Quantification of Hepatitis C Virus RNA in a multicentre study: implication for the management of HCV genotype 1 patients

Running title: Multicentre study for quantification of HCV RNA

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ABSTRACT

Assessing the viral load in hepatitis C virus (HCV) genotype 1 patients is critical before, during and after antiviral therapy. In patients achieving a Rapid Virological Response at week 4 of treatment, the viral load at baseline is considered a predictive criterion of a Sustained Virological Response 24 weeks after discontinuation of treatment. A \( >2\log_{10} \) drop in viral load at week 12 of treatment (Early Virological Response) triggers continuation of the therapy. We organized a multicentre study (MS) for diagnostic laboratories involved in the quantification of HCV RNA. Commercial assays, including two based on real-time RT-PCR (TaqMan system), and in-house methods were used by the sixty-one participants. The overall reproducibility of the commercial qNAT assays is acceptable. As the inter-method variability among commercial qNAT assays for HCV RNA is still present, manufacturers of these test kits should join efforts toward harmonisation in the quantification of HCV RNA. This study also shows that caution should be exercised when evaluating the baseline viral load and in the interpretation of the \( 2\log_{10} \) reduction after 12 weeks of therapy. Finally, this MS confirms the higher sensitivity of the commercial qNAT assays based on the Taqman system, making them the elective assays for monitoring the therapy.
INTRODUCTION

A combination treatment with peginterferon alfa and ribavirin is currently considered the therapy of choice for chronic patients infected with hepatitis C virus (HCV) (5). As HCV genotype 1 patients are less responsive to this therapy, especially in the presence of a high virus titre, the assessment of the viral load before, during and after antiviral therapy plays a crucial role in the management of these patients (6). The viral load at baseline is deemed important in HCV genotype 1 patients achieving a Rapid Virological Response (RVR), defined as undetectable serum HCV RNA (<50IU/mL) by a sensitive molecular assay at week 4 of treatment. In two studies (9, 15), a baseline viremia of <400,000 IU/mL in RVR patients, treated for only 24 weeks as opposed to the canonical 48 weeks, was shown to be a predictive criterion of achieving a Sustained Virological Response (SVR), defined as the absence of serum HCV RNA (<50IU/mL) by a sensitive molecular assay 24 weeks after cessation of therapy. With respect to the viral load at week 12 of treatment, this was shown to have an impact on the outcome of treatment in HCV genotype 1 patients as a failure to obtain a >2-log$_{10}$ drop in HCV RNA, namely an Early Virological Response (EVR), strongly associated with nonresponsiveness, i.e. a failure to achieve an SVR (4). Consequently, in the absence of an EVR, the treatment in these patients is considered ineffective and can be discontinued.

The quantification of HCV RNA requires easy, reliable, and standardized tests with high reproducibility in order to be used in the clinical routine (2, 10). Since the establishment in 1998 of the World Health Organization (WHO) HCV International Standard, expressed in International Units (IU) (12), the standardization of different quantitative assays based on nucleic acid amplification techniques (qNAT) has become feasible. Commercial qNAT assays based on two different technologies, i.e. non-competitive RT-PCR and branched DNA (bDNA) were initially developed. More recently, real-time RT-PCR assays, based on the Taqman system, were introduced on the market. Compared to the classical qNAT assays, this system has proved to be more precise with a broader range of quantification and more sensitive with lower limits of detection (10-15 IU/mL).
In spite of the adoption of a common unit of measurement, differences in the performances of these qNAT assays have been observed, mainly due to the different technologies used and to the different efficiency of HCV RNA hybridization to complementary probe/primer sequences (3, 14). The latter technical aspect may lead to method-related discrepancies in the quantification of the viral load. The intrinsic variability in the efficiency of qNAT assays is also accompanied by the day-to-day variability due to extrinsic factors (e.g. operators, instruments, sample processing, etc.). The introduction of systems to prevent carry over contamination (uracil N-glycosylase) has reduced the percentage of false positive results. Automation of the qNAT assays has also contributed to this reduction in cross contamination, with the additional benefit of minimising the intra/inter-assay variability.

For diagnostic laboratories, participation in a multicentre study (MS) represents a critical tool for assessing performance. With respect to quantification of HCV RNA by NAT, up to now only a few MSs have been done and none included the use of commercial assays based on the TaqMan system (1, 8, 11, 13).

