Immunofluorescence–based biosensor for the determination of dengue virus NS1 in clinical samples

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The sharp increase in incidence of dengue infection has necessitated the development of methods for the rapid diagnosis of this deadly disease. Here we report the design and development of a reliable, sensitive, and specific optical immunosensor for the detection of the dengue non-structural protein 1 (NS1) biomarker in clinical samples obtained during early stages of infection. The present optical NS1 immunosensor comprises a biosensing surface consisting of specific monoclonal NS1 antibody for immunofluorescence-based NS1 antigen determination using fluorescein isothiocyanate (FITC) conjugated to IgG antibody. The linear range of the optical immunosensor was from 15 – 500 ng mL⁻¹, with coefficient of determination (R²) of 0.92, high reproducibility (the relative standard deviation obtained was 2%), good stability for 21 days at 4 °C, and low detection limit (LOD) at 15 ng mL⁻¹. Furthermore, the optical immunosensor was capable of detecting NS1 analytes in plasma specimens from patients infected with the dengue virus, with low cross – reaction with plasma specimens containing the Japanese encephalitis virus (JEV) and Zika virus. No studies have been performed on the reproducibility and cross-reactivity regarding NS1 specificity, which is thus a limitation for optical NS1 immunosensors. In contrast, the present study addressed these limitations carefully where these two important experiments were conducted to showcase the robustness of our newly developed optical-based fluorescence immunosensor, which can be practically used for direct NS1 determination in any untreated clinical sample.

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1. Introduction

Dengue fever is a major public health crisis in tropical and subtropical regions of the world [1,2]. The rapid diagnosis of dengue virus (DENV) infection is crucial for limiting the spread of this disease [3,4]. ELISA (enzyme-linked immunosorbent assay) is currently the most frequently used diagnostic assay for DENV. This immunoassay is used to detect non-structural protein 1 (NS1), a DENV glycoprotein produced by infected host cells, or DENV-specific IgM and IgG antibodies (Abs) [5]. ELISAs are inexpensive and sensitive enough to detect analytes present at very low concentrations (0.1–2 μg L⁻¹) [6,7]. However, these assays are also time-consuming. For example, ELISAs measuring DENV specific IgM require about 1–2 days to perform. In addition, production of a chromogenic signal on enzyme-substrate interaction is a requisite in ELISA, and this enzyme-mediated reaction requires approximately 15 min to produce a detectable color change. However, this enzyme-mediated color change is not stable and can change indefinitely over a long period. Therefore, the resulting color intensity imprecisely reflects the quantity of primary antibody, and can yield false-positive results [6,8]. In traditional ELISA, enzymes such as horseradish peroxidase and alkaline phosphatase are often used for the amplification of the chromogenic signal. Nevertheless, the enzymes are usually costly and the catalytic activity of enzymes is sensitive to the environmental changes, e.g., temperature and pH. Enzyme denaturation caused by these environmental factors would compromise the accuracy of ELISA results and render them irreproducible [9,10]. Therefore, more stable indicators, such as fluorescence labels, are required to be developed. Fluorescence ELISAs [8] and immunospot assays [11] have been suggested as a means to overcome the limitations of conventional ELISAs. Fluorescence ELISAs have been conducted by Hosseini et al. using DENV – specific Ab bound to methacrylic microspheres via surface attachment.