LoopTag Real-Time PCR Probe System for Sensitive Pathogen Detection

H. Hanchmann1, T. Kramer1, S. Rödiger1,2, T. Juretzek1, M. Steidle1, C. Schneider1, P. Schwenk1, W. Lehmann1
1 Lausitz University of Applied Sciences, Germany, Stefan.Roediger@HS-Lausitz.de
2 Atomic GmbH, Germany
3 Laborarzt Sindelfingen, Germany
4 Carl-Thiem Kliniek ChemieGmbH, Germany
5 Laborarzt Sindelfingen, Germany

Introduction
In routine laboratory diagnostics quantitative real-time polymerase chain reaction (qPCR) is a powerful method to detect microorganisms in sample materials of different sources. We developed the patented LoopTag real-time PCR probe system [1] as a new qPCR technology for pathogen detection of isolated DNA from different sample materials (e.g., swabs, liquor cerebrospinalis, cultures, ticks). The LoopTag is a gene-specific bimolecular structure which forms during the hybridization of a labelled primer together with a labelled probe on the elongated strand. The design ensures an optimal FRET distance of the fluorescence labels for a good signal-to-noise ratio. The FRET signal is proportional to the formed amount of PCR product. The LoopTag system was tested for detection of clinical relevant pathogens (e.g., swabs, liquor cerebrospinalis, cultures, ticks).

Materials and Methods
We developed the patented LoopTag real-time PCR probe system [1] as a new technology for real-time PCR detection of pathogens isolated from different sample materials (e.g., swabs, liquor cerebrospinalis, cultures, ticks).

Detection of Mycobacterium tuberculosis. All members of the M. tuberculosis complex can be amplified with a detection limit of 2 gene copies per sample with a M. tuberculosis complex-specific primer pair (Figure 2A).

Detection of Borrelia spp. All members of the Borrelia sensu lato complex can be amplified with a detection limit of 1 to 70 gene copies per sample with a Borrelia sensu lato complex-specific primer pair (Figure 2B). Many Borrelia sensu lato species can be differentiated by melting curve analysis (Figure 2D). Only using huge amounts of DNA (10^4–10^6 copies per PCR reaction, except with B. hertmisii) false positive signals with non-pathogenic Borrelia strains and with Treponema strains are avoidable.

Discussion and Conclusion
LoopTag real-time PCR probe system is a new and reliable technology for the real-time PCR detection and characterization of pathogens. It uses cheap conventional fluorescence dyes, exhibits an easy primer design, has no restrictions regarding the PCR product lengths and is highly specific. The LoopTag system is applicable for multiplex real time PCRs and melting curve analysis over a large temperature range. The potential of melting curve analysis for differentiation of more Borrelia and Bordetella species has further to be studied.

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References