B191 Development of a High Resolution Real-Time Polymerase Chain Reaction (PCR) Melt Assay for Identifying "Legal High" Plant Material

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After attending this presentation, attendees will better understand plants that endogenously produce chemicals used as "legal highs," how genetic targets can be probed using PCR, and how "legal highs" can be identified using high-resolution melt assays.

This presentation will impact the forensic science community by providing results of the development of a multiplex to probe several "legal high" plant species as well as specificity, reproducibility, selectivity, and sensitivity data for the singleplex assays. This study presents assays that could help forensic laboratories identify trace quantities of plant material in comingled samples.

Mixture analysis has been a cornerstone of human DNA typing methods for several years. Forensic laboratories focus a significant portion of their caseload on detecting and identifying controlled substances and new drugs of abuse. The interpretation of trace material is complicated by the fact that the quantity of recoverable material can fall below the detection limits of instrumental methods. While microscopy is used to identify botanical material, this method has limitations when only trace material is present. Similarly, Gas Chromatography/Mass Spectrometry (GC/MS) is used to identify the active drug compounds contained in the plant material when sufficient material is present for detection and identification. As PCR amplifies the extracted genetic material, it has the advantage of detecting trace or low-template DNA. High resolution PCR melt assays have been developed to detect and identify four plant species: *Cannabis sativa* (marijuana), *Papaver somniferum* (poppy), *Ipomoea purpurea* (morning glory), and *Datura stramonium* (jimson weed). Marijuana and poppy are the two most prevalent internationally abused herbal highs; jimson weed and morning glory can be found in gardens and along roadsides. Reports have indicated that users consume the seeds or plant material whole or via teas or smoking.

To differentiate the plants, PCR primers specific for each plant were designed using public genome data from the National Center for Biotechnology Information (NCBI) and evaluated uniqueness using the Basic Local Alignment Search Tool (BLAST). Each set of PCR primers was designed to produce an amplicon from the plant of interest with a specific melt temperature that differs from amplicons produced with primers for the other plants. DNA was extracted from plant and seed material using the QIAGEN® DNeasy® Plant Mini Kit and primers were obtained from IDT. Using the Bio-Rad iTaq™ Universal SYBR® Green Supermix, the primers were tested for specificity, selectivity, sensitivity, and reproducibility in singleplex reaction assays. Agarose gel electrophoresis was used to confirm production of PCR amplicons of the designed sizes. Progress in multiplexing primer sets to simultaneously detect one or more of the species using an LCGreen mastermix will also be presented. The PCR melt assay is an inexpensive, quick, and specific method to detect and identify genetic material derived from "legal highs." It is expected that the assay could be employed by forensic laboratories to detect and identify "legal highs" in the current international drug market.

DNA, PCR, High-Resolution Melt

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