Increased Sensitivity of the Roche COBAS AMPLICOR HCV Test, Version 2.0, Using Modified Extraction Techniques

Michael Stuart Forman and Alexandra Valsamakis

From the Division of Microbiology, Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, Maryland

Processing modifications were made to the COBAS AMPLICOR HCV version 2.0 assay to enhance sensitivity. Two methods of specimen concentration, centrifugation ("ultraspin") and cationic detergent plus silica membrane ("ultracolumn"), were compared to the standard method. The effect of these changes on assay sensitivity and specificity was examined using commercial hepatitis C virus (HCV) preparations. The limits of detection (LOD, defined as detection of HCV RNA in $\geq 95\%$ of replicates) of genotype 1a were 50, 12, and 6 by standard method, ultraspin and ultracolumn, respectively. For genotype 1b, the LOD was 25 IU/ml, 12 IU/ml, and 3 IU/ml; for 2b, it was 50, 12, and 3; for 3a, it was 25, 12, and 1.5; for 4 it was 18, 4, and 2; for 5a, it was 38, 9, and 2; and for 6a it was 47, 6, and 3. No false positives were detected after ultraspin when controls containing high or low HCV concentrations were alternated with normal human plasma. Plasmas in which HCV RNA was not detected by the standard assay were re-tested with modified methods to assess the effect of altered processing in clinical specimens. Three of 152 specimens with no detectable HCV RNA by the standard method were positive by ultraspin and 2 of 109 were positive by ultracolumn, suggesting that these methods may increase assay sensitivity in clinical specimens. (*J Mol Diagn* 2004, 6:225–230)

Accurate, sensitive detection of hepatitis C virus (HCV) in serum or plasma is essential for diagnosis of HCV infections and assessing response to anti-HCV therapy. The standard, FDA approved COBAS AMPLICOR HCV version 2.0 assay has a reported LOD of 50 IU/ml for HCV genotype 1b.¹ This assay detects HCV RNA during active HCV infection.² It has been used to diagnose viremia^{3,4} and to assess treatment response.⁵

Therapy for chronic HCV infection has evolved from interferon alpha (IFN- α) alone, to second generation combination therapy with IFN- α plus ribavirin for either 24 or 48 weeks, to the latest generation of pegylated-interferon α with ribavirin (pegIFN- α /riba) for either 24

or 48 weeks. The lowest rates of response and highest rates of relapse were observed with IFN- α monotherapy.⁶ Treatment with pegIFN- α /riba resulted in the highest rates of response and the lowest rates of relapse.^{5,7} Variables that potentially affect rates of virologic relapse include treatment regimen, genetic variability of the virus, and HCV RNA detection assay sensitivity. The ability to detect very low levels of virus may prove useful in identifying patients who may relapse at end of treatment. Transcription-mediated amplification (TMA) has been used to assess the presence of residual HCV RNA in plasma from patients treated with pegIFN- α -2a and IFN- α -2a.⁸ In patients who relapsed after therapy, residual HCV RNA was detected in 7% of plasma samples at end of treatment with pegIFN- α -2a and 33% of samples following therapy with IFN- α -2a.

The aim of this study was to increase the sensitivity of COBAS AMPLICOR HCV version 2.0 through alteration of extraction methods. Two modifications were used to extract HCV RNA from one milliliter of plasma, representing a fivefold increase in volume compared to the standard assay. These methods included ultracentrifugation and a commercially available cationic detergent/spin column protocol. Using the standard method and these two modifications, the LOD for genotypes 1a, 2b, 3a, 4, 5a, and 6a (from a single manufacturer) and genotype 1b (from three different manufacturers) was determined. Residual RNA in supernatants after ultraspin was also assessed. The occurrence of false positives after ultraspin was evaluated by alternating high or low HCV concentration controls with normal human plasma. Finally, patient samples with no detectable HCV RNA after initial testing by the standard method were re-tested using the modified extraction methods to assess the performance of the modified extraction protocol on clinical specimens.

Materials and Methods

Viruses

HCV-positive samples were obtained from three different manufacturers (Teragenix Corporation, Fort Lauderdale, FL ["Manufacturer A"], AcroMetrix, Benicia, CA ["Manu-

Accepted for publication February 25, 2004.

