JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 1992, p. 2145–2149
0095-1137/92/082145-05$02.00/0

Vol. 30, No. 8

# Use of Aminotransferase, Hepatitis C Antibody, and Hepatitis C Polymerase Chain Reaction RNA Assays To Establish the Diagnosis of Hepatitis C Virus Infection in a Diagnostic Virology Laboratory

DAVID GRETCH, WILLA LEE, AND LAWRENCE COREY*

*The Departments of Laboratory Medicine, Microbiology, and Medicine, University of Washington, Seattle, Washington 98144*

Received 10 March 1992/Accepted 20 May 1992

**Clinical and therapeutic decisions for hepatitis C virus (HCV) infection depend on factors that include documentation of past infection as well as identification of those who might benefit from antiviral chemotherapy with systemic interferon. To evaluate the ability of a diagnostic laboratory to accurately identify such patients, we compared results obtained with serum transaminase assays, two HCV antibody assays (enzyme immunoassay [EIA] and immunoblot), and a polymerase chain reaction (PCR)-based assay for HCV RNA using a group of consecutively submitted samples within our university-based diagnostic virology laboratory and sera from a population of random blood donors. One hundred percent of specimens with *R* values of greater than 3.0 in the HCV EIA were positive in the confirmatory immunoblot. However, 25% of specimens with EIA *R* values of between 1.0 and 3.0 were not confirmed by either recombinant immunoblot assay (RIBA) or RNA PCR assay (false-positive specimens). A significant correlation ($P < 0.01$) between increasing reactivity in the RIBA and positivity in the RNA PCR assay was found. The incidence of HCV viremia, as determined by the RNA PCR assay, was 73% for confirmed seropositive specimens, 33% for seropositive specimens with indeterminate RIBA results, 12% for seronegative specimens obtained from infected patients, and 2.0% for seronegative specimens obtained from uninfected blood donors. In contrast, serum transaminase testing did not correlate with the RNA PCR assay for HCV. Use of the EIA and immunoblot assay followed by RNA PCR testing will identify most patients who are viremic with HCV.**

Accurate testing for hepatitis C virus (HCV) has both clinical and epidemiologic importance. Antibody testing has been used to screen blood and organ donors. More recently, HCV RNA detection by the polymerase chain reaction (PCR) has been utilized to diagnose chronic hepatitis C and to monitor patients who are undergoing therapy with systemic interferon (1, 2, 5).

Identification of persons at risk for transmitting or acquiring HCV infection and identifying candidates for therapy are important functions of a regional virology service. We assayed levels of transaminases and antibodies to HCV in serum using the currently licensed enzyme immunoassay (EIA), a recently developed recombinant immunoblot assay (RIBA), and PCR testing for HCV RNA.

## MATERIALS AND METHODS

**Patient population and specimen processing.** A total of 119 serum samples submitted to the University of Washington Medical Center Clinical Virology Laboratory for anti-HCV testing between 1 August and 1 September 1991 were studied. The sera chosen for this study were selected on the basis of the presence or absence of antibody to HCV as detected by EIA (Abbott Laboratories, Abbott Park, Ill.). A total of 42 consecutive EIA-negative serum samples, 32 consecutive serum samples with EIA ratio (*R*) values of between 1 and 3, and 45 consecutive serum samples with *R* values of greater than 3 were chosen for further study. Seventy-five percent of these serum samples had elevated levels of transaminase in serum, indicating that they were drawn from patients with biochemical evidence of hepatitis at the time of analysis. We also obtained 42 consecutive HCV-seronegative specimens from prospective blood donors at the Puget Sound Blood Center (Seattle, Wash.), representing a healthy (nonhepatitis) control population. The blood center uses volunteer donors whose histories are screened to yield those with a low risk of transmitting blood-borne infection. One hundred percent of the blood donor serum samples had normal serum transaminase levels. All serum samples were processed through the routine collection methods at the hospital as follows. Blood was drawn into Vacutainer tubes at a central processing facility and centrifuged at room temperature (maximum processing time, 2 h), and the serum samples were stored at 4°C prior to and during HCV serologic testing (average time at 4°C was 1 to 2 days); following this, aliquots were stored at −70°C until subsequent analysis for serum transaminase levels and HCV RNA. All testing was completed within a 6-week period following venipuncture; all HCV antibody assays, RNA PCR, and transaminase level studies were run without knowledge of the results from any other laboratory assays.

