Performance attributes of the LCx® HCV RNA quantitative assay

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Received 9 September 2003; accepted 2 October 2003

Abstract

The LCx® HCV RNA quantitative assay (Abbott Laboratories, North Chicago, IL) is designed to use competitive reverse transcriptase-polymerase chain reaction (RT-PCR) and microparticle enzyme immunoassay (MEIA), in combination with a modified Qiaxen sample preparation method, to measure the level of hepatitis C virus (HCV) in human plasma and serum. The assay provides quantitative results in international units (IU) of HCV RNA/ml, in copies of HCV RNA/ml, or their log (base 10) equivalents. A conversion study determined that 1 IU equals 4.3 copies. The LCx HCV assay detected HCV RNA transcripts representative of genotypes 1–6 with near equal efficiency. The assay did not cross-react with high concentrations of 21 potentially cross-reactive microorganisms or with 100 HCV-negative specimens. The lower limit of detection was demonstrated to be 23 IU/ml. The LCx assay had similar sensitivity to the Roche Amplicor HCV (version 2.0) qualitative assay when used to test panels containing 6, 12, 23, and 47 IU/ml. The assay linear range was shown to extend from 23 to 2.3 million IU/ml. The intra-assay standard deviation (S.D.) was ≤0.066 log IU/ml for the four HCV positive samples tested, while for the same samples the observed inter-assay S.D. was ≤0.075 log IU/ml. The overall mean assay quantitation value for seven HCV-positive WHO-standardized Acrometrix NAP linearity panel members was within 0.06 log IU/ml of the mean assigned value. The assay was demonstrated to correlate acceptably against the Roche Amplicor HCV monitor test (version 2.0). These data suggest that the assay is standardized appropriately against the WHO standard across its linear range and can be used for quantitation of HCV. In addition, with a sensitivity of 23 IU/ml, the assay can be used to determine if post-therapy viral clearance has occurred.

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Keywords: HCV; RT-PCR assay; Viral load

1. Introduction

Hepatitis C virus (HCV), a major etiological agent of hepatitis, is an enveloped virus with a single-stranded, positive sense RNA genome of approximately 9500 nucleotides (Choo et al., 1989; Kuo et al., 1989; Cuthbert, 1994; Clarke, 1997). Up to 85% of HCV-infected individuals develop chronic hepatitis, with approximately 20% of the chronically infected individuals developing cirrhosis (EASL, 1999). In patients with cirrhosis, the incidence of hepatocellular carcinoma is 1–4% per year (EASL, 1999). HCV infection can be cleared using interferon or peginterferon monotherapy, or using interferon/ribavirin or peginterferon/ribavirin combination therapy (Davis et al., 1998; McHutchinson et al., 1998; Lindsay et al., 2001; Manns et al., 2001; Fried et al., 2002). HCV anti-therapeutic decisions are made using a wide variety of information, including the clinical presentation of the patient, the results of liver biopsy, the genotype of the infecting HCV isolate, and the concentration of HCV present in a patient’s plasma or serum (EASL, 1999; NIH, 2002).

Current recommendations suggest that nucleic acid tests for quantitation or detection of HCV RNA in serum or plasma be used to determine HCV viral load at four different points—before the initiation of therapy, 3–6 months after the initiation of therapy, at the termination of therapy, and periodically after therapy-termination (EASL, 1999; NIH, 2002). Pre-therapy viral load information is used, in combination with the genotype of the infecting HCV isolate, to determine the duration of the anti-HCV therapy regimen. Twelve months of interferon/ribavirin combination therapy is recommended if the patient is infected with HCV genotype 1 and has a viral load greater than two million copies.
of HCV RNA/ml (800,000 IU/ml). A 6-month course of interferon/ribavirin combination therapy is recommended if the patient either has a genotype 2 or 3 infection or has a viral load lower than 800,000 IU/ml (EASL, 1999; Pawlotsky et al., 2000; NIH, 2002). Viral load information is used to monitor the response of patients to anti-HCV therapy. A sustained virological response (SVR) is the goal of anti-HCV therapy and is defined as virus concentrations less than 50 IU/ml 24 weeks after the end of therapy. However, therapy may be discontinued in patients who fail to achieve a two-log (base 10) drop in viral load after the first 12 weeks of therapy (EASL, 1999; NIH, 2002).