Address reprint requests to Alexandra Valsamakis, Division of Microbiology, The Johns Hopkins Medical Institutions, Meyer B1-193, 600 North Wolfe Street, Baltimore, MD 21287-7093. Email: avalsam1@jhmi.edu.

Table 1. Starting Concentrations of Hepatitis C Viruses

Genotype	Manufacturer	Concentration (IU/ml log ₁₀)
1a	A	5.4
1b	A	4.5
1b	B	3.7
1b	C	5.8
2b	A	5.4
3a	A	5.4
4	A	5.8 (6.0)*
5a	A	6.3 (5.9)*
6a	A	5.7 (5.9)*

*, Concentration obtained after re-quantification using Roche COBAS HCV Monitor 2.0.

facturer B"], and Boston Biomedica, Inc., West Bridgewater, MA [BBI, "Manufacturer C], Table 1). All viruses were originally quantified by the manufacturers using Amplicor HCV Monitor (Roche Diagnostics Corp., Indianapolis, IN). For these experiments, the viruses were re-quantified, using COBAS AMPLICOR HCV Monitor version 2.0 (three to six replicates per panel member) to verify the manufacturer's stated concentrations. New values were used to establish the LOD if the concentration observed after re-quantification differed from the expected value by a factor of 1.5-fold or greater. For LOD determination, all panel members were diluted to 100 IU/ml in defibrinated human plasma (BBI) and then further diluted in twofold increments down to 1.5 IU/ml. A sufficient volume of each dilution was prepared to allow extraction of 60 replicates (20 aliquots per test method).

HCV RNA Extractions

Standard Method

All samples were processed as per manufacturer's instructions. Briefly, plasma samples (200 μ l) or controls (200 μ l normal human plasma and 20 μ l control) were added to tubes containing lysis reagent with HCV internal control (400 μ l) as per manufacturer's protocol. The tubes were incubated at 60°C $\pm$ 2°C for 10 minutes. RNA was precipitated with isopropyl alcohol (600 μ l). The tubes were vortexed and centrifuged at 16,100 $\times$ *g* for 15 minutes at room temperature to pellet the RNA. The supernatant was carefully removed, and the pellet was washed with 70% ethanol. The tubes were again centrifuged at 16,100 $\times$ *g* for 5 minutes at room temperature, and the supernatant was removed and discarded. After a 3-to 5-second centrifugation to remove any residual ethanol, specimen diluent (200 μ l) was added to each tube. The pellets were disrupted with a micropipette tip and the tubes were then vortexed vigorously for 10 seconds.

Ultraspin

Plasma samples (1 ml) were centrifuged at 25,000 $\times$ *g* at 4°C for 90 minutes (Biofuge Stratos, Heraeus, Asheville, NC). A portion of the supernatant (800 μ l) was carefully removed and either discarded or tested for residual HCV RNA after extraction with the ultracolumn

method. The remaining 200 μ l of supernatant plus pellet was extracted using the standard method described above.

Ultracolumn

The protocol for the QIAamp UltraSens Virus Kit (QIAGEN, Inc., Valencia, CA) was followed. Briefly, 0.8-ml buffer AC containing cationic detergent was added to 1-ml plasma samples. Carrier RNA (supplied in the Qiagen kit) and HCV internal control (1.44 μ l/sample, supplied with the COBAS AMPLICOR HCV version 2.0 reagents) were placed into the tube lids. The tubes were first inverted, then vortexed for 10 seconds, and incubated at room temperature for 10 minutes. The samples were centrifuged at 1200 $\times$ *g* for 3 minutes. The cationic detergent forms complexes with nucleic acids that can be sedimented by low-*g* force centrifugation. The supernatants were removed, and a buffer containing chaotropic salt and proteinase K was added to the pellets. The tubes were incubated at 40°C for 10 minutes, and buffer AB containing ethanol was added. The sample lysates were then applied to QIAamp spin columns. The columns were washed twice with AW1 and AW2 to remove additional contaminants and inhibitors. RNA was eluted in 80 μ l of buffer AVE.