**Serologic and serum transaminase assays.** The EIA for antibody (Abbott Laboratories) utilizes a recombinant HCV antigen, designated C-100, which is a fusion protein containing an HCV nonstructural gene product combined with *Saccharomyces cerevisiae* superoxide dismutase (6). EIA was run daily according to the manufacturer's specifications. Sera were classified as EIA negative if the *R* values (ratios of the specimen optical densities over negative control sera optical densities) were less than 0.8, as EIA low positive if

* Corresponding author.

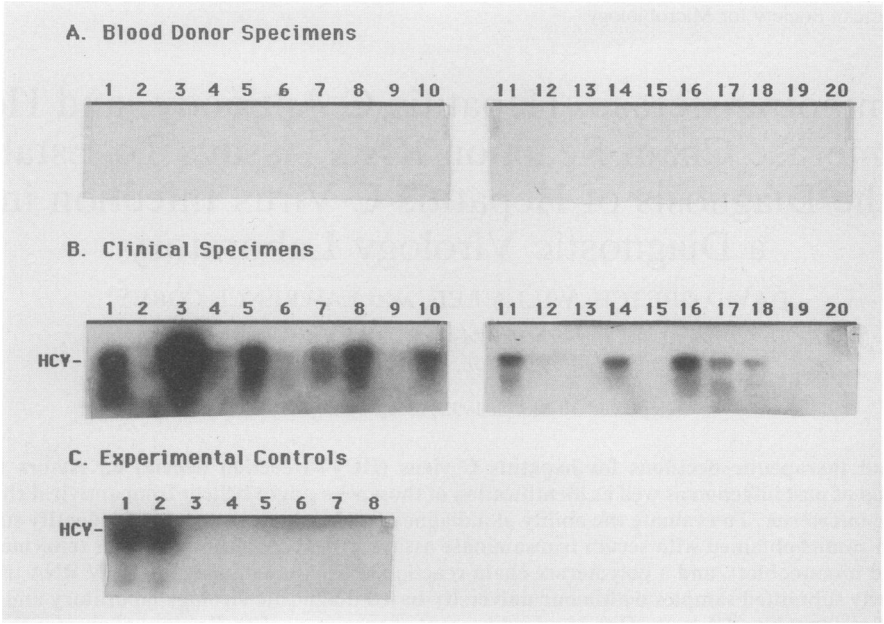


FIG. 1. Detection of HCV RNA by PCR plus liquid hybridization. (A) Sera from 20 HCV-seronegative blood donors with normal serum transaminase levels; (B) sera from patients with antibodies to HCV by both EIA and RIBA; (C) sera from two patients with documented active hepatitis C included as positive controls in each run (lanes 1 and 2). Negative controls included sera from two healthy blood donors previously shown to be HCV antibody and antigen negative (lanes 3 and 4), water in the cDNA reaction (lanes 5 and 6), and water in the PCR reaction (lanes 7 and 8).

the *R* values were between 1 and 3, and as EIA high positive if the *R* values were greater than 3. None of the specimens screened for this study were in the grey zone of 0.8 to 0.99. All specimens were also tested by an immunoblot assay, RIBA (Ortho Diagnostics, Ranton, N.J.), which consists of four recombinant HCV antigens immobilized on strips in an immunoblot format (10). The viral antigens are derived from HCV nonstructural genes (33c from NS-3 and c-100 plus 5-1-1 from NS-4) and the putative nucleocapsid (core) gene (c22-3). Additional antigens on the immunoblot strips include two strip positive controls (a strong-positive and a weak-positive control) and recombinant superoxide dismutase, which serves as a control for anti-superoxide dismutase antibodies. Patient sera, positive control sera, and negative control sera are reacted with the immunoblot strips and then with peroxidase-conjugated goat anti-human immunoglobulin G. For a viral band to be positive, it must have an intensity greater than that of the weak-positive strip control. A positive RIBA result is indicated by at least two positive viral bands, while a negative result occurs when no viral bands are reactive. An indeterminate result is indicated by a single positive viral band (see Fig. 2).

