Whole-Blood Hepatitis C Virus RNA Extraction Methods

We read the article by Cook et al. (3) with interest, since they compared their findings with our previous work (4–8; D. Brashcer, S. Taylor, J. Xiang, D. Klinzman, F. LaBrecque, M. Pfaller, B. Alden, D. LaBrecque, J. Stapleton, and W. Schmidt, 10th Triennial Int. Symp. Viral Hepatitis Liver Dis., abstr. C008, 2000; J. Xiang, F. LaBrecque, W. N. Schmidt, D. Klinzman, D. Brashcer, D. R. LaBrecque, M. J. Perino-Phillips, and J. T. Stapleton, 10th Triennial Int. Symp. Viral Hepatitis Liver Dis., abstr. F011, 2000). Cook et al. did not find that whole blood was a more sensitive source of hepatitis C virus (HCV) RNA than plasma. Careful review of their paper revealed several potential reasons for this discrepancy, including the following. (i) A different method of extracting RNA from whole blood was used (5). We did not use Trizol following extraction with Catrimox (4, 9). The pH of the phenol-guanidinium mixture in Trizol is not published. We found that the phenol pH is critical for optimal extraction (4, Brashcer et al., 10th Triennial Int. Symp. Viral Hepatitis Liver Dis.). In addition, we showed that extraction with Trizol was less sensitive than our Catrimox method (Xiang et al., 10th Triennial Int. Symp. Viral Hepatitis Liver Dis.). (ii) Cook et al. added carrier RNA to serum but not to whole blood (3), yet we have shown that the addition of carrier RNA to either plasma or whole blood increases sensitivity (Brashcer et al., 10th Triennial Int. Symp. Viral Hepatitis Liver Dis.). (iii) Cook et al. used different primers and thermocycler conditions than did we, and they used only single-round reverse transcription (RT)-PCR instead of nested RT-PCR (3, 4). (iv) Finally, the serum volume was not adjusted to the whole-blood volume (3), which we took into account (6).

As suggested by Cook et al., intracellular HCV RNA is a potential explanation for the improved sensitivity of HCV RNA detection in whole blood (3), yet we found that a significant portion of HCV RNA in the crude cellular pellet was not intracellular (6). Our study of 115 patients with HCV infection demonstrated that the improved sensitivity of whole-blood HCV RNA detection correlated with the presence and quantity of cryoglobulins (8). We also noted highly significant differences in the detection of HCV RNA for whole blood and plasma among 52 interferon-treated patients who were monitored throughout treatment (7). As was the case for the one patient studied by Cook et al. (3), among our 52 patients the clearance of intracellular virus occurred nearly simultaneously with the clearance of plasma virus during therapy (7).

The extraneous bands described by the authors (3) are not routinely present in our work (e.g., see Fig. 1, 3, and 5B in reference 4). In addition, Cook et al. stated that it was not tenable that our antibody-negative subjects were truly infected with HCV since we did not evaluate liver biopsy samples for HCV RNA (3). However, we proved that several HCV antibody-negative patients had HCV RNA present in multiple whole-blood and plasma samples collected over months and years, using primers from several different regions of the genome (4, 6). In addition, we confirmed our results by Southern blotting and sequence analysis of PCR products and by direct comparison with results obtained with the use of commercial HCV RNA assays (4, 6, 9). The finding of prolonged RNA positivity in the absence of detectable HCV antibody has been described by others (2), including after the experimental infection of chimpanzees (1). In conclusion, we believe that the difference between our work and that of Cook et al. (3) relates to differences in the whole-blood RNA extraction methodology.

REFERENCES


Authors’ Reply

Drs. Schmidt and Stapleton think that the difference between our work (3) and theirs (6) is related to differences in whole-blood RNA extraction methods due to a presumed difference in pH of the phenol-guanidinium. The Trizol U.S. patent documents state that the pH of Trizol is 4.0, the exact pH used in their method (D. Brashcer, S. Taylor, J. Xiang, D. Klinzman, F. LaBrecque, M. Pfaller, B. Alden, D. LaBrecque, J. Stapleton, and W. Schmidt, 10th Triennial Int. Symp. Viral Hepatitis Liver Dis., abstr. C008, 2000). Therefore, Trizol and the acid phenol-guanidinium mixture used by Schmidt and Stapleton are equivalent chemical environments and the fact that our data failed to confirm their findings is not explained by differences in extraction methods. Nor are the differences between PCR methodology, carrier RNA, and volume adjustment consequential. The competitive reverse transcription
We reiterate that the presence of HCV in blood cells without the demonstration of HCV RNA in the liver is untenable. If there were replication of HCV in blood cells, an idea that is controversial and therefore requires verification, then there must be replication in the liver. A verification of finding HCV in the blood cells but not in the serum of seronegative patients is detection of HCV RNA in the liver.

An improved method for detection of HCV RNA in blood samples would be welcomed in diagnostic clinical laboratories; unfortunately, we did not find that the Schmidt-Stapleton methodology provided any improvement over our current method.

REFERENCES


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