To meet the demand for these MSs from diagnostic laboratories, we organised a new study for quantification of HCV RNA by NAT. The state of the art of all the available commercial qNAT assays, in terms of detection limit and dynamic range, were taken into account in the development of the panels. Furthermore, the design of the study took into consideration the above-mentioned algorithms for the use of antiviral therapy in HCV genotype 1 patients.

MATERIALS AND METHODS

Negative and positive samples

Negative samples were prepared using a plasma pool made up of 20 donations negative for HCV, HIV and HBV by serological and NAT testing.
Two groups of positive samples were prepared from an anti-HCV/HCV RNA-positive donation (genotype 1b) tentatively calibrated by the ISS against the WHO HCV RNA International Standard (IS 96/798) using a qNAT assay (in-house real-time RT-PCR) and found to have a presumed titre of 6.69 log_{10} IU/mL. This donation was diluted with the above-mentioned negative plasma pool as follows:

- 1:15 for the High Titre Samples (HTS), chosen to have a final titre within the linearity range of the available commercial qNAT assays for HCV RNA
- 1:1500 for the Low Titre Samples (LTS), to simulate the minimal viral load decrease considered to be predictive of a positive response to antiviral treatment.

Positive samples with a concentration of 50 IU/mL were prepared by appropriately diluting the WHO HCV IS 96/798 (genotype 1).

A total of 1,600 vials, including negative and positive (HTS, LTS, 50 IU/mL) samples, were prepared and stored at -80°C, sequentially numbered from 0001 to 1600. The negative and positive status of the samples was confirmed by the ISS. To this aim, the vials to be thawed and tested were randomly selected.

qNAT assays

Four commercial qNAT assays for HCV RNA were used in this MS: the COBAS TaqMan (CTM) with automated (CAP/CTM) or manual extraction (HP/CTM), Roche Diagnostic GmbH, Mannheim, Germany; the COBAS Amplicor Monitor (CAM), Roche Diagnostic GmbH, Mannheim, Germany; the RealTime HCV (RealT), Abbott Laboratories, Abbott Park, IL; and the Versant HCV RNA 3.0 Assay (bDNA), Siemens Healthcare Diagnostics, Deerfield, IL. These commercial qNAT assays are listed in Table 1 along with their respective detection limit and dynamic range as stated by the kits’ manufacturers. Four independent in-house (INH) assays were also used in this MS and they were all grouped together. Two of these assays were completely developed in-house while the remaining two
were a combination of commercial test kits, one for the extraction (Qiagen column) and one for the amplification (Arthus kit).

Study design and composition of the panels

Each participant was sent two panels identified with a code number assigned randomly (e.g. A1, C2).

Each of the two panels was made up of eight samples: three HTS, three LTS, one 50-IU/mL sample and one negative sample, all of them randomly selected. According to the testing protocol, participants had to test the panels in two separate runs, 1-2 weeks apart, and to report the results in IU/mL. At the end of the study, each participant was due to receive a detailed technical report with all the results.

Participants

Fifty-eight Italian diagnostic laboratories, one Spanish diagnostic laboratory and two kits’ manufacturers (Roche, Germany and Abbott, USA) took part in this study for a total of 61 laboratories. A coded number identified each laboratory throughout the study.

Shipment

Panels were shipped on dry ice. Participants were asked to check the integrity of the parcel, the presence of dry ice and the frozen status of the samples and to fax this information to the ISS using the “Acknowledgment of receipt” sheet.

Analysis of results for HTS and LTS

The HTS and LTS IU/mL titres reported by the participants were converted to log_{10} titres. These values were then separately used for the following calculations:

- For each laboratory, the respective Geometric Mean/Laboratory expressed in log_{10} IU/mL and the standard deviation (GM (log_{10})±SD) were calculated for each panel (GM (log_{10})panel±SD: HTS n=3; LTS n=3) and for the two panels together (GM (log_{10})panel 1+2±SD: HTS n=6; LTS n=6). A Consensus mean Titre (C_{mean,T}) and the 95% confidence interval were then established for HTS and LTS using all the log_{10} titres obtained in the study.
These GM (log_{10}) values were then grouped by qNAT assay for each run, thus obtaining the respective GM (log_{10}) values. To evaluate the performance of the different qNAT assays used in this study, a range of acceptable values equal to GM (log_{10}) ± 0.15 was established taking into account the SD of all the commercial assays. For each qNAT assay, the Grubb’s test (7) was used to detect outliers. Then, the respective GM (log_{10}) overall ± SD for each qNAT assay and the respective coefficient of variation (CV) were calculated for HTS and LTS.

