Chronic hepatitis C: new diagnostic tools and therapeutic strategies

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INTERNATIONAL COLLABORATIVE STUDY ON THE SECOND EUROHEP HCV-RNA REFERENCE PANEL

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ABSTRACT

Eighty-six laboratories participated in a collaborative study and tested the second EUROHEP HCV-RNA reference panel. The coded panel comprised 4 HCV-RNA positive plasma samples (one weak positive), 6 HCV-RNA negative plasma samples and two dilution series of HCV-RNA genotype 1 and 3 plasma standards. The 86 laboratories submitted 136 coded data forms for evaluation. Of these data sets 99 were tested using a PCR assay developed in-house, 28 using a commercially available HCV-PCR test (AMPLICOR, Roche Diagnostic Systems) and 9 using other amplification methods. Twenty-two data forms (16%) had faultless results, 39 (29%) missed the weak positive sample only and 75 data sets (55%) had false positive and/or false negative results. Participants using the commercial HCV-PCR test tended to reach a sufficient quality score more often than investigators using assays developed in-house (64% versus 45%, p=0.11). The UNG system in the commercial HCV-PCR test did not prevent five laboratories generating false-positive results in the 6 HCV-RNA negative samples. Among the laboratories with satisfactory results, up to 10,000-fold differences in sensitivity were observed in the dilution series. The 50% and 90% laboratories detection endpoints in the dilution series of the HCV genotype 1 plasma standard were approximately 600 genome equivalents per ml (geq/ml) and 7750 geq/ml according to a standard applied in a signal amplification assay (bDNA, Chiron). Our results suggest that the detection efficiency for genotype 3 by commercial HCV-RNA assays is lower than by the in-house assays. Internationally characterized HCV-RNA plasma standards should be made available for validation and standardization of HCV-RNA assays for HCV diagnosis and virological safety testing of blood products.

Keywords: HCV-RNA detection; PCR; amplification assays; quality control
1. INTRODUCTION

The technique used most commonly to detect HCV RNA is reverse transcription of the viral RNA and subsequent amplification of the copy DNA (cDNA) by the polymerase chain reaction (PCR). However, other amplification techniques (Kievits et al., 1991) and a sensitive method involving hybridization with branched DNA probes (bDNA) are also used (Lau et al., 1993). In a person with antibodies against HCV, a negative PCR result may reflect a resolved infection while a positive PCR indicates ongoing infection (Alberti et al., 1992; Bresters et al., 1993; Marin et al., 1994). PCR is also used for monitoring the effect of antiviral treatment of HCV infection (Bresters et al., 1994; Kleter et al., 1993; Soni et al., 1995). Apart from sensitive, qualitative detection of HCV-RNA, quantitation and genotype determination of HCV-RNA has also become important (Davis, 1994). To date, PCR and other amplification tests are used routinely in many laboratories and have major consequences for the management of patients and blood donors. Recently, PCR has also been included in the virological safety testing of plasma products (Nübling et al., 1995; Yu et al., 1995). Since the monitoring of laboratory performance is a prerequisite for ensuring reliable amplification test facilities, the European Expert Group on Viral Hepatitis (EUROHEP) is continuing its program for the quality control and standardization of HCV-RNA detection. From quality control studies on PCR in leukaemia research and detection of the human immunodeficiency virus, it has become apparent that PCR results are often false-positive or false-negative (Hughes et al., 1990; Jackson et al., 1993). In the first EUROHEP quality control study for HCV-RNA detection in 1992, only 16% of the laboratories produced faultless results. Even these laboratories reported, in their analysis of the dilution series, 100-fold differences in sensitivity (Zaarier et al., 1993). In this larger EUROHEP HCV-RNA quality control study, organized in the same way, we examined whether the performance of HCV-RNA detection had improved. Since an international HCV-RNA reference preparation is not available we expressed the sensitivity of the different amplification test protocols with reference to standards applied in a commercially available signal amplification assay (Collins et al., 1995). Finally the EUROHEP reference panel was used to examine the variation between the currently available quantitative HCV-RNA detection methods.

