

Título: OPTIMIZATION OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION PROTOCOL FOR DETECTION OF THE FOUR DENGUE VIRUS SEROTYPES

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Dengue is an acute febrile disease caused by a virus of the same name, which has a single-stranded positive-sense RNA as a genome, belonging to the genus *Flavivirus*, family *Flaviviridae*. There are four serotypes of the virus, called DENV-1, DENV-2, DENV-3 and DENV-4. Dengue is the main vector-borne disease nowadays, occurring mainly in tropical and subtropical areas of the world. The World Health Organization (WHO) classifies the spectrum of the disease as dengue fever, dengue with warning signs and severe dengue. The Brazilian Ministry of Health recommends the differential diagnosis once other clinical syndromes, caused by other microorganisms, might show similar symptoms. Currently, the Dengue diagnostic is done through clinical examination, serological techniques (IgM antibody detection) and detection of the viral protein NS1. However, the advance of molecular biology techniques, as an important tool for the detection of pathogens is revolutionizing laboratory diagnostics. Among nucleic acid amplification techniques, the LAMP method - Loop-mediated Isothermal Amplification, a technique that uses four to six primers, with high sensitivity, specificity, low cost and fast results may represent an important alternative. Considering the above, in this project we aimed to standardize a protocol for diagnosis of dengue, based on LAMP methodology. Firstly, samples representing all four serotypes were isolated in C6/36 cells (*Aedes albopictus*), and confirmed by a previously published semi-nested PCR protocol. Once the four dengue virus serotypes were isolated, the amplification tests with the LAMP technique were performed. Different temperatures were evaluated (62°C; 63°C; 64°C; 65°C; 66°C and 67°C), as well as different incubation period (45, 60, 90 minutes). Different magnesium sulfate concentrations (4mM and 8mM) were also evaluated. The best results were achieved when the reaction was conducted at 65°C over a one-hour period with 8mM of magnesium sulfate. Other studies are being carried out to confirm the specificity and sensibility of this protocol. Moreover, a colorimetric protocol using hydroxy naphthol blue (HNB), is under evaluation for visual detection of the results by naked-eye, without the necessity of agarose gel electrophoresis.

Keywords: Dengue, LAMP, molecular diagnosis

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