The differences in the titres between the first and the second run and vice versa were calculated for HTS and LTS.

RESULTS

Overall, 130 panels were distributed to the 61 laboratories that participated in the study. Four laboratories tested the panels using a second qNAT assay for a total of 65 data sets. All participants completed the study and returned the reporting form. Testing of the two panels was finalized within 20-45 days from receipt of the panels. No deviations from the protocol were observed with the exception of one of the four INH users that reported the results expressed in copies/mL instead of IU/mL. Consequently, the results provided by this laboratory were not included in the statistical analysis.

The GM (log_{10}) values, grouped by qNAT assay, are plotted in Fig. 1 for HTS and LTS. As no statistically significant differences were observed between the CAP/CTM and the HP/CTM assays, these results were analysed together under CTM. All the GM (log_{10}) values for bDNA and RealT fell within the respective range of acceptability (GM (log_{10}) ± 0.15) both for HTS and LTS. With respect to the other qNAT assays, a total of seven GM (log_{10}) values falling outside the range of acceptability were observed both for HTS (3 CTM, 2 CAM and 2 INH) and LTS (4 CTM, 1 CAM and 2 INH).
HTS titre

386 quantitative HCV results out of 390 samples were reported by the participants as three results from CTM users were invalid and one from a CAM user was aberrant. The latter, due to a mistake in the sample pre-dilution phase, was not included in the statistical analysis. The GM (log_{10})_{\text{assay}}\pm SD along with the percentages of individual results (n=1) falling within the range of acceptability of each qNAT assay are reported in Table 2 for each run.

The GM (log_{10})_{\text{overall}}\pm SD values and the respective CV, calculated for each qNAT, are reported in Table 3 along with the C_{\text{mean}} T value (5.62 log_{10} IU/mL \pm 0.16; 95% confidence interval 5.58-5.67 log_{10} IU/mL).

LTS titre

388 quantitative HCV results out of 390 samples were reported by the participants as two invalid results were reported by CTM users. The GM (log_{10})_{\text{assay}}\pm SD along with the percentages of individual results (n=1) falling within the range of acceptability of each qNAT assay are reported in Table 2 for each run.

The GM (log_{10})_{\text{overall}}\pm SD values and the respective CV, calculated for each qNAT, are reported in Table 3 along with the C_{\text{mean}} T value (3.61 log_{10} IU/mL \pm 0.19; 95% confidence interval: 3.56-3.66 log_{10} IU/mL).

2 -log_{10} difference in HCV RNA between HTS and LTS

All the possible combinations between HTS and LTS in the two panels (HTS_{\text{Panel1}}-LTS_{\text{Panel2}} and HTS_{\text{Panel2}}-LTS_{\text{Panel1}}) were taken into account for each participant obtaining a total of 1097 differences. The INH users were excluded from this evaluation due to the high variability of the GM(log_{10})_{\text{assay}} (see above). For each assay method the mean of these combinations was very close to the expected value of 2.0 log_{10}, with an SD varying from 0.09 to 0.17 (Table 4).

50-IU/mL samples
With respect to the 130 low-titre samples, only the 30 CTM and RealT users were expected to correctly identify them based on the detection limit of these qNAT assay, for a total of 60 positive results. Instead, 58 positive results were provided by these participants as one CTM user missed the 50-IU/mL sample in both panels (data not shown). Though below the detection limit of the assays, one CAM and five bDNA users reported a 50-IU/mL sample as positive in one of the two panels. INH users were not capable of detecting the 50-IU/mL samples with the exception of one laboratory (using a combination of two commercial kits) that correctly identified one of them.

Negative samples
Finally, 129 results out of 130 negative samples (one invalid result from a CTM user) were reported by the participants. Five bDNA users reported a positive result for these samples. Four of these users were the same that reported a positive result for the 50-IU/mL samples.

