K49 Aligning With the National Safety Council's Recommendations: Redesigning the Enzyme-Linked Immuno-Sorbent Assay (ELISA) Screen Testing Scope and Improving Sensitivity for Driving Under the Influence of Drugs (DUID) Investigation Cases

Ayako Chan-Hosokawa, MS, NMS Labs, 3701 Welsh Road, Willow Grove, PA 19090*

After attending this presentation, attendees will better understand how to evaluate prevalency data, the changes in drug trends, and observed blood drug concentrations in impaired driving investigation cases to optimize the routine immunoassay screen ELISA.

This presentation will impact the forensic science community by providing information regarding constantly changing drug trends and the drug screen positivity rates over the years in DUID investigations and by demonstrating the need to update the ELISA drug screen.

With an increasing awareness of the high prevalence of drug use in drivers, both the Driving Under the Influence of Drugs, Alcohol and Medicines (DRUID) and the National Safety Council (NSC) published a set of standardized guidelines for toxicological investigation of drug and impaired driving and motor vehicle fatalities. Based on the 2012 recommendations by the NSC, the scope and sensitivity of DUID screening and confirmation procedures at NMS Labs were evaluated and optimized.

The scope of screen and confirmation tests as well as cut-off concentrations for blood, urine, and oral fluid in NMS Lab's DUID panel were assessed and determined to be mostly in agreement with the new recommended guidelines. To align with the NSC's recommendations, a few improvements were proposed: (1) removal of propoxyphene from the ELISA screen; (2) inclusion of zolpidem and carisoprodol to the ELISA screen; (3) improvement of the ELISA screen sensitivity for low-dose benzodiazepines such as lorazepam and clonazepam; and, (4) improvement of confirmation cut-offs for opiates (i.e., 6-monoacetylmorphine, oxymorphone, and hydromorphone). Each of the proposed improvement projects was further supported by the prevalence study, as well as observed blood drug concentrations and/or therapeutic drug concentrations.

The use of propoxyphene has significantly decreased since 2010, when the compound was banned by the Food and Drug Administration (FDA). The positivity rate of 0.11% (44 out of 39,260 cases) in the past five years (since January 2010) was the lowest among the compounds included in the ELISA screen. Thus, propoxyphene was removed from the ELISA screen scope and moved to an expanded therapeutic drug screening scope using a mass spectrometry technique.

In addition, the popularity of the compounds zolpidem, carisoprodol and its metabolite meprobamate in DUID cases was determined by calculating positivity rates from the expanded therapeutic drug screening. Of 167 compounds included in the scope of the analysis, zolpidem, meprobamate, and carisoprodol were the fifth (5.78%), seventh (5.41%), and eighth (5.22%) most frequently reported compounds during this study period. The list of the top 15 drugs found in 2013 DUID cases provided by the Pennsylvania Traffic Safety Resource Prosecutor (TSRP) also included carisoprodol and zolpidem. Based on these prevalence studies, both carisoprodol and zolpidem were upgraded to the ELISA screen scope.

Traditionally, benzodiazepine ELISA plates are designed using oxazepam as a reference and relying on cross-reactivity of other benzodiazepines to trigger a confirmation. Due to known poor cross-reactivity of clonazepam and lorazepam, a cut-off concentration of 100ng/mL of oxazepam was not sensitive enough to detect blood drug concentrations within the therapeutic ranges for these compounds. Based on the data analysis using both screening methodologies (ELISA and **Liquid Chromatography/Time-of-Flight**/Mass Spectrometry (LC/TOF/MS)), it was also determined that approximately 3% of benzodiazepines positive cases were initially not detected. A cut-off concentration of 20ng/mL was achieved by utilizing a calibration standard in 5-folds dilution. As a result, the ELISA benzodiazepine positivity rate increased from 13.9% to 23.5% in the first month, making benzodiazepines the second most-prevalent compound after cannabinoids in DUID investigation cases.

Lastly, opiates confirmatory analysis was redeveloped on Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS) to accommodate the low cut-off concentrations that are consistent with the therapeutic ranges for oxymorphone and hydromorphone as well as low 6-monoacetylmorphine blood concentrations detected due to rapid metabolism. The cut-offs were improved from 10ng/mL to 5.0ng/mL for codeine, dihydrocodeine/hydrocodol, hydrocodone, morphine, and oxycodone and to 1.0ng/mL for 6-monoacetylmorphine, oxymorphone, and hydromorphone. As expected, the significant increase in positivity rates was observed for many compounds: oxymorphone (1.00% to 13.8%), morphine (38.4% to 48.9%), and 6-monoacetylmorphine (2.10% to 12.2%).

Drug-impaired driving has been a noted problem for many years with guidelines that were developed back in the 1980s; however, it was recognized that this is not a static problem and therefore toxicology laboratories must evaluate and adjust their testing scopes to maintain relevancy to modern times.

DUID Drug Screen, Standardized Guideline, ELISA