapy in HCV/HIV co-infected individuals. Recent studies have shown that patients who do not obtain a 2 log₁₀ drop in HCV RNA 12 weeks after initiating therapy have a very low likelihood (~3%) of responding to pegylated interferon and ribavirin therapy. There is increased interest in using real-time PCR assays for the management of HCV infected patients because they are very sensitive and have a broader linear range than conventional PCR assays. Currently, there are a limited number of published studies on the performance characteristics of real-time HCV PCR assays. Two such assays, the HCV RNA ASR (Abbott Laboratories, Abbott Park, IL) and the COBAS TaqMan48 HCV RUO (Roche Diagnostics, Indianapolis, IN) were evaluated in this multi-laboratory study. The parameters that were assessed included limit of detection, linear range, reproducibility, and agreement between the two assays.

Methods

Standard: Panels were produced by the DAIDS VQA laboratory (Rush University Medical Center). Plasma was obtained from an HCV infected individual (genotype 1b) following an IRB approved protocol. The concentration of HCV RNA in the plasma sample was determined using the COBAS Amplifier HCV Monitor v2.0 Test (Roche Diagnostics). The sample was diluted so that the viral load values obtained were within the linear range of the assay. This stock material was then diluted with normal human plasma to concentrations of 1 to 7 log₁₀ IU/ml. Aliquots were stored at -70°C prior to testing. **Real-Time PCR Assays:** The HCV RNA ASR (Abbott Laboratories) and the COBAS TaqMan48 HCV RUO (Roche Diagnostics) were performed following the manufacturers' recommendations with the exception of the nucleic acid extraction procedure. For both assays, nucleic acid was extracted from 500µl of plasma using the QIAamp MinElute Virus Vacuum Kit (QIAGEN, Valencia, CA). Samples were eluted in 75µl and 50µl was used in the amplification assays. **Study Design:** For the Roche assay, each of 4 laboratories tested 7 replicates of each panel member (total of 28 replicates for each panel member). Three laboratories performed the Abbott assay, with 1 laboratory testing 2 panels, so a total of 4 panels (28 replicates for each member) were tested. For the Abbott assay, the data were initially analyzed using version 1.0 of the manufacturer's software. Once available the data were re-analyzed using version 3.0 of the software.

Statistical Analysis: Quantitative HCV viral loads were log₁₀ transformed for analysis. The linear range was examined by comparison to a 45° line. Crude within-laboratory standard deviations were tools for detecting departure from linear range. Invalid specimens were excluded from the analysis.

Figure 1: Linear range of the Roche RUO (Fig 1A) and Abbott ASR v3.0 (Fig 1B) assays.

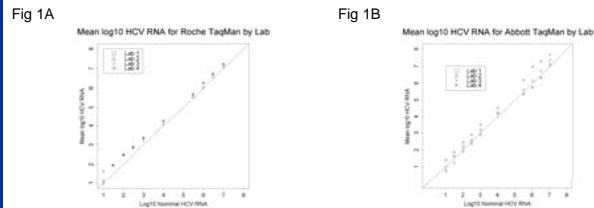


Figure 2: Plot of the mean residual values for the Roche RUO (Fig 2A) and Abbott ASR v3.0 (Fig 2B) assays. Mean residual values are expressed as real-time value minus the nominal Concentration.

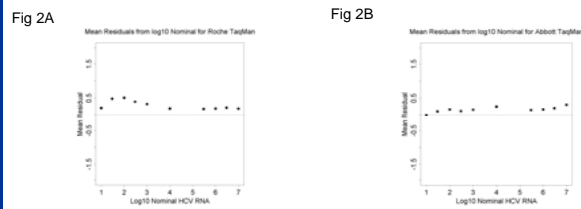
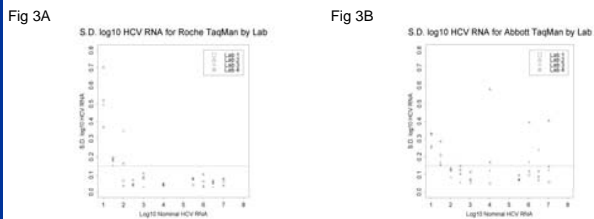


Figure 3: Standard deviation of the Roche RUO (Fig 3A) and Abbott ASR v3.0 (Fig 3B) assays.



Results

Both the Roche RUO and Abbott ASR (v1.0 and v3.0) were able to detect viral RNA in all 28 replicates of the 1.0 log₁₀ IU/ml dilution. The HCV RNA-free control had false positive readings in 1/28 replicates in the Abbott ASR assay (v1.0 and v3.0) and 0/28 replicates in the Roche RUO assay. There were 3 and 4 invalid results using the Abbott ASR v1.0 and v3.0 assays, respectively. There were no invalid results using the Roche RUO assay.

Table 1. Mean Viral Load Values for the Roche RUO and Abbott ASR Assays

| Nominal Concentration (Log ₁₀ IU/ml) | Roche RUO (Log ₁₀ IU/ml) | Abbott ASR v3.0 (Log ₁₀ IU/ml) | Abbott ASR v1.0 (Log ₁₀ IU/ml) |
|---|-------------------------------------|---|---|
| 1.00 | 1.17 | 0.99 | 1.20 |
| 1.48 | 1.96 | 1.59 | 1.54 |
| 2.00 | 2.51 | 2.17 | 1.96 |
| 2.48 | 2.87 | 2.59 | 2.24 |
| 3.00 | 3.32 | 3.16 | 3.11 |
| 4.00 | 4.19 | 4.25 | 3.90 |
| 5.48 | 5.65 | 5.63 | 5.00 |
| 6.00 | 6.19 | 6.17 | 5.52 |
| 6.48 | 6.69 | 6.68 | 6.01 |
| 7.00 | 7.19 | 7.30 | 6.66 |

Conclusions

The Roche RUO and Abbott ASR (v1.0 and v3.0) assays have a lower limit of detection of at least 1.0 log₁₀ IU/ml (10 IU/ml).

The Roche RUO and Abbott ASR (v3.0) assays are linear to at least 7.0 log₁₀ IU/ml (10 million IU/ml).

Overall, the Roche RUO assay is more reproducible than the Abbott ASR v1.0 and v3.0 assays, and all assays are less reproducible at lower viral load values.

The Abbott ASR v1.0 assay consistently under quantifies samples with viral load values greater than 4.0 log₁₀ IU/ml.

For the Roche RUO assay, all laboratories could reliably detect 5-fold changes in viral load for values ≥2.5 log₁₀ IU/ml. For the Abbott ASR v3.0 assay this level of reproducibility was obtained in 3 of the 4 laboratories.

The sensitivity and linear range of the Roche RUO and Abbott ASR v3.0 assays enable them to be used for both HCV diagnostics as well as therapeutic monitoring.