JOURNAL OF CLINICAL MICROBIOLOGY, July 2003, p. 3046–3050 Vol. 41, No. 7
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.7.3046–3050.2003

Quantification of Hepatitis C Virus (HCV) in Liver Specimens and Sera from Patients with Human Immunodeficiency Virus Coinfection by Using the Versant HCV RNA 3.0 (Branched DNA-Based) DNA Assay

Rosamaria Tedeschi,[1]* Eliana Pivetta,[1] Stefania Zanussi,[1] Ettore Bidoli,[2] Mirna Ros,[1] Giampiero di Gennaro,[3] Guglielmo Nasti,[3] and Paolo De Paoli[1]

Microbiology-Immunology and Virology Department,[1] Epidemiology Unit,[2] and Medical Oncology A,[3] Centro di Riferimento Oncologico, IRCCS, I-33081 Aviano, Italy

Received 24 February 2003/Returned for modification 11 April 2003/Accepted 28 April 2003

The new generation assay Versant HCV RNA 3.0v (Bayer Diagnostics) was evaluated to quantify hepatitis C virus (HCV) RNA levels in liver biopsy specimens from patients with HCV and human immunodeficiency virus (HIV) coinfection. A total of 25 liver biopsies and sera collected at the time of liver biopsy were used. The efficiency of HCV RNA recovery from spiked samples was between 38.6 and 50.7%, and reproducible measurements of viral load were observed (the intra- and interrun coefficients of variation were 0.5 to 13% and 3.5 to 24.7%, respectively), with good specificity and sensitivity. Linearity was evaluated in the range of 96,154 to 769 IU/μg by using a serially diluted high-titer sample. Coinfected patients had high HCV RNA viral loads in serum and liver (498,471 IU/ml and 231,495 IU/μg, respectively), and both levels were correlated ($r = 0.63$; $P < 0.01$). The amount of hepatic HCV RNA was significantly higher among patients with genotype 1 than among patients with genotype 3 ($P < 0.01$). The virological end-of-treatment response in the serum was associated with a lower pretreatment intrahepatic HCV viral load ($P = 0.03$). The new version of b-DNA is a sensitive, specific, and reproducible method for quantitating HCV RNA in the liver. Given its positive analytical performance, the assay will be used to evaluate the HCV RNA levels in the serum and liver during follow-up of patients treated with an anti-HCV therapeutic regimen.

Molecular-biology-based assays are invaluable tools for the management of chronic viral hepatitis, and hepatitis C virus (HCV) viral load testing is one of the most common procedures done in molecular biology laboratories. Although the HCV viral load in serum does not correlate with the severity of the hepatitis or with a poor prognosis, like human immunodeficiency virus (HIV) viremia, it does correlate with the likelihood of response to antiviral therapy (9, 17, 23). The liver represents the primary site of HCV replication, and therefore it may be important to quantitate HCV RNA in livers of chronic hepatitis C patients; several studies assessing the clinical relevance of hepatic viral load reported some associations between viral loads in the liver and serum (3, 14, 15, 28).

Coinfection of HIV with HCV leads to increased HCV replication, with high HCV RNA levels (22), and to a faster and more severe histopathological course (10). However, the longer survival of HIV-infected patients as a result of highly active antiretroviral treatments means that there will remain a high prevalence of HIV-HCV-coinfected people in the near future, with many of them being potential candidates for receiving specific treatment for HCV infection.

The availability of effective antiviral therapy for hepatitis C has increased the need for molecular detection of the virus. In a recent study, we assessed the feasibility of tribavirin plus interferon alfa-2b combination therapy and evaluated the sustained virological response in the peripheral blood of a small cohort of coinfected patients (19).

Two commercial test technologies are commonly used for HCV viral load testing, although other kit-based assays and proprietary "homebrew" assays are also available. Among the commercial assays, the branched DNA (b-DNA) relies on the use of signal amplification (Bayer Quantiplex HCV RNA b-DNA), while another uses PCR to amplify target portions of the viral genome (Roche Amplicor Monitor assay).