Initial versions of the Roche Amplicor Monitor and Bayer Quantiplex assays, the most widely commercially available quantitative HCV RNA nucleic acid tests, did not produce easily comparable HCV RNA quantitation values (Pawlotsky, 1997). Lack of result comparability was caused by unequal quantitation of HCV genotypes 1–6, by lack of result-unit standardization, and for the Roche Monitor assay, by underquantitation of specimens containing greater than approximately 500,000 IU/ml (Pawlotsky, 1997). The comparability of HCV RNA quantitation values produced using these two assays has been improved considerably through implementation of assay modifications and the development of the World Health Organization (WHO) First International Standard 96/790 (Detmer et al., 1996; Mellor et al., 1999; Saldanha et al., 1999; Martinot-Peignoux et al., 2000; Beld et al., 2002; Germer et al., 2002; Ross et al., 2002; Trimoulet et al., 2002).

Neither quantitative assay, however, is sensitive enough to determine if viral clearance has occurred and, therefore, both manufacturers have developed sensitive qualitative assays for this purpose (Lee et al., 2000; Ross et al., 2001).

The LCx® HCV RNA Quantitative assay uses competitive reverse transcription PCR (RT-PCR) followed by microparticle enzyme immunoassay (MEIA) detection to quantitate HCV in plasma and serum specimens. An internal standard (IS) transcript RNA is introduced into each specimen at the start of sample preparation and is used in the quantitative algorithm. The assay was designed to quantitate HCV RNA in IU/ml or copies/ml, and also have sufficient sensitivity to determine if viral clearance has occurred. In this paper, we describe the performance of the LCx HCV RNA quantitative assay in studies performed at Abbott Laboratories.

2. Materials and methods

2.1. Panels, samples, and microbiological isolates

The genotype 1 CLB Pelicheck HCV RNA standard was purchased from the Central Laboratory of the Blood (CLB) Transfusion Service (Amsterdam, The Netherlands). The HCV genotype 1 World Health Organization (WHO) First International Standard 96/790 was kindly provided by Dr. John Saldanha at the National Institute for Biological Standards and Control (South Mimms, UK) (Saldanha et al., 1999). The genotype 1 NAP nucleic acid panel was purchased from Acrometrix (Berkeley, CA); this panel is calibrated against the WHO First International Standard for HCV (Jorgensen and Neuwald, 2001). HCV-positive plasma and serum specimens were purchased by Abbott Laboratories, while Candida albicans (ATCC 2091), flavivirus Dengue type 4 (ATCC VR-1490), rhinovirus 16 (ATCC VR-283), Pneumocystis carinii (ATCC 50381), Staphylococcus aureus (ATCC 4012) and Staphylococcus epidermidis (ATCC 146) were obtained from the American Tissue Culture Collection. Nucleic acid from these species was purified and quantitated using standard methodologies. Purified nucleic acid representing the following microbiological species was purchased from Advanced Biotechnologies Inc. (Columbia, MD): human adenovirus type 5, human cytomegalovirus, Epstein-Barr virus, hepatitis B virus, human herpes virus type 6, herpes simplex virus type 1, human immunodeficiency virus type 1 (HIV-1), HIV type 2 (HIV-2), human T-cell lymphotropic virus type I (HTLV-I), HTLV-II, Mycobacterium avium, vaccinia virus, and varicella-zoster virus.

2.2. Primer and probe sequences and RNA transcripts

The PCR target sequence is in the 5′ untranslated region (UTR) of the HCV genome. The anti-sense reverse primer is 22 nucleotides long and is labeled at its 5′ terminus with the hapten carbazole. The sense forward primer is 22 nucleotides long and is unlabeled. The sense HCV-specific probe is 18 nucleotides long and is labeled with the hapten carbazole at its 5′ end with the hapten carbazole.

The HCV RNA transcript and the IS transcript are both 1426 bases long. The HCV RNA transcript and IS RNA transcript are identical except for the probe-binding sites. The transcripts were produced as previously described except that the HCV plasmid and the IS plasmid were linearized with Sca I prior to RNA transcription (Johnson et al., 2001). RNA transcripts were quantitated using hyperchromicity (Collins et al., 1995).

