Performance evaluation of the new Roche COBAS AmpliPrep/ COBAS TaqMan HCV Test, v2.0 for the detection and quantification of HCV RNA

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Summary

To evaluate the analytical performance and explore the clinical applicability of the new Roche COBAS AmpliPrep/COBAS TaqMan HCV Test, v2.0 (CAP/CTM v2.0), a platform comparison was performed with the Roche COBAS AmpliPrep/COBAS TaqMan HCV Test (CAP/CTM v1.0), the Siemens Versant HCV RNA 3.0 (bDNA) test, the Abbott m2000 RealTime HCV assay (Realtime assay) and the Siemens Versant HCV TMA test (TMA assay) on panels and diagnostic samples. The analytical performance of the CAP/CTM v2.0 on WHO and Acrometrix panels and clinical specimens of genotype 1-6 patients relative to the CAP/CTM v1.0 was significantly improved. In a qualitative comparison of the CAP/CTM v2.0 relative to the TMA on Genotype 1-4 samples the CAP/CTM v2.0 test proved to be almost equally sensitive. Response guided therapy in one out of five Genotype 4 HCV infected patients previously tested with the CAP/CTM v1.0 would have significantly changed when tested with the CAP/CTM v2.0. In conclusion the Roche CAP/CTM v2.0 has significantly better performance characteristics as compared to the former CAP/CTM HCV v1.0 and the bDNA and comparable performance characteristics to the Realtime assay.
Introduction

Hepatitis C virus (HCV) is a major public health problem and a leading cause of chronic liver disease. An estimated 170 million individuals are infected worldwide. Diagnostic assays for HCV include serological assays for the detection of HCV specific antibodies and molecular assays aiming at sensitive detection of HCV RNA in plasma or serum. The detection of HCV RNA is important to distinguish between acute, chronic and resolved infection. Quantitative and qualitative detection of HCV RNA is furthermore used to determine treatment response.

Decision points in the treatment of HCV require both qualitative results generated with an assay with a lower limit of detection of at least 15 IU/mL and quantitative results. Due to the increase of global availability of medication and changes of the epidemiology of HCV genotypes in the Western world these assays would preferably be equally sensitive for all different HCV genotypes(3, 14). Current methods for the quantitative detection of HCV RNA include end-point RT-PCR, real-time RT-PCR, branched DNA and for qualitative results, the transcription-mediated amplification (TMA) method (4-5, 12, 15).

Quantitative end-point RT-PCR are increasingly displaced by real-time RT-PCR because the latter in general have an increased dynamic range, are less prone to contamination and easier to automate(13). An alternative to real-time RT-PCR is a combination of testing with bDNA and TMA where the bDNA is used to quantify before start of treatment and at weeks 4 and 12 and TMA is used for sensitive qualitative detection at weeks 12 and beyond although testing algorithms are more complex as compared to testing with sensitive real-time RT-PCR.

After the introduction of the COBAS AmpliPrep/COBAS TaqMan HCV Test v1.0 (CAP/CTM v1.0) it became apparent that this test, despite its good performance
characteristics on most genotype 1-3 strains, under-quantifies certain genotype 4 strains and a small percentage of genotype 1 patients (1-2, 8, 12, 15). Recently results obtained with a prototype of a second version of the CAP/CTM HCV test have been described (16), demonstrating that after redesign of primers and addition of a second probe under-quantification of certain genotype 4 HCV strains was solved. This redesign was used for the development of the CAP/CTM v2.0 Test. We set out to determine the quantitative and qualitative detection of HCV by the CAP/CTM HCV v2.0 test relative to its major commercially available competitors.
Material and methods