HCV RNA Detection

All processed samples and controls were amplified and detected with the COBAS AMPLICOR analyzer, an automated instrument that combines a thermal cycler, incubator, wash station, and photometer. The analyzer interprets results by comparing the HCV and internal control (IC) absorbance values to established cut-offs. Results were reported as positive if the HCV *A*₆₆₀ values were $\geq$ 1.0, negative if the HCV *A*₆₆₀ values were $<$ 0.3 and the IC *A*₆₆₀ values were $\geq$ 0.3, inhibited if both HCV and IC *A*₆₆₀ values were $<$ 0.3, and equivocal (gray zone) if the HCV *A*₆₆₀ values were $\geq$ 0.3 and $<$ 1.0. Equivocal results were resolved by re-testing in duplicate, as stated in the package insert. The LOD was defined as the concentration at which $\geq$ 95% of replicates were detected.

Clinical Samples

A total of 261 random plasma samples were chosen from specimens that had at least one milliliter remaining and previously had no detectable HCV RNA after testing with the standard COBAS AMPLICOR HCV version 2.0 assay. Residual volume was insufficient to allow extraction by both methods, with the exception of a single specimen. The samples were frozen at -70°C until further processing. Chart review was performed to ascertain whether tests had been ordered to diagnose active HCV infection or to monitor therapeutic response. These experiments were performed following guidelines for human subjects experimentation and this protocol was approved by the Johns Hopkins Medicine Institutional Review Board.

Table 2. LOD of Different HCV Genotypes

Genotype	Method	HCV concentration						
		100 IU/ml	50 IU/ml	25 IU/ml	12 IU/ml	6 IU/ml	3 IU/ml	1.5 IU/ml
1a	FDA		20/20	12/14 [†]	4/10			
	Spin			19/19 [‡]	15/15	10/11	4/17	
	Column					20/20	17/18	12/17
1b*	FDA		20/20	18/19	10/20	6/20	3/20	2/18
	Spin		20/20	19/20	19/20	12/20	10/20	
	Column		20/20	20/20	20/20	20/20	17/17	
2b	FDA		17/17	10/14	2/9			
	Spin				19/19	11/16	5/12	
	Column					20/20	18/19	15/19
3a	FDA		20/20	18/18	14/15	7/14		
	Spin				20/20	17/18	8/12	
	Column						20/20	19/19
				36 IU/ml	18 IU/ml	9 IU/ml	4 IU/ml	2 IU/ml
4 [§]	FDA			20/20	20/20	16/19	11/17	
	Spin				20/20	20/20	18/19	14/20
	Column							20/20
		38 IU/ml	19 IU/ml	9 IU/ml	4 IU/ml	2 IU/ml	1 IU/ml	
5a [§]	FDA	20/20	17/20	15/19				
	Spin			18/19	16/17	10/18		
	Column					20/20	14/16	
				47 IU/ml	23 IU/ml	11 IU/ml	6 IU/ml	3 IU/ml
6a [§]	FDA			20/20	16/18	16/18		
	Spin					19/19	18/18	14/19
	Column							20/20

Twenty replicates were tested at each concentration. *n* < 20 represents equivocal results that were excluded from analysis. Values in **bold** indicate concentrations at which ≥95% of replicates were detected. *, All virus preparations were obtained from Manufacturer A except for HCV 1b (Manufacturer B). †, Internal control not detected for one replicate. ‡, Specimen spilled. §, Requantitation of HCV preparations yielded concentrations that varied greater than 1.5-fold from the concentration stated by the manufacturer. Concentrations based on requantitation are shown above the genotype.

Results

LOD Studies

To determine whether protocol modifications increased the sensitivity of the AMPLICOR version 2.0 assay, the LOD of HCV genotypes 1 (subtypes a and b), 2b, 3a, 4, 5a, and 6a were determined after standard, ultraspin, and ultracolumn extraction (*n* = 20 for each virus preparation). LODs were calculated by excluding all samples with equivocal results since resolution of these data by repeat testing in duplicate, as per the package insert, had minimal effect on LOD results for all genotypes after extraction by the standard and ultraspin methods.

Specifically, data obtained after testing using the standard method were unchanged after resolution of equivocal. Ultraspin data demonstrated a small and conflicting change in LOD for genotypes 1a and 3a when equivocal results were included (LOD decreased twofold for genotype 1a and increased twofold for genotype 3a). Because resolution of equivocal had minimal effect on LOD after standard extraction and ultraspin modification, equivocal data obtained after testing of samples extracted by ultracolumn was not further resolved.

