Chronic hepatitis C: new diagnostic tools and therapeutic strategies
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STABILITY OF HEPATITIS C VIRUS RNA DURING SPECIMEN HANDLING AND STORAGE PRIOR TO NASBA AMPLIFICATION


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ABSTRACT

The influence of different anticoagulants and pre-amplification storage conditions on the stability of hepatitis C virus (HCV)-RNA as detected by the quantitative HCV NASBA assay (NASBA-QT), was studied. The HCV-RNA load remained stable for at least 15 months when serum or plasma samples (EDTA and heparin) were directly frozen at -70°C in lysis buffer. At 4°C, the HCV-RNA load in serum or plasma stored with lysis buffer did not decline for at least 14 days. At 30°C, however, the load declined significantly after 7 days. When clotted, whole blood was stored at 4°C, the HCV-RNA load was stable for 72 h. However, when EDTA-anticoagulated whole blood was stored at 4°C, the HCV-RNA load declined significantly after 48 hours. In paired plasma and serum samples at baseline the HCV-RNA levels were similar. Heparin did not influence the efficiency of the HCV NASBA-QT assay.

Clotted blood as well as EDTA or heparin anticoagulated blood can be used for quantifying HCV-RNA using the NASBA-QT assay. Blood samples should be stored at 4°C after collection and serum or plasma separated within 24 h. Preferably, after separation, samples should be frozen in lysis buffer at -70°C until NASBA-QT analysis.

Key words: HCV-RNA; Stability; Lysis buffer; Storage conditions; NASBA-QT.
1. INTRODUCTION

In anti-hepatitis C virus (HCV) positive patients, detectable HCV-RNA in blood has been shown to correlate with the presence of chronic persistent or chronic active hepatitis (Alberti et al., 1992; Nalpas et al., 1995; Prieto et al., 1995). Furthermore, the disappearance of HCV-RNA from blood and/or liver has been shown to be an important endpoint in the treatment of chronic hepatitis C (Rumi et al., 1996; Schiffman et al., 1996; Brouwer et al., 1997). It has become apparent that the HCV-RNA load at the start of treatment is an important predictor of the outcome of treatment with interferon (IFN) (Weiland et al., 1995; Yuki et al., 1995; Brouwer et al., 1997).

Several assays for the quantification of HCV-RNA have been described (Detmer et al., 1996; Hawkins et al., 1997; Melsert et al., 1997) and some of these have been commercialized. The principle of these assays is based on different techniques: either branched DNA (bDNA) signal amplification (Quantiplex HCV-RNA Assay, Chiron, Emmeryville, CA) (Detmer et al., 1996), template amplification with PCR, co-amplifying an internal calibrator (AMPLICOR HCV MONITOR Kit, Roche Diagnostic Systems, Branchburg, NJ) (Hawkins et al., 1997) or quantitative NASBA (HCV NASBA-QT) (van Gemen et al., 1995; Melsert et al., 1997). The accuracy of these assays is dependent on the sensitivity and reproducibility of the amplification procedure, the choice of primers (Cuypers et al., 1992; Hawkins et al., 1997), the efficiency of the isolation of nucleic acids (Cheung et al., 1994), and the handling of blood samples before nucleic acid isolation. The last issue has been studied for the HCV-PCR (Busch et al., 1992; Cuypers et al., 1992; Wang et al., 1992; Cheung et al., 1994) and the bDNA assay (Davis et al., 1994; Halfon et al., 1996) but not for the HCV NASBA-QT assay.

The current study addresses variations of HCV-RNA load, as measured with NASBA-QT, due to different handling and storage conditions of the clinical samples. The influence of different anticoagulants on the HCV-RNA load was determined by testing paired samples from the same HCV-infected individual. The HCV-RNA load in whole blood stored at 4°C was studied at different times after collection, to provide data relevant to specimens that are stored in the refrigerator immediately after collection. Furthermore, the stability of HCV-RNA in plasma and serum was studied during storage in lysis buffer (pH 6.2) at -70°C for 15 months and at 4 and 30°C for 2 weeks, to assess maximal duration of storage before analysis and/or transportation to a referral laboratory.