There are several reports and numerous assays on viral load detection in serum or plasma and, furthermore, quantitation of liver HCV RNA has been performed, by standardized techniques, such as as b-DNA and the Roche PCR assay (1, 14, 15, 26), other quantitative PCR methods (11, 25), or dot blot PCR (16). However, only a few studies on intrahepatic HCV viral load in HIV-HCV-coinfected patients have been reported (3, 20, 28).

We sought to investigate the applicability of the new, updated version of the b-DNA assay, currently available to evaluate HCV viral load in serum, to quantitate HCV RNA in paired serum-liver biopsies from HIV-HCV-coinfected patients. We therefore conducted a laboratory study in which the specificity, reproducibility, and linearity of the test were analyzed. At the same time, we also investigated the relation of intrahepatic viral load with HCV RNA levels in serum and with viral genotypes.

* Corresponding author. Mailing address: Microbiology-Immunology and Virology Department, Centro di Riferimento Oncologico, IRCCS, via Pedemontana Occ. 12, I-33081 Aviano, Italy. Phone: 39-0434-659421. Fax: 39-0434-659402. E-mail: rtedeschi@cro.it.

TABLE 1. Evaluation of efficiency of HCV RNA recovery

Sample	HCV RNA				
	IU log_{10} added	Method	IU log_{10} detected (b-DNA)	Δlog_{10}	Recovery (%)
HCV RNA	5.86		5.70		
	5.86 + 4.7 mg of tissue (HCV neg)	RNA extraction	5.29	0.41	38.6
Serum HCV positive	5.86		5.65		
	5.86 + 4.7 mg of tissue (HCV neg)	RNA extraction	5.94	0.29	50.7

MATERIALS AND METHODS

Patients. A total of 25 consecutive HIV-positive patients with chronic hepatitis C (17 males and 8 females; mean age, 46 years) were enrolled in the study.

These patients were selected for a clinical trial designed to treat chronic hepatitis C in HIV-coinfected persons. The employed scheduled therapy was pegylated interferon alfa-2a administered subcutaneously (1.5 μg/kg, weekly), plus tribavirin (800 to 1,200 mg/day as a function of body weight), for 24 weeks. The HCV and HIV virological parameters were monitored every 3 months throughout the study period. All patients had antibodies to HCV (as detected by a third-generation test, HCV Axsym [Abbott] and as confirmed by INNO-LIA HCV [Innogenetics]) and serum HCV RNA, as detected by the Bayer HCV RNA qualitative assay (Versant HCV RNA [TMA; Bayer]). HCV genotyping was done by using the reverse hybridization assay after amplification with the PCR assay (LIPA [Innogenetics]). The following genotypes were identified: 1b (n = 2), 1a (n = 5), 3a (n = 13), and 4 (n = 3) (two samples were undetermined).

The patients had never received anti-HCV therapy and had no hepatitis B surface antigen (HbsAg) or other indication of chronic liver disease. All patients were receiving antiretroviral therapy at the time of the study. Three HIV-negative HCV-positive patients and one HIV-positive HCV-negative HbsAg-positive patient were also evaluated as controls. Two HIV-positive, HCV-positive patients, who in our previous study (19), achieved clearance of serum HCV RNA after 24 weeks of therapy based on combination of interferon alfa-2b and tribavirin and still HCV RNA negative (long-term response patients), were also included in the present study.

All patients had given informed consent to liver biopsy, which was obtained for the routine pathological assessment by standard procedures, before starting therapy (T0). A part of each of the same liver biopsy was placed on dry ice within a few minutes of removal from the liver and then weighed and stored at −80°C before use for molecular biology analysis. In addition, a liver biopsy (28 mg) from one patients who was HCV negative (kindly provided by P. Pontisso, University of Padua) was also used to develop and validate the method for viral quantification in the liver. Simultaneous serum samples were available for all of the patients and were stored at −80°C.

