ORT.09 - Long template RT-PCR optimization to amplify the first complete genome of Hepatitis C Virus subtype 2b from Latin America

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Introduction: Hepatitis C virus (HCV) is an important human pathogen affecting nearly 3% of the world's population, and is a leading cause of chronic liver diseases including cirrhosis and hepatocellular carcinoma. HCV is a rapidly evolving RNA virus that has been classified into seven genotypes and numerous subtypes. In the last years, HCV subtype 2b has been detected in different geographic regions of Brazil. However, no complete genome of this subtype from the Latin America was obtained until now, limiting studies on the diversity and molecular epidemiology of the virus. Furthermore, amplification of large HCV genomic fragments is challenging, since reverse transcription polymerase chain reaction (RT-PCR) must overcome low template concentrations and high target sequence diversity.

Objective: The aim of this study is to perform the molecular characterization of the first HCV subtype 2b full-length genome from the Latin America by optimization of a long template RT-PCR technique.

Methodology: First, viral RNA was extracted from 200 μL of serum by gently manipulating the sample, and then total RNA was precipitated with sodium acetate (3 M) and resuspended in a volume of 9.5 μL. The cDNA was synthetized using SuperScript IV Reverse Transcriptase and a nested PCR was done with Platinum Taq DNA Polymerase. Sequencing was performed using the Sanger method.

Results: A complete genome, with two overlapping fragments of 3,388 and 4,541 base pairs (bp) in length, was successfully amplified. Phylogenetic analysis confirmed that both PCR fragments belonged to subtype 2b. Surprisingly, the full-length genome presented a total size of 7,298 bp, showing a deletion of 2,022 bp (genome position 965 - 2986) covering most of the E1, E2, p7 and the 5′ end of NS2 genomic regions. To investigate the presence of viral subpopulations without the deletion, we designed oligonucleotides flanking this region. A fragment of 2.047 bp was amplified, demonstrating coinfection with wild-type HCV populations.

Conclusion: In conclusion, we obtained the first complete genomes of HCV subtype 2b from Latin America and developed a method that should prove useful for molecular, epidemiological and clinical studies of HCV where complete virus sequence is required.

Keywords: HCV; Long RT-PCR; Complete Genome