ORT_09 - Usefulness of rapid molecular assay to detect hepatitis C virus

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Introduction: It is estimated that 58 million people are chronically infected with hepatitis C virus (HCV). HCV active infection diagnosis is currently performed through quantitative reverse transcription polymerase chain reaction (RT-qPCR). Despite PCR-based assays can provide results relatively fast, these techniques require capable professionals, specific equipment, and adequate infrastructure. A single point temperature technique named reverse transcription loop-mediated isothermal amplification (RT-LAMP) which combines a simple visualization of amplification products, fast procedures and no necessity of a real-time thermal cycler could be an alternative to RT-qPCR especially in places with geographical conditions of difficult access profile.

Objectives: To optimize RT-LAMP method for HCV RNA detection in samples from patients with chronic liver disease.

Methodology: A total of 89 serum samples were obtained from hepatitis C patients referred to Viral Hepatitis Ambulatory. All of them had HCV RNA detectable by RT-qPCR. Sixty-three samples were also genotyped by HCV NS5B nucleotide sequencing (~370 bp). The study also enrolled 30 individuals who had HCV RNA undetectable at RT-qPCR. HCV RNA extraction was done using a commercial kit. For RT-LAMP methodology, primers were used for 5′ untranslated region (UTR) amplification. Before amplification reaction, a preheating stage at 95ºC was done to dismount 5′ UTR RNA secondary structures. RT-LAMP reaction was standardized at 63ºC for 60 minutes and enzyme inactivation at 80ºC for 10 minutes. Amplification products were fractionated by 3% agarose gel electrophoresis.

Results: As expected, Sanger sequencing identified a higher prevalence of HCV genotype 1 (56/63; 88.9%) followed by genotypes 3 (4/63; 6.3%) and 4 (3/63; 4.8%). All sequenced samples had a positive result at RT- LAMP. Samples with viral RNA detectable by RT-qPCR (n=89) had a mean viral load of 5.8 ± 0.76 Log IU/mL. Referring to RT-LAMP, it was observed a sensitivity of 91% (81/89) and specificity of 100% (30/30) since all negative samples tested by RT-qPCR were also negative at RT-LAMP. Test accuracy was 93% (81+30/119) with a respective 95% confidence interval (95% CI) of 0.8711 to 0.9674.

Conclusion: RT-LAMP assay was capable to detect HCV RNA in serum samples with high sensitivity and specificity. Also, the test was able to detect most prevalent HCV genotypes circulating in Brazilian samples demonstrating its significant potential for use in clinical routine as a screening diagnosis.

Keywords: Hepatitis C virus, RT-LAMP, diagnosis