Liver tissue processing and HCV RNA extraction. The weight of the biopsies ranged from 1.0 to 17.9 mg (mean, 5.7 mg; median, 4.4 mg). Total RNA from the biopsy was extracted by using the procedure of Chomczynski and Sacchi (5) with minor modifications. In particular, frozen liver tissue was maintained on dry ice and homogenized rapidly in 0.65 ml of cold guanidinium-thiocyanate-based solution (4 M guanidinium thiocyanate, 25 mM sodium citrate [pH 7], 0.1 M 2-mercaptoethanol, 0.5% sarcosyl) by using a pellet pestle mixer Ultraturrax T25 (Janke and Kunkel, IKA Laboratecanik) until the tissue was completely disrupted (ca. 30 s, twice). To precipitate RNA, isopropyl alcohol plus glycogen (2 mg/ml) was added to the homogenized liver tissue mixture, which was then vortexed vigorously and incubated 1 h at −20°C. Precipitated RNA was collected by centrifugation in a microcentrifuge at 12,000 × g for 10 min at 4°C. The pellet was washed with 75% ethanol and, after aspiration of the supernatant, the remaining liquid was removed by evaporation. The pellet was resuspended in 30 μl of RNase-free water and stored at −80°C until used.

Total RNA was measured by spectrophotometric analysis at 260 and 280 nm (GeneQuant II; Pharmacia Biotech).

Determination of HCV RNA levels in serum samples and liver tissue. The Versant HCV RNA b-DNA 3.0 version assay (Bayer Diagnostics, Berkeley, Calif.) was used to quantitate HCV RNA in both extracted liver tissue and paired serum samples. The assay has a reported quantification range of 3.2×10^3 to 40×10^6 copies/ml. The software reports test results in copies/milliliter and World Health Organization International Units (IU)/milliliter by using a conversion factor of 5.2 copies/IU, as recommended by Bayer Corp.

Serum samples were processed according to the manufacturer's instructions, while a modified protocol suggested and validated at the Chiron Corp. by using previous versions of the assay (26) was used for the biopsy samples. Briefly, 220 μl of lysis working reagent, followed by 110 μl of RNase-free water, was added to the liver-extracted RNA suspension and then incubated in a heat block for 10 min at 63°C. The sample was then vortexed and pipetted (150 μl) into wells in duplicate. From this point on, it was processed in the same manner as for the serum samples. RNA extracted from a negative biopsy was included in each run as a negative control.

The results, in IU/milliliter, that were obtained automatically using the Quantiplex computerized program were converted and corrected to give quantitation values as HCV RNA IU/microgram of total extracted RNA from liver tissue.

Statistical analysis. The results are expressed as median values, and a log_{10} transformation was used to adjust for nonhomogeneous variability.

HCV RNA load variations of <3-fold (±0.5 log_{10}), which may be related to the intrinsic variability of the assays, were not considered significant (21). The variability between replicate tests was described by using the percent coefficient of variation (CV) for the log_{10}-transformed values.

The Spearman correlation coefficient (r) was used to analyze the correlation between expected and observed HCV RNA values. Liver and serum HCV RNA IU between genotypes were compared by using the nonparametric Mann-Whitney U test for unpaired data. All P values were two sided.

Odds ratios (ORs) and their corresponding 95% confidence intervals (95% CI) were obtained by unconditional multiple logistic regression models (4). The dependent variable was the virological end of the treatment response (T6) (yes or no). The covariates were levels of HCV RNA IU in serum and liver before therapy (T0), and they were entered as continuous variables with the unit set to 20,000 IU of HCV RNA.

The statistical analyses were performed by using the SAS software (version 8.2; SAS Institute, Inc., Cary, N.C.).

