IVD_04 - Development and evaluation of recombinant biomolecules for the immunological diagnosis of hepatitis C

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Introduction: Hepatitis C virus (HCV) infection is a major public health problem worldwide. In Brazil, approximately 1.88 billion reais were spent to control viral hepatitis in 2021. Due to the asymptomatic cases, many of the infected individuals can evolve to cirrhosis and hepatocarcinoma when not diagnosed early.

Objective: In this scenario, the present study aims to develop biomolecules with potential use in diagnostic assays for hepatitis C.

Methodology: First, multiepitope recombinant proteins of HCV were designed *in silico* based on the sequences of the three most prevalent genotypes in Brazil (1a, 1b and 3a). The recombinant genes were synthesized chemically in expression vector pET21a, which were used in transformations of *E. coli* Bl21. In order to increase the yield of the recombinant protein, different concentrations (0.5 mM to 5 mM) of the inducer IPTG were evaluated. After expression and subsequent purification by affinity chromatography, the integrity of the recombinant proteins was evaluated by SDS-PAGE, and the structures were confirmed by circular dichroism. The immunogenic potential of these proteins were confirmed by indirect ELISA using serum from chronic HCV cases and health subjects as samples and the anti-human IgG/HRP as secondary antibody. To develop the assay, some aspects were evaluated: i) concentrations of recombinant protein as capture biomolecule (2 µg/mL up to 30 µg/mL), ii) dilutions of the serum samples (1X up to 10X in PBS-BSA) and the secondary antibody (1:20.000 up to 1:60.000 in PBS-BSA), and iii) addition of BSA-blocking steps, were evaluated in search of a higher sensitivity in the ELISA assays.

Results: The SDS-PAGE analysis demonstrated that recombinant proteins are more expressed using 5mM of IPTG, in addition, ELISA tests were more sensitive using 10 µg/mL of recombinant protein, with serum samples diluted 10X, and the secondary antibody diluted 1:30.000. On the other hand, no additional blocking steps provided the best results for the ELISA tests. For comparative evaluation, a total of 84 serum samples from anti-HCV positive and negative patients were used, achieving results similar to those obtained using Murex anti-HCV (version 4.0) comercial kit.

Conclusion: Taken together these data suggest the potential applicability of these biomolecules in diagnostic tests for the detection of anti-HCV antibodies in serum samples.

Keywords: Hepatitis C; Diagnosis; Multiepitope proteins