IVD.16 - Development and Validation of Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) for Rapid Detection of ZIKV in Human Samples

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Introduction: Zika virus (ZIKV) has emerged as a major global public health concern in the last years due to its link as a causative virus of congenital neurological disease. In countries where there is a circulation of other arboviruses, such as Dengue and Chikungunya, such as Brazil, the clinical diagnosis of ZIKV infection becomes extremely difficult since the signs and symptoms are very similar. Therefore, laboratory diagnosis is of fundamental importance. Currently, the reverse transcriptase reaction followed by quantitative polymerase chain reaction (qRT-PCR) is the gold standard for molecular diagnostic of ZIKV in human samples. However, the technique presents high cost and limitations for Point-of-care (POC) diagnostics. Given the lack of approved vaccines and antivirals against ZIKV, rapid and reliable POC diagnostic test for detection of ZIKV is urgently required to facilitate initiation of control and preventive measures, especially for pregnant women who are at a risk of infection, as well as to increase the diagnostic capacity of ZIKV-affected low-resource scenarios.

Objective: In this context, the aim of this work was to develop and validate a diagnostic platform based on the reverse transcriptase technique followed by isothermal loop-mediated amplification (RT-LAMP) for detection of ZIKV in human samples.

Methodology: In all experiments, the ZIKV strain named PE243/2015 was used. Initially, it was determined the capacity of RT-LAMP to detect ZIKV in biological samples, including serum, urine, saliva and semen under controlled conditions, including experimentally infected biological samples. In addition, the analytical specificity and analytical sensitivity of the assay were evaluated. Subsequently, the validation of the RT-LAMP assay was performed with 40 clinical samples from patients who presented clinical symptoms similar to those caused by ZIKV in the State of Pernambuco, Brazil. Finally, the value *per* reaction was calculated based on the cost of all the reagents.

Results: Regarding the results, the RT-LAMP assay was highly specific for detection of ZIKV in 20 minutes and was up to 10,000 times more sensitive than qRT-PCR for detection of ZIKV in human samples without RNA extraction. The RT-LAMP had a sensitivity of 100%, specificity of 88.46 %, and overall accuracy of 93.48%, highlighting the potential of RT-LAMP for detection of ZIKV in clinical samples. As for the cost of each reaction of the RT-LAMP, the value was one Real (R$ 1.00).

Conclusion: Based on the results obtained in this work, the ZIKV RT-LAMP assay described here represents a potential alternative and inexpensive tool for the molecular diagnosis and routine screening of ZIKV human infections. It could also be useful in monitoring the efficacy of ZIKV eradication and control programs, especially in ZIKV endemic countries which present low resource settings.

Keywords: Diagnostic; Low cost diagnostic tools; ZIKV