IVD_02 - New approaches for recombinant protein VP1-2A of HAV production and characterization based on liquid microarray assay: application for developing a point-of care diagnostic test

Michel Vergne Felix Sucupira[1]; Leila Botelho Rodrigues da Silva[1]; Marcelle Bral de Mello[1]; Christiane de Fátima Silva Marques[1]; Mariana Miguez Tardelli Garcia[1]; Ana Paula Corrêa Argondizzo[1]; Anna Erika Vieira de Araujo[1]; Renata Chagas Bastos[1]; Vanessa Salete de Paula[2]; Luciane Almeida Amado Leon[2].
[1]Fiocruz/Bio-Manguinhos;
[2]Fiocruz/IOC.

Introduction: The current WHO guidelines highlight the importance of Point-of-Care (POC) platforms, as a viable strategy to facilitate and expand diagnosis programs, such as viruses infections, allowing the access of rural populations and regions of difficult access. Current gold standard immunoassays used for hepatitis A diagnosis employ a long time of execution.

Objective: Our aim was to express, purify and characterize the recombinant VP1-2A HAV protein to be used in the development of a POC test.

Methodology: The VP1-2A protein was expressed in *E. coli* system using commercial plasmids (pET-TOPO®). For improvement of protein expression, solubility, and purification, fusion tags were added at the recombinant protein. The induction of protein expression with IPTG was standardized, and then, the proteins were submitted to homogenization and pressurization, followed by a purification step through immobilized metal ion affinity chromatography (IMAC) and quantification by the bicinchoninic acid (BCA) method. The protein solubility was evaluated, as well as its electrophoretic profile and isoelectric point (pI). Multi-antigen print immunoassay (MAPIA) was used to determine the ideal protein concentration for use in developing of a rapid test. In addition, we applied a microarray assay to evaluate the potential of the VP1-2A protein to detect specific anti-HAV antibodies in serum samples (n=15), previously characterized (by chemiluminescence) from patients with different titers of IgG anti-HAV. First, 50µg of HAV protein were coupled to a magnetic bead using three buffers: MES, NaHCO3, and PBS that presented the best coupling conditions.

Results: The optimal expression condition was established (IPTG 0.4mM for 4 hours at 37°C). The VP1-2A protein was obtained mainly from insoluble portion. Its pI was 6.45 and achieved a concentration of 1.09 mg/mL. The strategies used for expression and the improvement achieved in the concentration were probably due the homogenization approach used. Through the SDS-PAGE and western blot it was possible to characterize the recombinant VP1-2A concerning its molecular size and antigenicity. The bead-based assay clearly discriminated between positive and negative samples for anti-HAV, showing a sensitivity, specificity, and accuracy of 80%, 100% and 86.67%. Through the MAPIA, it was observed that the ideal amount of protein to be printed was 40ng/mm at least.

Conclusion: Our findings demonstrated an efficient process to obtaining large amounts of HAV protein recombinant from *E. coli*. In addition, it was showed the feasiability of the microarray platform as a useful tool for screening recombinant protein.

Keywords: Microarray; HAV VP1-2A; Diagnosis