Plasmids containing sequences representing the HCV 5′ untranslated region (UTR) from genotypes 1a, 1b, 2a, 2b, 3a, 4, 5, and 6a were constructed by Dr. Johnson Lau (University of Florida, Gainesville). RNA transcripts were produced using the above methodology.

2.3. Sample preparation

Sample preparation is performed using a modified Qiagen RNA purification procedure. One ml of specimen
(ACD plasma, CPD plasma, EDTA plasma, or serum) or control (Negative Control, Low Positive Control 2.37 log IU/ml of HCV RNA transcript, or High Positive Control 5.37 log IU/ml of HCV RNA transcript) is added to 1 ml of guanidine hydrochloride-based lysis buffer that contains approximately 3.68 log copies of IS RNA transcript. After addition of 125 μl of protease to each sample (specimen or control), the reactions are incubated for 10 min at 70°C, and then cooled for 10 min at room temperature. After adding 1 ml of ethanol, each reaction is added to a Quagen spin column that has been placed onto a 24-position vacuum manifold. Each sample is passed through the spin column matrix via application of a vacuum (20–28 in. of Hg). HCV genomic RNA, HCV transcript RNA, and IS transcript RNA are captured on the spin column matrix. Four vacuum-mediated 500 μl washes (two with AW1 buffer followed by two with AW2 buffer) are performed to remove residual sample contaminants. The spin columns are removed from the vacuum manifold and centrifuged (4 min at 13,000 × g) to remove residual AW2 buffer. RNA is eluted from each spin column by addition of 120 μl of RNAse-free water (Buffer AVE), followed by centrifugation (4 min at 13,000 × g) into an RNAse-free collection tube.

2.4. Competitive RT-PCR

Fifty microliters of eluted sample are added to an amplification vial that contains 100 μl of pre- aliquoted amplification reagent (100 mM bicine pH 8.25, 16% glycerol, 230 mM K+, 300 μM of each deoxynucleoside triphosphate (dATP, dCTP, dGTP, dTTP), 30–70 nM HCV-specific adamantane-labeled probe, 100 nM internal standard-specific dansyl-labeled reverse primer, 100 nM HCV-specific carbazole-labeled forward primer, 100 mM HCV-specific adamantane-labeled probe, 0.02 mg/ml acetylated BSA, 0.125% Tween 20, 0.04% sodium azide and 10.5 units recombinant *Thermus thermophilus* DNA polymerase). Thus, each final reaction contains approximately 3.31 log copies of IS transcript RNA, plus HCV RNA that was present in the sample or controls. 50 μl of activation reagent (10 mM manganese chloride, 0.045% sodium azide, 0.01% xylenol orange dye) is added to each amplification vial and activated reactions are maintained in a chilled rack prior to being placed into a Thermal Cycler (Perkin Elmer 480). Competitive RT-PCR is performed using the following thermal cycling program:

1. Reverse transcription (1 cycle: 94°C for 1 min, 62°C for 30 min, 94°C for 2 min) in which the carbazole-labeled reverse primer initiates reverse transcription from both the IS transcript RNA, as well as HCV genomic RNA, if present.
2. Lower stringency PCR (5 cycles: 94°C for 15 s, 58°C for 20 s with automatic extension of 1 s per cycle).
3. Higher stringency PCR (40 cycles: 94°C for 15 s, 62°C for 25 s with automatic extension of 1 s per cycle).
4. Oligonucleotide hybridization (1 cycle: 97°C for 5 min, 15°C for 5 min) in which the HCV-specific probe and the IS-specific probe bind to their respective amplimers.
5. Storage prior to detection (15°C soak).

