Title: Performance of the Abbott RealTime HCV Genotype II RUO Assay

Running Title: Abbott RealTime HCV Genotype II RUO Assay

Rebecca C. Shinol¹, Howard B. Gale¹, Virginia L. Kan¹

¹Infectious Diseases Laboratory, Medical Service, Veterans Affairs Medical Center, Washington, D.C.

Corresponding author: Rebecca Shinol. Mailing address: Infectious Diseases Section (151B), VA Medical Center, 50 Irving Street, NW, Washington, DC 20422. Phone: 202-745-8000 ext. 7998. Fax: 202-745-8432. Email: Rebecca.Shinol@va.gov
Abstract (50 words)

The Abbott HCV Genotype II RUO was evaluated using the automated Abbott RealTime m2000 system. Concordance was 98% (81/83) with samples previously typed using Versant HCV Genotype 2.0 RUO with manual extraction. Total assay time was reduced from 10.5 to 6.0 hours and hands-on time from 13 to 4 minutes/patient sample.
Hepatitis C (HCV) is a significant medical problem worldwide, as acute infection is typically asymptomatic and up to 85% of patients develop chronic infection which may lead to cirrhosis and hepatocellular carcinoma decades later if not treated [1, 7]. The prevalence of chronic HCV infection has been higher among veterans [5], making the Veterans Health Administration (VHA) the largest single provider of HCV care in the US to more than 207,000 veterans in 2011 [9]. Veterans screened for HCV have a prevalence of 4%, which is more than 3-fold higher than the national rate of 1.3% [12].

Among the 6 HCV genotypes, chronic infections within the US [3] and VHA [12] have been predominately with genotype (GT) 1. Genotyping prior to HCV treatment has been important, as patients with GT 1 and GT 4 have had lower sustained virologic response to ribavirin and pegylated interferon-alpha therapy compared to those with GT 2 and GT 3 [3]. More recently, the addition of a direct-acting NS3 protease inhibitor of HCV GT 1 to standard dual therapy has been shown to improve the sustained virologic response [3] for these treatment-naïve [6, 8] and treatment-experienced [2, 13] patients.

Since 2004, our laboratory has performed HCV quantitation and genotyping for the VA Medical Centers in Washington, DC, Baltimore, MD, and Martinsburg, WV. Among clinical samples from these sites, we found the following distribution: 89% GT 1, 7% GT 2, 3% GT 3, and 1% GT 4. We evaluated the correlation between the Versant HCV Genotype 2.0 RUO line probe assay (LiPA) and Abbott RealTime HCV Genotype II RUO assay during July and August, 2011 and also assessed the time requirements and cost differences for these assays.

The samples chosen for comparison had been previously typed for clinical indications using the Versant LiPA on the AutoBlot 3000 (Siemens Healthcare Diagnostics Inc., Tarrytown, NY) with a manual extraction (QIAamp Viral RNA Mini Kit, Qiagen Inc., Valencia, CA).
assayed 80 unique samples that included: (a) 67 patient sera or K2EDTA-plasma, (b) 3 dual-positive, pooled-patient controls of 1a+2b, 1b+2a and 3+4, (c) 4 controls for GT 1, 2, 3 and 4 (AcroMetrix Corporation, Benicia, CA), (d) 4 controls for GT 3, 4, 5 and 6 (SeraCare Life Sciences, West Bridgewater, MA), and (e) 2 proficiency samples for GT 2 and 4 (obtained through the College of American Pathologists), as shown on Table 1. One each of patient samples with GT 1a, 1b, 2, 3 and 4 was diluted to HCV RNA concentration of 500 IU/mL. Manufacturers’ controls were diluted 2- to 10-fold before extraction. All dilutions were prepared in Dilution Matrix (Life Technologies Corporation, Carlsbad, CA). A minimum volume of 600-µL serum or plasma centrifuged at 2000 g for 5 minutes prior to extraction was required for the Abbott RealTime assay and 140-µL for LiPA.

