IVD_07 - Development and standardization of the PAN–FLAVI assay for the detection of flaviviruses with epidemiological importance in Brazil

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Introduction: Orthoflaviviruses cause a series of diseases in humans and animals, resulting in social and economic issues. The main viruses circulating in the country, due to their severity and potential for dissemination, are yellow fever virus, Zika virus, dengue virus and West Nile virus. However there are other orthoflaviviruses that have also advanced to other regions, where their circulation had not beendetected yet, causing new outbreaks. Given the need for efficient epidemiological surveillance and the implementation of more efficient control measures, it is necessary to improve virological diagnosis in humans, vertebrate hosts and arthropod vectors. For this, a molecular assay was developed using genus-specific primers and probes (PAN-FLAVIVIRUS), which was capable of screening several members of the genus at once, directing and assisting in the species-specific diagnosis.

Objectives: Develop an RT-qPCR for simultaneous detection of members of the *Orthoflavivirus* genus with public health importance, to be used in epidemiological surveillance.

Methodology: The best set of the system was established with primers and probes for the *Orthoflavivirus* genus, located in the NS5 region, the most conserved of the genus. The standardization of the PAN FLAVI assay by RT- qPCR was carried out using ZIKV, DENV 1-4, YFV and WNV in different concentrations to evaluate specificity, repeatability and sensitivity of the methodology, in addition to testing true positive samples with different viruses from different locations, dates and hosts. All development was completed at LAMOL in Bio-Manguinhos.

Results: The PAN-FLAVI molecular assay showed high specificity and sensitivity, being able to detect 42 samples of Yellow Fever, Zika, Dengue, West Nile fever, Ilheus fever and Saint Louis Encephalitis, whose Cts were compared with the results of species-specific tests.

Conclusion: With the satisfactory performance of the assay, it can be considered an excellent tool for monitoring studies on viral vectors and reservoirs and screening of flaviviruses in blood bags and blood products and be able to carry out screening with the detection of several members of the genus at once, targeting and assisting in species-specific diagnosis.

Keywords: Orthoflavivirus; RT-qPCR; Epidemiological-surveillance