

Customer Evaluation of Thermo Scientific™ CEDIA™ Mitragynine (Kratom) Assay in Drug Courts and Reference Laboratories

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INTRODUCTION

Opiate addiction has reached epidemic proportions in the United States and people have turned to natural alternatives for self-management of pain. One such alternative is kratom (*Mitragyna speciosa*), a tree indigenous to Southeast Asia. The main alkaloids in kratom include mitragynine, speciogynine, speciociliatine, paynantheine, and 7-hydroxymitragynine. Studies have identified 3 major metabolites for mitragynine: 9-desmethylmitragynine, 16-carboxymitragynine, as well as 7-OH mitragynine. Kratom is commonly ingested by chewing or smoking kratom leaves, or drinking tea brewed using Kratom leaves. In the United States, kratom can be purchased in various forms, including capsules, powders, e-liquid, and chocolate bars. Kratom is currently not a federally controlled substance but is listed as a "drug of concern" by the US Drug Enforcement Administration. Kratom is banned by several cities, counties and states within the US.

OBJECTIVES

We recently developed the CEDIA Mitragynine (Kratom) Assay to detect mitragynine, the main alkaloid of kratom, in human urine. The assay was provided to drug courts and reference laboratories for evaluation. The assay is currently intended for Criminal Justice and Forensic Use Only.

MATERIALS AND METHODS

CEDIA technology is based on the bacterial enzyme β -Galactosidase which has been genetically engineered into two inactive fragments, Enzyme Acceptor (EA) and Enzyme Donor (ED). These fragments spontaneously re-associate to form an active enzyme. In the absence of analyte from the sample, the specific antibody binds to the ED-drug conjugate causing a decrease in enzyme activity. The free drug in the sample will compete for the limited number of antibody binding sites, making the ED-drug conjugate available for complementation to form an active enzyme. This phenomenon creates a direct relationship between the drug concentration in urine and enzyme activity. The enzyme activity is then determined spectrophotometrically at 570 nm.

The CEDIA Mitragynine (Kratom) reagents are provided in lyophilized form, while the calibrators and controls are liquid ready-to-use. The assay uses 5 calibrators (0, 20, 50, 100 and 200 ng/mL), with 50 ng/mL as the cutoff calibrator. The controls are at \pm 25% of the cutoff (37.5 and 62.5 ng/mL). The assay was provided to drug courts and reference laboratories, together with draft application parameters for Thermo Scientific™ Indiko™ and Beckman Coulter™ AU™ series automated analyzers.

The CEDIA Mitragynine (Kratom) Assay is a screening test. Confirmation of positive results were performed by LC-MS/MS.

RESULTS

A total of 1446 patient samples were tested from 11 laboratories from 7 states, including Ohio, Alabama, California, Oklahoma, Maryland, Massachusetts and Florida.

Graph 1. Patient Samples Distribution by States

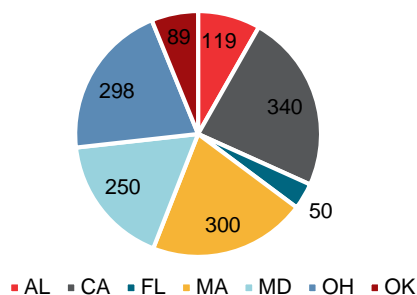


Table 1. CEDIA Mitragynine (Kratom) Assay Customer Evaluation Results

Sample Size (N=)	Screened Positive (\geq 50 ng/mL)	Screened Negative (N=)
1446	49	1397

Table 2. LC-MS/MS Confirmation Results for Mitragynine

Screened Positive (\geq 50 ng/mL)	LCMS Confirmed (\geq 50 ng/mL)	Discrepant (Contains Mitragynine)
49	42	7

Table 3. Mitragynine Concentration in Discrepant Samples

Sample ID	CEDIA Mitragynine Immunoassay result (ng/mL)	LC-MS/MS Mitragynine concentration (ng/mL)
ACT 37	168.4	5.75
705764	110.1	9.85
1812120273	77.9	15.8
1812260201	200	35
1812110126	53.7	47
V970099	53	27
V970181	123	16.55

CONCLUSIONS

The Thermo Scientific CEDIA Mitragynine (Kratom) Assay was able to detect mitragynine in human urine accurately when tested in drug courts and reference laboratories, with no false positives observed from other commonly prescribed or abused drugs.

ACKNOWLEDGEMENTS

The following reference laboratories and drug courts participated in the evaluation:

1. ACT Innovations
2. Frederick County Drug Courts
3. Preferred Laboratory LLC
4. Friends Medical Laboratory, Inc.
5. DTPM/Family Life Center, Inc.
6. Mt Vernon Municipal County
7. Fairfield County Municipal Drug County
8. NextGen Laboratories
9. National Labs
10. The Compliance Resource Group, Inc.
11. San Diego Reference Laboratory

TRADEMARKS/LICENSING

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NOTE

The assay is for Criminal Justice and Forensic Use in the USA Only. The assay is not FDA 510(k) cleared. The assay is not registered nor is it approved for sale in Europe.