DISCUSSION
This study reports the results of an MS for quantification of HCV RNA where participants used in-house NAT assays and commercial qNAT assays, including two assays based on real-time RT-PCR (Taqman system) recently introduced on the market. HCV RNA quantification is critical in genotype 1 patients before, during and after antiviral therapy. To simulate the $2\log_{10}$ viral load drop in patients after 12 weeks of antiviral therapy, which triggers the continuation of the treatment, samples with a $2\log_{10}$ difference in HCV RNA (HTS and LTS) were included in two panels that were sent to each participant to be tested in two separate runs.

Of the 61 laboratories participating in the study, 57 used a commercial qNAT assay (CAM, CTM, RealT, and bDNA) while four used an in-house assay (INH). One of the INH users was excluded due to the reporting of the results in copies/mL rather than in IU/mL. With respect to HTS, the precision was quite variable among the qNAT assays (Table 2). This appears to be especially true for INH, CTM and CAM which, consequently, gave lower percentages of results within the range of
acceptability with respect to RealT and bDNA (22-78% vs 99-100%) (Table 2). However, all methods showed a satisfactory reproducibility (CV<3.0%) with the exception of INH assays (CV 5.9%) (Table 3). In particular, the worst CV was exhibited by the INH assay completely developed in-house. In reference to the mean titres obtained with the four commercial qNAT assays, differences up to 0.33 log\textsubscript{10} IU/mL were observed (CTM vs bDNA). Based on this, a blood viral load of approximately 850,000 IU/mL obtained with CTM would correspond to 400,000 IU/mL with bDNA. In HCV genotype 1 RVR patients, this discrepancy could influence the evaluation of the therapy’s outcome (SVR). Therefore, a clinician should always take into account the qNAT assay used by a laboratory when evaluating the viral load at baseline. Also in the case of LTS, lower percentages of results within the range of acceptability were observed for INH, CTM and CAM with respect to RealT and bDNA (33-79% vs 93-97%) (Table 2). The precision was more variable with respect to HTS as none of the assays showed 100% of results within the range of acceptability. Furthermore, all methods showed a somewhat less satisfactory reproducibility (CV<5.0% vs CV<3.0% for HTS) with a peak of CV 10.9% for INH (Table 3). Once again, the worst CV was exhibited by the INH assay completely developed in-house. With respect to the mean titres, differences among the four commercial qNAT assays were observed also in the LTS case (up to 0.38 log\textsubscript{10} IU/mL between CAM and bDNA).

Considering the precision of the results obtained, the range of acceptable values established in this MS, namely GM (log\textsubscript{10})\textsubscript{assay}±0.15, proved to be more appropriate than the one used in previous studies, namely GM (log\textsubscript{10})\textsubscript{assay}±0.50.

HTS and LTS were included in both panels in order to simulate testing of HCV genotype 1 patient’s samples taken at the beginning and after 12 weeks of antiviral therapy. This design allowed us to evaluate the ability of the diagnostic laboratories to detect a 2-log\textsubscript{10} difference in HCV RNA. It is worth noting that in this simulated scenario a high percentage (~40%) of all the 1097 differences were <2.0 log\textsubscript{10}. In the clinical practise this would lead to an incorrect discontinuation of the therapy even in the presence of a real 2-log\textsubscript{10} drop in the viral load. Therefore, the qNAT assay used for
quantification of HCV RNA in clinical samples should be taken into consideration also when
evaluating the efficacy of the therapy. For example, if in the above-mentioned simulated scenario we
assume an uncertainty of 2SD, the percentage of unacceptable values (<2.0 log_{10}) decreases from
~40% to ~2% (Table 4). Based on these observations, patients should be monitored for the entire
cycle of the antiviral therapy using the same qNAT assay.

In patients achieving an RVR and an SVR, the blood is expected to contain undetectable amounts of
the viral RNA using a sensitive test with a lower limit of detection of 50 IU/mL (10). Therefore, to
assess the ability of diagnostic laboratories to detect low levels of HCV RNA, 50-IU/mL samples
were included in the panels. This concentration is close to the lowest detection limit of most
commercial assays, namely threefold the CTM and RealT values and just below the bDNA and CAM
ones. As expected, 97.7% of the laboratories using CTM and RealT quantified the 50-IU/mL samples
correctly. With respect to bDNA and CAM users, six of them found one or more of these samples
positive. As the detection limit of bDNA and CAM is much higher than 50 IU/mL (615 and 600
IU/mL, respectively), and the titres reported by these laboratories are much higher than 50 IU/mL
(>700 IU/mL), this strongly suggests that these were false positive results due to either cross-
contamination or carry-over events.