Patient samples and test materials

Leftover EDTA plasma samples sent to the diagnostic units of the Department of Virology of the Erasmus MC and the Department of Clinical Microbiology of the Amsterdam Medical Center for HCV diagnostics were used in this study. Written informed consent was obtained from each patient and the study was approved by the Medical Ethical Committee. In addition plasma samples previously used in a study to validate the performance of the Siemens VERSANT HCV Genotype 2.0 Products (LiPA), (Siemens Healthcare Diagnostics, Den Haag, The Netherlands) on HCV genotypes 5 and 6, were included(10). Plasma samples were stored at -80°C until use. For platform comparison plasma samples were diluted 10 to 200 times with a pool of HCV-RNA negative, RNAse free plasma using a CAS4200 (Qiagen, Hilden, Germany). Diluted samples were aliquoted into sufficient aliquotes to allow testing on all platforms and stored at –80°C until use. Aliquotes tested at the Academic Medical Centre Amsterdam were sent on dry-ice to prevent multiple freeze thaw cycles. For each test a separate aliquot was used to prevent multiple freeze-thaw cycles. Plasma samples were selected to have a HCV RNA load between 115 IU/mL and 100.000 IU/mL after dilution. Genotyping was performed with the Siemens Versant HCV Genotype 2.0 Products (LiPA) (Siemens Healthcare Diagnostics, the Hague, the Netherlands) on an Autolipa48 (Innogenetics, Gent, Belgium) at the Erasmus MC and by sequencing part of NS5B at the Amsterdam Medical Centre(9). In order to compare HCV RNA quantification of the different platforms internationally recognised standards were used: the 2nd WHO International standard (06-100, NIBSC, Potters Bar, United Kingdom) and the Acrometrix HCV genotyping panel (Optiqual 1-4, Acrometrix, Benicia, USA).
primary analysis of the data an outlier analysis was performed. Samples that after retesting or dilution were below the lower limit of quantification (LLOQ) in all platforms tested were excluded from the analyses. Samples that differed more than 0.5 log in one platform as compared to the other two platforms tested were repeated in the platform that differed from the other two. The result of the re-test procedure was used in the study.

Quantitative and qualitative HCV RNA assays

In the present study, all testing procedures for the CAP/CTM HCV v1.0 and v2.0 tests (Roche Molecular Diagnostics, Almere, the Netherlands) were performed at the Erasmus MC. Siemens Versant HCV RNA 3.0 assay (bDNA) (Siemens Healthcare Diagnostics, the Hague, the Netherlands), Abbott m2000 RealTime HCV assay (Abbott Diagnostics, Hoofddorp, the Netherlands) and Siemens Versant HCV qualitative assay were performed at the Amsterdam Medical Centre. During routine diagnostics quantitative detection of HCV was performed with the COBAS AMPLICOR HCV MONITOR Test using the manual High Pure system (HPS/CA) and qualitative detection was performed with the COBAS AmpliPrep/COBAS AMPLICOR HCV Test, version 2.0 (CAP/CA) (Roche Molecular Diagnostics, Almere, the Netherlands). All assays were performed according to the manufacturer’s instructions as described in the respective package inserts.

Data analysis.

Data were analyzed using Microsoft Office Excel 2003 and SigmaPlot for Windows, version 10.0.
Results

Validity of testing procedure

To study whether the dilution procedure and the matrix used to dilute the plasma samples might influence the results of the study, a selection of genotype1-4 samples (10 samples for each genotype) was tested in the CAP/CTM v2.0 neat and diluted and results were compared. None of the tested samples differed more than 0.5log and the regression coefficient was close to 1 ($R^2 = 0.9925$) validating the dilution procedure used in this study (data not shown).