Biochemical tests for aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were performed by a COBAS FARA system (Hoffmann-La Roche, Nutley, N.J.), a fully automated multienzyme analyzer in the Clinical Chemistry Laboratory at the University of Washington Medical Center. The normal range for AST is 9 to 27 U/liter; the ALT normal range is 6 to 32 U/liter.

**RNA PCR and liquid hybridization assays for HCV genomic sequences.** The RNA PCR assay for HCV was performed by a modification of the procedure described by Han et al. (4), which takes advantage of the highly conserved nucleotide sequences within the 5′ noncoding region of the HCV genome. RNA was extracted from 100 μl of sera by the proteinase K-sodium dodecyl sulfate method, followed by phenol, phenol-chloroform, and chloroform extractions. cDNA was synthesized with the JHC51 antisense primer and amplified with the JHC52 and JHC93 PCR primers by a method described previously (4). Oligonucleotide primers were synthesized according to the published HCV sequences by using an Applied Bio Systems DNA Synthesizer. PCR amplification products were detected by liquid hybridization (3). Briefly, 15 μl of viral amplification product was directly mixed with 5 μl of $^{32}$P-end-labeled HCV-specific oligonucleotide probe A89 (4) in 60 mM NaCl and 30 mM EDTA. Approximately $2 \times 10^5$ cpm of probe per reaction mixture was used for optimum results. The product-probe mixtures were heated to 95°C for 7 min to dissociate DNA hybrids and then to 55°C for 18 min to allow reannealing. The product-probe mixtures were immediately analyzed by electrophoresis in 6 to 10% polyacrylamide gels and then autoradiographed. In a series of studies that used in vitro dilution experiments to evaluate specimens from 20 consecutive patients with chronic hepatitis C, we demonstrated that the liquid hybridization assay is approximately as sensitive as the nested-set PCR; it has the advantages of being quicker and less subject to contamination (3). To avoid contamination of specimens with amplified DNA, a separate containment facility was utilized to isolate PCR products from the serology laboratory and PCR setup areas, in addition to other recommended precautions (7). For each RNA PCR experiment, negative controls were added at the RNA extraction, cDNA synthesis, and PCR amplification steps.

## RESULTS

**Detection of HCV RNA by one-stage PCR plus liquid hybridization.** Figure 1 demonstrates a representative liquid hybridization assay for detecting HCV genomic sequences amplified by one-stage RNA PCR from the sera from 20 random seronegative blood donors (Fig. 1A) and 20 patients

TABLE 1. Association between HCV antibody testing by EIA and RIBA and HCV RNA testing by PCR among 119 patients with suspected hepatitis C and 42 normal blood donors

| Specimen source and EIA result | No. (%) | | | | | |
|---|---|---|---|---|---|---|
| | Total specimens | EIA negative | RIBA negative | RIBA indeterminate | RIBA positive | HCV PCR |
| Clinical specimens | | | | | | |
| Negative EIA ($R < 0.8$) | 42 | 42 (100) | 37 (88) | 4 (10) | 1 (2) | 5 (12) |
| Low-positive EIA ($R$, 1 to 3) | 32 | | 8 (25) | 5 (16) | 19 (59) | 16 (50) |
| High-positive EIA ($R > 3$) | 45 | | 0 (0) | 0 (0) | 45 (100) | 32 (71) |
| Normal blood donors | 42 | 42 (100) | 39 (93) | 3 (7) | 0 (0) | 1 (2) |

with HCV confirmed by EIA and RIBA (Fig. 1B). All 20 blood donors lacked evidence of HCV RNA in their sera, while 12 of 20 seropositive patients tested positive in the RNA PCR assay (Fig. 1B, lanes 1, 3, 4, 5, 7, 8, 10, 11, 14, 16, 17, and 18).

