Institute of Molecular Pathogenesis, Friedrich-Loeffler-Institut (FLI),
Naumburger Straße 96a, 07743 Jena

DEVELOPMENT OF A NOVEL GENUS-SPECIFIC REAL TIME PCR FOR DETECTION OF MYCOBACTERIA IN INFECTED TISSUE

J. Nieter, K. Sachse, I. Moser

Purpose

It is the aim to establish a rapid diagnostic tool for detection of members of the genus *mycobacterium* in infected tissue

Methods

The 16S rRNA gene was selected as target gene for a genus-specific real time PCR. Primers and probes were designed according to suitable positions in the conserved region of the gene. The specificity was tested using DNA extracts from pure cultures of 35 members of the genus *Mycobacterium* and representatives of 18 non-mycobacterial species. The cut-off was set at 39 PCR cycles.

Results

All the members of the genus *Mycobacterium* included in the study and two representatives of the non-mycobacterial genera *Rhodococcus* and *Gordonia* yielded Ct-values < 39. The real time PCR was further evaluated using DNA extracts from 22 tissue samples of animals with known or suspected mycobacterial infections which had been examined before using a MTC-specific real time PCR and mycobacterial culture. For 68.1% of the tissue samples, culture and 16S rDNA PCR yielded corresponding results. For 13.7% of the samples, PCR was less sensitive than culture, whereas for 18.2% of the samples, PCR was more sensitive than culture. The detection limit was 0.21 genome units /µl DNA extract from infected tissue.

Conclusion

The 16S rRNA gene real time PCR is a useful tool to detect DNA of non-tuberculous mycobacteria in infected tissue. However, due to their genetic similarity to mycobacteria members of the genera *Gordonia* and *Rhodococcus* are also detected.

Corresponding author

Dr. Irmgard Moser
Institute of Molecular Pathogenesis
Friedrich-Loeffler-Institut (FLI)
Naumburger Straße 96a
07743 Jena, Germany
E-Mail: irmgard.moser@fli.bund.de

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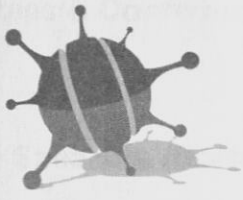

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DVG Service GmbH
Friedrichstr. 17 · 35392 Giessen
Tel.: +49 (0)641 24466 · Fax: +49 (0)641 25375
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