RESULTS

Efficiency of HCV RNA recovery. The amount of extracted RNA ranged from 0.3 to 3.7 μg/mg of liver tissue (median, 1.38 μg/mg). The efficiency of HCV RNA recovery from liver tissue after extraction was evaluated by adding to a noninfected liver tissue sample a known quantity of HCV RNA previously extracted and quantified by the b-DNA assay or a serum sample with a known HCV load. The efficiency was calculated by comparing the amount added initially with the amount recovered at the end, after the liver specimens were processed according to the standard protocol of RNA extraction.

When a liver homogenate was processed after the addition of 1 μg of HCV RNA previously assessed by b-DNA as 730,769 IU, the mean recovery of HCV RNA was 38.6%. The mean recovery of HCV RNA from the liver homogenate was 50.7% when a serum with the same load was added. HCV RNA load variations after RNA extraction were, in both cases, <0.5 log_{10} (Table 1).

Specificity of the b-DNA assay. Three different tests were carried out to assess the specificity of the b-DNA assay signal.

(i) In every experiment, a liver tissue specimen from the same HCV-negative patient was always run, and HCV RNA was never detectable. Furthermore, HCV RNA was not de-

TABLE 2. Evaluation of HCV RNA linearity[a] of the b-DNA assay on liver biopsy samples

Dilution	HCV RNA linearity				
	Expected value		Observed value		$\Delta\log_{10}$
	IU/μg	IU $\log_{10}$	IU/μg	IU $\log_{10}$	
	96,154	4.98	102,596	5.01	0.03
1:2	48,077	4.68	56,127	4.75	0.07
1:4	24,038	4.38	28,587	4.46	0.08
1:8	12,019	4.08	14,858	4.17	0.09
1:16	6,010	3.78	6,452	3.81	0.03
1:32	3,005	3.48	2,671	3.43	−0.05
1:64	1,502	3.18	791	2.90	−0.28
1:128	751	2.88	1,057	3.02	0.15

[a] The Spearman correlation coefficient between expected and observed values was 0.98 ($P < 0.01$).

tected in the biopsy from the HBV-positive HIV-positive HCV-negative control patient.

(ii) Three HCV RNA-positive specimens were processed by the standard protocol and tested by the b-DNA assay without target and captures probes to mediate capture: no specimens yielded positive signals.

(iii) To verify whether cellular RNA could have any quenching effect on the b-DNA signal, reducing specific hybridization, a serum sample of 19,231 HCV RNA IU/ml was tested in duplicate alone and with increasing amounts (0.5 to 8 μg) of RNA extracted from an HCV-negative liver biopsy. No statistically significant difference was detected ($P = 1.00$) between HCV RNA load of the serum sample and the samples for which increasing amounts of RNA were added, with a constant difference of <0.5 $\log_{10}$.

Linearity and sensitivity of the b-DNA assay. HCV RNA extracted from a liver sample and quantified as 96,154 IU/μg of RNA was diluted in a series of twofold step dilutions with RNase-free water, and seven prepared dilutions were assessed in duplicate and in parallel with a high-titer HCV RNA routine clinical serum sample.

A good linearity was observed when the expected and experimental values were compared ($r = 0.98$; $P < 0.01$) and the difference ranged from -0.28 to 0.15 $\log_{10}$ (Table 2). The b-DNA assay on liver biopsy samples provided a detection sensitivity of ca. 1,000 HCV RNA IU/μg of RNA.

Reproducibility of the b-DNA assay. In order to evaluate the reproducibility of HCV quantification in liver tissue, multiple aliquots of RNA extracted from biopsies from six seropositive patients were tested. The results were highly reproducible: the intrarun CV was between 0.5 and 13.0% and interrun variability was between 3.5 and 24.7%.

HCV RNA in liver biopsy and serum samples. HCV-specific detection and quantification was obtained in all liver biopsies tested, including the three samples from the HIV-negative HCV-positive patients, which were evaluated as controls. The two HIV-positive HCV-positive patients with a virological sustained response had no detectable HCV RNA on liver, and they will be further analyzed by a qualitative assay with lower sensitivity (TMA; Bayer).