2. METHODS

2.1. HCV-RNA reference panel

The panel consisted of 26 coded plasma samples with and without HCV-RNA: 10 undiluted samples and 2 dilution series of 8 samples each. The 10 undiluted samples originated from 6 HCV-RNA negative donors and 4 HCV-RNA positive patients. Two reference laboratories tested the samples before release of the panel. Table 1 summarizes the characteristics of the undiluted samples in the reference panel. The participants were not able to deduce the HCV-RNA status from the anti-HCV status, since 5 out of the 6 HCV-RNA negative samples were reactive for anti-HCV. The HCV-RNA levels in the EUROHEP genotype 1 and 3 plasma standards used for the dilution series are summarized in Table 2. The following dilutions were included in the reference panel: genotype 1 plasma: $10^2$, $10^3$, $4 \times 10^3$, $16 \times 10^3$, $64 \times 10^3$, $256 \times 10^3$, $10.2 \times 10^3$, $41 \times 10^3$; genotype 3 plasma: $10^1$, $10^2$, $10^3$, $4 \times 10^3$, $16 \times 10^3$, $64 \times 10^3$, $256 \times 10^3$, $10.2 \times 10^3$. Genotype detection was undertaken at the Central Laboratory of the Blood Transfusion Service (CLB) using restriction fragment length polymorphism (RFLP) analysis (McOmith
Table 1
Characteristics of the undiluted samples in the second EUROHEP HCV-RNA reference panel.

<table>
<thead>
<tr>
<th>Sample</th>
<th>RIBA-3 result (^a)</th>
<th>PCR result (^b)</th>
<th>bDNA (^c) (geq/mlx10^5)</th>
<th>Genotype (RFLP) (^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pos</td>
<td>pos</td>
<td>75.9</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>pos</td>
<td>pos</td>
<td>&lt;3.5</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>pos</td>
<td>w.pos (^e)</td>
<td>&lt;3.5</td>
<td>nd (^e)</td>
</tr>
<tr>
<td>4</td>
<td>pos</td>
<td>pos</td>
<td>12.3</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>pos</td>
<td>neg</td>
<td>&lt;3.5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>ind</td>
<td>neg</td>
<td>&lt;3.5</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>pos</td>
<td>neg</td>
<td>&lt;3.5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>ind</td>
<td>neg</td>
<td>&lt;3.5</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>ind</td>
<td>neg</td>
<td>&lt;3.5</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>neg</td>
<td>neg</td>
<td>&lt;3.5</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) 3rd generation recombinant immunoblot assay (Chiron Corporation); pos = positive; ind = indeterminate; neg = negative.

\(^b\) In-house PCR results according to reference laboratory 1 and 2.

\(^c\) HCV-bDNA assay (Quantiplex HCV-RNA Assay, Chiron Corporation).

\(^d\) Restriction Fragment Length Polymorphism analysis (McOmish et al., 1994).

\(^e\) nd = not detectable

\(^f\) w. pos = weak positive

et al., 1994). Quantitative detection of HCV-RNA was carried out at CLB with 3 assays: the bDNA assay (Quantiplex HCV-RNA Assay, Chiron Corporation, Emmeryville, CA, USA) (Lau et al., 1993), the AMPLICOR HCV MONITOR Kit (Roche Diagnostic Systems, Branchburg, NJ, USA) and an experimental quantitative self-sustaining isothermal RNA amplification method (QTNASBA) (Kievits et al., 1991).

2.2. Participants and quality score
The panel together with result and method forms were sent to 97 investigators of whom 86 submitted results (1-4 data sets per laboratory). The participants came from Europe (n=65), North-America (n=9), South-America (n=2), Japan (n=4), Asia (n=2), Africa (n=2) and

Table 2
Quantitative HCV-RNA test results of the Eurohep genotype 1 and 3 plasma standard used for the dilution series.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>bDNA (geq/mlx10^5)</th>
<th>Roche MONITOR assay (cps/mlx10^5)</th>
<th>Experimental QT NASBA (cps/mlx10^5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype 1</td>
<td>36.4 (n=6)(^a)</td>
<td>4.9 (n=4)</td>
<td>165 (n=3)</td>
</tr>
<tr>
<td>Genotype 3</td>
<td>≤3.5 (n=4)</td>
<td>0.065 (n=3)</td>
<td>24 (n=3)</td>
</tr>
</tbody>
</table>

