IVD.17 - Development of Surface Plasmonic Biosensor for Rapid and Large-scale Diagnosis of Hepatitis A

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Introduction: In Brazil, the improvements in sanitary conditions and the recent inclusion of hepatitis A vaccine resulted in an increase in the number of individuals susceptible to the disease. These facts together with the circulation of the virus in the environment, increases the occurrence of epidemic outbreaks. Therefore, a test that enables a rapid and large-scale diagnosis of acute cases may be a promising alternative to the currently available immunoenzymatic assays. In this way, Biosensor assay may help in the adoption of appropriate measures to contain hepatitis A outbreaks, as well as, in the detection of susceptible individuals involved in the outbreak that may be candidates for vaccination, avoiding the spread of the infection

Objective: The objective of this study was to standardize an immunosensor of reflectance applied to the large-scale immunodiagnosis of hepatitis A.

Methodology: This methodology was based on the development of a reflectance biosensor for the detection of IgM and IgG anti-HAV, which allows the detection of antibodies at low detection limits (nanoscale) and monitor the antigen-antibody reactions in real time, without the use of conjugated developers and, with high selectivity and low cost. For detection of anti-HAV IgM and IgG antibodies, the chip sensor surface was adsorbed with different concentrations of recombinant HAV VP1 protein (0.001 μg - 0.5 μg) in run buffer. Purified anti-HAV IgM and IgG in different concentrations (3.5nM to 219pM) were applied in triplicate, to evaluate the interaction of anti-HAV antibodies with HAV VP1 and the limit of detection of the assay. After the step of interacting the specific antibodies with the VP1 protein and recording the data in a sensorgram, the surface of the sensor chip was regenerated, so that a second interaction with antibodies could occur. A panel of anti-HAV IgG and IgM reactive and non-reactive serum samples were used to evaluate the sensitivity, specificity and reproducibility of this assay.

Results: Purified anti-HAV IgM and IgG were detected in different concentrations (0.02μg to 0.17μg). The increase in the detection signal was proportional to the increase in anti-HAV concentrations. It was possible to define the binding affinity (1.85nM and 1.20nM) and the maximum response (86,42 RU and 90,33 RU) to IgM and IgG, respectively. The linearization of the antibodies concentration curves generated a saturation constant, which allowed inferring the amount of specific antibodies in the anti-HAV positive serum compared to the negative serum. Through the panel of serum samples, it was observed that the detection signal of the anti-HAV positive serum samples was three times greater than the signal of the negative serum samples.

Conclusion: These preliminary results demonstrated that the biosensor was able to identify with high sensitivity, the presence of specific anti-HAV antibodies in sera of patients.

Keywords: Hepatitis A; Diagnosis; Biosensor