**Comparison of EIA, RIBA, and serum transaminase and RNA PCR assays.** The results of HCV antibody testing by EIA and RIBA for the 119 clinical specimens and the 42 control blood donor serum samples are presented in Table 1. All 45 specimens from patients with suspected hepatitis C which had EIA $R$ values of greater than 3 were also positive in the confirmatory RIBA; 82% had antibodies to all four viral proteins (Fig. 2, lane 3), 14% had antibodies to three viral proteins, and 5% had antibodies to two viral proteins. Among patients with a high-positive EIA antibody result, 84% had antibodies to 33C, 93% had antibodies to c22-3, and 100% had antibodies to c-100 and 5-1-1. A total of 32 (71%) of these 45 patients also had HCV RNA detected by RNA PCR.

Specimens with a low degree of reactivity in the EIA assay ($R$ values of 1 to 3) had a lower prevalence of HCV RNA detection and lower rate of RIBA confirmation than those that were high positive by EIA. Fifty percent of the low-positive HCV antibody serum samples had HCV RNA detected by PCR ($P < 0.05$ [comparison with high-positive group]). In addition, of the 32 specimens from patients with suspected hepatitis C who had low-positive results in the EIA for antibody, 19 were RIBA positive ($P < 0.05$ [comparison with high-positive specimens]), 8 were RIBA negative, and 5 were RIBA indeterminate. Examples of specimens giving indeterminate RIBA results are shown in Fig. 2, lanes 4 (C22-3 indeterminate) and 5 (C100-3 indeterminate).

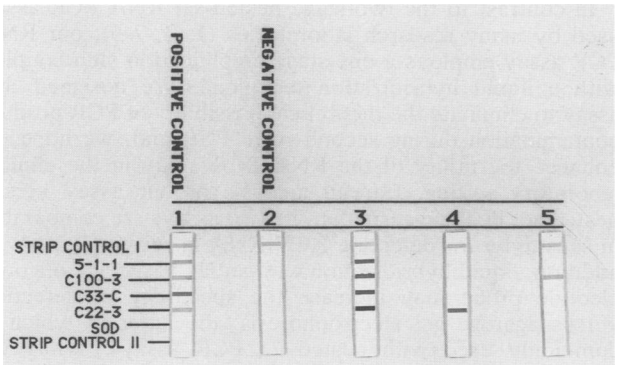


FIG. 2. RIBA for antibody to HCV. Lanes 1 and 2, positive and negative controls, respectively; lane 3, a positive RIBA profile from a patient who was RNA PCR positive; lanes 4 and 5, RIBA-indeterminate results from patients who were RNA PCR positive and negative, respectively. SOD, superoxide dismutase.

Within the low-positive EIA category, specimens with higher reactivities in the RIBA had greater incidences of positivity in the RNA PCR assay; 0% of RIBA-negative, 40% of RIBA-indeterminate, and 73% of RIBA-positive specimens tested positive by RNA PCR. Thus, an EIA value of greater than 3 or a positive RIBA result identified a group of patients with HCV RNA in their sera. In addition, it appears that 25% of the low-positive EIA results were false positive, as evidenced by their negativity in both the RIBA and the RNA PCR assay.

The correlation among HCV antibody assays, serum transaminase assays, and RNA PCR for HCV is presented in Table 2. Among the 64 EIA-positive, RIBA-positive specimens, serum transaminase levels were elevated in 52 (81%) and were elevated to greater than twice the upper limits of normal in 37 (58%). HCV RNA was detected in 46 (72%) of these specimens. HCV RNA was detected in 4 of 12 (33%) RIBA-indeterminate specimens versus 4 of 84 (5%) RIBA-negative specimens ($P < 0.01$), suggesting that in our population, a RIBA-indeterminate assay identifies patients with ongoing HCV infection. Serum transaminases were elevated in 6 of 12 (50%) RIBA-indeterminate specimens versus 31 of 84 (37%) RIBA-negative specimens ($P > 0.5$).