\(^a\) n = number of measurements.
Australia (n=2). In total 144 data sets were received, of which 136 were suitable for evaluation. To ensure confidentiality, all laboratories sent their results to Prof. S.W. Schalm (University Hospital Rotterdam), who assigned a code to each participant. The anonymous results were analyzed at the coordinating laboratory (CLB). Results of the laboratories were sorted by a four-point ‘quality score’ system, as was used in preceding EUROHEP studies (Quint et al., 1995; Zaaijer et al., 1993). The score was as follows: one point for 9 correct results in undiluted samples, one point for, in addition to the above, a correct result in the weak positive sample and one point for each correctly tested dilution series. Dilution series were considered to be tested correctly if one or more of the least diluted samples were found to be positive and all higher dilutions were found to be negative.

2.3. Statistics
Differences in performance were calculated using a Chi-square test with Yates correction or the Fisher’s exact test. A p-value ≤ 0.05 was regarded to be significant. The 50% and 90% detection end points of the different PCR methods were calculated by probit analysis (Finney, 1971). Briefly, the method is as follows: in the dilution series the proportion of laboratories with a positive result is plotted against the log of the dilution. From this dose-response curve the 50% and 90% detection rates are calculated. The potency between the genotype 1 and 3 curves as measured in the different PCR protocols, can be determined if the curves are parallel. Curves were considered to be parallel if the results of the Pearson Goodness-of-fit test and Pearson Parallelism test were not significant (p>0.05).

3. RESULTS

3.1. Amplification test protocols
In total 136 data forms suitable for evaluation were submitted by 86 laboratories. The following PCR protocols were used: in 99 data sets cDNA-PCR developed in-house, in 28 the AMPLICOR HCV test (Roche Diagnostic Systems, Branchburg, NJ, USA) and in 3 the HCV bDNA assay (Chiron Corporation, Emmeryville, CA, USA). In 6 cases other protocols were used, such as experimental NASBA protocols or combinations of different protocols.

The methods used in the 99 in-house PCR protocols were as follows: RNA extraction: 56 data sets used denaturation in a chaotropic agent followed by phenol/chloroform extraction, 11 used proteinase K digestion followed by phenol/chloroform extraction, 6 used guanidine isothiocyanate denaturation and absorption-purification on silica particles and 18 used commercially available extraction methods. In 8 sets other extraction protocols or combinations of the above protocols were applied. Amplification: 80 data sets used nested PCR and 19 single round PCR. All PCR protocols except 2 used primers in the 5' TR region. Detection: in 66 data sets (67%) gel electrophoresis followed by ethidium bromide staining and UV-visualization was applied to analyze the PCR products, in 19 (29%) data sets gel electrophoresis followed by hybridization with a labelled probe was applied, and in 14 cases other methods were used such as liquid oligomer hybridization, electrochemiluminescence, antibody detection and combinations of different methods. Quality assurance: In 93 data sets PCR was carried out in 3 different rooms and in 2 cases the uracil N-glycosylase (UNG) system (Udaykumar et al., 1993) was used in addition. In 98 data sets a negative control sample was tested in every test run and in 70 cases a weak positive control sample was used. A statistically significant correlation was not found between the quality of performance of
the in-house PCR assays with the different protocols used for extraction, amplification, detection and measures for quality control.

3.2. Performance on 2nd EUROHEP HCV-RNA reference panel

Analysis of the results in the 10 undiluted samples revealed the following results: faultless 30/136 (22%); only weak positive sample missed 64/136 (47%); false-positive results 28/136 (21%); false-negative results 17/136 (12.5%); false-positive and/or false-negative results 42/136 (31%). In 71/136 (52%) of the data sets a correct sequence in both dilution series was reported, in 34/136 (25%) one of the two dilution series was correct.

Taking the panel as a whole (Table 3), 22/136 result forms (16%) were fully correct and 39/136 (29%) only missed the weak positive sample. In 75/136 data forms (55%) false-positive and/or false-negative results were reported in the undiluted samples or the dilution series.