At the end of this MS we were able to quantify the HTS and LTS included in the panels. Based on
preliminary testing by the ISS and by two kits manufacturers (Roche Diagnostics, Germany and
Abbott Molecular Inc. USA), provisional titres of 5.68 log_{10} IU/mL and of 3.65 log_{10} IU/mL had been
assigned to these samples. From the overall mean of the individual data received from the
participants, consensus titres of 5.62 log_{10} IU/mL and of 3.61 log_{10} IU/mL were calculated for HTS
and LTS, respectively, satisfactorily confirming the provisional titres.

In conclusion, the results of the present study are satisfactory with respect to the commercial qNAT
assays currently used for quantification of HCV RNA. In fact, the overall reproducibility, slightly
better for HTS than for LTS, proved to be acceptable (CV<5%). In particular, regarding the two
qNAT assays based on the Taqman system, CTM and RealT, the latter shows a greater precision (CV=1.06-2.34%) than the former (CV=2.41-4.04%) though there could be a bias due to the smaller number of RealT users. It is worth noting that in spite of the establishment of the WHO HCV International Standard, with a viral content expressed in IU/mL, the inter-method variability among commercial qNAT assays for HCV RNA is still present. Therefore, it would be advisable for the manufacturers of these kits to join efforts toward harmonisation in the quantification of HCV RNA. Such collaboration is particularly desirable in light of the clinical relevance of the qNAT assays. This study also shows that caution should be exercised both when evaluating the baseline viral load and in the interpretation of the 2log_{10} reduction of the viral load after 12 weeks of therapy. Finally, this MS confirms the higher sensitivity of the commercial qNAT assays based on the Taqman system (CTM and RealT), consistently capable of detecting low levels of HCV RNA, which makes them the elective assays to establish RVR/SVR response.

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REFERENCES


TABLE 1. Commercial qNAT assays used in this MS

<table>
<thead>
<tr>
<th>qNAT assay</th>
<th>No. of data sets</th>
<th>Detection limit (IU/mL)</th>
<th>Dynamic range (IU/mL)</th>
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<tr>
<td>COBAS Ampliprep/COBAS Taqman HCV test (CAP/CTM)</td>
<td>18</td>
<td>15</td>
<td>43-69,000,000</td>
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<tr>
<td>High Pure/COBAS Taqman HCV test (HP/CTM)</td>
<td>3</td>
<td>25</td>
<td>25-391,000,000</td>
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<tr>
<td>COBAS Amplicor HCV Monitor v2.0 test (CAM)</td>
<td>5</td>
<td>600</td>
<td>600-700,000</td>
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<tr>
<td>ABBOTT Real Time HCV test (RealT)</td>
<td>9</td>
<td>12</td>
<td>12-100,000,000</td>
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<tr>
<td>Versant HCV RNA 3.0 Assay (bDNA)</td>
<td>27</td>
<td>615</td>
<td>615-7,700,000</td>
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**TABLE 2. Intra-assay precision for HTS and LTS, grouped by qNAT, for Runs 1 and 2**

<table>
<thead>
<tr>
<th>Sample</th>
<th>qNAT (provisional titre in log_{10} IU/mL)</th>
<th>Run 1</th>
<th>Run 2</th>
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<tbody>
<tr>
<td></td>
<td>No. of data sets</td>
<td>GM(log_{10} assay)±SD</td>
<td>Percentage of results within GM(log_{10} assay)±0.15</td>
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<tr>
<td>HTS (5.68)</td>
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<tr>
<td>CTM</td>
<td>63</td>
<td>5.77±0.13</td>
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<tr>
<td>CAM</td>
<td>15</td>
<td>5.76±0.18</td>
<td>66</td>
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<tr>
<td>RealT</td>
<td>27</td>
<td>5.63±0.06</td>
<td>100</td>
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<tr>
<td>bDNA</td>
<td>81</td>
<td>5.46±0.05</td>
<td>99</td>
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<tr>
<td>INH</td>
<td>9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5.31±0.36</td>
<td>44</td>
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<tr>
<td>LTS (3.65)</td>
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<td></td>
<td></td>
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<tr>
<td>CTM</td>
<td>62&lt;sup&gt;e&lt;/sup&gt;</td>
<td>3.76±0.16</td>
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<tr>
<td>CAM</td>
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<td>9&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.29±0.18</td>
<td>44</td>
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<sup>a</sup> All GM(log_{10} assay) values are expressed in log_{10} IU/mL.