The LOD of the standard method ranged from 18 IU/ml for genotype 4 to 50 IU/ml for genotypes 1a and 2b (Table 2). The LOD after the ultraspin modification de-

creased 2- to 8-fold depending on the genotype, compared to standard AMPLICOR version 2.0. For example, the LODs of genotype 1a and 6a were 12 IU/ml and 6 IU/ml after ultraspin modification, compared to 50 IU/ml and 47 IU/ml for standard method (Table 2). The LOD after ultracolumn extraction decreased from 8- to approximately 20-fold depending on genotype compared to standard AMPLICOR version 2.0 (Table 2). The LODs of genotypes 1a and 5a were 6 IU/ml and 2 IU/ml after ultracolumn extraction compared to 50 IU/ml and 38 IU/ml after testing by the standard method (Table 2). Similar results were obtained after diluting genotype 1b virus in pooled human plasma (data not shown), suggesting that the increase in assay sensitivity was not due to the use of defibrinated plasma as a diluent.

The greater sensitivity of the ultracolumn method compared to ultraspin suggested that residual HCV RNA remained in the supernatant after ultraspin. To test whether this was true, three dilutions (12, 6, and 3 IU/ml) of an HCV 1b panel member were centrifuged and supernatants (800 µl) were extracted using the ultracolumn method. Ultraspin pellets and ultracolumn eluates were then tested by COBAS AMPLICOR HCV version 2.0. Residual HCV RNA was found in the supernatants of all three concentrations tested (Table 3). However, the number of replicates with detectable residual HCV RNA in

Table 3. HCV RNA Detection in Pellet and Supernatant Fraction of Samples Processed by Ultraspin

Sample fraction	IU/ml		
	12	6	3
“pellet”	10/10 (100%)	6/8 (75%)	4/7 (57%)
supernatant	5/6 (83%)	2/4 (50%)	2/9 (22%)

An HCV 1b panel member (Manufacturer B) was diluted to 12, 6, and 3 IU/ml. Replicates ($n = 10$) were centrifuged using the ultraspin protocol. The supernatants from 10 replicates were processed using the ultracolumn protocol. The pellet was extracted according to the standard COBAS AMPLICOR v. 2.0 protocol. $n < 10$ represent equivocal results that were excluded from analysis.

supernatants was always less than the number of replicates that contained detectable HCV RNA in pellet fractions and, at lower concentrations, fewer ultraspin samples had eluates with detectable HCV RNA (Table 3).

To ascertain whether alteration in extraction protocol had an equivalent effect on the detection of HCV preparations from different commercial sources, HCV 1b viruses from two additional manufacturers (A and C) were tested after extraction by standard and ultraspin methods. The LODs of these viruses (Table 4) were similar to that of Manufacturer B (Table 2) and were dependent on the RNA extraction method. Using the standard method, HCV1b viruses from Manufacturers A and C were detected in 100% of 50 IU/ml replicates and in 93% and 94% of 25 IU/ml replicates, respectively (Table 4). The ultraspin modification decreased the LOD of the virus preparations from Manufacturer A and Manufacturer C, similar to the effect observed with the HCV 1b virus obtained from Manufacturer B. AMPLICOR HCV version 2.0 detected 95% and 100% of 12 IU/ml replicates of HCV 1b obtained from Manufacturer A and C after ultraspin (Table 4).

Specificity of Ultraspin

Increased specimen extraction complexity and assay sensitivity could theoretically result in an increased rate of false-positive test results. This was investigated by assessing the incidence of false-positive results after ultraspin processing. The optical densities (ODs) of samples were recorded from experiments containing 5 high-positive ($\geq 1 \times 10^6$ IU/ml) and 10 low-positive (50 to 100 IU/ml) controls interspersed among negative controls. No false-positive results were obtained (Figure 1). ODs of negative samples ($n = 60$) fell between 0.00 and 0.05. Two of 15 positive samples (both with 50 to 100 IU/ml) had ODs between 2.0 and 2.49. The remaining 13 positive samples had ODs > 3.5 .

Figure 1. OD values of negative controls and intermittent positive controls. **White bar**, negative controls. **Black bars**, low-positive and high-positive controls.

