K37 Performance Characteristics of the Neogen ELISA Screening Assay for the Detection of Synthetic Cannabinoids in Urine

Eliani Spinelli, PhD, 251 Bayview Boulevard, 05A505, Baltimore, MD 21224; Allan J. Barnes, BS, 5500 Nathan Shock Drive, Rm 373, Baltimore, Maryland 21224; Jose Luiz Da Costa, PhD, 251 Bayview Boulevard, Ste 200, Rm 05A721, Baltimore, MD 21224; and Marilyn A. Huestis, PhD, Chemistry & Drug Metabolism, Intramural Research, NIDA, NIH, 251 Bayview Boulevard, Rm 05A721, Baltimore, MD 21224*

After attending this presentation, attendees will become familiar with the validation and performance characteristics of an Enzyme Linked Immunosorbent Assay (ELISA) urine screening procedure for detection of synthetic cannabinoids in urine.

This presentation will impact the forensic science community by improving interpretation of synthetic cannabinoid immunoassay results and guiding selection of appropriate cutoff concentrations.

Cannabis is the oldest drug of abuse in the world. Synthetic cannabinoids are touted as a legal and safer alternative to cannabis as routine urine drug screening methods do not detect synthetic cannabinoids, making them attractive to users who must undergo random or regular urine drug screenings. The recent emergence of these new legal variants on the market and their widespread availability make it difficult for laboratories to identify and regulatory agencies to prohibit. The goal of this research is to demonstrate that the Neogen® JWH-018 (SPICE) ELISA kit is a highly sensitive and selective method for the rapid detection of JWH-018 N-pentanoic acid in urine.

The Neogen® (SPICE) ELISA kit contained all components required for routine analysis and the assay was performed without modifications according to manufacturer's instruction. Automated analysis was performed on a Freedom EVO® 100 platform configured with a microtiter plate washer and reader.

Performance was evaluated by analyzing results from 2,469 authentic urine samples. Two cutoff concentrations (5 and 10µg/L) were evaluated to classify the samples as positive or negative and establish diagnostic efficiencies. Performance challenges at ±25 and ±50% of these cutoff levels also were investigated to determine intra- and inter-plate imprecision. All 2,469 urine samples were assayed by a qualitative LC/MS/MS for 29 synthetic cannabinoids. Immunoassay results were compared to LC/MS/MS results to determine true positive (TP, positive in both assays for any synthetic cannabinoid), true negative (TN, negative in both assays), false positive (FP, positive in the Neogen® assay but negative for all synthetic cannabinoids by LC/MS/MS), and false negative (FN, negative in the Neogen® assay but positive by LC/MS/MS). Sensitivity was determined as (TP/TP + FN)*100, specificity as (TN/TN +FP)* 100, and efficiency as ((TP + TN)/(TP +TN + FP + FN))*100.

The Neogen® assay was linear from 1-250µg/L with a calculated limit of detection of 0.53µg/L. Intra-plate imprecision (N=7) was <4%, while inter-plate imprecision (N=34) was <9%. Sensitivity, specificity, and efficiency results with the 5µg/L cutoff were 79.9%, 99.7%, and 97.4% and with the 10µg/L cutoff were 69.3%, 99.8%, and 96.3%, respectively. Eighteen of 73 individual, synthetic cannabinoids (25%) exhibited moderate to high cross-reactivity to the target compound. No interferences were present from 94 common drugs of abuse, metabolites, co-administered drugs, over-the-counter medications, or structurally similar compounds.

The absence of extensive sample preparation requirements and short incubation times prove that analysis using the Neogen® (SPICE) ELISA kit, with a high-speed automated system, is a viable method for screening synthetic cannabinoids in urine targeting JWH-018 N-pentanoic acid.

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Synthetic Cannabinoids, ELISA, Legal Highs

 ** Presenting Author*