K4 Detection of Trace Buprenorphine and Norbuprenorphine in Human Hair Using Enzyme-Linked Immuno-Sorbent Assay (ELISA)

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After attending this presentation, attendees will be able to develop, validate, and implement an ELISA method in their forensic toxicology laboratories for detecting buprenorphine and its metabolite, norbuprenorphine, in human hair.

This presentation will impact the forensic science community by introducing a sensitive, robust, and short turn-around-time method to detect both the parent drug and the metabolite to support surveillance of compliance with opioid dependence treatments.

Buprenorphine (BUP) is a partial mu opioid agonist with kappa opioid antagonist property that has been used as a substitution drug for opioid dependence treatment; however, the drug has potential for abuse and is more easily obtained than other substitution drugs such as methadone. A few studies have suggested that hair analysis of BUP and norbuprenorphine (norBUP), the major N-dealkylated metabolite, can complement urine drug analysis to monitor the drug intake in a detection window of up to three months. Combined BUP and norBUP were reported to accumulate to more than 20pg/mg in hair at a dose as low as 0.2mg/week maintained for two to three months.

Currently at this study's laboratory, only a Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS) method has been implemented to quantitate both BUP and norBUP in human hair with a Lower Limit Of Quantitation (LLOQ) of 8pg/mg for each analyte. Only ~30% of the hair samples in the laboratory reported out with quantitated results had BUP/norBUP ratio greater than 1.0 (BUP 8 — 1,517pg/mg, norBUP none-detectable — 1,295pg/mg), and the rest had norBUP as the predominant analyte, including those in which only norBUP was quantitated (BUP none-detectable — 775pg/mg, norBUP 19 — 2,192pg/mg).

An ELISA method was sought to be utilized as the initial detection method, which ideally should detect both the parent drug and its metabolite at the desired analytical sensitivity.

An ELISA kit targeting BUP was validated according to the Scientific Working Group for Forensic Toxicology (SWGTOX) guidelines. An approximate 20mg aliquot of 1.5-inch hair segment proximal to scalp was washed once with acetone, pulverized, and then sonicated with heat in 1.5mL methanol for two hours. The methanol mixture was centrifuged and 1.0mL of the resulting supernatant was evaporated and reconstituted with the diluent provided by the ELISA kit. Laboratory-determined volume of the final reconstituted hair extract was then pipetted for ELISA development according to the manufacturer's package insert. The assay principle is heterogeneous-competitive ELISA, where the intensity of the developed color is inversely proportional to the sample drug concentration. The absorbance of each sample well was normalized to that of the negative controls (B/B0) within the same batch. The desired cut-off level was 20pg/mg of BUP in hair.

Controls at five concentrations (cut-off, ±25% and ±50% of cut-off) were prepared for analysis of precision and a control at 100pg/mg was included to determine extended assay linearity. Coefficients of variation for the measured B/B0 were 4.6%-11.4% within-run (n=4) and 7.6%-11.7% between-run (five runs, n=20). The B/B0 mean ±2 Standard Deviations (SDs) for concentrations at ±50% of cut-off were well separated from the mean B/B0 at cut-off. Correlation coefficient R2 of B/B0 versus concentrations (expressed in logarithm) was 0.9953, demonstrating satisfactory linearity. The mean B0 — 3.3×SDs determined limit of detection to be 6.3pg/mg. The ELISA did not present hook effect and carry-over at least at 2,000pg/mg. The assay did not show interference from common over-the-counter or prescription drugs at 25ng/mg.

The previously analyzed hair samples in-house for BUP and norBUP by LC/MS/MS were de-identified and randomly chosen based on their quantitated results for ELISA analysis. The hair samples were categorized into three groups: (1) High BUP group with >30pg/mg of both BUP (32 — 1,517pg/mg) and norBUP (155 — >2,000pg/mg) (n=10); (2) Borderline BUP group with non-detectable — 21.7pg/mg BUP and 27.3 — 123pg/mg norBUP (n=8); and, (3) Negative BUP group with non-detectable BUP and norBUP (n=9). All High BUP and seven Borderline hair samples were determined positive, and all Negative hair samples were determined negative by the ELISA method. One Borderline hair sample (BUP=17.9, norBUP=54.2pg/mg) was equivocal to cut-off controls. With this limited sampling size, the ELISA demonstrated to detect both parent drug and its metabolite, achieving ≥94.4% sensitivity and 100% specificity.

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To provide forensically defensible toxicology results, an initial detection of substances should be confirmed whenever possible by a second technique based on a different chemical principle. The ELISA developed and validated herein satisfies the laboratory's needs to be implemented as an appropriate initial test method to complement the currently used confirmatory LC/MS/MS method.

Buprenorphine, Hair Testing, ELISA

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