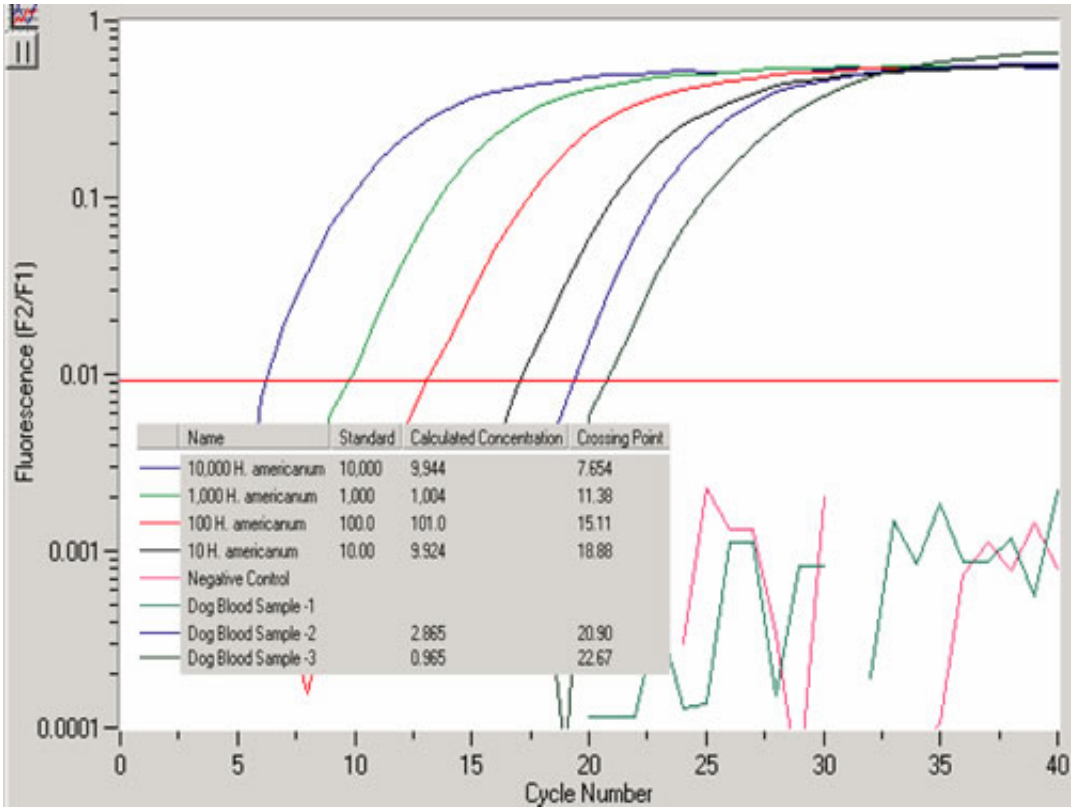
AUBURN UNIVERSITY
COLLEGE OF
VETERINARY MEDICINE

# Sensitivity

All real-time PCR methods in the Auburn University Molecular Diagnostics Laboratory are validated for robust amplification of single copies of target nucleic acid in a high background of unrelated nucleic acids.

- For each assay, sensitivity is optimized by limiting dilution of nucleic acid standards and specimens, and by multiple PCR assays of each sample dilution.
- At dilutions with - on average - single target copies in the PCR sample aliquot, some PCRs will be positive while others will remain negative (Poisson sampling distribution).
- Amplification efficiency of single-target samples is equal to efficiency of samples with high target copies (similar slope of amplification curve).
- This approach ensures that amplification even after many PCR thermal cycles remains powerful so that even single target copies can be strongly amplified.




**Robust real-time PCR methodology for reliable amplification of single target copies.** Three aliquots of nucleic acids extracted from EDTA blood of a dog chronically infected with very low numbers of *Hepatozoon americanum* are examined by amplification of *Hepatozoon* spp. 18S rRNA gene target sequences. The graph displays fluorescence intensity at each amplification cycle. During the exponential amplification phase, low target copy numbers are correlated with delayed appearance of the signal. The robustness of the assay is indicated by the fact that samples with 1 or 3 target copies display as efficient amplification as the standards with high copy numbers while samples without *Hepatozoon americanum* target DNA show only a background signal below the detection threshold.