K27 Enzyme Linked Immunosorbent Assay (ELISA) for Detection of Use of the Synthetic Cannabinoid Agonists UR-144 and XLR-11 in Human Urine

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After attending this presentation, attendees will be able to describe the method validation parameters used to asses this ELISA kit as well as the performance characteristics established for each parameter during the validation. Attendees will also be able to evaluate the applicability of the UR-144/XLR-11 Direct ELISA kit for screening large-scale populations.

This presentation will impact the forensic science community by providing information about a novel validated ELISA and its performance designed to detect two of the currently most prevalent synthetic cannabinoids in urine.

The purpose of this project was to develop and validate an ELISA designed to test for metabolites of the synthetic cannabinoid UR-144 and its fluorinated homolog XLR-11 in urine.

The dynamic synthetic cannabinoid drug market has created the need to continually revisit and update approaches for screening for use of these drugs in urine. Prior to use for forensic purposes, these approaches need to be thoroughly validated. The current federal regulations regarding these compounds and the inability to detect synthetic cannabinoids on traditional marijuana immunoassay tests have created the need for sensitive cost-effective assays capable of screening large-scale populations. As recently as 2012, tetramethylcyclopropylindoles, including UR-144 and its closely related analog XLR-11 began to appear on the Novel Psychoactive Substance (NPS) market. Like other synthetic cannabinoids, UR-144 and XLR-11 act on the cannabinoids receptors (CB1 and CB2) in the central and peripheral nervous system to elicit effects similar to delta-9-tetrahydrocannabinol (THC).

In this study, the UR-144/XLR-11 ELISA kit was validated for use on human urine samples, although it is also suitable for use on other matrices. The principle of the ELISA kit was based on the competitive binding of the analyte and a UR-144 pentanoic acid metabolite-peroxidase conjugate, to a proprietary antibody coated on the 96-well microplate. The assay was calibrated at 5ng/mL with the pentanoic acid metabolite of UR-144. The cut-off of 5ng/mL was selected to reflect the concentrations of metabolites encountered in actual subject samples from a large screening population tested in-house. The performance of the assay below the cutoff concentration of 5ng/mL was not challenged.

The UR-144/XLR-11 assay has no significant cross-reactivity with most other commonly encountered synthetic cannabinoids and their metabolites, such as JWH-018, AM-2201, JWH-210, and others. However, the assay does cross-react with UR-144-5-OH metabolite (100%), UR-144-4-OH metabolite (50%), and XLR-11-4-OH metabolite (50%) in addition to UR-144 pentanoic acid, which is used as the calibrator. Besides a broad range of synthetic cannabinoids and their metabolites, a range of therapeutic drugs and several commonly used drugs of abuse including benzoylecgonine, cocaine, codeine, EDDP (a metabolite of methadone), MDMA, methamphetamine, methadone, morphine, and PCP in addition to THC, 11-nor-9-carboxy-THC, 11-hydroxy-THC, and cannabidiol were evaluated to determine potential interference with the assay. None produced any positive results at a concentration of 20,000ng/mL.

The long-term stability of the kit is currently under investigation. To date, the assay appears to be stable and performed acceptably after 4.3 months. With respect to intraplate and interplate precision, the UR-144/XLR-11 kit was precise with a coefficient of variation of less than 15% for the negative (3.75ng/mL), positive (6.25ng/mL), and cutoff (5.0ng/mL) controls. Both carryover and plate drift were examined using the UR-144 pentanoic acid calibrator. The assay was free from carryover at a concentration of 1,000ng/mL and no plate drift was observed.

One hundred blind controls were prepared for screening by the assay. Forty were negative controls, verified by LC/MS/MS, to which no drug or metabolite was added. Sixty other positive controls that contained UR-144-4-OH, UR-144-5-OH, and UR-144 pentanoic acid were spiked at varying concentrations ranging from 0-100ng/mL. The assay yielded 40 true negatives and 60 true positives, giving an overall sensitivity, specificity, and accuracy of 100% for the assay.

The rapidly changing synthetic cannabinoid market and continuous regulation of these compounds, including UR-144 and XLR-11, has created a need for rapid screening techniques capable of handling high volumes of samples. Using the UR-144 Direct ELISA assay kit, screening urine samples is both highly cost effective, specific, and had appropriate sensitivity for actual patient populations.

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