2.5. Detection

Following RT-PCR, the amplification vials are placed into an LCx Analyzer, a 24-position automated batch analyzer. In the LCx Analyzer, an aliquot of each sample is transferred to a reaction cell, where HCV and IS amplification products are captured by anti-carbazole-coated microparticles via the carbazole-labeled reverse primer. The microparticle complexes are transferred to a glass fiber matrix to which the microparticle complexes bind irreversibly. After washing, the bound microparticle complexes are incubated with a mix of anti-adamantane alkaline phosphatase conjugate and anti-dansyl beta-galactosidase conjugate. The anti-adamantane alkaline phosphatase conjugate binds only to the HCV-specific probe while the anti-dansyl beta-galactosidase binds only to the IS probe. The detection of IS amplification product is accomplished through cleavage of the substrate, 7-beta galactopyrano-nosyl coumarin-4-acetic acid-(2-hydroxyethylamide) (AUG) by beta-galactosidase to produce a fluorescent product, 7-hydroxy-4-acetic acid-2-hydroxyethylamide coumarin (AU), that is measured by the LCx Analyzer optical assembly. After measuring the signal generated by AU, the LCx Analyzer washes the glass fiber matrix and adds 4-methylumbelliferyl phosphate (MUP) for the detection of HCV amplification product. The MUP is cleaved by alkaline phosphatase to 4-methyl umbelliforone (MU). The LCx Analyzer optical assembly measures the resultant fluorescent signal. At the completion of detection, the LCx Analyzer delivers two reagents (a chelating metal complex and an oxidizing reagent) into the LCx reaction cells. Addition of these two reagents results in a millionfold reduction of the nucleic acids present (Hu et al., 1996).

2.6. Calibration and result calculation

Two types of runs are performed using the LCx Analyzer—calibration and mode 2. Both types of runs contain one replicate each of negative control, low positive control, and high positive control. Calibration runs also contain two replicates each of six different calibrators. Unlike controls, calibrators are not processed through sample preparation. Rather, 50 μl of each calibrator, containing 2000 copies (log 3.31 copies/ml) of IS transcript RNA and various concentrations of HCV transcript RNA (equivalent to 0, 1.67, 2.67, 3.67, 4.67, and 6.67 log IU/ml), are added to an amplification vial, followed by addition of 50 μl of activation reagent.
Fig. 1. (a) Example rates used to generate an LCx HCV RNA quantitative assay calibration curve, demonstrating the competitive relationship between internal standard rates (AUG signal) and HCV rates (MUP signal). (b) Example of a calibration curve generated by taking the log (HCV rate/IS rate) from the data in Fig. 1a.

reagent. The calibrators are matched to the CLB Pelicheck HCV RNA standard. After amplification and detection, the log rate ratio (LRR) of each calibrator replicate is calculated (log_{10} (HCV rate/IS rate)). Because the RT-PCR reaction is competitive, samples with low HCV concentrations produce high AUG signals and low MUP signals, while samples with high HCV concentrations produce low AUG signals and high MUP signals (Fig. 1a). The mean LRR of each calibrator level is stored within the LCx Analyzer software (Fig. 1b). Specimens can be run on Calibration runs (maximum of nine specimens) or on mode two runs (maximum of 21 specimens). To determine their concentration, the LRR of each specimen is calculated and compared against the LRR values of the stored calibration curve. Because the concentrations of the calibrators are known, the specimen LRR values can be converted into concentration values. Use of conversion factors permits HCV RNA concentration results to be reported in copies/ml or IU/ml, or their logarithmic equivalents. It is important to note that the LCx Analyzer only reports specimen results when the run controls, and the internal control for the sample in question, are valid.
3. Results

3.1. Standardization and lower limit of detection (LLD)

Seven replicates each of the CLB genotype 1 Pelicheck HCV-RNA standard and the WHO HCV genotype First International Standard were quantitated on the same assay run, and a conversion factor to compare the two standards was determined to be one WHO IU equals 4.3 CLB copies (95% confidence interval of 3.7–5.1). The lower limit of detection (LLD) of the assay was assessed using three reagent lots and three instrument pairs (LCx Thermal Cycler and LCx Analyzer). Each reagent lot and instrument pair was used to test 10 replicates of panel members containing 46.5 IU/ml and 23.3 IU/ml, 15 replicates of panel members containing 11.6 IU/ml and 5.8 IU/ml, and 35 HCV-negative specimens. The HCV positive panel members were created by dilution of the CLB standard. The upper limit of the negative population (ULN) was defined as the LRR value that encompasses 99% of the assay values resulting from the testing of the HCV-negative specimens. The assay lower limit of detection (LLD) was calculated for each reagent lot and instrument pair, and was defined as the concentration of HCV where 95% of the results were above the ULN. The reagent lot and instrument pair LLD values were 5.8, 10.7, and 6.0 IU/ml. To ensure that the LLD could be maintained with all reagent lots, the assay software reports quantitative results if the HCV concentration is greater than or equal to 23 IU/ml, or 1.37 log IU/ml. Because quantitation values below 23 IU/ml are not reported by the assay software, a specimen containing exactly 23 IU/ml would be quantitated 50% of the time.

