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Development and validation of low-cost portable biosensors for dengue diagnosis

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Despite technological advances around the world and in the most diverse areas, developing countries still face numerous challenges in the area of health. Clinical and laboratory diagnoses are generally limited and inaccessible for the majority of patients, which can result in high rates of mortality due to neglected diseases. (1) A promising alternative in the diagnostic scenario is the use of biosensors due to their simplicity, precision and high sensitivity characteristics. (2) Despite increasing advances in the academic field in relation to biosensors, aiming to increase the sensitivity of the technique and make it cheaper comparing to the chemiluminescence, gold standard method for Dengue, there are still no commercial tests using this methodology on the market. In this project we aimed to develop and validate a disposable device based on electrochemical techniques. Ten anti-Dengue NS1 from three different companies, national and international, were tested with ELISA to select the best antibodies capable of detecting Dengue NS1 positive and negative control samples. Then, carbon screen printed electrodes containing Carbon Black were modified with films containing a redox probe Ferrocene (Fc) 4 mg mL-1 and a solution containing chitosan (Qs) 5 mg mL-1. After the film electrodes modification, the two antibodies selected by ELISA were then immobilized over the film using three different conjugation methods: simple absorption, glutaraldehyde covalent bonding and EDC/NHC covalent bonding. The glutaraldehyde covalent bonding showed better results and therefore was selected for the next tests. At that moment was also selected the best antibody, from an international company. Further, were tested antibody concentration, in a range of 50 to 150 μg mL-1, being selected 100 μg mL-1, and some other electrode optimization parameters, such as time for antibody immobilization, in a range of 2 to 14 hours, being selected 2 hours as the best. The electrochemical characterization was made by using cyclic voltammetry and differential pulse voltammetry techniques and the results were compared to chemiluminescence values, used to characterized previously the commercial positive and negative controls, in a confidence level of 95%. The biosensors were characterized by electronic and vibrational spectroscopy techniques (UV-VIS and FT-IR) and microscopy. The validation is ongoing and so far, the test presented a good repeatability and reproducibility, a high sensitivity compared to the tests available on the market, with LOD of 0,01 ug mL-1 NS1 protein and a low cost, estimated price less than two dollars. The next step is testing the prototype with positive and negative clinical samples and perform shelf-life stability tests. In summary, protocols and techniques used in the biosensor preparation were optimized in order to have simpler and faster methodologies aiming at obtaining specific, selective, sensitive and low-cost point-of-care test for detection of dengue disease.

Palavras-chave: Screen-printed electrodes; Dengue; POC test.

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