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Development of absolute quantification method for genotype-specific *Babesia microti* using Real-Time PCR and practical experimental tips of Real-Time PCR

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Abstract

Babesia microti, a rodent babesia, is known as a pathogen of zoonosis, human babesiosis, is composed of several genotypes of small subunit ribosomal RNA gene (SSUrDNA) and different genotypes have been suggested to have different infectivity and pathogenicity to humans. We established a real-time PCR assay using SYBR Green I, which allows specific detection and absolute quantification for each SSUrDNA-type-*B. microti* of four SSUrDNA-types found in Japanese rodents even in mixed infection. In this assay, four genotype-specific primer pairs targeted on internal transcribed spacer 1 or 2 sequences were used. Primer pairs have the characteristics for a high specificity for homologous genotype DNA. The calibration curves of cycle threshold (Ct) values versus log concentrations of DNA for all four genotypes were linear over 10^7 fold range of DNA concentrations with correlation coefficient from 0.95 to 1 and sufficient amplification efficiency from 90% to 110%. The standard curves for all four genotypes were not changed even in the presence of heterologous DNA.

In this paper, we introduce how to establish and perform the genotype-specific real-time PCR and our practical experimental tips to be recommended.