**Lack of correlation between serum transaminase values and HCV RNA detection.** Table 3 summarizes the frequency of HCV viremia detected by RNA PCR in all clinical and blood donor specimens tested, stratified by increasing RIBA reactivity and by serum transaminase results. The percentage of specimens for which HCV RNA could be detected by PCR

TABLE 2. Correlation between HCV antibody assays, serum transaminase assays, and PCR for HCV RNA

| Test results | No. (%) RNA PCR positive | No. RNA PCR negative |
|---|---|---|
| EIA+, RIBA+ | | |
| AST and/or ALT+ | 38 (73) | 14 |
| AST and ALT− | 8 (66) | 4 |
| EIA+, RIBA− | | |
| AST and/or ALT+ | 0 | 7 |
| AST and ALT− | 0 | 1 |
| EIA+, RIBA indeterminate | | |
| AST and/or ALT+ | 1 (25) | 3 |
| AST and ALT− | 1 (100) | 0 |
| EIA−, RIBA indeterminate | | |
| AST and/or ALT+ | 1 (50) | 1 |
| AST and ALT− | 1 (20) | 4 |
| EIA−, RIBA− | | |
| AST and/or ALT+ | 3 (13) | 21 |
| AST and ALT− | 1 (2) | 51 |
| EIA−, RIBA+ | | |
| AST and ALT+ | 1 (100) | 0 |

TABLE 3. Detection of HCV nucleic acid in 119 clinical specimens and 42 blood donor serum samples by RNA PCR assay

| Results of RIBA and serum transaminase assay (AST and ALT) | No. of specimens | No. (%) positive for HCV by RNA PCR assay |
|---|---|---|
| RIBA negative, AST and ALT normal | 53 | 1 (2) |
| RIBA negative, AST and/or ALT elevated | 31 | 3 (10) |
| RIBA indeterminate, AST and ALT normal | 6 | 2 (33) |
| RIBA indeterminate, AST and/or ALT elevated | 6 | 2 (33) |
| RIBA positive, AST and ALT normal | 12 | 8 (67) |
| RIBA positive, AST and/or ALT elevated | 53 | 39 (74) |

was associated with the level of reactivity as determined by the RIBA but not that determined by transaminase testing. For example, the frequency of detecting HCV RNA by PCR rose from 4 of 84 (4.8%) in RIBA-negative specimens to 47 of 65 (72%) in RIBA-positive specimens. Transaminase levels had no impact on the percentage of specimens with a positive RNA PCR result.

**Specificity of HCV assays.** To evaluate the specificity of the HCV serologic assays, we analyzed two groups of specimens with negative HCV EIA test results by RIBA and RNA PCR. The first group included 42 consecutive samples from patients with suspected hepatitis C who were EIA negative. Abnormal serum transaminase assay values were present in 28 (67%) of 42. The second group included 42 consecutive blood donor serum samples, of which none had biochemical evidence of hepatitis. Of the EIA-negative specimens from patients with suspected hepatitis C, 37 (88%) were RIBA negative, 4 (10%) were RIBA indeterminate, and 1 (2%) was RIBA positive. Five (12%) of these specimens had HCV RNA in serum: one was RIBA positive, one was RIBA indeterminate, and three were RIBA negative. These five serum samples were rerun in the HCV RNA assay by both liquid hybridization and the nested-set PCR technique and remained positive in repeat assays. Among the specimens obtained from healthy blood donors, 39 (93%) were RIBA negative, 3 were RIBA indeterminate, and 0 were RIBA positive. One of these specimens (RIBA indeterminate) had HCV RNA in serum. Thus, the specificity of EIA for detecting HCV RNA in serum was 93%. Calculation of the specificity of the RIBA for detection of HCV antibodies depends on the interpretation of indeterminate-RIBA results. If an indeterminate RIBA is considered a positive test result, the specificity of this assay was 94%. If an indeterminate RIBA is considered negative, the test specificity was 100%. Since there is a correlation between indeterminate-RIBA results and PCR positivity, we feel that the 94% value is the correct one.

If one uses the RNA PCR assay and RIBA as "gold standards" for identifying HCV-positive individuals, the sensitivities of EIA, RIBA, and the serum transaminase assays in our infected population were 92, 94, and 80%, respectively. The specificities of these tests for infected specimens were 80% for EIA, 100% for RIBA (by assumption), and 32% for serum transaminase assays.