2. MATERIALS AND METHODS

2.1. Blood samples for storage experiments

All blood samples were taken from HCV-RNA positive individuals who had not received anti-viral treatment. The samples for storage experiments in NASBA lysis buffer (pH 6.2) were processed within 3 h after venipuncture. Serum was prepared from whole blood without anticoagulant and plasma was prepared from EDTA and heparin anticoagulated blood.

2.2. Storage of whole blood

From four HCV-RNA positive individuals, blood was drawn into a 10 ml standard potassium EDTA vacutainer tube and three 5 ml vacutainer tubes without anticoagulant. Six 1.5 ml aliquots of EDTA-anticoagulated whole blood were placed in empty dry-glass
tubes. From one tube, plasma was separated immediately by centrifugation and duplicate aliquots of 100 µl plasma were mixed with 900 µl lysis buffer (pH 6.2). These baseline samples were stored at -70°C (t=0). The five remaining tubes were stored at 4°C for 5, 8, 24, 48, and 72 h, respectively. At the indicated time-points, a tube was centrifuged and duplicate aliquots of 100µl plasma were mixed with 900 µl lysis buffer and stored at -70°C before NASBA-QT analysis (within 30 days).

One of the three tubes of clotted whole blood was centrifuged immediately and two aliquots of 100 µl serum were mixed with 900 µl lysis buffer and stored at -70°C (t=0).

Subsequently, the remaining clotted whole blood in the first tube was stored at 4°C for another 5 h. The other two tubes were stored at 4°C for 8, 24, and 48, 72 h, respectively. At the times indicated, the relevant tube was centrifuged and two aliquots of 100µl serum in 900 µl lysis buffer were prepared and stored at -70°C before NASBA-QT analysis (within 30 days). The storage experiment of whole blood is summarized in Fig. 1.

**Fig. 1.**
Flow diagram of the storage experiments in whole blood and lysis buffer.

### Storage in whole blood

1. **4 HCV-RNA positive patients**
   - Drawing of EDTA-anticoagulated whole blood and clotted whole blood.
   - Preparing baseline (t=0) samples.
   - Storage at 4°C.
   - Sampling of plasma and serum at t=5, 8, 24, 48 and 72 hours and mixing with lysis buffer, subsequent storage at -70°C.
   - HCV NASBA-QT analysis.

### Storage in lysis buffer

1. **6 HCV-RNA positive patients**
   - Drawing of EDTA-anticoagulated whole blood, heparin-anticoagulated whole blood and clotted whole blood.
   - Preparing of plasma and serum samples and mixing with lysis buffer.
   - Preparing baseline (t=0) samples with and without lysis buffer.
   - Short-term experiment: Storage at 4°C and 30°C for 2, 7 and 14 days and subsequent storage at -70°C.
   - Long-term experiment: Storage at -70°C for 6, 12 and 15 months.
   - HCV NASBA-QT analysis.

### 2.3. Storage of plasma/serum in lysis buffer (pH 6.2)

From six HCV infected individuals blood was drawn into one standard potassium EDTA vacutainer tube, one 10 ml heparin vacutainer tube and one 10 ml vacutainer tube without anticoagulant. Plasma or serum was separated within 3 h after venipuncture.

Duplicate aliquots of 100 µl EDTA plasma, heparin plasma and serum were stored at -70°C in 1.5 ml vials (baseline, storage without lysis buffer). Fourteen 100 µl aliquots of EDTA plasma, heparin plasma and serum, respectively, from each patient were mixed with 900 µl lysis buffer (pH 6.2) in 1.5 ml vials. Two vials of EDTA plasma, heparin plasma and serum...
in lysis buffer from each patient were stored immediately at -70°C (baseline, storage in lysis buffer). The remaining vials were used for storage experiments as described below. For the short-term experiment, 12 vials of EDTA plasma, heparin plasma and serum per patient were stored at +4°C (six vials) or +30°C (six vials), respectively. Two vials of EDTA plasma, heparin plasma and serum were removed from +4 and +30°C storage at 2, 7 and 14 days and stored at -70°C until analysis (within 30 days). For the long-term experiment, another six vials of EDTA plasma, heparin plasma and serum from four patients were stored at -70°C. Two vials of EDTA plasma, heparin plasma and serum were removed from storage at 6, 12 and 15 months and immediately analyzed in the NASBA-QT assay. The short- and long-term storage experiments in lysis buffer are summarized in Fig. 1.

