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Reverse transcription loop mediated isothermal amplication for low cost HIV-1 viral load qualification in resources limited settings

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Abstract

Background: A novel, isothermal nucleic acid amplification method, RT-LAMP, presents potential for nucleic acid amplification-based diagnostics in resource-limited settings. Low-cost HIV-1 viral load monitoring will improve access to ART for HIV-1-infected individuals present in settings where on-site viral load testing is unavailable.

Aim: The aim of this dissertation was to develop an RT-LAMP HIV-1 viral load assay by combining the RT-LAMP reaction with colorimetric amplification detection by hydroxy-naphthol blue dye.

Methods: Different approaches for HIV RNA extraction from patient plasma and culture supernatant were studied to obtain template for RT-LAMP. Reaction products for 4 different RT-LAMP primer sets were analysed using agarose gel electrophoresis and restriction digestion.

Results: The first 3 primers sets produced persistent off-target amplification. The fourth primer set, designed against culture supernatant DU179, produced a target-specific colour change from violet to blue after 1 hour, following optimisation of amounts of Mg_2SO_4 and AMV RT. Further studies showed HNB detection sensitivity to template copy number.

Conclusions: Initial reaction conditions pertaining to an RT-LAMP based, colorimetric HIV-1 viral load assay were established. Further work is required to determine the reaction duration at which the colour change represents a viral load of ≥1000 copies HIV RNA per ml plasma.