In theory, detection of HCV-RNA in one (4-fold) higher dilution step than the first negative dilution is possible because at low nucleic acid concentrations the chance of HCV-RNA being amplified is determined by the Poisson distribution. Indeed, some investigators reported an intermission in the positive results at the end of their dilution series. If we had considered such results as correct, 1 data set would have had 3 points instead of 1, 8 sets 3 points instead of 2 and 5 sets 4 points instead of 3. The performance on the whole panel after correction for such inconsistent results at the end of the dilution series would become: 27/136 (20%) faultless; 48/136 (35%) only weak positive sample missed and 61/136 (45%) erroneous results. The intermissions in the dilution series mentioned above were distributed equally among the different amplification test protocols.

Table 3
Performance of 86 laboratories on the second EUROHEP HCV-RNA reference panel.

<table>
<thead>
<tr>
<th>2 dilution series</th>
<th>10 undiluted samples</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 correct</td>
<td>weak positive incorrect</td>
</tr>
<tr>
<td>2 series correct</td>
<td>22 (16%)</td>
<td>39 (29%)</td>
</tr>
<tr>
<td>1 series correct</td>
<td>7 (5%)</td>
<td>16 (12%)</td>
</tr>
<tr>
<td>both incorrect</td>
<td>1 (1%)</td>
<td>9 (6%)</td>
</tr>
<tr>
<td>Total</td>
<td>30 (22%)</td>
<td>64 (47%)</td>
</tr>
</tbody>
</table>

*The 86 laboratories submitted 136 result sets suitable for evaluation.

3.3. Comparison of in-house PCR protocols and a commercially HCV-PCR test

In-house nested-PCRs tended to reach maximum quality scores more often than single round PCRs developed in-house, but the difference was not statistically significant (Table 4). If the performance of the commercial HCV-PCR test (AMPLICOR) is compared with the different in-house PCR protocols (Table 4), 64% of data sets obtained by the commercial assay and 45% of the data sets obtained by in-house PCR had a sufficient quality score (3 or 4 points). This difference is not statistically significant (p=0.11). Examining the result
sets of the laboratories with an insufficient quality score, it was noticed that false-positive results in the 6 undiluted HCV-RNA negative samples occurred not only in the in-house PCR tests (17/99) but also in the commercial assay (5/28). Twenty-one laboratories applied both the commercial assay and their in-house PCR assay. In these laboratories it was found that 5/13 (38%) performed sufficiently with their in-house PCR (3 or 4 points), but insufficiently with the commercial assay. Conversely, 4/8 (50%) performed inadequately with their in-house PCR, but sufficiently with the commercial assay.

### Table 4
Comparison of quality scores in different PCR protocols

<table>
<thead>
<tr>
<th>Quality score</th>
<th>Single-PCR (n=19)</th>
<th>Nested-PCR (n=80)</th>
<th>Total in-house PCR (n=99)</th>
<th>Commercial PCR (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0 (0%)</td>
<td>15 (19%)</td>
<td>15 (15%)</td>
<td>6 (21%)</td>
</tr>
<tr>
<td>3</td>
<td>7 (36%)</td>
<td>23 (29%)</td>
<td>30 (30%)</td>
<td>12 (43%)</td>
</tr>
<tr>
<td>2</td>
<td>6 (32%)</td>
<td>16 (20%)</td>
<td>22 (22%)</td>
<td>5 (18%)</td>
</tr>
<tr>
<td>&lt;2</td>
<td>6 (32%)</td>
<td>26 (32%)</td>
<td>32 (33%)</td>
<td>5 (18%)</td>
</tr>
</tbody>
</table>

* Difference not significant: P=0.07.

b Commercial: AMPLICOR HCV test, Roche Diagnostic Systems.

### 3.4. Quantitative results of participants

Thirteen laboratories reported quantitative results in 18 data sets. Results were reported in 7 different units. Therefore we were only able to compare all 13 laboratories in a rank order analysis of the dilution series: 10/13 laboratories had a correct rank order in their quantitative results. Eight laboratories reported their quantitative results in concentration units. The quantitative results of these laboratories differed up to 1000-fold.

