



**P 72 *Borrelia burgdorferi* sensu lato species identification using RT-PCR and *MluI*-LRFP**

T. Cerar<sup>a</sup>, J. Ferdin<sup>a</sup>, F. Strle<sup>b</sup>, E. Ružič-Sabljić<sup>a</sup>

<sup>a</sup> Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Slovenia

<sup>b</sup> Dept. of Infectious Diseases, University Medical Center Ljubljana, Slovenia

Several molecular methods have been employed for *Borrelia* species identification, but information on direct comparison of their findings is limited.

In the present study, we assessed and compared 2 approaches for *Borrelia burgdorferi* sensu lato species identification: (i) a real-time polymerase chain reaction (RT-PCR) based on differences in the sequence of the *hbb* gene and T<sub>m</sub> determination, which combines simultaneous amplification, detection, and differentiation of strains in one PCR run, and (ii) a classical approach based on *MluI* restriction of the entire borrelial chromosome (*MluI*-large restriction fragment patterns, LRFP). Both methods were used for identification of 347 borrelia strains isolated from human clinical samples.

*MluI*-LRFP differentiated all examined *Borrelia* strains into 3 species with several subtypes within particular species. Out of 347 borrelia strains, 177 were identified as *B. afzelii* (174 as Mla1, 2 as Mla2, 1 as Mla3), 147 as *B. garinii* (9 as Mlg1, 112 as Mlg2, 12 as Mlg3, 11 as Mlg4, 2 as Mlg6, 1 as Mlg7), and 22 as *B. burgdorferi* sensu stricto (15 as Mlb2, 7 as Mlb8).

RT-PCR identified a unique T<sub>m</sub> for all strains identified by *MluI*-LRFP to be *B. afzelii* (T<sub>m</sub> 66.11°C) or *B. burgdorferi* sensu stricto (T<sub>m</sub> 68.18°C). The large majority of *B. garinii* strains shared T<sub>m</sub> 51.42°C, while *MluI*-LRFP subtype Mlg4 was characterized by T<sub>m</sub> 56.87°C.

Both methods allowed the identification of the individual species within the *B. burgdorferi* sensu lato complex. The major advantage of RT-PCR was that it was easy to perform and that the results were obtained within a few hours, whereas the time-consuming *MluI*-LRFP enabled identification of the subtypes within particular species. RT-PCR gave a unique T<sub>m</sub> for all strains and completely concordant results with *MluI*-LRFP concerning *B. afzelii* and *B. burgdorferi* sensu stricto species, but revealed 2 clusters within *B. garinii*.