## DISCUSSION

Using consecutive serum specimens submitted to our Clinical Virology Laboratory for evaluation of suspected hepatitis C, we compared a screening serologic assay (EIA), a confirmatory serologic assay (RIBA), biochemical tests for active hepatitis (ALT and AST), and a molecular assay for HCV genomic sequences (RNA PCR). Our data comparing EIA with RNA PCR suggest that the sensitivity of the first-generation EIA may be as high as 92% for detecting active HCV infection in infected patients. However, it was apparent that a negative EIA result did not rule out HCV infection in these patients or in uninfected blood donors, since 14% of EIA-negative clinical specimens and 2.5% of random blood donors were positive by the RNA PCR assay. The current study also demonstrates that a high-positive *R* value in the HCV EIA is very likely to be a true-positive value on the basis of RIBA and RNA PCR, while at least 25% of low-positive EIA results (*R* value of between 1 and 3) represent false-positive results. The second-generation RIBA appeared to be only slightly more sensitive than the first-generation EIA for the detection of HCV infection, since 11% of RIBA-negative specimens were positive in the RNA PCR assay. However, 72% of RIBA-positive specimens were positive in the RNA PCR assay, compared with 61% of EIA-positive specimens, confirming that RIBA is more specific than EIA for detecting active HCV infection.

Of the nine clinical specimens identified with indeterminate RIBAs, three (33%) tested positive in the RNA PCR assay. In addition, one EIA-negative blood donor specimen was RIBA indeterminate and positive by RNA PCR assay. Although the sample size is not large enough for firm conclusions to be drawn, the data suggest that the indeterminate RIBA may identify some patients with active HCV infections. The RNA PCR assay positivity rates of 72% for RIBA-positive and 33% for RIBA-indeterminate clinical specimens are very similar to those in the findings of Weiner et al. (11) with blood donor sera. Additional clinical studies are necessary to precisely define the predictive value of an indeterminate RIBA in individual patients and the continued specificity of HCV RNA PCR testing.

While the majority of RNA PCR-positive specimens had elevated serum transaminase levels, 9 of 56 (16%) had normal AST and ALT levels, despite a positive RNA PCR assay. This suggests that a significant percentage of patients with active HCV infections lack biochemical evidence of hepatitis. Whether this finding represents subclinical hepatitis or possibly a second site of HCV replication (other than the liver) remains undetermined. Since serum transaminase values may oscillate between normal and low positive during chronic hepatitis (1), a single determination would not be as sensitive as repetitive testing.

In contrast to the two-stage nested-set RNA PCR assay used by many research laboratories (1, 2, 7–9), our RNA PCR assay employs a one-stage amplification step coupled with a liquid hybridization technique. We designed this assay to eliminate the theoretical possibility of PCR product contamination during second-stage PCR and, we hope, to enhance the utility of the RNA PCR assay in the clinical laboratory setting. Careful analysis of our assay versus nested-set PCR suggests that the two assays are comparable in sensitivity for detecting HCV RNA in serum (3, 10a). In addition, liquid hybridization with an HCV-specific oligonucleotide probe may increase the specificity of detection versus agarose gel electrophoresis, the latter of which is commonly used with nested-set PCR assays. While we cannot with assurance eliminate the possibility of an occasional false-positive RNA PCR result, the repeated positivity in two separate assays and the strong association between PCR results and both HCV antibody status and clinical source of the specimen (blood donor versus diseased patient)

all favor the accuracy of our results. The specificity of our PCR assay can be inferred from the high rate of positivity with RIBA-positive specimens (72%), the low rate of positivity with blood donor specimens (2.5%), and the absence of positive results in all negative-control specimens analyzed to date (data not shown). Thus, it appears that confirmation of current EIA results with the recently developed RIBA constitutes a reasonable method for the screening of patients with ongoing HCV infections. Such patients are candidates for potential referral for antiviral chemotherapy. However, it should be remembered that occasional patients with RIBA-indeterminate values may be HCV RNA PCR positive; the clinical course and response to therapy of this group warrant further study.

## ACKNOWLEDGMENTS

We thank Diana Ishak for assistance in preparing samples for RNA PCR, the University of Washington Central Processing Area, Clinical Virology Laboratory, Clinical Chemistry Laboratory personnel for processing samples, Chuck Rohrer for performing serum transaminase determinations, Robert Coombs and Joel Gibson for technical advice, and Nancy Coomer for assistance in preparation of the manuscript. The protocol for HCV RNA PCR analysis was kindly provided by Jang Han and Amy Weiner, Chiron Corporation, Emeryville, Calif., to whom we are grateful.

