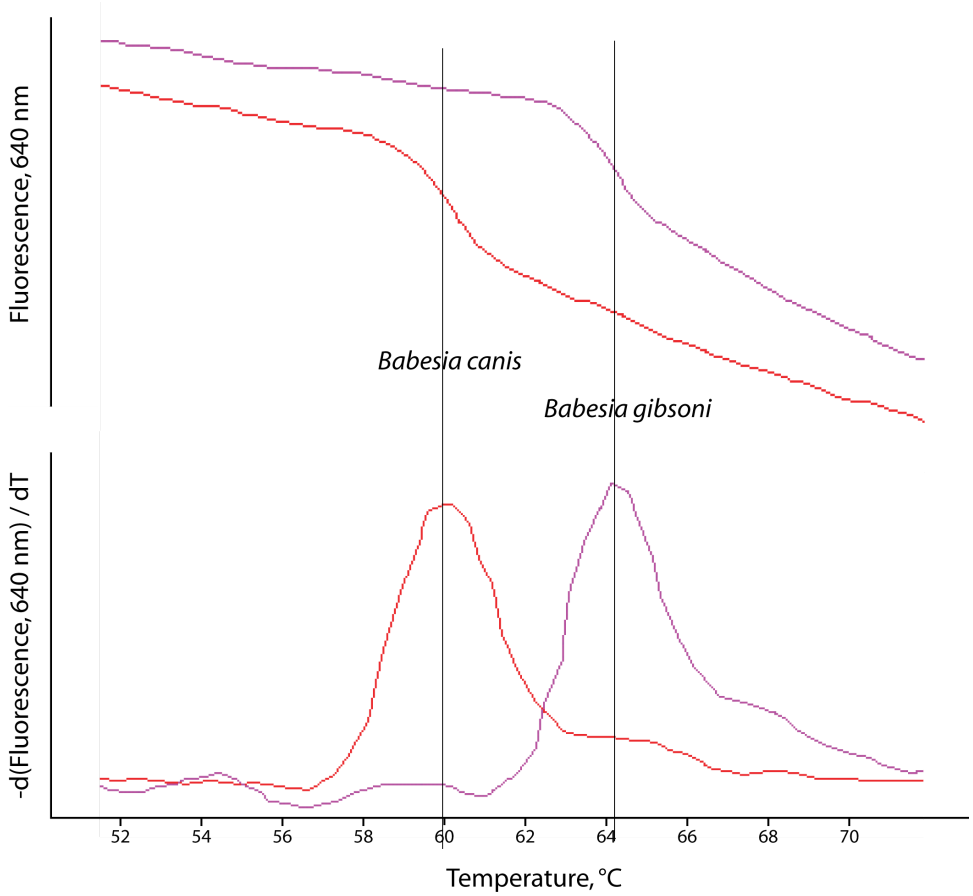
AUBURN UNIVERSITY
COLLEGE OF
VETERINARY MEDICINE

Differentiation

Determination of genetic polymorphisms of the amplified pathogen sequences allows typing of pathogens or detection of mutations associated with genetic diseases.

- The melting curve of amplification product (upper curves in the melting graph) indicates the temperature at which the probes separate from the amplification product.
- The peak of the negative first derivative (lower curves in the melting graph) of the melting curve identifies the temperature at which maximum probe separation occurs = melting point (T_m).
- Single base mismatches reduce the melting point by ~2°C and allow for reliable detection and typing of mutations.



Melting point determination of the amplification products of *Babesia gibsoni* and *Babesia canis*. The *Babesia gibsoni/canis* real-time PCR assay amplifies the target sequence of both species, but the sequences to which the green fluorescent donor probe attaches differ by 2 bases between *B. gibsoni* and *B. canis*. These differences result in a Tm of 64.2°C for *B. gibsoni* and of 60.0 °C for *B. canis* and allow clear identification of each species by melting curve analysis in the same PCR.

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