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Quantification of Hepatitis C Virus RNA in Patients Sera by Competitive RT-nested PCR

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The quantification of serum levels of Hepatitis C virus RNA in chronic hepatitis C has been considered as an important indicator for the outcome of interferon therapy. In recent some HCV-RNA quantitative assays were developed, but their baseline cut-off levels were around 2 x 10^6 copies/ml. For more sensitive quantification of HCV-RNA, we investigated new competitive RT-nested PCR methods.

RNA was extracted from 50 l serum using the RNAzol kit. To determine the amount of HCV-RNA, we used two types of mutagenized HCV-RNA as standars comprtitor, one containing *EcoR* I site (point mutant, PM) and the other being deleted 120 nt sequences (deletion mutant, DM). Two pair of primers used for reverse-transcription and nested PCR were deduced from a well conserved 5 noncoding region: 5-CTG TGA GGA ACT ACT GTC TT-3 (outer sense; nt 43-52), 5-CAT GAT GCA CGG TCT ACG AGA C-3 (outer anti-sense; nt 311-332), 5-TTC ACG CAG AAA GCG TCT AG-3 (inner sense; nt 51-60) and 5-CAC TCG CAA GCA CCC ATA CAG GC-3 (inner anti-sense; nt 279-301). Bioneers premix RT-PCR and PCR kit were used in RT-nested PCR. The amount of HCV-RNA was determined by 2% agarose gel electrophoresis with ethidium bromide staining.

The detection rates of HCV RNA by these competitive RT-nested PCRs were from 10-103 copies per 50 l serum. No differences were found when viral load was assayed using either PM or DM as the standard competitor.

Thus, these competitive RT-nested PCR assays appear sufficiently sensitive for the evaluation of HCV RNA and would be useful for the diagnosis and management of HCV infection.

Key Words: Hepatitis C virus, Competitive RT-nested PCR