High HCV RNA concentrations were observed both in serum samples (median, 498,471; range, 69,235 to 3,816,553 IU/ml) and liver samples (median, 231,495; range, 16,353 to 1,240,513 IU/μg). The relationship between HCV RNA levels in liver and serum was determined in all liver-serum pairs, and a positive statistically significant correlation was observed, although it was not strong ($r = 0.63$; $P < 0.01$).

A virological end-of-treatment response (after 24 weeks of therapy [T6]) in serum was associated with a lower pretreatment intrahepatic HCV viral load ($P = 0.03$) (Table 3). A direct association, although not statistically significant, emerged between an increase of 20,000 IU of liver HCV RNA and no virological response (OR = 1.10; 95% CI = 0.99 to 1.22) (Table 3).

When the different genotype groups were analyzed, the median levels of liver HCV RNA were statistically higher in patients with genotype 1 (including genotypes 1a and 1b; 749,852 IU/μg) compared to genotype 3a (192,737 IU/μg). No statistical difference was found when HCV RNA levels in serum among the other different genotype groups were compared (Table 4).

DISCUSSION

The performance of the Versant HCV RNA 3.0 b-DNA assay has been recently assessed to quantitate HCV RNA levels in serum specimens (27). In the new b-DNA assay, isoC and isoG nucleotides are used in the amplification molecules to reduce background signals and to allow stronger amplification.

In the present study, we evaluated the performance characteristics of this updated assay for detecting HCV viral load in liver biopsy samples. Liver specimens from HIV-HCV-coinfected patients were used to develop a method for the extraction of viral RNA for subsequent quantification by b-DNA.

TABLE 3. Comparison of HCV RNA levels in serum before (T0) and at the end of therapy (T6) and in liver (T0) among HIV-HCV-coinfected patients with or without a virological end-of-treatment response

Patient group (n)	HCV RNA[a] level in:			
	Serum (IU/ml) at:			Liver (IU/μg) at T0[d]
	T0[b]	T3[c]	T6	
Patients with a virological response (7)	523,252	<615	<615	192,737
Patients with no virological response (6)	1,465,203	35,645	149,319	666,355

[a] P values were calculated by using the Mann-Whitney U test. The OR value is that of the no-virological-response patients relative to an increase of 20,000 IU of HCV RNA.
[b] $P = 0.25$; OR = 1.01; 95% CI = 0.99 to 1.04.
[c] $P = 0.01$.
[d] $P = 0.03$; OR = 1.10; 95 CI = 0.99 to 1.22.

TABLE 4. Median HCV RNA loads in liver and serum grouped according to HCV genotype and comparison of viral loads across genotypes[a]

Genotype (*n*)	Median HCV RNA load in:	
	Liver (IU/μg)	Serum (IU/ml)
Genotype 1 (7)	749,852	1,465,203
Genotype 3 (13)	192,737	358,080
Genotype 4 (3)	510,341	473,689

[a] Comparisons (calculated by using the Mann-Whitney U test) were as follows for liver and serum, respectively: genotype 1 versus 3, $P < 0.01$ and $P = 0.11$; genotype 1 versus 4, $P = 0.21$ and $P = 0.12$; and genotype 3 versus 4, $P = 0.25$ and $P = 0.74$.

Regarding the choice of method for the extraction of RNA from tissue, there are conflicts between maximizing purity and maximizing nucleic acid recovery: techniques designed to yield highly purified RNA, because of their complexity, can lead to reduced quantitative recovery. The purity required for b-DNA amplification is less than that required for PCR, since inhibitors of *Taq* polymerase must be removed from the liver for PCR but not for b-DNA (13). The guanidinium thiocyanate–phenol-chloroform procedure is a simple and efficient method of extraction from liver tissue (7, 26), and the amount of extracted HCV RNA obtained was in agreement with previous reports (2).