The LCx HCV RNA Quantitative assay sensitivity was compared to that of the Roche Amplicor HCV (version 2.0) qualitative test that has a claimed sensitivity of 50 IU/ml. The CLB standard was diluted in negative plasma to 47, 23, 12, and 6 IU/ml (assuming that 1 IU equals 4.3 copies). The LCx assay was used to quantitate 12 replicates of each of the panels at Abbott Laboratories, and each panel member was quantitated 12 times using the Roche Amplicor test at a clinical laboratory. The data are summarized in Table 1. These data show that the LCx assay quantitated 100% (12/12) of the replicates at 47 IU/ml and 58.3% (7/12) of the replicates at 23 IU/ml. The Roche Amplicor HCV qualitative test detected 66.6% (8/12) of the replicates at 47 IU/ml and 58.3% (7/12) of the replicates at 23 IU/ml.

3.2. Linear range and ability to quantitate a therapy panel

The linear range of the assay was assessed using three different reagent lot-instrument pairs. Each reagent lot-instrument pair was used to test ten replicates each of panel members containing the following HCV concentrations: 1.07, 1.37, 1.67, 2.37, 3.37, 4.37, 5.37, 6.37, 6.67, and 6.97 log IU/ml. Illustrated in Fig. 2 are representative linearity

![Linear Range](image-url)

Fig. 2. Correlation between observed concentrations and actual concentrations of HCV-positive samples, as measured by the LCx HCV RNA quantitative assay.

**Table 1** Comparative sensitivity of the LCx’s HCV RNA quantitative assay and the Roche Amplicor HCV qualitative test when used to test the same panels

<table>
<thead>
<tr>
<th>Panel concentration (IU/ml)</th>
<th>Percentage of positive (≥LLD) Abbott LCx HCV results (%)</th>
<th>Percentage of positive Roche Amplicor HCV qualitative test results (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>47</td>
<td>100 (12/12)</td>
<td>66.7 (8/12)</td>
</tr>
<tr>
<td>23</td>
<td>58.3 (7/12)</td>
<td>75 (9/12)</td>
</tr>
<tr>
<td>12</td>
<td>0 (0/12)</td>
<td>8.3 (1/12)</td>
</tr>
<tr>
<td>6</td>
<td>0 (0/12)</td>
<td>0 (0/12)</td>
</tr>
</tbody>
</table>

The data are summarized in Table 1. These data show that the LCx assay quantitated 100% (12/12) of the replicates at 47 IU/ml and 58.3% (7/12) of the replicates at 23 IU/ml. The Roche Amplicor HCV qualitative test detected 66.6% (8/12) of the replicates at 47 IU/ml and 58.3% (7/12) of the replicates at 23 IU/ml.
Table 2

Intra-assay and inter-assay precision of the Abbott LCx HCV RNA quantitative assay

<table>
<thead>
<tr>
<th>Panel member</th>
<th>N</th>
<th>Mean concentration (log IU/ml)</th>
<th>Intra-assay S.D. (log IU/ml)</th>
<th>Inter-assay S.D. (log IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>1.92</td>
<td>0.066</td>
<td>0.075</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>2.62</td>
<td>0.031</td>
<td>0.036</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>3.33</td>
<td>0.055</td>
<td>0.070</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>5.71</td>
<td>0.051</td>
<td>0.057</td>
</tr>
</tbody>
</table>

data for one reagent lot and instrument pair. Fig. 2 illustrates that the quantitative assay response flattens above 6.37 log IU/ml and quantitative assay results are therefore reported from the LLD to 6.37 log IU/ml. If a sample contains greater than 6.37 log IU/ml, the sample can be diluted 1:50 and retested to provide a result within the linear range of the assay, thus extending the linear range to 8.07 log IU/ml.

