Development and evaluation of a quantitative competitive reverse transcription polymerase chain reaction (RT-PCR) for hepatitis C virus RNA in serum using transcribed thio-RNA as internal control

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Abstract

A method for quantitation of hepatitis C virus (HCV) RNA was developed based on competitive reverse transcription polymerase chain reaction (RT-PCR) using in vitro transcribed mutated thio-RNA as a competitor template. The thio-RNA is more resistant to RNase and is stable over a year. This assay was compared with the commercially available Roche Amplicor HCV Monitor assay V 2.0 and real time PCR using SYBR green I dye method. A total of 18 pre-therapy serum samples from chronic hepatitis C cases were tested in parallel by the three assays. All samples could be quantitated using the in-house competitive RT-PCR and real time PCR and there was a significant correlation in the virus titer (P<0.05). However, 8 (44%) samples could not be quantified by Amplicor HCV Monitor assay, which has a lower detection range (10(2) to 10(5.5) copies/ml). The in-house method of competitive RT-PCR showed a detection range of 10(3) to 10(10) copies/ml. In the patients the mean viral titer was found to be (9.66+/−9.3)x10(6) copies/ml. Ten (55%) of the samples, assessed by the Amplicor HCV Monitor assay showed a mean viral titre of (1.13+/−0.75)x10(6) copies/ml, which was lower than the other two tests. The competitive PCR method and real time PCR could amplify all prevalent genotypes. This in-house quantitative competitive RT-PCR method is simple, cheap, reproducible and useful for estimation of HCV RNA load.