B114 Identification of Kratom (*Mitragyna Speciosa*) DNA Using a Real-Time Polymerase Chain Reaction High-Resolution Melt (PCR HRM) Assay

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After attending this presentation, attendees will be able to evaluate how a newly developed real-time PCR assay coupled with post-PCR HRM can be used for kratom identification when dealing with degraded or mixture plant samples in forensic casework.

This presentation will impact the forensic science community by demonstrating a new method to identify kratom from other illegal and "legal high" plants that may be in forms indistinguishable by eye and through microscopic techniques. This process could be used as a cost-saving alternative to chemical analysis currently in use, such as Fourier Transform Infrared (FTIR) or Gas Chromatography/Mass Spectrometry (GC/MS). In addition, equipment required for this screening is readily available to forensic DNA scientists in major crime labs.

Kratom (*Mitragyna speciosa)* is a plant that is a member of the family Rubiaceae, or coffee family, and is commonly used for the analgesic, opiate-like, and stimulant properties from the alkaloids it produces. The most famous and widely cited of these alkaloids is mitragynine as it is the most abundant metabolite, although there are more than 40 alkaloids produced by the plant, including 7-hydroxymitragynine. While it is widely referred to as kratom worldwide and in its native Southeast Asia, it is also referred to by other names in various countries, including biak-biak or ketum in Malaysia, krathom, kakuam, ithang, or thom in Thailand, and mambog in the Philippines. Its popularity among users primarily relies on its stimulant and pain-relieving properties, which have been reported to be 17 times more potent than morphine.

The first country to formally control the use of kratom was Thailand in 1943. In recent years, its use as a recreational drug has increased worldwide and numerous case studies have been reported in the literature documenting various effects on users and in the extreme instances, including its presence in cases of death in which the drug was taken in combination with other legal and illegal substances. Western nations have responded to this recent increase of prevalence by all-out banning, controlling, and/or imposing restrictions to only be used with a medical prescription. The United States Drug Enforcement Administration (DEA) currently includes kratom on the list of Drugs and Chemicals of Concern. As of September 2016, the DEA reopened the comment period to consider labeling kratom as a Schedule I substance; thus, its legality currently defers to a state-by-state basis.

Current methods of identification seen in the literature include visualization techniques such as colorimetric tests and microscopy and quantitation through alkaloid chemical analysis such as Hydrogen-1 Nuclear Magnetic Resonance (^{1}H NMR), GC/MS, FTIR, High-Performance Liquid Chromatography (HPLC), and Direct Analysis in Real-Time Mass Spectrometry (DART®-MS), to name a few. Recent DNA techniques have relied on Restriction Digest Length Polymorphism (RFLP) assays.

In this study, primers designed for the PCR-HRM assay targeted the Secologanin Synthase 2 (SLS2) and Strictosidine Beta-D-Glucosidase (SGD) genes within the *M. speciosa* genome. Amplification of SLS2 and SGD genes produced amplicons of 168bp and 66bp, respectively. The SLS2 primer set was specific for *M. speciosa* against other "legal high" plant species such as *Cannabis sativa*, *Datura wrightii*, and *Papaver orientale* with a melt curve of 77.09°C. Further sensitivity testing indicated continued detection of SLS2 amplicon at concentrations as low as 0.05µg/mL.

Kratom, Real-Time PCR, High-Resolution Melt

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