### 3.5. Sensitivity in dilution series

Fig. 1 shows the detection rates in the genotype 1 and 3 dilution series of 56 data sets that were selected because the investigator used a well described PCR for amplification and performed faultlessly or missed the weak positive sample only. The results of nested-PCR developed in-house (n=33), single-PCR (n=7) and the commercial assay (AMPLICOR) (n=16) were analyzed separately. As shown in Fig. 1, nested amplification protocols tended to be more sensitive than single round PCR protocols. The individual end point titres achieved by participants varied between 1:10 to 1:100 and 1:64000 to 1:1024000 with genotypes 3 and 1, respectively. Table 5 shows the dilutions of the genotype 1 and 3 standards at the 90% and 50% detection endpoints with the different PCR protocols, as calculated by probit analysis. The 90% genotype 1 detection rate was found at a higher dilution by the commercial assay than by the in-house assays. By contrast, the 90% genotype 3 detection rate was found at a lower dilution by the commercial assay. Table 5 also shows the calculated HCV-RNA concentrations at the 90% and 50% genotype 1 detection endpoints as quantified using the bDNA assay (Chiron Corporation). Taking all PCR protocols together a 50% genotype 1 PCR detection endpoint was found at an HCV-RNA
level of approximately 600 geq/ml. The 50% detection endpoint would be 13x lower (45 cps/ml) if the HCV-RNA concentration was expressed as quantified by the AMPLICOR MONITOR assay (Roche Diagnostic Systems), whereas a 4.5x higher value (2,700 cps/ml) was found when using an experimental quantitative NASBA assay (Table 2).

Five result forms of sufficient quality could not be used in the sensitivity analysis: one participant carried out an experimental qualitative NASBA. Potentially this assay seems to be as sensitive as PCR since HCV-RNA was detectable down at 1:16,000 and 1:1,000 dilution levels of the genotype 1 and 3 plasma respectively. Two result sets were based on an experimental quantitative PCR assay. The results of two other data sets were interpreted from a combination of different PCR protocols.

Fig. 1
Detection rates in the genotype 1 and 3 dilution series of 56 result sets scoring faultless or missing the weak positive sample. n-PCR = in-house nested-PCR; s-PCR = in- house single-round PCR; AMPLICOR = AMPLICOR HCV test, Roche Diagnostic Systems.
The three participants using the bDNA assay did not detect HCV-RNA in any of the diluted samples, and 2 of the 4 undiluted HCV-RNA positive samples had HCV-RNA levels below the detection limit ($\leq 3.5 \times 10^5$ geq/ml). This indicates that the bDNA assay is approximately 1,000-fold less sensitive than PCR.

Table 5

<table>
<thead>
<tr>
<th>PCR protocol</th>
<th>Genotype 1 standard</th>
<th>Genotype 3 standard</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dilution 90% detection rate:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>geq/ml 90% detection rate:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dilution 50% detection rate:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>geq/ml 50% detection rate:</td>
<td></td>
</tr>
<tr>
<td>nested-PCR</td>
<td>1:346</td>
<td>1:282</td>
</tr>
<tr>
<td>(n=33)</td>
<td>10520</td>
<td>1:2978</td>
</tr>
<tr>
<td>single-PCR</td>
<td>1:729</td>
<td>1:566</td>
</tr>
<tr>
<td>(n=7)</td>
<td>4993</td>
<td>1:1614</td>
</tr>
<tr>
<td>commercial PCR$^b$</td>
<td>1:1137</td>
<td>1:117</td>
</tr>
<tr>
<td>(n=16)</td>
<td>3201</td>
<td>1:1197</td>
</tr>
<tr>
<td>All $^b$</td>
<td>1:624</td>
<td>1:270</td>
</tr>
<tr>
<td>(n=56)</td>
<td>7738</td>
<td>1:2299</td>
</tr>
</tbody>
</table>

$^a$ bDNA (Quantiplex HCV assay 1.0, Chiron Corporation); calculated from the HCV-RNA level in the undiluted sample (Table 2)

