



K28 Evaluation of the Lin-Zhi International Benzodiazepine Enzyme Immunoassay for the Detection of Benzodiazepines and Their Metabolites in Urine

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The goal of this presentation is to inform the toxicology community and others of the performance of the Lin-Zhi International Benzodiazepine Enzyme Immunoassay for the detection of benzodiazepines and their associated metabolites in urine.

An evaluation of the performance of the Lin-Zhi International Benzodiazepine Enzyme Immunoassay will impact the forensic science community by providing the field of toxicology with an alternative choice for the rapid detection of benzodiazepines and their associated metabolites in urine.

An evaluation of a new Benzodiazepine Enzyme Immunoassay [BEI] (Lin-Zhi International, Inc., Sunnyvale, CA) for the detection of benzodiazepines and their major metabolites in urine will be presented. The Lin-Zhi assay is based on competitive antibody binding between benzodiazepines in urine and glucose-6-phosphate dehydrogenase labeled oxazepam. When benzodiazepines and/or their metabolites are present in urine, active unbound enzyme reduces the co-enzyme NAD to NADH that results in an increase of measured absorbance at 340 nm. The assay is calibrated with oxazepam.

The BEI was evaluated by testing 1409 urine specimens collected from pain management patients. All 1097 specimens were tested with the assay in an ADVIA 1200 Chemistry System auto-analyzer (Bayer Health Care, Diagnostics Division, Tarrytown, NY) with calibrators containing 0, 200 (cut-off calibrator) and 600 ng/mL of benzodiazepine. Controls containing 0 ng/mL of oxazepam and -25% (negative control) and +25% (positive control) of the 200 ng/mL cut-off calibrator (Bio-Rad Laboratories, Irvine, CA) were analyzed with each batch of samples. All urines were then analyzed by a GC/MS for alprazolam, hydroxy-alprazolam, diazepam, nordiazepam, lorazepam, oxazepam and temazepam at a cut-off concentration of 75 ng/mL.

Approximately, 30% (315) of the 1097 specimens yielded positive results by the BEI assay. Of these specimens, GC/MS confirmed the presence of Benzodiazepines at 75 ng/mL in 306 specimens, indicating 9 false positive results. However, 36 specimens yielding negative BEI results were found to contain Benzodiazepines above the GC/MS cut-off of 75 ng/mL. Therefore the overall agreement of BEI and GC/MS results was 96%. From the presented study, the sensitivity of the BEI was 0.895 and the selectivity 0.988. Testing at 1,000 mg/mL of other drugs of abuse or their metabolites such as amphetamine, benzoylecgonine, morphine and phencyclidine, BEI demonstrated no cross reactivity. The within-run precision of BEI was determined by the absorbance rates of the negative and positive controls was CV=1% (n=16); while the between-run precision of the controls was CV=<6% (n=16). The assay was found linear from -50% to 150% of cut-off concentration. The Lin-Zhi BEI provides a precise, reliable method for the routine detection of benzodiazepines and/or their metabolites in urine specimens.

Enzyme Immunoassay, Benzodiazepines, Urine Drug Testing