Platform comparison

To test the standardization of the different platforms on International standards, the Acrometrix genotype panel and a dilution series of the 2nd WHO international standard were tested in monoplo with the CAP/CTM v2.0, Realtime and the bDNA assays (Table 1). No major differences of more than 0.7log10(6) from the expected value were observed between the test platforms except lower values of the Realtime test on the WHO international standard and the Acrometrix Optiqual genotype 2 sample. The performance of the CAP/CTM v1.0, CAP/CTM v2.0, Realtime assay and the bDNA assay on a genotype diverse panel of HCV RNA positive samples was tested. Due to limited availability of samples not all samples were tested in all platforms. As a result 151, 190, 189 and 190 samples were tested in the CAP/CTM v1.0, CAP/CTM v2.0, Realtime assay and the bDNA assay respectively. For platform comparison, a regression analysis was performed on data plots generated similarly to Bland-Altman plots (plotting the mean of results from the two assays against the difference between
results from the two assays) (Figure 1). For the calculation of the average viral load per
test and regression analysis, only samples tested on all platforms were used and
samples recorded below the LLOQ of the CAP/CTM v1.0 (15 IU/ml), the CAP/CTM v2.0
(15 IU/ml) and the Realtime test (12 IU/ml) or below the LLOD of the bDNA (615 IU/ml)
were assigned the LLOQ and LLOD value respectively (Table 2). The average viral loads
for the CAP/CTM v1.0, CAP/CTM v2.0, Realtime test and the bDNA assay were 5.25log,
4.91log, 4.90log, and 4.74log respectively. The slopes of the regression curves in the
Bland-Altman plots were close to zero, -0.07, -0.07 and -0.06 for comparison of the
CAP/CTM v2.0 with CAP/CTM 1.0, Realtime and bDNA tests, respectively. The y-
intercepts were slightly higher than 0 for all three comparisons (0.12, 0.40, and 0.46 for
the CAP/CTM 1.0, Realtime and bDNA tests, respectively). The regression coefficients
of the linear regression analysis were 0.8957, 0.9648 and 0.9372 for the CAP/CTM 1.0,
Realtime and bDNA tests, respectively. Samples recorded below the LLOQ by the
Realtime test (1 sample) and the LLOD of the bDNA assay (18 samples) had a viral load
around the LLOQ of the corresponding test, while samples recorded below the LLOQ by
the CAP/CTM v1.0 (4 samples) had a significantly higher viral load (mean 3.10E4
IU/mL). 5'UTR region of the latter 4 samples were sequenced and all four sequences
obtained proved to have the previously reported G145A in combination with A165T
mutation in the probe region (data not shown)(16). None of these four samples were
recorded below the LLOQ by the CAP/CTM v2.0 or in any of the other test platforms.
Both the Realtime and the CAP/CTM v2.0 proved to have similar averages on different
genotypes (Table 2). The CAP/CTM v1.0 proved to have higher averages on genotype
1, 3, 5 and 6 while lower average viral loads were recorded for genotype 2 and 4. The
bDNA test recorded on average similar viral loads for genotypes 1 and 5 while lower averages were observed for genotypes 2, 3, 4 and 6.

Qualitative analysis and genotype 4 response guided therapy
To determine the sensitivity of the CAP/CTM v2.0 test for the qualitative detection of HCV RNA, samples from genotype 1-4 infected patients receiving therapy were processed with the TMA assay and the CAP/CTM v2.0 (Table 3). Samples were selected that were below the LLOD of the bDNA and either TMA negative or TMA positive in our routine diagnostic procedure and subsequently retested with the CAP/CTM v2.0. An equal distribution of genotype 1-4 infected patients was selected in both groups although due to limited availability of sample only few TMA positive samples from genotype 2 infected patients could be tested. Samples may be positive or negative for HCV RNA but blow the LLOQ and thus unquantified by the CAP/CTM v2.0. These results are reported as <15 IU/ml. Qualitative results are reported as “target detected” and “target not detected” by the CAP/CTM v2.0 for samples below the LLOQ. The TMA result was compared with the CAP/CTM v2.0 qualitative (Target detected or target not detected) and the quantitative result (<15 IU/mL or >15 IU/mL). The negative predictive value of the CAP/CTM v2.0 results on the TMA negative samples was 100% for all genotype samples. The positive predictive values of the quantitative and qualitative results was 70% and 91% respectively with a lower performance on genotype 4 samples as compared to genotype 1 and 3.