3.3. Precision

Assay precision was assessed with two sets of reagent lot and instrument pairs. Each reagent lot and instrument pair was used to test five replicates each of a four-member precision panel on 5 different days. The data are presented in Table 2. For the combined reagent lot and instrument pair data, the intra-assay standard deviation (S.D.) for each panel member was ≤0.066 log IU/ml, while the inter-assay S.D.s (includes intra-assay S.D.) were ≤0.075 log IU/ml.

3.4. Confirmation of standardization and analytical performance

To verify the standardization, LLD, and linear range, the genotype 1 NAP nucleic acid panel from Acrometrix was tested using one reagent masterlot-instrument pair. Each of the seven HCV-positive panel members (1.70, 2.70, 3.70, 4.70, 5.30, 5.70, and 6.30 log IU/ml) was tested eight times. The LCx assay quantitated all eight replicates of each panel member, including the panel member at 1.7 log IU/ml. In Table 3, it can be seen that quantitation differences between the expected panel member log IU/ml values and the mean observed log IU/ml values ranged from −0.18 to 0.21 log IU/ml. The overall mean difference was −0.06 log IU/ml and the correlation coefficient (r) was 0.999. These data support that the assay is standardized appropriately against the WHO standard and can meet the claimed LLD and ULO.

3.5. Cross-reactivity and specificity

To assess potential assay cross-reactivity, 6.00 log copies/ml of each microorganism, or its equivalent in purified genomic nucleic acid, was processed through sample preparation prior to performing one replicate of assay testing. No cross-reactivity was observed when the following microorganisms were tested: human adenovirus type 5, *Candida albicans*, human cytomegalovirus, Epstein–Barr virus, *Escherichia coli*, the flavivirus, Dengue type 4, hepatitis A virus, hepatitis B virus, human herpes virus type 6, herpes simplex virus type 1, human immunodeficiency virus type 1 (HIV-1), HIV type 2 (HIV-2), Human T-Cell Lymphotropic Virus Type I (HTLV-I), HTLV-II, *Mycobacterium avium*, *Pneumocystis carinii*, rhinovirus 16, *Staphylococcus aureus*, *Staphylococcus epidermidis*, vaccinia virus, and variella-zoster virus. In addition, 100 anti-HCV antibody-negative specimens were negative when tested by the assay.

3.6. Genotype detection

To assess whether the assay detects HCV genotypes with equal efficiency, purified RNA transcripts representative of HCV genotypes 1a, 1b, 2a, 2b, 3, 4, 5, and 6 were diluted to 2.37 log IU/ml and tested in the assay. Each genotype transcript was added directly to six different amplification vials before amplification and detection. The data presented in Fig. 3 show that the reported assay mean log IU/ml values were 2.38, 2.39, 2.39, 2.43, 2.42, 2.42, 2.49, and 2.57 for genotypes 1a, 1b, 2a, 2b, 3, 4, 5, and 6, respectively.
Correlation vs. Roche Monitor

\[ y = 0.8439x + 0.5458 \]

\[ r = 0.900 \]

Fig. 4. Correlation of the Abbott LCx HCV RNA quantitative assay with Roche Amplicor HCV monitor test, version 2.0.

3.7. Correlation with available commercially quantitative assays

Assay correlation was determined by testing purchased HCV-positive specimens that had been quantitated using the Roche Monitor v. 2.0 test \((n = 42)\). These specimens had also been genotyped (five 1a, five 1b, one 2a/2c, four 2b, two 3a, one 3e, 17 4, one 4a, one 4b/4c, one 4c/4d, two 5a, and two 6a). Comparing the LCx HCV log IU/ml quantitation values and the Roche log IU/ml values (Fig. 4), the correlation coefficient, \(r\), was 0.9 and the line equation was \(y = 0.8439x + 0.5458\). All but one of the LCx and Roche quantitation log IU/ml values were within one log of each other. One genotype 2b sample was quantitated 1.5 log IU/ml lower by the LCx assay than by the Roche assay. This specimen was quantitated using the Bayer version 3.0 assay and it produced a quantitation value within 0.1 log IU/ml of the LCx assay value. The mean LCx HCV log IU/ml value was 0.32 log lower than the mean Roche quantitation value.