<sup>b</sup> Three invalid results, see Results.

<sup>c</sup> One aberrant result, see Results.

<sup>d</sup> Three data sets from one laboratory excluded, see Results.

<sup>e</sup> One invalid result, see Results.
<table>
<thead>
<tr>
<th>qNAT assay</th>
<th>No. of data sets</th>
<th>HTS GM (log_{10})overall±SD</th>
<th>CV%</th>
<th>LTS GM (log_{10})overall±SD</th>
<th>CV%</th>
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<td>CTM</td>
<td>21</td>
<td>5.80±0.14</td>
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<td>3.78±0.15</td>
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<td>CAM</td>
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<td>5.74±0.17</td>
<td>2.96</td>
<td>3.81±0.18</td>
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<td>RealT</td>
<td>9</td>
<td>5.65±0.06</td>
<td>1.06</td>
<td>3.64±0.08</td>
<td>2.34</td>
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<tr>
<td>bDNA</td>
<td>27</td>
<td>5.47±0.05</td>
<td>0.91</td>
<td>3.43±0.05</td>
<td>1.46</td>
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<tr>
<td>INH</td>
<td>3</td>
<td>5.45±0.32</td>
<td>5.90</td>
<td>3.26±0.36</td>
<td>10.92</td>
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<tr>
<td>CmeanT value</td>
<td>65</td>
<td>5.62±0.16</td>
<td>2.99</td>
<td>3.61±0.19</td>
<td>5.20</td>
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</table>

a All GM (log_{10})overall values are expressed in log_{10} IU/mL.
TABLE 4. 2 log$_{10}$-difference in HCV RNA between HTS and LTS

<table>
<thead>
<tr>
<th>qNAT assay</th>
<th>No. of HTS and LTS combinations</th>
<th>Mean of the 2-log difference</th>
<th>SD</th>
<th>No. of unacceptable values (%)</th>
<th>No. of unacceptable values (%) after 2SD correction</th>
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</thead>
<tbody>
<tr>
<td>CTM</td>
<td>362</td>
<td>2.03</td>
<td>0.16</td>
<td>145</td>
<td>13</td>
</tr>
<tr>
<td>CAM</td>
<td>87</td>
<td>1.94</td>
<td>0.17</td>
<td>58</td>
<td>9</td>
</tr>
<tr>
<td>RealT</td>
<td>162</td>
<td>2.01</td>
<td>0.09</td>
<td>85</td>
<td>0</td>
</tr>
<tr>
<td>bDNA</td>
<td>486</td>
<td>2.03</td>
<td>0.09</td>
<td>148</td>
<td>12</td>
</tr>
<tr>
<td>Total no. of combinations</td>
<td>1097</td>
<td>NA$^a$</td>
<td>NA$^a$</td>
<td>436 (40)</td>
<td>25 (2)</td>
</tr>
</tbody>
</table>

$^a$ Not applicable.
Legend to Fig. 1

For each laboratory, the GM ($\log_{10}$panel1+2) (± SD) values, expressed in $\log_{10}$ IU/mL and grouped by qNAT assays, are reported with respect to HTS and LTS. Each point represents the mean with the standard deviation. For each group, the respective GM ($\log_{10}$assay) value is also reported (solid line) with the established ±0.15 range of acceptability (dashed lines).
ERRATUM

Quantification of Hepatitis C Virus (HCV) RNA in a Multicenter Study: Implications for Management of HCV Genotype 1-Infected Patients

Giulio Pisani, Karen Cristiano, Francesco Marino, Francesca Luciani, Guillermo M. Bisso, Claudio Mele, Daniela Adriani, Giuliano Gentili, and Maria Wirz

Volume 47, no. 9, p. 2931–2936, 2009. Any mention of an assay based on the TaqMan system should be considered throughout the paper as solely referring to the Cobas TaqMan assay by Roche, as the RealTime HCV assay by Abbott does not depend on TaqMan exonuclease activity to generate a signal.