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Establishment of Simultaneous Detection and Quantitation of HCV-RNA by Third Generation Intercalating Dye Real-time PCR

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Introduction: Rapid quantification of hepatitis C virus is helpful in determining and monitoring of the disease progression and nature of the virus replication. The aim of the present study was to establish a fast, specific and sensitive tool for HCV-RNA quantification. **Materials and Methods:** A total of 50 serum samples, comprising of 40 HCV-positive and 10 HCV-negative, were included in our study. RNA was extracted, reverse transcribed, and then subjected to real-time PCR amplification. Real-time PCR using EvaGreen dye and primers targeting a 5'UTR was carried out. Reference samples with known viral load were treated similarly to the unknown samples and used to create the standard curves. **Results:** Our method showed a high level of analytical specificity and accuracy, with a low limit of detection (~2 IU/ml). It yielded repeatable results with less than 4% of intra- assay variation. The assay covered a broad dynamic range of quantification, ranging from 0.34 to 6 log IU/ml. The diagnostic sensitivity, specificity, and accuracy were all 100%, indicating neither false positive nor false negative results were obtained. **Conclusion:** The developed real time PCR using EvaGreen dye has demonstrated a highly analytical and diagnostic performance for HCV quantification, suggesting its potential in clinical diagnosis and management.