Quantitation of extracted HCV RNA by the b-DNA approach was sensitive, with a viral load detected in as little as 1 mg of liver tissue, and specific as no signal was found in any of the HCV-negative controls. We also showed that cellular RNA does not interfere with the specific signal by artificially increasing or decreasing HCV RNA titers.

When precision was evaluated, the HCV RNA 3.0 assay yielded reproducible results with intra- and interassay CVs similar to those reported for the standard serum procedure (27).

A linear response between observed and expected HCV RNA concentrations was observed when a dynamic quantification range of 96,154 to 769 IU/μg was considered.

An accurate measurement of viral load may be important when pretreatment predictors of response to antiviral treatments are evaluated since HCV eradication in the liver is also an important endpoint of therapy. However, studies utilizing viral quantitation in the liver are limited by the absence of reliable standards necessary for comparison between studies and between serum and liver compartments. In fact, using the different quantitative HCV RNA assays, the levels of intrahepatic HCV RNA have been reported to vary widely, depending on how liver viral load measurements have been expressed: the number of copies per milligram or per gram of liver tissue or the number of copies per microgram of total RNA (2, 25, 26, 28). Furthermore, data on the correlation between concentrations in liver and concentrations in serum or plasma and on the viral load ratios of the liver versus serum (2, 6, 12, 14, 18, 24, 25, 28, 29) also conflict, mostly because no accurate numerical constant or conversion factor has been defined (16, 26). It must also be taken into account that these discrepancies might be due to the different series and numbers of patients involved and to the different techniques used, which possibly shared diverging patterns of sensitivity and reliability when tested with different samples (plasma or serum or liver biopsy tissue). The question of how to express HCV viral load obtained from tissues remains still open. Since uniform standards have not been identified for determining viral intrahepatic levels, since there is less accuracy in weighing these small biopsies versus determining the concentration of extracted RNA from liver tissue, and since there are different relative proportions of weight contributed by nonparenchymal cells and hepatocytes from each biopsy (8, 26), we believe that correlating HCV viral load to the total extracted RNA from the tissue may limit the disadvantages inherent to quantitative molecular assays.

The present study demonstrated that the Versant HCV RNA 3.0 assay was effective in the quantitation of HCV RNA levels in the liver. By applying this assay, we found that patients who were HCV RNA negative in the serum after 6 months of therapy were characterized by lower intrahepatic pretreatment virus levels compared to patients who did not respond to treatment. Furthermore, we also showed that patients infected with genotype 1 had a higher tissue viral load than patients infected with other HCV genotypes tested. The reasons and the possible prognostic implications of these observations are not clear at the moment.

In summary, we have described a simple and efficient method for extracting HCV RNA from liver tissue that can then be reliably detected and quantified by the Versant b-DNA 3.0 method. A prospective study is ongoing to evaluate the levels in serum and intrahepatic levels in patients treated with anti-HCV therapeutic regimens that may contribute to understand the pathogenesis of HCV-associated liver disease and thus yield valuable insight into the clinical management of HIV-positive patients with chronic hepatitis.

ACKNOWLEDGMENT

We thank Laura Catozzi (Bayer Diagnostics, Milan, Italy) for providing helpful advice.