2.4. Nucleic acid isolation and NASBA-QT
The quantitative HCV NASBA assay (HCV NASBA-QT) is an isothermal nucleic acid amplification method in which three calibrator RNAs (Qa, Qb and Qc) are used as internal standards (Melsert et al., 1997). In this procedure, nucleic acids were isolated according to the method described by Boom et al. (1991). Briefly, samples of 100 μl plasma or serum in 900 μl lysis buffer (pH 6.2; 5 mol/l guanidine thiocyanate, Triton X-100, Tris/HCl) were thawed and three calibrator RNAs were added together with 50 μl of silica suspension, to bind the released nucleic acids. After washing and drying, the nucleic acids were dissolved in 50 μl elution buffer. The isolated RNA was amplified as described (Melsert et al., 1997). In each run of 10 samples a control sample was included.

2.5. Statistics
All statistical analyses were performed on the log converted values of the HCV-RNA levels in copies per millilitre (log/ml). To test the stability of HCV-RNA at different temperatures, storage periods and conditions, as well as to test differences between EDTA plasma, heparin plasma and serum, the Wilcoxon Signed Rank Test was carried out.

3. RESULTS

3.1. Reproducibility of the HCV NASBA-QT assay
The precision of the HCV NASBA-QT assay was determined by assessing the mean difference of the 196 duplicate measurements. The mean difference in duplicate measurements was 0.031 log/ml, with a S.D. of 0.16 log/ml. This is in accordance with reports using NASBA-QT for measurement of HIV-RNA (Schuurman et al., 1996; Vandamme et al., 1996; Bruisten et al., 1997). In this study, only differences in HCV levels that exceeded 0.32 log/ml (twice the S.D.) were considered to be relevant. Furthermore, in all 35 runs undertaken for this study, a control sample was included for quality control. The mean HCV-RNA level of this control sample was 6.18 log/ml and the precision, defined as the S.D., was 0.11 log/ml.

3.2. Stability of HCV-RNA in clotted whole blood and EDTA-anticoagulated whole blood
The effect on HCV-RNA load of storage at 4°C for 5, 8, 24, 48 and 72 h is presented in Table 1 and Fig. 2. The HCV-RNA load in clotted whole blood did not decline significantly, but the HCV-RNA load in EDTA-anticoagulated whole blood was significantly less after 24 h of storage than at baseline (mean difference 0.18 log/ml). This decline was relevant after
48 and 72 h of storage (decrease in HCV-RNA level of 0.38 and 0.45 log/ml, respectively; \(P=0.02\)). The time course of HCV-RNA load was comparable in the four patients studied.

Table 1
Stability of HCV-RNA at 4°C in clotted whole blood and EDTA-anticoagulated whole blood.

<table>
<thead>
<tr>
<th>Compartment</th>
<th>h</th>
<th>log/ml HCV-RNA: Median (Range)</th>
<th>Mean difference</th>
<th>(P^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotted</td>
<td>0</td>
<td>6.85 (6.13-7.35)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6.81 (6.22-7.15)</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>6.93 (6.14-7.17)</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>6.82 (6.14-7.14)</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>6.85 (6.23-7.12)</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>6.66 (6.13-7.63)</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>EDTA</td>
<td>0</td>
<td>6.75 (6.18-7.11)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6.68 (6.13-7.17)</td>
<td>0.07</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>6.59 (6.18-6.93)</td>
<td>0.12</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>6.64 (6.00-6.99)</td>
<td>0.18(^b)</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>48</td>
<td>6.55 (5.36-6.88)</td>
<td>0.38</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>6.51 (5.21-6.87)</td>
<td>0.45</td>
<td>0.02</td>
</tr>
</tbody>
</table>

\(^a\) Wilcoxon Signed Rank Test: difference from t=0 was tested. NS = not significant, \(p>0.05\).