Detection of HCV RNA in Clinical Specimens by Ultraspin and Ultracolumn Column

Low HCV viral loads may be present in acute infection⁹ and during treatment, particularly with IFN- α monotherapy,⁸ when viral replication is apparently suppressed but virus is not fully cleared. COBAS AMPLICOR version 2.0 may not effectively detect HCV in specimens with such low RNA concentrations, resulting in false-negative test results. To determine whether processing modifications could potentially increase the clinical sensitivity of the COBAS AMPLICOR version 2.0 assay, sequential clinical specimens that had previously been found to have undetectable levels of HCV RNA were re-tested after extraction by ultraspin or ultracolumn methods. A total of 261 clinical specimens were re-tested (152 by ultraspin and 109 by ultracolumn). Of the specimens tested after ultraspin, 130 of 152 were sent for diagnostic purposes and 22 of 152 were submitted for therapeutic monitoring. HCV RNA was detected by ultraspin in three samples that were sent for therapeutic monitoring and in none of the samples that were sent for diagnostic purposes. Of the samples tested by ultracolumn, 89 of 109 were sent for diagnostic purposes and 20 of 109 were sent for therapeutic monitoring. HCV RNA was detected by ultracolumn in two samples that were sent for therapeutic monitoring and in no samples that were sent for diagnostic purposes. Only one sample had a sufficient volume of plasma to allow testing after extraction by ultraspin and ultracolumn. This specimen was positive by both methods.

Table 4. LOD Comparison of HCV 1b Viruses from Other Manufacturers

Method	Manufacturer	IU/ml			
		50	25	12	6
Standard	A	20/20 (100%)	13/14 (93%)	9/17 (53%)	
	C	20/20 (100%)	17/18 (94%)	13/19 (68%)	
Ultraspin	A			18/19 (95%)	17/20 (85%)
	C			19/19 (100%)	12/18 (67%)

Effect of Processing Modification on PCR Inhibition Rates

Alteration of specimen processing protocol modifications could potentially inhibit the downstream PCR reaction, either by altering the specimen or introducing interfering substances. An internal control RNA molecule that contains a combination of HCV and unique sequences is added to each amplification reaction to identify specimens that inhibited PCR. Internal control failure rates were determined to assess whether specimen-processing modifications altered the incidence of PCR inhibition. The rate of internal control failure was 3.2% for the standard method (11 internal control failures for 336 non-detectable HCV RNA test results from 10 runs), 10% (15 of 152) for ultraspin, and 39% (43 of 109) for ultracolumn.

Discussion

Our data demonstrate that alteration of specimen processing increased the analytical sensitivity of the Roche AMPLICOR version 2.0 assay. This was observed when a number of different variables were examined including different genotypes, subtypes (1a and 1b), manufacturers, and diluents. The ultracolumn protocol appeared to be the most sensitive since LODs were lowest for all genotypes with this method and it effectively detected HCV RNA in the supernatant fraction after ultraspin. Information on the LOD of genotype 6 using the AMPLICOR version 2.0 assay has not been reported previously in the literature. Our data show that the LOD of this genotype is comparable to the others using the standard assay and both processing protocol modifications.

Our data also suggest that extraction protocol alterations increased the clinical sensitivity of the assay since HCV was detected in patient specimens that were found to have no detectable HCV RNA after testing by the standard method. These specimens likely contained < 50 IU/ml of HCV, the LOD of the standard method.¹ In these experiments, only one replicate was tested, reflecting current laboratory practice on routine runs. A definitive statement about the comparative sensitivity of these methods in clinical specimens could not be made since, in most instances, there was insufficient residual plasma volume. Theoretically this question would be optimally investigated with a hit rate study that would test at least 20 replicates per method. In reality, such a study would be difficult to execute since it would necessitate the prospective collection of approximately 45-ml of plasma, corresponding to 90-ml of whole blood per patient.

It is not possible to formally exclude that the results obtained by processing clinical specimens with the ultrasensitive modifications are not false positives since insufficient residual volume precluded confirmatory testing. Nonetheless, it is likely that these are true positive results since they were observed in HCV-infected patients who were being treated. The lack of false-positive results in ultraspin experiments with intermittent positive specimens suggests that the sensitivity of the AMPLICOR ver-

sion 2.0 assay can be increased without decreasing specificity.

