A80 The Development of a Screening Method for Biological Samples Using a Real-Time PCR Assay for HLA-DQA1

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The goal of this presentation is to describe the development of a screening method for biological samples using a real - time PCR assay for HLA-DQA1.

This presentation will impact the forensic community by providing an analytical method to those in the forensic biology community that will screen DNA samples for possible common origin.

In laboratories with limited resources, a DNA screening test to identify potential samples from a common origin would be of great benefit. Differentiation of bloodstains from different sources on a sample of biological evidence is often based on bloodstain pattern analysis which could be problematic to forensic biologists who may not have the experience or training to make the correct interpretation. The development of a DNA screening test using real-time PCR was attempted by examining differences in the slopes of melting curves and first derivative melting curves, and the melting temperature of HLA-DQA1 amplicons from samples from individuals with varying HLA-DQA1 genotypes. HLA-DQA1 was chosen as a screening locus to develop the screening method because its polymorphism is based on sequence creating the likelihood of melting curve and melting temperature variation as a function of genotype. In addition, polymorphisms from HLA-DQA1 are well established considering that the HLA-DQA1 locus was once routinely used in forensic DNA typing. The HLA-DQA1 locus was amplified in buccal swabs from several individuals with known HLA-DQA1 genotypes using a real-time PCR assay utilizing SYBR green to measure PCR product through fluorescence. Samples were amplified on a Corbett RotorGene 6000 using the following PCR parameters: 95^0C hold for 10 minutes to activate the polymerase, 95^0C for 15 seconds, 55^0C for 30 seconds, and 72^0C for 30 minutes for 40 cycles. Subsequent examination of melt curves and the melting temperature of amplified product show that variation does occur with HLA-DQA1 genotype and replicate results from the same genotype are reproducible. Results thus indicate that HLA-DQA1 can serve as a screening locus to help locate biological samples from different sources prior to the development of a DNA profile. Additional study is also being conducted to determine the possibility that this assay could simultaneously quantitate high copy DNA samples such as bloodstains.

DNA Screening Method, Real - Time PCR, HLA-DQA1

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