Polymerase Chain Reaction for the Diagnosis of Viral Hepatitis C in Clinical Medicine

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Dear Sir,

The polymerase chain reaction (PCR) using amplification of reversely transcribed RNA is considered the 'gold standard' for the detection of viral nucleic acids in serum and tissue samples and is thought to indicate active infection with the hepatitis C virus (HCV) [1,2]. This sensitive method is now increasingly employed for the diagnosis of replica-tive HCV infection in high-risk groups such as patients on hemodialysis or recipients of multiple blood transfusions [3]. However, it is open to question whether this highly sophisticated and expensive method is applicable for routine clinical practice [4,5].

When working with PCR techniques several sources of error exist. In general, the detection of RNA viruses such as the HCV is much more difficult than that of DNA viruses. This is due to the fact that contamination with RNase, which is ubiquitous, very stable and highly active at even very low concentrations, may result in enzymatic degradation of any RNA present [5]. Thus, if serum specimens are handled in a way that may allow contamination as is the case when collection, centrifugation and pipetting are done under nonsterile conditions, reverse transcription to complementary DNA may not occur. This would cause 'false'-negative results.

Also, blood samples need to be rapidly cooled to 4°C, since RNase remains very active at room temperature [5]. This requires the use of a refrigerated centrifuge and immediate storage of samples at temperatures below -70°C.

We have recently detected hepatitis C viremia in 48 hemodialysis patients using PCR analysis (duplicate controls on each patient plus standards with negative and positive controls) following the above-mentioned strict sampling criteria. To investigate the incidence of 'false'-negative results due to incorrect handling of samples by doctors and other hospital personnel, we reexamined blood from all these patients 2 weeks later. Whole blood was obtained by puncture of the patients' arteriovenous fistula. All blood samples were collected and centrifuged by nurses of the dialysis unit without special instructions and knowledge of the type of investigation. Vials were not centrifuged and/or cooled immediately after collection. Twelve of 48 previously positive blood samples (25%) now gave a negative result. Since a loss of hepatitis C viremia is unlikely during this short period of time, we suggest that

negative results are due to RNase contamination caused by inappropriate handling of blood and serum samples.

We conclude that PCR for the detection of HCV may provide very variable results depending on the type of blood collection and processing. Thus, several recommendations need to be followed by the clinician to avoid false-negative results: (1) the use of sterile gloves by persons handling the samples from bedside to laboratory, and (2) rapid processing of whole blood samples in a refrigerated centrifuge and immediate storage of serum at -70°C until analysis.

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