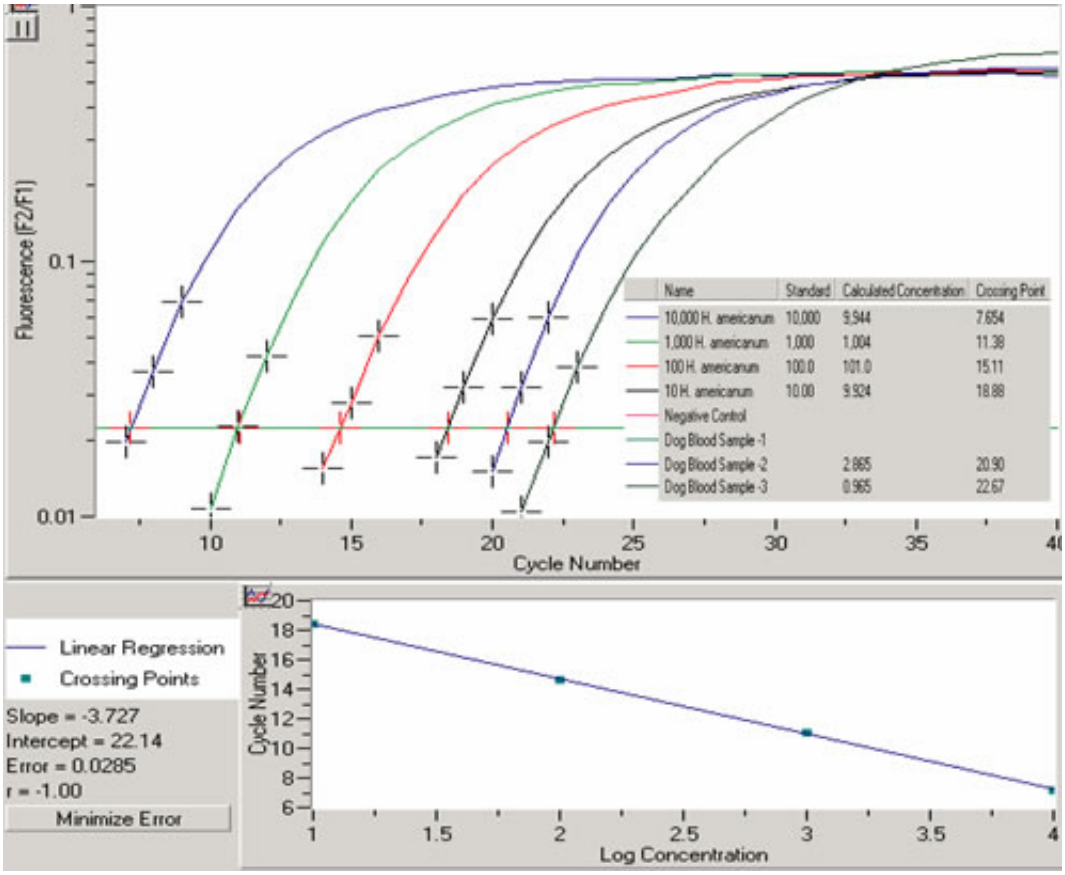
AUBURN UNIVERSITY
COLLEGE OF
VETERINARY MEDICINE

Quantification

The intensity of the fluorescent signal is proportional to the amount of amplification product and is measured at each thermal PCR cycle.

- The number of thermal PCR amplification cycles required for the threshold fluorescent signal increases with low target copy numbers.
- A linear regression for the relation between threshold cycle number and logarithm of copy number per standard target is constructed.
- The threshold cycle for positive samples is converted into starting target copy numbers by use of the standard regression.



Quantification of target nucleic acids in real-time PCR. Three aliquots of nucleic acids extracted from EDTA blood of a dog chronically infected with very low numbers of *Hepatozoon americanum* are examined for monitoring of the response to therapy. The cycle number at which the fluorescent signal reaches the threshold level is negatively correlated to the logarithm of target copies in the sample. Standards from 10,000 to 10 copies of the *Hepatozoon* spp. 18S rRNA gene target are used to compute a linear regression between target copy number and threshold cycle. The copy number in the unknown samples is calculated from the threshold cycle using this regression equation. The graph displays fluorescence intensity at each amplification cycle.