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Use of reverse transcription-loop mediated isothermal amplification (RT-LAMP) to identify HIV positive patients with a detectable viral load on antiretroviral therapy

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A novel nucleic acid technique currently being explored to identify pathogens is reverse transcription loop mediated isothermal amplification (RT-LAMP). LAMP amplifies nucleic acid isothermally in a simple heating block within an hour, and is therefore a rapid, cost effective alternative to conventional PCR, making this technique suitable for resource poor areas. The reaction and product detection take place in a single tube by including a colorimetric dye thereby reducing the risk of contamination. This study aims to test and optimize the RT-LAMP assay, a semi-quantitative colorimetric assay, to distinguish between a detectable (≥1 000 copies/ml) and undetectable (<1 000 copies/ml) viral load in HIV positive patients on antiretroviral therapy (ART). RNA was extracted from the DU179 HIV positive control and used for RT-LAMP to determine if HIV-1 RNA could be detected. RT-LAMP primers were designed against highly conserved sequences located within the Gag gene and a published primer for the p24 gene region (Curtis et al., 2008). Conventional RT-PCR showed amplification of the target gene in the DU179 positive control. After incubation at 60°C, the RT-LAMP reaction product changed colour from purple to pale blue, while the negative control remained purple. Agarose gel electrophoresis of this product showed a typical ladder like pattern of bands. We are collecting HIV patient samples for the second phase of this work; where RT-LAMP will be performed on plasma samples with a low (<1000 copies/ml) or high (≥ 1000 copies/ml) viral load.

Biography

Jeyalakshmi Kandhavleu, completed her PhD from the University of Camerino, Italy. She is presently an NRF Postdoctoral fellow within the Department of Internal Medicine, University of the Witwatersrand, South Africa. Previous postdoctoral research was undertaken at the Dr. G. Venkatawamy Eye Research Institute in India. Dr Kandhavleu has published 10 peer-reviewed papers in the field of clinical molecular biology.

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