

Title: DEVELOPMENT AND EVALUATION OF A DIAGNOSTIC TEST FOR THE DETECTION AND DIFFERENTIATION OF THE DENGUE VIRUS SEROTYPES.

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Abstract:

The dengue virus (DENV), the etiologic agent of Dengue Fever (DF) and Dengue Hemorrhagic Fever (DHF) is transmitted by the bite of arthropod vectors, and the *Aedes aegypti*, is the main vector. Dengue is an acute fever disease caused by infection with any of the four serotypes of dengue (DENV1-4) that can cause variables clinical presentment. Its clinical manifestations are similar to those of other infections, becoming so an easily underdiagnosed disease. Over the past 20 years the DENV and its main vector is spread in Brazil and outbreaks have occurred in all regions of the country, the Northeast region is one of the most affected. In the northeastern states, Maranhão is one that has shown highest number of dengue cases, including the circulation of serotype 4, and São Luis, the capital, is the most affected. Although different serologic techniques have been developed and evaluated for the diagnosis of dengue, none have been standardized for routine laboratory use, making it necessary to review other methods, such as molecular techniques based on amplification of viral genetic material. The ELISA test for capturing IgM antibodies is available in the Central Public Health Laboratories across the country, but it is a test that detects only the patient's immune response, which is generally used for epidemiological studies. In this context, this study aimed to evaluate a diagnostic method, the Reverse Transcription technique associated with the Polymerase Chain Reaction (RT-mPCR) in single step. For this, primers were designed able to detecting and differentiating the four serotypes of DENV. The standardization of the technique was successfully performed using reference strains, and it is possible getting a different electrophoretic pattern for each serotype of the virus. Were screened 150 serum samples previously analyzed by ELISA-IgM test in CPHL-MA (Central Public Health Laboratory of Maranhão), which obtained a rate of 26% of positive samples, and 56, 41% presented to serotype 4; 12,82% of serotype 1; 7, 69% serotype 3 and 2,56%, of serotype 2, having further 20,51% of the samples presented coinfection profile. These results confirm the movement of the four viral serotypes in the Maranhão, and DENV-4 is the most prevalent in the last two years. The results obtained so far point to RT-mPCR reaction as a promising technique for the diagnosis of Dengue use.

Key words: Diagnosis; dengue; multiplex-RT-PCR

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