Evaluation of the COBAS TaqMan HCV Test with Automated Sample Processing Using the MagNA Pure LC Instrument

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Hepatitis C virus (HCV) infection is a leading cause of chronic liver disease in the United States. The prevalence of HCV infection has been estimated to be 1.8% in the general population, resulting in a conservative estimate of 2.7 million cases of chronic HCV infection in the United States (2). Current anti-HCV treatment algorithms continue to rely on the direct detection and quantitation of HCV RNA in serum or plasma for confirmation of chronic HCV infection and determining duration of anti-HCV therapy. Significant decreases in HCV RNA viral load and clearance of HCV RNA during the course of therapy are also important predictors of sustained virologic response with current anti-HCV therapies. As a result, there is a growing clinical need for rapid automated molecular techniques capable of providing both sensitive detection of HCV RNA and accurate quantitation of HCV RNA over a broad dynamic range.

The COBAS TaqMan HCV Test (TaqMan HCV; Roche Molecular Systems Inc., Branchburg, N.J.) is a real-time nucleic acid amplification assay for the qualitative and quantitative detection of HCV RNA in human serum or plasma. Like the TaqMan HCV analyte specific reagent (TaqMan HCV ASR; Roche Molecular Systems), this assay has been developed for use with the recently introduced COBAS TaqMan 48 Analyzer (CTM 48; Roche Molecular Systems). While use of the TaqMan HCV ASR requires the testing laboratory to establish and validate the actual test method and provide appropriate test controls, TaqMan HCV is a complete test kit currently designated for research use only in the United States. Currently, TaqMan HCV is comprised of two products, the High Pure System viral nucleic acid kit (Roche Molecular Systems) and the COBAS TaqMan HCV Test kit, which can be purchased separately. The High Pure System viral nucleic acid kit is a generic, manual specimen preparation method configured for processing multiple samples in batches. Specimen preparation is followed by automated reverse transcription (RT)-PCR amplification and real-time detection of cleaved dual fluorescent dye-labeled oligonucleotide (TaqMan) probes that allow the specific and simultaneous detection and quantitation of the target sequence as well as an internal HCV quantitation standard with the CTM 48.

The potential benefits of nucleic acid amplification and detection with real-time PCR have been well documented (6, 14, 16). Among the benefits are substantial reductions in labor as well as decreased test turnaround time. The disadvantages of manual specimen preparation methods have also been well documented. Labor-intensive manual sample preparation methods have been reported to account for the majority of the hands-on time required to perform current commercially available PCR-based assays for HCV (1, 12, 13). In many cases, sample preparation is the most technically demanding portion of the assay and a potential source of run-to-run variability as well as sample contamination. Implementation of an automated sample processing system in clinical diagnostic laboratories provides a labor-saving approach to reducing the number of failed preparations and limiting the occurrence of specimen-to-specimen contamination during processing. Other important advantages of automated specimen processing include reduced laboratory space...
TABLE 1. Analytical sensitivity of TaqMan HCV combined with sample processing by MP

<table>
<thead>
<tr>
<th>HCV RNA concn (IU/mL)</th>
<th>No. of replicates</th>
<th>No. of positive replicates</th>
<th>% positive (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>10</td>
<td>10</td>
<td>100 (69–100)</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td>10</td>
<td>100 (69–100)</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>6</td>
<td>60 (26–88)</td>
</tr>
<tr>
<td>2.5</td>
<td>10</td>
<td>3</td>
<td>30 (7–65)</td>
</tr>
<tr>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0 (0–31)</td>
</tr>
</tbody>
</table>

TABLE 2. Precision of TaqMan HCV combined with sample processing by MP

<table>
<thead>
<tr>
<th>HCV RNA concn (IU/mL)</th>
<th>Observed mean HCV RNA titer (IU/mL)</th>
<th>No. of replicates</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>22</td>
<td>10</td>
<td>46</td>
</tr>
<tr>
<td>50</td>
<td>102</td>
<td>10</td>
<td>29</td>
</tr>
<tr>
<td>500</td>
<td>770</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>5,000</td>
<td>4,958</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>50,000</td>
<td>40,100</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>500,000</td>
<td>345,600</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>5,000,000</td>
<td>2,452,000</td>
<td>10</td>
<td>19</td>
</tr>
</tbody>
</table>

TABLE 3. Clinical sensitivity and specificity of TaqMan HCV with sample processing by MP compared to COBAS AMPLICOR

<table>
<thead>
<tr>
<th>TaqMan HCV result</th>
<th>No. of specimens with COBAS AMPLICOR result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>41</td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
</tr>
</tbody>
</table>

Y = 1.0837x - 0.2944
R² = 0.99
Man HCV working master mix and 24 open COBAS TaqMan kinetic reaction tubes (K-tubes) contained in a K-tube holder (K-carrier) were placed onto a prototype (noncooling) postelution handling block specifically designed to hold a K-carrier (containing K-tubes) in the MP. The addition of working master mix (50 μl) and sample eluate (50 μl) to each K-tube (followed by a mixing step) was automatically performed by the MP. K-carriers were removed from the MP and individual K-tubes were manually sealed prior to loading onto the CTM 48. Amplification and detection were performed according to the manufacturer’s instructions for TaqMan HCV with the CTM 48 with AMLILINK software v 3.0.1 (Roche Molecular Systems).