\(^b\) Not relevant, within inter-assay variation.

Samples of clotted blood (n=8) and EDTA anti-coagulated blood (n=8) were drawn from four HCV-infected individuals and serum/plasma was separated after storage at 4°C for the indicated number of hours. The HCV-RNA load was measured with the HCV NASBA-Q1 assay. Time 0: between 0-3 h after venipuncture.

**Fig. 2.**
Stability of HCV-RNA in clotted whole blood and EDTA-anticoagulated whole blood from four HCV-RNA positive patients. The median HCV-RNA load of eight samples is expressed in log/ml.
3.3. Stability of HCV-RNA in serum, EDTA plasma and heparin plasma in lysis buffer

The effect on HCV-RNA load of storage in lysis buffer at 4 and 30°C during 2, 7 and 14 days is presented in Table 2 and Fig. 3. Storage of serum at 4°C resulted in a statistically significant decline of HCV-RNA load after 7 and 14 days. However, the magnitude of decrease of the HCV-RNA load was within the range considered to be irrelevant. Storage of EDTA plasma and heparin plasma at 4°C did not result in a decline of HCV-RNA load (Fig. 3).

Storage of EDTA plasma, heparin plasma and serum at 30°C resulted in a statistically significant decline after 7 and 14 days. The decrease of HCV-RNA in EDTA plasma after storage at 30°C for 14 days was also relevant (decrease of 0.39 log/ml; P=0.0005). The time course of HCV-RNA load at all temperatures was comparable in the six individual patients. The effect on HCV-RNA load of storage in lysis buffer at -70°C during 6, 12 and 15 months is presented in Table 3. None of the specimens showed a relevant decline in HCV-RNA load. Only in EDTA plasma was a statistically significant decrease of HCV-RNA observed after 12 months of storage and this decrease may be attributed to chance, because values at 15 months were similar to baseline.

Table 2

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Days</th>
<th>4°C</th>
<th>30°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HCV-RNA:</td>
<td>HCV-RNA:</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mean (range)</td>
<td>mean (range)</td>
</tr>
<tr>
<td>serum</td>
<td>0</td>
<td>6.62 (5.59-6.97)</td>
<td>6.62 (5.59-6.97)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>n.t.</td>
<td>6.50 (5.51-7.05)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>6.53 (5.50-6.92)</td>
<td>6.52 (5.44-6.86)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>6.51 (5.51-6.92)</td>
<td>6.26 (5.28-6.69)</td>
</tr>
<tr>
<td>EDTA plasma</td>
<td>0</td>
<td>6.48 (5.57-7.16)</td>
<td>6.48 (5.57-7.16)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>n.t.</td>
<td>6.58 (5.45-7.05)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>6.45 (5.54-7.01)</td>
<td>6.34 (5.26-6.91)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>6.46 (5.45-6.95)</td>
<td>6.21 (5.10-6.64)</td>
</tr>
<tr>
<td>heparin plasma</td>
<td>0</td>
<td>6.43 (5.46-7.06)</td>
<td>6.43 (5.46-7.06)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>n.t.</td>
<td>6.55 (5.49-7.15)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>6.49 (5.48-7.17)</td>
<td>6.35 (5.18-7.01)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>6.49 (5.35-7.06)</td>
<td>6.35 (5.04-6.79)</td>
</tr>
</tbody>
</table>

*Wilcoxon Signed Rank Test: difference from t=0 is tested. NS = not significant; P>0.05.

b Not relevant, within inter-assay variation.