REFERENCES

1. **Adinolfi, L. E., A. Andreana, R. Utili, R. Zampino, E. Ragone, and G. Ruggiero.** 1998. HCV RNA levels in serum, liver, and peripheral blood mononuclear cells of chronic hepatitis C patients and their relationship to liver injury. Am. J. Gastroenterol. **93:**2162–2166.
2. **Ballardini, G., A. Manzin, F. Giostra, R. Francesconi, P. Groff, A. Grassi, L. Solforosi, S. Ghetti, D. Zauli, M. Clementi, and F. B. Bianchi.** 1997. Quantitative liver parameters of HCV infection: relation to HCV genotypes, viremia and response to interferon treatment. J. Hepatol. **26:**779–786.
3. **Bonacini, M., S. Govindarajan, L. M. Blatt, P. Schmid, A. Conrad, and K. L. Lindsay.** 1999. Patients coinfected with human immunodeficiency virus and hepatitis C virus demonstrate higher levels of hepatic HCV RNA. J. Viral Hepatol. **6:**203–208.
4. **Breslow, N. E., and N. E. Day.** 1980. Statistical methods in cancer research, vol. I. The analysis of case-control studies. IARC scientific publication no. 32. International Agency for Research on Cancer, Lyon, France.
5. **Chomczynski, P., and N. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162:**156–159.
6. **Coelho-Little, E., L. J. Jeffers, M. Bartholomew, K. R. Reddy, E. R. Schiff, and P. J. Dailey.** 1995. Correlation of HCV-RNA levels in serum and liver of patients with chronic hepatitis C. J. Hepatol. **22:**508.
7. **Cox, R. A.** 1968. The use of guanidinium chloride in the isolation of nucleic acids. Methods Enzymol. **12B:**120–129.
8. **Dash, S., R. Saxena, J. Myung, T. Rege, H. Tsuji, P. Gaglio, R. F. Garry, S. N. Thung, and M. A. Gerber.** 2000. HCV RNA levels in hepatocellular carcinomas and adjacent non-tumorous livers. J. Virol. Methods **90:**15–23.
9. **Davis, G. L., R. Esteban-Mur, V. Rustgi, J. Hoefs, S. C. Gordon, C. Trepo, M. L. Shiffman, S. Zeuzem, A. Craxi, M. H. Ling, J. Albrecht, et al.** 1998. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. N. Engl. J. Med. **339:**1493–1499.
10. **Eyster, M. E., L. S. Diamondstone, J. M. Lien, W. C. Ehmann, S. Quan, J. J.**