Statistical analysis. Observed results of HCV analytical standards tested by TaqMan HCV were compared with the expected results by linear regression, while the lower limit of detection with 95% confidence interval was determined by probit analysis (95% hit rate) (8). The precision of TaqMan HCV was estimated by determining the coefficients of variation at seven different HCV RNA concentrations (25 to 5 × 10⁶ IU/ml) tested over a total of 5 days. Correlation and strength of agreement between TaqMan HCV and bDNA results were determined by linear regression and Bland-Altman plot (4), respectively. Potential differences in target amplification efficiency (quantitation) by TaqMan HCV were examined further by comparing the slopes of the linear regression lines from 12 clinical specimens (HCV genotypes 1 to 6) tested undiluted and at 1:100 and 1:1,000 dilutions.

RESULTS

The lower limit of detection or analytical sensitivity of TaqMan HCV combined with sample processing by MP was determined based on the results of replicate testing of HCV analytical standards at concentrations of 25, 10, 5, 2.5, and 0 IU/ml (Table 1). With probit analysis with a 95% hit rate, the lower limit of detection was 8.1 IU/ml (95% confidence interval, 6.1 to 15.2 IU/ml). Excellent linearity ($R^2 = 0.99$) was also present across a wide range of HCV RNA levels extending...
from 25 to $5 \times 10^6$ IU/ml (Fig. 1), with interassay coefficients of variation ranging from 10% to 46% among the seven concentrations tested over 5 days (Table 2).

When the results of TaqMan HCV combined with sample processing by MP were compared to those of COBAS AMPLICOR among the 83 clinical serum specimens, the clinical sensitivity and specificity of TaqMan HCV combined with sample processing by MP were 100% (95% confidence interval, 91% to 100%) and 95% (95% confidence interval, 84% to 99%), respectively. TaqMan HCV detected two more HCV RNA-positive specimens than COBAS AMPLICOR (Table 3). These two discordant specimens were also tested by the VERSANT HCV RNA qualitative test, with both specimens yielding positive HCV RNA results.

The group of 93 well-characterized specimens consisted of HCV genotype 1 ($n = 61$), genotype 2 ($n = 13$), genotype 3 ($n = 8$), genotype 4 ($n = 4$), genotype 5 ($n = 2$), and genotype 6 ($n = 2$) as well as three HCV RNA-positive specimens that could not be genotyped, presumably due to low viral titer (862 to 5,720 IU/ml by bDNA). Comparison of TaqMan HCV and bDNA results among this group of specimens demonstrated strong correlation ($R^2 = 0.95$) of HCV RNA quantitative results obtained by TaqMan HCV and bDNA (Fig. 1). Bland-Altman plotting showed that differences between the $\log_{10}$ IU/ml results obtained from TaqMan HCV and bDNA were within $\pm 0.5 \log_{10}$ IU/ml of the averaged $\log_{10}$ results of the two tests for 98% of the specimens tested (Fig. 3). The two specimens with differences of $> 0.5 \log_{10}$ IU/ml contained HCV genotypes 4 and 6. Repeat testing (TaqMan HCV and bDNA) of the specimen containing HCV genotype 4 yielded similar results, while the specimen containing HCV genotype 6 could not be retested due to insufficient volume. The mean slope of the linear regression lines derived from the 12 clinical specimens tested undiluted, and at 1:100 and 1:1000 dilutions was $-1.4$, with the mean genotype-specific slope ranging from $-1.1$ for genotype 4 to $-1.6$ for genotypes 1 and 6.

The hands-on setup time for the MP was $\approx 20$ min. The time required to prepare and manually load specimens into the MP sample cartridge prior to automated processing was also $\approx 20$ min. With the postelution handling option, the total time required for processing 24 reactions by MP was 192 min, an increase of 37 min relative to the estimated time required to...
perform the manual method. However, the actual hands-on time was reduced from an estimated 145 min required by the manual method to just 57 min with MP sample processing and postelution handling, including two periods (93 and 42 min) of hands-off time (Fig. 4). The total duration for the entire procedure (21 samples plus three controls), including sample preparation, MP sample processing, and amplification/detection, was 6 h, of which 1 h was actual hands-on time. During the course of this study, there were no failures of sample processing associated with the MP, and the overall rate of TaqMan HCV failure (an invalid test result as defined by the APLILINK 3.0.1 software) was 1% (2 of 313) over the 17 runs completed for this study.