Serum (n=12), EDTA plasma (n=12), and heparin plasma samples (n=12) were prepared from six HCV-infected individuals and added to lysis buffer at time 0. HCV-RNA load was measured with the HCV NASBA-QT assay after storage at 4 or 30°C for the indicated number of days. Time 0: between 0-3 h after venipuncture. n.t.: not tested.
Fig. 3.
Stability of HCV-RNA in lysis buffer at 4°C (a) and 30°C (b) in serum/plasma from six HCV-RNA positive patients. The median HCV-RNA load of 12 samples is expressed in log/ml.

(a) Median log/ml

(b) Median log/ml

3.4. Impact of processing of blood and chaotropic lysis
Comparison of the baseline samples (presented in Tables 1-3) revealed no relevant differences and no statistically significant differences between EDTA plasma, heparin plasma and serum, as determined with the Wilcoxon Signed Rank test.
We also compared the effect on HCV-RNA level of storage of plasma or serum at -70°C in lysis buffer and storage of plasma or serum frozen as such at -70°C. For this purpose, samples frozen in lysis buffer were tested (shown in Tables 2 and 3) as well as samples frozen as such, at baseline. The median baseline value of all samples frozen in lysis buffer
was 6.62 log/ml and of all samples frozen as such was 6.49 log/ml. The mean difference of 0.07 was not relevant, but was statistically significant (P=0.035, Wilcoxon Signed Rank Test).

Table 3: Stability of HCV-RNA at -70°C in lysis buffer (pH 6.2).

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Months</th>
<th>log/ml HCV-RNA: Median (Range)</th>
<th>Mean difference</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>serum</td>
<td>0</td>
<td>6.76 (5.59-6.97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6.75 (5.40-7.09)</td>
<td>+0.01</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6.73 (5.31-7.17)</td>
<td>0.008</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>6.79 (5.60-7.24)</td>
<td>0.08</td>
<td>NS</td>
</tr>
<tr>
<td>EDTA plasma</td>
<td>0</td>
<td>6.84 (5.57-7.16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>6.79 (5.50-7.27)</td>
<td>0.02</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6.66 (5.47-7.08)</td>
<td>0.13b</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>6.68 (5.64-7.16)</td>
<td>0.06</td>
<td>NS</td>
</tr>
<tr>
<td>heparin</td>
<td>0</td>
<td>6.79 (5.46-7.06)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>plasma</td>
<td>6</td>
<td>6.72 (5.38-7.04)</td>
<td>0.05</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>6.73 (5.49-7.12)</td>
<td>+0.01</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>6.74 (5.58-7.08)</td>
<td>0.006</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Wilcoxon Signed Rank Test: difference from t=0 is tested. NS, not significant; P>0.05.

b Not relevant, within inter-assay variation.

Serum (n=8), EDTA (n=8) and heparin plasma samples (n=8) were prepared from four HCV-infected individuals and added to lysis buffer at time 0. HCV-RNA load was measured with the HCV NASBA-QT assay after storage at -70°C for the indicated number of months. Time 0: between 0-3 h after venipuncture.

4. DISCUSSION

In the present study the detectability of HCV-RNA after various sample handling and storage conditions was assessed in order to obtain an optimal protocol for sample preparation and storage for the HCV NASBA-QT assay.

The HCV-RNA load was found to be stable in clotted whole blood during storage for 72 h at 4°C. During storage of EDTA-anticoagulated whole blood for 72 h at 4°C, there was a statistically significant decrease in the HCV-RNA level after 24 h and this decline was relevant after 48 h. In a previous study on storage conditions of blood samples it was found that storage of EDTA-anticoagulated whole blood and serum at room temperature for 2 weeks was associated with a considerable decrease in HCV-RNA load as measured with cDNA-PCR (Cuypers et al., 1992). Storage of serum at 4°C for 2 weeks had no detectable effect on the HCV-RNA load. Comparable results have been presented in other studies using cDNA-PCR (Busch et al., 1992; Quan et al., 1992), the Roche MONITOR assay (Miskovsky et al., 1996) and bDNA (Davis et al., 1994; Halfon et al., 1996) for detection of HCV-RNA. In EDTA-anticoagulated whole blood HCV-RNA is more likely to come into contact with lytic enzymes as a result of granulocyte activation. Probably for this reason,
HCV-RNA is more stable in clotted whole blood. In a study on storage conditions prior to HIV NASBA-QT, a statistically significant decline in HIV-RNA load was found after 6 h of storage at 4°C in EDTA-anticoagulated whole blood, but no relevant decline was found for at least 72 h. The stability of HIV-RNA in EDTA-anticoagulated whole blood was even better during storage for 72 h at 25°C (Bruisten et al., 1997).