4. Discussion

The LCx HCV RNA Quantitative assay was designed to produce accurate and reproducible quantitation results for HCV genotypes 1–6. The assay primers and probes were designed to hybridize to sequences within the 5′ untranslated region of HCV, the most highly conserved region of the HCV genome. To help achieve reproducible genotype quantitation, the first five PCR thermal cycles are carried out at a lower stringency than the subsequent PCR cycles. The LCx HCV assay quantitated genotypes 1–6 HCV RNA transcripts equally, and produced similar quantitation values as the Roche Monitor v. 2.0 test when used to assay 42 patient specimens containing representatives of genotypes 1–6. This is significant because the Roche Amplicor HCV Monitor test (version 2.0) test has been demonstrated to quantitate HCV genotypes 1–6 with equality (Mellor et al., 1999; Lee et al., 2000). The LCx HCV RNA quantitative assay did not cross-react with high concentrations of microorganisms that might be present in a plasma or serum specimen, and produced a specificity of 100% when used to test HCV negative plasma samples. In combination, these data suggest that the LCx HCV assay
quantitates HCV genotypes 1–6 equally with excellent specificity.

The LCx HCV assay is linear from the LLD of 1.37 log IU/ml to the ULQ of 6.37 log IU/ml. The relatively wide linear range is achieved by using the ratio of the HCV signal divided by the internal standard signal. The assay linear range encompasses the 4.9 log IU/ml (800,000 IU/ml) threshold value for therapy (Pawlotsky et al., 2000). Some specimens will contain a concentration of HCV greater than the ULQ of the LCx HCV RNA Quantitative assay, but these specimens can be diluted 1:50 and retested.

The intra-assay S.D. of the LCx HCV RNA quantitative assay was ≤0.15 log IU/ml for all four HCV positive samples tested, which is in line with the intra-assay S.D. of ≤0.15 log copies/ml required to differentiate half-log changes in viral load (Yen-Lieberman et al., 1996). Unlike monitoring of HIV anti-viral therapy, where the ability to detect a half log shift in viral load is important, a two-log decrease in viral load is considered clinically significant in HCV anti-viral therapy monitoring (NIH, 2002).

The LCx HCV assay calibrators were standardized against the CLB genotype 1 Pelicheck HCV RNA copies per ml standard and an assay conversion factor between the CLB copies per ml and the WHO IU per ml was established to be 4.3 copies to 1 IU. The 95% confidence limits of this conversion factor were 3.7–5.1, encompass the conversion factor of 3.8 copies to 1 IU reported by the developers of the CLB and WHO standards (Saldana et al., 2000). Further confirmation of the accuracy of this LCx HCV conversion factor was obtained by running the WHO-standardized Acrornetics linearity panel (Jorgensen and Neuwald, 1999). The overall mean LCx HCV RNA quantitative assay result differed from the overall expected value by −0.06 log IU/ml.

The correlation data between the LCX HCV RNA quantitative assay and the Roche Amplicor HCV monitor test (version 2.0) are limited. However, the high observed correlation coefficient of 0.90 (LCX–Roche) suggest that both assays perform well to quantitate genotypes 1–6 with equality, and produce precise results over their claimed linear ranges. The mean Roche Amplicor HCV monitor test (version 2.0) log IU/ml value was 0.32 log (or approximately two-fold) higher than that produced by the LCX HCV RNA quantitative assay, which could be explained by use of, in this study, archived instead of newly generated Roche quantitation values, by lot-to-lot variability, or by differences in standardization strategy. One report suggested the Roche Amplicor HCV monitor test (version 2.0) over-quantitated the WHO standard by 0.47–0.49 log IU/ml (Psimi et al., 2002).

The Acrornetics panel testing data support that the LCx HCV RNA quantitative assay has a LLOQ of 1.37 log IU/ml (23 IU/ml), and thus could be used to determine if HCV viral clearance had occurred. HCV is considered cleared if virus levels fall below 50 IU/ml of HCV RNA/ml (NIH, 2002). Additionally, the sensitivity of the LCx HCV RNA quantitative assay was compared to that of the Roche HCV Amplicor qualitative assay and had similar sensitivity for samples containing 47 IU/ml. These data suggest that the LCx HCV RNA quantitative may be used both to quantitate HCV in plasma and serum, and to determine if anti-HCV therapy has resulted in clearance of HCV.

References