DISCUSSION

Sensitive, accurate detection and quantitation of HCV RNA is essential for the diagnosis and management of chronic HCV infection. With projected increases in the diagnosis of chronic HCV infection in the United States over the next several decades (3), clinical laboratories will face growing pressure to efficiently accommodate increasing test volumes, while continuing to provide both sensitive detection and accurate quantitation of HCV RNA in clinical specimens. The results of this study have demonstrated that TaqMan HCV used in conjunction with sample processing by MP is an efficient and reliable method capable of providing both qualitative and quantitative results with a single test.

Our analytical data suggest that TaqMan HCV, when used in combination with sample processing by MP (with a lower limit of detection of 8.1 IU/ml), is more sensitive than COBAS AMPLICOR, which has a reported lower limit of detection ranging from 50 to 75 IU/ml (9, 15). Our clinical data also suggest that TaqMan HCV combined with sample processing by MP was more sensitive than COBAS AMPLICOR (Table 3), although the difference in clinical sensitivity between the two assays was not statistically significant ($P = 0.438$; one-tailed Fisher’s exact test). When used in conjunction with MP, TaqMan HCV yielded a lower limit of detection comparable to that of the VERSANT HCV RNA qualitative test, which has been shown to reliably detect HCV RNA at concentrations of <10 IU/ml (11). The increased sensitivity of these ultrasensitive assays could be important in the management of HCV-infected patients, since the increased sensitivity of transcription-mediated amplification has been shown to improve the detection of extremely low levels of HCV RNA in end-of-treatment specimens and improve the prediction of treatment failure or virologic relapse in patients receiving anti-HCV therapy (5, 10, 17, 18).

In the current study, evaluation of HCV RNA quantitation by TaqMan HCV was limited to the analysis of analytical standards ranging from 25 to $5 \times 10^6$ IU/ml and a group of 93 well-characterized serum specimens previously tested by bDNA. While our study was limited in scope and did not evaluate the entire reportable range of TaqMan HCV (10 to $2 \times 10^8$ IU/ml) defined by the assay manufacturer, it did...
demonstrate that the results of TaqMan HCV were in good agreement with those of bDNA and that there were no substantial differences in quantitation among the various HCV genotypes. In addition, TaqMan HCV provides a broader dynamic range than bDNA (615 to 7.69 × 10⁷ IU/ml). The increased dynamic range of TaqMan HCV improves clinician’s ability to monitor changes in HCV viral load in patients undergoing anti-HCV therapy, particularly when HCV RNA titers are <615 IU/ml or >7.69 × 10⁷ IU/ml.

During the course of this study, we experienced no significant problems related to MP performance. The lower limit of detection and precision of TaqMan HCV with sample processing performed by MP were comparable to those reported in a previous evaluation of TaqMan HCV used in conjunction with the High Pure System viral nucleic acid kit (J. Birkett, K. Demartin, C. Hakleroad, D. Romo, D. Liao, A. Maxwell, H. Sandhu, and S. Tsang, Abstr. 19th Annual Clinical Virology Symposium, abstr. M43, 2003). While our study was not specifically designed to evaluate specimen contamination with exogenous nucleic acids, we found no evidence of sample-to-sample contamination during evaluation of either the analytical or clinical samples, despite the processing of both HCV RNA-negative and high-titer samples within the same run on the MP. In addition to these findings, the hands-on time required for sample processing by MP, compared to manual sample processing, was reduced by ~1 h with or without use of the postelution handling capability of MP (run size of 24). Importantly, with MP sample processing and postelution handling, there are two extended periods of hands-off time, which allows technologists time to perform other laboratory duties.

Although the MP performed well in this evaluation with a run size of 24, the efficiency of the complete process may be effected by other factors, such as laboratory test volume. Differences in instrument configuration limit the overall efficiency of MP specimen processing for TaqMan HCV. The MP instrument was originally designed to work with the LightCycler instrument (Roche Applied Science), and it efficiently processes eight specimens simultaneously with a maximum batch size of 32, such that processing batches of 8, 16, or 32 samples is most efficient. However, TaqMan HCV is performed most efficiently in batch sizes of 12, 24, and 48 samples on the CTM 48. It would be advantageous to use multiple MP instruments in clinical laboratories with high specimen throughput demands. Alternatively, omission of postelution handling by the MP in favor of manual K-tube loading could further decrease the total time required for MP sample processing and increase specimen throughput.

In summary, this study demonstrated that the MP is a versatile, automated, and labor-saving sample processing platform useful for the reliable performance of TaqMan HCV. Our analytical and clinical data suggest that TaqMan HCV combined with sample processing by MP may be more sensitive than COBAS AMPLICOR for the qualitative detection of HCV RNA. In this study, TaqMan HCV also provided quantitative HCV RNA results that were in good agreement with those of bDNA. Our findings, generated with a research-use-only test kit (TaqMan HCV), suggest that the MP would also be suitable for use with the TaqMan HCV ASR. However, the performance characteristics of assays utilizing TaqMan HCV ARS in conjunction with MP sample processing require further validation by individual clinical diagnostic laboratories.

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REFERENCES