Longitudinal samples from 21 HCV genotype 4 infected patients who started on PEG-IFN with ribavirin were re-analyzed with the CAP/CTM v2.0. Samples from 16 patients had previously been tested with the CAP/CA and HPS/CA and samples from five...
patients with the CAP/CTM v1.0. Re-testing resulted in three changes in response interpretation relative to the previous performed test. One week 4 sample from a patient with high baseline viral load (6.9Log IU/ml) and a cEVR, previously tested HCV RNA positive with the CAP/CA (interpretation no RVR) tested negative in the CAP/CTM v2.0 (interpretation RVR). Both results would have resulted in 48 weeks treatment advice according to treatment guideline(7). Another week 4 sample from a patient also with high baseline viral load (6.2Log IU/ml) previously tested negative with the CAP/CA re-tested positive (1.17x10^3 IU/mL) in the CAP/CTM v2.0. Also in this case both results would have indicated 48 weeks of treatment. The result of a third sample that was discrepant after re-testing would have had a significant effect on therapy duration. The week 24 sample of a patient with no-RVR and an EVR was previously tested negative with the CAP/CTM v1.0 and therefore was advised 72 weeks therapy under which the patient experienced a relapse. The week 24 sample tested positive after re-testing with the CAP/CTM v2.0 and would therefore according to treatment have been advised to stop treatment after 24 weeks.
Discussion

In the present manuscript we show that the CAP/CTM v2.0 has significantly improved analytical and clinical performance characteristics as compared to the previous version. Furthermore the sensitivity of the CAP/CTM v2.0 was close to that of the TMA on genotypes 1-4. Good precision and high sensitivity on HCV genotype 1-6 indicate that CAP/CTM v2.0 is a good test for monitoring HCV viral load during treatment.

Recently an improved prototype version of the CAP/CTM HCV real-time quantitative RT-PCR was described (16). CAP/CTM v1.0 has been shown to under-quantify certain genotype 4 samples while overestimating the viral load of other genotypes (1). In addition two polymorphisms in the region where the probe anneals could also result in false negative results (16). In the present manuscript we set out to analyze the quantitative analytical performance of the CAP/CTM v2.0 test relative to the Realtime test and the bDNA assay. In addition the qualitative performance relative to the TMA test and the clinical performance in a small cohort of genotype 4 infected individuals treated with PEG-IFN plus Ribavirin was analyzed.

No significant differences in quantification were observed relative to the Realtime test on any of the six genotypes. The genotype quantification of the bDNA assay relative to the Realtime and the CAP/CTM v2.0 was variable where the bDNA test seemed to under-quantify genotype 2, 3 and 6 while similar results were obtained relative to its comparators on genotype 1, 4 and 5. Similar to previously published data the CAP/CTM v1.0 overestimated HCV viral loads in genotype 1, 3, 5 and 6 samples while under-estimating viral loads in genotype 2 and 4 samples (1). Also in our analysis 4 samples were identified (1 genotype 1 and 3 genotype 4) that carried the mutation G145A in combination with A165T in the 5'UTR which seriously compromised the quantitation by
the CAP/CTM v1.0. The CAP/CTM v2.0 correctly quantified these samples relative to the Realtime and bDNA tests. Currently the most sensitive test for the detection of HCV RNA is the qualitative TMA with a LLOD of approximately 5 IU/mL although a head to head comparison with the Realtime and CAP/CTM v1.0 and v2.0 has not been published yet (4, 11). In our analysis only 3 out of the 34 bDNA negative, TMA positive samples from genotype 1-4 patient on treatment, were not detected by the CAP/CTM v2.0 in the qualitative dataset. In our qualitative dataset the CAP/CTM v2.0 had a slightly lower sensitivity on genotype 4 relative to the TMA while performance on genotype 1 and 3 was similar relative to the TMA test. Given the fact that quantitative and qualitative detection of genotype 4 HCV RNA relative to the Realtime assay is comparable it seems unlikely that the slightly lower sensitivity below the LLOQ of the CAP/CTM v2.0 (15 IU/mL) relative to the TMA on genotype 4 will have a significant impact on clinical performance characteristics. Current guidelines for the treatment of HCV recommend response guided therapy monitored with a sensitive real-time RT-PCR based assay. Given the sensitivity of the qualitative results and the good genotype inclusivity of the quantitative results it may be assumed that the CAP/CTM v2.0 can be used world wide for response guided therapy of HCV. Robustness of the assay in diverse laboratory settings and genotypes encountered world wide still needs to be elucidated however. In a small cohort of genotype 4 HCV infected patients on PEG-IFN+ribavirin therapy of which 16 had previously been monitored with the CAP/CA and 5 with the CAP/CTM v1.0, the CAP/CTM v2.0 performed comparable to the CAP/CA but better than the CAP/CTM v1.0. In one patient monitoring with the CAP/CTM v2.0 instead of the CAP/CTM v1.0 would have led to earlier prediction of therapy failure and thus to shortening of therapy.
Acknowledgement