## REFERENCES

1. **Farci, P., H. J. Alter, D. Wong, R. H. Miller, J. W. Shih, B. Jeff, and R. H. Purcell.** 1991. A long term study of hepatitis C virus replication in non-A, non-B hepatitis. N. Engl. J. Med. **325:**98–104.
2. **Garson, J. A., R. S. Tedder, M. Briggs, P. Tuke, J. A. Glazebrook, A. Trute, A. Parker, J. A. J. Barbara, M. Contreras, and S. Aloysius.** 1990. Detection of hepatitis C viral sequences in blood donations by "nested" polymerase chain reaction and prediction of infectivity. Lancet **335:**1419–1422.
3. **Gretch, D. R., J. J. Wilson, R. L. Carithers, and L. Corey.** 1992. A liquid hybridization assay for the detection of hepatitis C viremia: comparison with two stage nested set PCR. Gastroenterology **102:**A815.
4. **Han, J. H., V. Shyamala, K. H. Richman, M. J. Braur, B. Irvine, M. Urdea, P. Tekamp-Olson, G. Kuo, Q.-L. Choo, and M. Houghton.** 1991. Characterization of the terminal regions of hepatitis C viral RNA: identification of conserved sequences in the 5′ untranslated region and poly (A) tails at the 3′ end. Proc. Natl. Acad. Sci. USA **88:**1711–1715.
5. **Houghton, M., A. Weiner, J. Han, G. Kuo, and Q.-L. Choo.** 1991. Molecular biology of hepatitis C viruses: implications for diagnosis, development and control of viral disease. Hepatology **14:**381–388.
6. **Kuo, G., Q.-L. Choo, H. G. Alter, G. I. Gitnick, A. G. Redeker, R. H. Purcell, T. Miyamara, J. L. Deinstag, M. J. Alter, C. E. Stevens, G. E. Tegtmeier, F. Banino, M. Colombo, W.-S. Lee, C. Kuo, K. Berger, J. R. Shuster, L. R. Overby, D. W. Bradly, and M. Houghton.** 1989. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science **244:**362–364.
7. **Kwok, S., and R. Higuchi.** 1989. Avoiding false positives with PCR. Nature (London) **339:**237–238.
8. **Miller, R. H., and R. H. Purcell.** 1990. Hepatitis C virus shares amino acid sequence similarity with pestiviruses and flaviviruses as well as members of two plant virus supergroups. Proc. Natl. Acad. Sci. USA **87:**2057–2061.
9. **Okamato, H., S. Okada, Y. Sugiyama, T. Tanaka, Y. Sugai, Y. Akahane, A. Machida, S. Mishiro, H. Yoshizawa, Y. Miyakawa, and M. Mayumi.** 1990. Detection of hepatitis C virus RNA by a two-stage polymerase chain reaction with two pairs of primers deduced from the 5′-noncoding region. Jpn. J. Exp. Med. **60:**215–222.
10. **Van der Poel, C. L., H. T. M. Cuypers, H. W. Reesink, A. J. Weiner, S. Quan, R. DiNello, J. J. P. van Boven, I. Winkel, D. Mulder-Folkerts, P. J. Exel-Oehlers, W. Schaasberg, A. Leentuaar-Kuypers, A. Polito, M. Houghten, and P. N. Lelie.** 1991. Confirmation of hepatitis C virus infection by new four-antigen recombinant immunoblot assay. Lancet **337:**317–319.

10a. **Weiner, A.** Personal communication.

11. **Weiner, A. J., M. A. Truett, J. Rosenblatt, J. Han, S. Quan, A. J. Polito, G. Kuo, Q.-L. Choo, and M. Houghton.** 1991. HCV: immunologic and hybridization-based diagnostics, p. 360–363. *In* F. B. Hollinger, S. M. Lemen, and H. S. Margolis (ed.), Viral hepatitis and liver disease. Williams & Wilkins, Baltimore.