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Simultaneous detection of HIV and HTLV by mediator displacement loop-mediated isothermal amplification

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Nucleic acid amplification tests (NAAT) are not only a powerful tool for early diagnosis of HIV infections, NAAT are also a reliable method for HIV viral load measurements during the monitoring of antiretroviral therapy. Additionally, NAAT allow simultaneous (multiplex) detection of different targets enabling the detection of HIV/HTLV co infections. Loop-mediated isothermal amplification (LAMP) [1, 2] emerges as a convenient alternative to polymerase chain reaction (PCR) for rapid amplification of target DNA and RNA. As an isothermal NAAT, LAMP does not require expensive equipment for thermo cycling and is therefore especially suitable for point-of-care testing [3]. However, available multiplex detection techniques for LAMP suffer from elaborate assay design as well as time-consuming optimization work. Here we present the first multiplex reverse transcription (RT) LAMP for identification of HIV/HTLV co-infections [4]. The quantitative real-time assay is based on universal mediator and reporter molecules [5] generating a fluorescence signal in the presence of target sequences. During amplification of target DNA the mediator is displaced (step 1, Figure 1). The released mediator hybridizes to the reporter generating a fluorescence signal (step 2, Figure 1) which can be detected. [4].

Recent Publications:

1. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, Hase T (2000) Loop-mediated isothermal amplification of DNA. Nucleic Acids Research 28: e63i-vii.
2. Nagamine K, Hase T, Notomi T (2002) Accelerated reaction by loop-mediated isothermal amplification using loop primers. Molecular and Cellular Probes 16:223-229.
3. Njiru ZK (2012) Loop-mediated isothermal amplification technology: Towards point of care diagnostic. PLOS Neglected Tropical Diseases 6:e1572:1-4.
4. Becherer L, Bakheit M, Frischmann S, Stinco S, Borst N, Zengerle R, von Stetten F (2018) Simplified real-time multiplex detection of loop-mediated isothermal amplification using novel mediator displacement probes. Analytical Chemistry 90: 4741−4748.
5. Faltin B, Wadle S, Roth G, Zengerle R, von Stetten F (2012) Mediator Probe PCR: A novel approach for detection of realt-ime PCR based on label-free primary probes and standardized secondary universal fluorogenic reporters. Clinical Chemistry 58:1546-1556.

Biography

Lisa Becherer studied chemistry (BSc and MSc) and is currently working on her PhD in Microsystems Engineering at the University of Freiburg at the Laboratory for MEMS Applications, IMTEK. Her research involve nucleic acid analysis with focus on isothermal amplification and digital amplification on centrifugal microfluidic cartridges.

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