Goedert, et al. 1993. Natural history of hepatitis C virus infection in multitransfused hemophiliacs: effect of coinfection with human immunodeficiency virus. J. Acquir. Immune Defic. Syndr. **6:**602–610.
11. **Fong, T. L., M. Shindo, S. M. Feinstone, J. H. Hoofnagle, and A. M. Di Bisceglie.** 1991. Detection of replicative intermediates of hepatitis C viral RNA in liver and serum of patients with chronic hepatitis C. J. Clin. Investig. **88:**1058–1060.
12. **Hasui, T., H. Shimomura, H. Tsuji, M. Wato, and T. Tsuji.** 1994. Quantitation of hepatitis C virus RNA in liver tissue as a predictive marker of the response to interferon therapy in chronic hepatitis C. Acta Med. Okayama **48:**151–157.
13. **Higuchi, R. G., and H. Ochman.** 1989. Production of single-stranded DNA templates by exonuclease digestion following the polymerase chain reaction. Nucleic Acids Res. **17:**5865.
14. **Idrovo, V., P. J. Dailey, L. J. Jeffers, E. Coelho-Little, D. Bernstein, M. Bartholomew, L. Alvarez, M. S. Urdea, M. L. Collins, and E. R. Schiff.** 1996. Hepatitis C virus RNA quantification in right and left lobes of the liver in patients with chronic hepatitis C. J. Viral Hepatol. **3:**239–246.
15. **Martin, J., S. Navas, J. A. Quiroga, G. Colucci, M. Pardo, and V. Carreno.** 1998. Quantitation of hepatitis C virus in liver and peripheral blood mononuclear cells from patients with chronic hepatitis C virus infection. J. Med. Virol. **54:**265–270.
16. **McGuinness, P. H., G. A. Bishop, D. M. Painter, R. Chan, and G. W. McCaughan.** 1996. Intrahepatic hepatitis C RNA levels do not correlate with degree of liver injury in patients with chronic hepatitis C. Hepatology **23:** 676–687.
17. **McHutchison, J. G., S. C. Gordon, E. R. Schiff, M. L. Shiffman, W. M. Lee, V. K. Rustgi, Z. D. Goodman, M. H. Ling, S. Cort, J. K. Albrecht, et al.** 1998. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. N. Engl. J. Med. **339:**1485–1492.
18. **Nakagawa, H., H. Shimomura, T. Hasui, H. Tsuji, and T. Tsuji.** 1994. Quantitative detection of hepatitis C virus genome in liver tissue and circulation by competitive reverse transcription-polymerase chain reaction. Dig. Dis. Sci. **39:**225–233.
19. **Nasti, G., G. Di Gennaro, M. Tavio, L. Cadorin, R. M. Tedeschi, R. Talamini, A. Carbone, and U. Tirelli.** 2001. Chronic hepatitis C in HIV infection: feasibility and sustained efficacy of therapy with interferon alfa-2b and tribavirin. AIDS **15:**1783–1787.
20. **Neau, D., P. Trimoulet, M. Winnock, B. Le Bail, E. Schvoerer, E. Legrand, J. M. Ragnaud, M. Dupon, H. Fleury, and M. E. Lafon.** 2001. Impact of protease inhibitors on intrahepatic hepatitis C virus viral load. AIDS **15:** 1736–1738.
21. **Pawlotsky, J. M.** 2002. Molecular diagnosis of viral hepatitis. Gastroenterology **122:**1554–1568.
22. **Poles, M. A., E. A. Lew, and D. T. Dieterich.** 1997. Diagnosis and treatment of hepatic disease in patients with HIV. Gastroenterol. Clin. N. Am. **26:**291–321.
23. **Poynard, T., P. Marcellin, S. S. Lee, C. Niederau, G. S. Minuk, G. Ideo, V. Bain, J. Heathcote, S. Zeuzem, C. Trepo, J. Albrecht, et al.** 1998. Randomised trial of interferon α2b plus ribavirin for 48 weeks or for 24 weeks versus interferon α2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet **352:**1426–1432.
24. **Sakamoto, N., N. Enomoto, M. Kurosaki, F. Marumo, and C. Sato.** 1994. Detection and quantification of hepatitis C virus RNA replication in the liver. J. Hepatol. **20:**593–597.
25. **Sugano, M., Y. Hayashi, S. Yoon, M. Kinoshita, T. Ninomiya, K. Ohta, H. Itoh, and M. Kasuga.** 1995. Quantitation of hepatitis C viral RNA in liver and serum samples using competitive polymerase chain reaction. J. Clin. Pathol. **48:**820–825.
26. **Terrault, N. A., P. J. Dailey, L. Ferrell, M. L. Collins, J. C. Wilber, M. S. Urdea, B. N. Bhandari, and T. L. Wright.** 1997. Hepatitis C virus: quantitation and distribution in liver. J. Med. Virol. **51:**217–224.
27. **Trimoulet, P., P. Halfon, E. Pohier, H. Khiri, G. Chene, and H. Fleury.** 2002. Evaluation of the Versant HCV RNA 3.0 assay for quantification of hepatitis C virus RNA in serum. J. Clin. Microbiol. **40:**2031–2036.
28. **Trimoulet, P., D. Neau, B. Le Bail, A. Rullier, M. Winnock, T. Galperine, E. Legrand, E. Schvoerer, M. Dupon, J. M. Ragnaud, P. Bioulac-Sage, G. Chene, H. Fleury, and M. E. Lafon.** 2002. Intrahepatic HCV RNA loads in 37 HIV-HCV coinfected patients with controlled HIV infection. J. Med. Virol. **67:**143–151.
29. **White, P. A., Y. Pan, A. J. Freeman, G. Marinos, R. A. Ffrench, A. R. Lloyd, and W. D. Rawlinson.** 2002. Quantification of hepatitis C virus in human liver and serum samples by using LightCycler reverse transcriptase PCR. J. Clin. Microbiol. **40:**4346–4348.