R11. A LUMINEX-BASED SINGLE DNA FRAGMENT AMPLIFICATION ASSAY AS A PRACTICAL TOOL FOR DETECTING AND SEROTYPING DENGUE VIRUS.

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INTRODUCTION Dengue is a mosquito-borne virus which may evolve from subclinical to severe forms of disease. Early recognition, during initial primary and secondary infections, correlates with reduced case-fatality rate in susceptible groups.

OBJECTIVE The aim of this study was to standardize a DNA hybridization assay using the Luminex system for detecting and serotyping of dengue virus (DENV).

METHODOLOGY Reference DENV of all four serotypes recognized to date was used as positive controls to standardize the methodology. Additionally, a total of 16 blinded DENV isolates were used to evaluate the reproducibility of the assay. The viral isolates were kindly provided by the WHO / PAHO Reference Center for Arbovirus Reference and Research at the Department of Arbovirology and Hemorrhagic Fevers, Instituto Evandro Chagas, Ananindeua (PA), Brazil. Human whole blood samples were obtained from patients clinically suspected of having dengue fever at the University Hospital Clementino Fraga Filho/ Federal University of Rio de Janeiro (HUCFF/ UFRJ), Brazil. All samples were tested in accordance with protocols approved by ethics in research committee of the HUCFF/UFRJ. Twentyfour DNA sequences DENV deposited in GenBank were analyzed for primers and probes design. After alignment, a fragment of approximately 159 bp was selected to design the primers and five probes. Among the five probes, four are serotype specific and one is common to all DENV serotypes. The reverse primer was biotinylated at the 5' end, and the probes were synthesized with a 5'end amino-modified group and linked to a 12-carbon linker. Viral RNA was extracted from 250 μL of cell culture supernatant or human whole blood samples, according to the manufacturer's recommendations. The coupling procedure was performed with capture probes amine-modified at the 5'end and

magnetic carboxylated microspheres (MagPlex® microspheres, Luminex Corp) using a covalent protocol described by the manufacturer. Complementary 5' biotinlabeled PCR amplicons were hybridized with the serotypespecific magnetic microsphere - capture probes. The assay was performed in a 96-conical well plate. Human whole blood samples were used to evaluate the methodology after a validating step comparing with the reference standard seminested RT-PCR.

RESULTS Using established conditions, the Luminex assay was able to correctly identify all 16 DENV isolates. In the evaluating step we obtained 86.7% of comparison with Semi-nested RTPCR. This is the first description wherein specific probes for each DENV serotype were defined from a single DENV DNA sequence and used in hybridization assays.

CONCLUSION The RT-PCR / Luminex system designed in this study proved to be an additional tool for early and faster detection and serotyping of the DENV. New optimization and validation of the RT-PCR/Luminex system will be produced to broaden the application of this methodology as an aid for the differential diagnosis between arboviruses such as DENV, Zika virus and Chikungunya virus

KEYWORDS Dengue virus, molecular diagnosis, RTPCR / Luminex system.