TITLE: Implementation of the Luciferase Immunoprecipitation System for the Detection of Antibodies against Zika Virus NS1 Protein

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ABSTRACT

Zika virus (ZIKV) is a single-stranded RNA neurotrophic flavivirus capable of causing neurological complications such as Guillain-Barré syndrome and fetal microcephaly. According to data from the Brazilian Ministry of Health, 7.387 probable cases of Zika were registered in 2020 in the country. The immunodiagnosis of the disease is mainly based on the NS1 protein of ZIKV, a multifunctional glycoprotein expressed during viral replication that dimerizes after post-translational modifications. The detection of specific NS1 proteins antibodies is possible between 4 to 8 days after the initial exposure to different flaviviruses. Because of cross-reactivity with other flaviviruses, clinical diagnosis can be further hampered by co-infection with other arboviruses in endemic areas. Therefore, new quantitative, more sensitive and specific diagnostic alternatives are needed. Antibody detection using the Luciferase Immunoprecipitation System (LIPS) is a fast and low-cost technology as an alternative to the main antibody detection methods. In this technique, the antigen is expressed in mammalian cells as a recombinant polypeptide in fusion with the FLAG epitope and *Renilla* luciferase (Ruc), and crude extracts are obtained and used without purification. However, in the *Flaviviridae* family viruses, there are no studies in the literature describing the use of this strategy as an alternative to serological diagnosis for the NS1 protein of ZIKV. The objective here is to implement LIPS as an alternative serological method for the detection of IgG and IgM for NS1 of ZIKV. For this, we cloned the insert of the NS1 protein coding region (obtained from the plasmid pSF-ZKV-Honduras-NS1-Puromycin, New York State Department of Health, Slingerlands, NY, USA) into the pREN2 vector that contains the FLAG epitope followed by the *Renilla* luciferase sequence to generate the pREN2-ZIKV-NS1 construct. At present, the expression of the FLAG-*Renilla*-Zika-NS1 polypeptide is being evaluated in HEK293 mammalian cells transfected with the construct to obtain cell extracts and for the analysis of luciferase activity. Serum samples from patients not infected or infected with ZIKV, Dengue and Chikungunya will be tested. We consider that the LIPS assay can be a promising alternative tool for screening, diagnosing and monitoring ZIKV, especially for its differential in detecting antigens containing post-translational modifications such as NS1.

Keywords: Zika virus, NS1, luciferase, immunodiagnosis

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