$^b$ Commercial: AMPLICOR HCV test, Roche Diagnostic Systems

4. DISCUSSION

The results of the second EUROHEP HCV-RNA quality control study show that 45% of the laboratories has serious problems with the correct detection of HCV-RNA. In the undiluted samples in the study panel, 21% of the laboratories had false-positive results with 6 HCV-RNA negative samples, whereas 12.5% had false-negative results with 3 HCV-RNA positive samples. Taking the panel as a whole, only 16% of the participants had faultless results both with the 10 undiluted samples and the 2 dilution series. Similar results were found in two earlier EUROHEP quality control studies on HCV-RNA (Zaaijer et al., 1993) and HBV-DNA detection (Quint et al., 1995). In a quality control study on Mycobacterium Tuberculosis comparable sensitivity and specificity problems were reported (Noordhoek et al., 1994). By contrast, in a French quality control study on HCV-RNA detection, more satisfactory results were reported, which improved in a consecutive study (French study group for the standardization of hepatitis C virus PCR, 1994).

As was the case with the first EUROHEP quality control study on HCV-RNA detection, a significant correlation was not found between the quality of performance and a particular PCR protocol. In the present study, 28 data sets were obtained from participants using a commercial HCV-PCR test (AMPLICOR). The results from the laboratories using the commercial test were slightly better than those of laboratories using PCR protocols developed in-house, although the difference was not statistically significant. Among the 10
laboratories with erroneous results obtained by the commercial assay, 5 had false-positive results with the 6 HCV-RNA negative samples. Obviously, the UNG system is not a guarantee for preventing false-positive results. As long as sample extraction is not separated physically in an automatic procedure and amplicons are not both inactivated and contained we recommend complete separation of the isolation, reagents preparation, amplification and detection steps. Moreover, training of technicians, and the use of quality control samples to monitor sensitivity and specificity of test runs are very important.

In the dilution series large differences (up to 10,000-fold) in PCR detection end points were observed between individual laboratories (Fig. 1). Although in a few laboratories very low HCV-RNA concentrations were detected with nested-PCR, there was a slight, and not significant, difference between nested-PCR protocols and commercial PCR (AMPLICOR) if the dilutions are compared at the 50% PCR detection endpoints. At the 90% detection endpoint the commercial assay tended to be more sensitive than nested-PCR protocols in the genotype 1 standard dilution series, but less sensitive in the genotype 3 standard dilution series. The differences in sensitivity did not reach statistical significance. The results suggest that HCV-RNA genotype 1 and 3 are detected with different efficiency in the PCR protocols studied. This finding may be important for the development of future assays and the design of international standards to be used for validation of HCV-RNA assays (Saldanha and Minor, 1996).

By probit analysis on the dilution series, it was possible to calculate from the shift between the parallel lines, the potency between the genotype 1 and 3 standard curves in three PCR protocols (nested-PCR, single-PCR, commercial). The potency of the genotype 1 standard compared to the genotype 3 standard was 2.8 if calculated from the nested-PCR results and 4.7 if calculated from commercial PCR test results (data not shown). The concentration difference between the EUROHEP genotype 1 and 3 standards as measured by three quantitative assays (bDNA, Roche MONITOR assays and experimental NASBA) was more than 10-, 44- and 6.9-fold respectively (Table 2). Also, after repeated testing by a newer version of the bDNA assay (Quantiplex HCV Assay, version 2.0) the concentration difference was approximately 10-fold (data not shown). In a recent study on the preparation and quantitation of in-vitro HCV-RNA standards, only a slight difference in detection efficiency of the bDNA assay between HCV-RNA of genotype 1 and 3 was claimed (1.0 - 1.6 fold difference) (Collins et al., 1995). However, the HCV-RNA concentration difference between the EUROHEP genotype 1 and 3 standard of approximately 10-fold in the two generations of the bDNA assay could not be confirmed by the 2.8 fold difference according to the collaborative nested-PCR results. Since we believe that limiting dilution nested-PCR is the most accurate and least genotype dependent quantitative method, the commercial assays may underestimate the genotype 3 standard by a factor 3 or more.

The detection efficiency of different HCV-RNA genotypes needs further study before guidelines can be developed for validation of HCV-RNA assays based on international standards.

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