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### Table 1 Panels tested with the respective platforms for Quantitative HCV viral load testing

<table>
<thead>
<tr>
<th>Panel sample</th>
<th>Expected</th>
<th>CAP/CTM v2.0</th>
<th>Real-time</th>
<th>bDNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO 1/10</td>
<td>1.55E+04</td>
<td>1.08E+04</td>
<td>5.71E+03</td>
<td>7.40E+03</td>
</tr>
<tr>
<td>WHO 1/100</td>
<td>1.55E+03</td>
<td>1.20E+03</td>
<td>6.26E+02</td>
<td>9.83E+02</td>
</tr>
<tr>
<td>WHO 1/1000</td>
<td>1.55E+02</td>
<td>2.72E+02</td>
<td>9.40E+01</td>
<td>&lt;6.15E+02</td>
</tr>
<tr>
<td>WHO 1/10.000</td>
<td>1.55E+01</td>
<td>&lt;1.50E+01</td>
<td>&lt;1.20E+01</td>
<td>&lt;6.15E+02</td>
</tr>
<tr>
<td>Optiqual GT1</td>
<td>3.18E+04</td>
<td>2.67E+04</td>
<td>1.31E+04</td>
<td>2.31E+04</td>
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<tr>
<td>Optiqual GT2</td>
<td>4.04E+04</td>
<td>2.20E+04</td>
<td>7.38E+03</td>
<td>1.94E+04</td>
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<tr>
<td>Optiqual GT3</td>
<td>3.99E+04</td>
<td>3.12E+04</td>
<td>1.25E+04</td>
<td>1.41E+04</td>
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<tr>
<td>Optiqual GT4</td>
<td>4.86E+04</td>
<td>5.49E+04</td>
<td>1.78E+04</td>
<td>5.15E+04</td>
</tr>
</tbody>
</table>

### Table 2 Number of samples and average viral load per genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of samples tested</th>
<th>Average viral load</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CAP/CTM v1.0</td>
<td>CAP/CTM v2.0</td>
</tr>
<tr>
<td>GT1-6</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>GT1</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>GT2</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>GT3</td>
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<td>25</td>
</tr>
<tr>
<td>GT4</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>GT5</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>GT6</td>
<td>35</td>
<td>35</td>
</tr>
</tbody>
</table>

*Viral load expressed as Log10 HCV RNA IU/ml

### Table 3 Qualitative analysis of HCV RNA testing with the CAP/CTM HCV v2.0 versus the TMA test

<table>
<thead>
<tr>
<th>TMA Negative</th>
<th>TMA Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAP/CTM v2.0</td>
<td></td>
</tr>
<tr>
<td>HCV v2.0</td>
<td></td>
</tr>
<tr>
<td>&lt;1.18Log10 IU/mL</td>
<td>GT1</td>
</tr>
<tr>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>&gt;1.18Log10 IU/mL</td>
<td>0</td>
</tr>
<tr>
<td>Target not detected</td>
<td>11</td>
</tr>
<tr>
<td>Target detected</td>
<td>0</td>
</tr>
</tbody>
</table>
Legend to figure 1
Bland-Altman analysis of the platform comparisons. In each plot the CAP/CTM HCV v2.0 is compared with the CAP/CTM HCV v1.0 (A), Realtime (B) and tDNA (C). The mean of the CAP/CTM HCV v2.0 with the comparator is plotted on the X-axis and the CAP/CTM HCV v2.0 value minus the comparator value is plotted on the Y-axis. Solid line indicates the regression line of the Bland-Altman data. Dashed line indicates samples that differ more than 0.7log10 from the regression line.