Laboratories that use COBAS AMPLICOR version 2.0 may consider using the ultraspin over the ultracolumn method when ultrasensitive HCV detection is requested. The ultraspin method was less sensitive than the ultracolumn method, however the ultraspin method is less labor intensive, is more easily incorporated into routine runs, and has a much lower rate of internal control failure in clinical specimens. In the ultraspin method, this failure may represent either PCR inhibition or aspiration of the RNA pellet during washing steps. In the ultracolumn method, it was likely due to PCR inhibition since there is no pellet formed during RNA extraction. The cause of this phenomenon is unclear. Increased rates of internal control failure were observed only in clinical specimens and not in commercial preparations that were diluted in defibrinated, detergent-washed human plasma. It is possible that concentration of plasma, either by high-speed centrifugation or cationic detergent, may also increase the effective concentration of PCR inhibitors in clinical specimens. Whether performance of this modification on serum specimens would lead to a lower rate of PCR inhibition is unknown. One final technical problem that we observed primarily during extraction of plasma specimens by ultracolumn was the relative insolubility of specimens after lysis and precipitation with detergent containing Buffer AC, despite low speed centrifugation. This problem is likely due to precipitated plasma proteins.

Ultracentrifugation of plasma has been used to increase the sensitivity of assays for HCV and other viruses such as HIV.¹⁰ Our data are in general agreement with studies that used ultracentrifugation of one milliliter of plasma that was collected at end of treatment and a home brew reverse-transcription/PCR detection assay to identify patients with residual HCV RNA who relapsed after completing standard IFN- α monotherapy.¹¹ The analytical performance of this modified user-defined assay was not described.

Catrimox-14, a cationic surfactant, has been used to precipitate HCV RNA from plasma and whole blood.¹² Our data demonstrating increased analytical and clinical sensitivity of HCV RNA detection in plasma using the ultracolumn cationic detergent modification are not in agreement with previously published data suggesting that treatment of plasma with catrimox-14 does not enhance the clinical sensitivity of HCV RNA detection.¹³ One difference between the two studies is the method of HCV RNA detection. Stapleton et al¹³ used a user-developed reverse transcription PCR assay whose performance characteristics were described as being equivalent to the standard COBAS AMPLICOR version 2.0 assay, while we used the latter assay. A second potential difference is the cationic detergent. Whether the QIAamp UltraSens Virus Kit employs catrimox-14 is unclear. The constituents of the detergent reagent are proprietary and are described only as a Nonidet P-40 substitute in the material safety data sheet. Of note, in the literature, the data on the effects of catrimox-14 on the detection of HCV RNA in whole blood are also discrepant.^{13,14}

Qualitative HCV RNA detection assays have been approved for the diagnosis of HCV infection, but these tests are also used to assess response to antiviral therapy, due to their low LODs compared to assays that quantitate HCV RNA. The utility of highly sensitive qualitative assays such as the modified COBAS AMPLICOR version 2.0 and Versant HCV RNA Qualitative Assay ([TMA, LOD = 2 to 10 IU/ml]^{15,16} in the setting of therapeutic monitoring is not yet clear. However, data in the literature suggest potential roles for these types of tests. Sarrazin et al⁸ showed that 7% of patients who relapsed after cessation of pegIFN- α monotherapy could be successfully identified as non-responders at end of treatment using the TMA assay. However, 4% of sustained responders were also found to be positive by TMA at end of treatment. Highly sensitive assays are not likely to be as useful at end of treatment with the current commonly used protocol of combination pegIFN- α /riba because relapse occurs less frequently with this regimen.⁵ In addition, most non-responders can be effectively identified after 12 weeks of combination therapy (negative predictive value of less than 2 log drop in HCV RNA at week 12 is 97% and the positive predictive value of a decline in virus concentration of 2 logs or greater at week 12 of therapy is approximately 65%).⁵ Whether ultrasensitive assays can be used during combination therapy to improve the positive predictive value of testing at week 12 of therapy with pegIFN- α /riba awaits further study.

Acknowledgments

We thank Lori Brisbin for technical assistance and advice, Qiagen Incorporated, Roche Molecular Systems, and Roche Diagnostics Corporation for the reagents provided, John Ticehurst for valuable comments on the data, and William Merz and Karen Carroll for insightful comments on this manuscript.

