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## VALIDATION OF FELINE, BOVINE, EQUINE AND CERVID QUANTITATIVE PCR ASSAYS

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In recent years, animal DNA analysis has been increasingly used in forensic investigations. While many of the procedures are similar to those in human casework, laboratories analyzing animal DNA face additional challenges. One of the largest is addressing the diverse species that are present in forensic casework. Although many species-specific genotyping panels have been developed, methods for quantifying the DNA of those various species have not. The advantages of quantifying target DNA for genotyping applications have been thoroughly demonstrated. By quantifying the DNA, the amount used in subsequent analyses can be optimized to yield high-quality results while limiting the consumption of valuable samples. A real-time PCR assay for the quantification of canine DNA was published previously, but the need for quantitative PCR assays for other species has not been met. Four quantitative-PCR assays have been developed to accurately quantify feline, bovine, equine, and cervid DNA—all species of forensic interest. Each Taqman®-based assay has two targets: one set of primers targets a portion of the *Melanocortin-1 Receptor (MC1R)* gene unique to the target species and is detected with a FAM-labeled TaqMan MGB probe; a second set of primers target a piece of synthetic DNA that acts as an internal positive control (IPC) allowing determination of inhibition using a VIC-labeled TaqMan MGB probe. For each species, these two primer-probe sets are run in duplex allowing quantification and determination of inhibition in one assay. The assays were optimized to allow accurate quantification of a target species' DNA in mixed-species samples that are encountered in animal-related casework. Developmental validation was carried out for each assay following the revised guidelines of the Scientific Working Group on DNA Analysis Methods (SWGDAM). The validation included verification of optimal primer and probe concentrations, precision, accuracy, lower detection limit determination, reproducibility and species specificity. Studies on the effects of inhibition and DNA mixtures were completed. Upon validation, the assays were incorporated into the Standard Operating Procedures for casework. The incorporation of these assays into forensic casework analysis has both conserved laboratory resources as well as optimized genotyping of samples being profiled. Examples in the application of these assays to animal forensic casework are also presented.