The Abbott RealTime HCV Genotype II RUO assay (Abbott Molecular Inc., Abbott Park, IL) was performed according to manufacturer’s instructions in conjunction with Abbott’s m2000 automated real-time PCR platform, the m2000sp and m2000rt. The m2000 platform performed the RNA extraction using the mSample Preparation System reagents and added the amplification reagents to the extractions. Four primer sets and corresponding genotype specific probes confirmed the presence of HCV RNA and identified HCV GT 1 through 6, targeting the 5’UTR region of the HCV genome and subtypes 1a and 1b from the NS5B region [10]. An unrelated, non-competitive RNA served as the internal control for each reaction. The kit’s dual positive control (GT 1a and 4) and negative control were processed in the same manner as clinical samples. The plate was then transferred manually to the m2000rt instrument for RT-PCR amplification and detection. Validity checks, data analysis, and a result report were generated by the m2000rt.
From the 80 samples, 83 GT were identified, as there were 3 dual-positive, pooled-patient controls. Agreement between the two methods was 100% for the controls, proficiency samples and patient samples diluted to 500 IU/mL. As shown in Table 2, overall concurrence was 98% (81/83). Identification of GT 1 was 100% and for subtypes 1a and 1b were 100% and 95%, respectively. There were two non-concordant results from patient sera which could not be further analyzed for verification.

As shown in Figure 1, Abbott RealTime had less hands-on time and instrument time compared with LiPA. Our total assay time was reduced from 10.5 hours to 6.0 hours. For each patient sample, hands-on time from the technologist decreased from 13 to 4 minutes. Time on the Abbott instruments was 4.5 hours compared with 6.5 hours for LiPA. The Abbott procedure would enable one technologist to complete the assay and report results for up to 22 patient samples well within an 8-hour shift. In our laboratory, the estimated cost/sample using LiPA was US $107 and US $109 for Abbott RealTime.

We confirmed the Abbott RealTime HCV Genotype II RUO assay for clinical use in identifying HCV GT 1 to 6 and subtypes 1a and 1b in clinical samples as previously reported [4]. In our study, the RealTime assay identified GT 1a, 1b, 2, 3 and 4 in patient sera at a minimum HCV RNA concentration of 500 IU/mL and distinguished dual-positive GT samples. Fewer GT 1 subtyping failures were found than previously described [11]. Importantly, Abbott’s automated extraction and assay compared to Versant LiPA with manual extraction reduced both hands-on time and assay time, thus affording a substantial gain in productivity without significant extra cost.
Acknowledgements

We thank Karen Rexroth, BS for her technical support.

Abbott Molecular Inc. provided the Sample Prep and Amplification Kits for this correlation project.

This work was presented in part at the 28th Annual Clinical Virology Symposium and Annual Meeting of the Pan American Society for Clinical Virology in, Daytona Beach, FL during April 20-25, 2012 (Abstract M40).

The views expressed are those of the authors and do not reflect the views or policies of the Department of Veterans Affairs.

All authors report no conflicts of interest.
References


Table 1. Sources of the 80 unique samples for 83 genotype identification.

<table>
<thead>
<tr>
<th></th>
<th>Serum/Plasma</th>
<th>Serum/Plasma Dual-positive</th>
<th>SeraCare</th>
<th>Acrometrix</th>
<th>CAP</th>
<th>Total GT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Plasma</td>
<td>1a+2</td>
<td>1b+2</td>
<td>3+4</td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>17</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>1b</td>
<td>21</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>18</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>
Table 2. Comparison of HCV Genotype RUO Assays

<table>
<thead>
<tr>
<th>HCV Genotype (Versant 2.0 RUO LiPA or Manufacturers' Controls)</th>
<th>Abbott Real Time HCV Genotype II RUO</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>1a</td>
</tr>
<tr>
<td>1b</td>
<td>21 *1</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>17 **1</td>
</tr>
<tr>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

*Not concordant and not repeated for confirmation

** Not concordant but repeated for confirmation using Abbott RealTime
Figure 1. Comparison of Abbott RealTime versus Versant LiPA HCV Genotype RUO Assays for Instrument and Hands-on Time in hours.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>6.0 hours</td>
</tr>
<tr>
<td>LiPA</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>10.5 hours</td>
</tr>
</tbody>
</table>

Legend:
- Hands on Time (hour)
- Instrument time (hour)

8 hour shift

on October 22, 2017 by guest