The HCV-RNA load was found to be stable in serum and plasma (EDTA and heparin) added to lysis buffer (pH 6.2) during storage at -70, 4 and 30°C. At 4°C the HCV-RNA load remained stable for 14 days in plasma and showed a statistically significant, but not relevant decline in serum after 7 days of storage. At 30°C the HCV-RNA load remained stable for 2 days both in serum and plasma. After 7 days a significant, but not relevant, decline was observed in EDTA plasma, heparin plasma and serum, but after 14 days this decline only became relevant for EDTA plasma. During storage at -70°C the HCV-RNA load remained stable for a period of 15 months in serum and plasma. The results of our study are comparable with the results obtained in the study on storage of HIV-RNA in EDTA plasma and serum in lysis buffer (pH 7.2) (Bruisten et al., 1997). HIV-RNA levels were also stable during storage at 4°C for 14 days and at -70°C for 6 months. During storage at 30°C a statistically significant and relevant decline of HIV-RNA load was observed after 7 days in serum or EDTA plasma, suggesting that HCV-RNA is somewhat more stable in lysis buffer at 30°C than HIV-RNA.

In several studies on HIV-RNA detection it was found that in paired plasma and serum samples, HIV-RNA levels are significantly lower in serum (Holodniy et al., 1995; Todd et al., 1995; Bruisten et al., 1997). This could be explained by the activation of lytic enzymes, capable of destroying viral RNA during the coagulation process, or by trapping viral particles in the clot. However, in the present study on HCV-RNA detection, we observed no difference in HCV-RNA level in paired serum and plasma samples and this observation is in accordance with a previous study using limiting dilution cDNA-PCR (Cuypers et al., 1992). Possibly, HCV-RNA is less susceptible to the coagulation process than HIV-RNA.

The nearly complete inhibitory effect of heparin on cDNA-PCR amplification has been extensively documented for several amplification templates (Beutler et al., 1990; Holodniy et al., 1991; Wang et al., 1992; Willems et al., 1993; Satsangi et al., 1994). In the present study, it was shown that NASBA amplification of HCV-RNA, collected in heparin plasma added to lysis buffer, was not inhibited after extraction of nucleic acid with the silica method (Boom et al., 1990). HIV-RNA levels, as measured with HIV NASBA-QT in heparin plasma added to lysis buffer, were only slightly lower than in paired EDTA plasma samples (Bruisten et al., 1997). Two explanations for successful application of heparin plasma in NASBA amplification can be considered. The silica extraction method is efficient in removal of heparin from the nucleic acid or NASBA amplification is insensitive to inhibition by heparin.

The results of this and other studies support the following procedures to ensure accurate measurements of HCV-RNA load with the NASBA-QT assay. Serum, EDTA plasma and heparin plasma all are appropriate for this assay. Plasma or serum samples should be prepared on the day of sampling. Serum or plasma should be added to lysis buffer and subsequently be stored at a temperature that preserves HCV-RNA until the day of analysis with NASBA-QT. Plasma or serum added to lysis buffer can be stored at -70°C for at least 15 months, at 4°C for at least 14 days, or at room temperature for a maximum of 7 days. Storage in lysis buffer at -20°C is not advised since irreversible coagulation can occur after thawing (manufacturer's instructions on the HIV-1 RNA NASBA-QT kit). Since the silica
extraction method yields an extremely purified nucleic acid extract, it may be of interest to study whether this method, in combination with cDNA-PCR, gives similar results to those of this study.

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