References

1. Lee SC, Antony A, Lee N, Leibow J, Yang JQ, Soviero S, Gutekunst K, Rosenstraus M: Improved version 2.0 qualitative and quantitative AMPLICOR reverse transcription-PCR tests for hepatitis C virus RNA: calibration to international units, enhanced genotype reactivity, and performance characteristics. *J Clin Microbiol* 2000, 38:4171–4179
2. Nolte FS, Fried MW, Shiffman ML, Ferreira-Gonzalez A, Garrett CT, Schiff ER, Polyak SJ, Gretch DR: Prospective multicenter clinical evaluation of AMPLICOR and COBAS AMPLICOR hepatitis C virus tests. *J Clin Microbiol* 2001, 39:4005–4012
3. Albadalejo J, Alonso R, Antinazzi R, Bogard M, Bourgault AM, Colucci G, Fenner T, Petersen H, Sala E, Vincelette J, Young C: Multicenter evaluation of the COBAS AMPLICOR HCV assay, an integrated PCR system for rapid detection of hepatitis C virus RNA in the diagnostic laboratory. *J Clin Microbiol* 1998, 36:862–865
4. Castro FJ, Sauleda S, Esteban JL, Viladomiu L, Martell M, Dragon E, Esteban R, Guardia J: Evaluation of hepatitis C virus RNA RT/PCR qualitative and quantitative second generation assays. *J Virol Methods* 2001, 91:51–58
5. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncalves Jr FL, Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J: Peginterferon α -2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002, 347:975–982
6. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, Goodman ZD, Ling MH, Cort S, Albrecht JK: Interferon α -2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C: Hepatitis Interventional Therapy Group. *N Engl J Med* 1998, 339:1485–1492
7. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK: Peginterferon α -2b plus ribavirin compared with interferon α -2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001, 358:958–965
8. Sarrazin C, Hendricks DA, Sedarati F, Zeuzem S: Assessment, by transcription-mediated amplification, of virologic response in patients with chronic hepatitis C virus treated with peginterferon α -2a. *J Clin Microbiol* 2001, 39:2850–2855
9. Beld M, Penning M, van Putten M, van den Hoek A, Damen M, Klein MR, Goudsmit J: Low levels of hepatitis C virus RNA in serum, plasma, and peripheral blood mononuclear cells of injecting drug users during long antibody-undetectable periods before seroconversion. *Blood* 1999, 94:1183–1191
10. Piowar-Manning EM, Henderson TA, Brisbin L, Jackson JB: A modified ultrasensitive assay to detect quantified HIV-1 RNA of fewer than 50 copies per milliliter. *Am J Clin Pathol* 2003, 120:268–270
11. McHutchison JG, Blatt LM, Ponnudurai R, Goodarzi K, Russell J, Conrad A: Ultracentrifugation and concentration of a large volume of serum for HCV RNA during treatment may predict sustained and relapse response in chronic HCV infection. *J Med Virol* 1999, 57:351–355
12. Schmidt WN, Klinzman D, LaBrecque DR, Macfarlane DE, Stapleton JT: Direct detection of hepatitis C virus (HCV) RNA from whole blood, and comparison with HCV RNA in plasma and peripheral blood mononuclear cells. *J Med Virol* 1995, 47:153–160
13. Stapleton JT, Klinzman D, Schmidt WN, Pfaller MA, Wu P, LaBrecque DR, Han J, Phillips MJ, Woolson R, Alden B: Prospective comparison of whole-blood- and plasma-based hepatitis C virus RNA detection systems: improved detection using whole blood as the source of viral RNA. *J Clin Microbiol* 1999, 37:484–489
14. Cook L, Ross AM, Knight GB, Agnello V: Use of whole blood specimens for routine clinical quantitation of hepatitis C virus RNA does not increase assay sensitivity. *J Clin Microbiol* 2000, 38:4326–4331
15. Hendricks DA, Friesenhahn M, Tanimoto L, Goergen B, Dodge D, Comanor L: Multicenter evaluation of the VERSANT HCV RNA qualitative assay for detection of hepatitis C virus RNA. *J Clin Microbiol* 2003, 41:651–656
16. Krajden M, Ziermann R, Khan A, Mak A, Leung K, Hendricks D, Comanor L: Qualitative detection of hepatitis C virus RNA: comparison of analytical sensitivity, clinical performance, and workflow of the Cobas Amplicor HCV test version 2.0 and the HCV RNA transcription-mediated amplification qualitative assay. *J Clin Microbiol* 2002, 40:2903–2907