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

Akrahm M. Saleh Habi1, Hairul Aini Hamzah1, Muhammad Imad Al-Deen Mustafa1, Norlelawati A. Talib2, Siti Nurul Fazlin Abdul Rahman1

1Department of Basic Medical Science, Kulliyyah of Medicine, International Islamic University Malaysia
2Department of Pathology and Laboratory Medicine, Kulliyyah of Medicine, International Islamic University Malaysia

Presenter: Hairul Aini Hamzah

**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.