K13 Evaluation of Lin-Zhi International EDDP Enzyme Immunoassay for the Determination of Methadone Metabolite in Urine

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The goal of this presentation is to inform attendees of the performance of the Lin-Zhi International EDDP [2-Ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine] Enzyme Immunoassay for the detection of this methadone metabolite in urine.

This presentation will impact the forensic science community by providing an evaluation of the performance of the Lin-Zhi International EDDP Enzyme Immunoassay offering the field of toxicology alternative choices for the rapid detection of methadone metabolite in urine.

In this presentation, an evaluation of a new EDDP [2-Ethylidene-1, 5-dimethyl-3, 3-diphenylpyrrolidine] Enzyme Immunoassay [EDDPI] for the detection of this methadone metabolite in urine is presented. The Lin-Zhi assay is based on competitive antibody binding between EDDP in urine and glucose-6-phosphate dehydrogenase labeled EDDP. When EDDP is present in urine, active unbound enzyme reduces the co-enzyme NAD to NADH resulting in an increase of measured absorbance at 340 nm.

The EDDPI was evaluated by testing 362 urine specimens collected from pain management clients and substance abuse treatment patients. All specimens were tested with the assay in a chemistry system auto-analyzer using two different calibrator sets. One calibration set contained 0 and 150ng/ml (cut-off calibrator) of EDDP and the other set contained 0 and 300ng/ml of EDDP. Controls containing 0ng/ml of EDDP, and -25% (negative control) and +25% (positive control) of the 150ng/ml and 300ng/ml cut-off calibrators (Lin-Zhi) were analyzed with each batch of specimens. All urine specimens were then analyzed by HPLC/MS/MS for EDDP with a 25ng/ml LOQ.

Approximately 42% (151) of the 362 specimens yielded positive results by the EDDPI at 150ng/ml and/or 300ng/ml cut-off values. Of these specimens, HPLC/MS/MS confirmed the presence of EDDP above 25ng/ml in 151 specimens; however, at the 300ng/mL EDDPI cut-off, nine specimens yielded positive results when EDDP was present at <300ng/mL as determined by HPLC/MS/MS. Similarly, at the 150ng/mL EDDPI cut-off, three of the nine specimens also yielded positive results when EDDP was present at <150ng/mL by HPLC/MS/MS. These three specimens contained 121, 68, and 29ng/mL EDDP. No specimen yielding a negative EDDPI result contained EDDP above 25ng/ml by HPLC/MS/MS. Therefore, when applying a 150ng/ml cut-off, the EDDPI demonstrated a sensitivity of 1.00, a specificity of 0.986, and an overall agreement with HPLC/MS/MS results ≥150ng/ml of >99%. When applying a 300ng/ml cut-off, the EDDPI demonstrated a sensitivity of 1.00, a specificity of 0.959, and an overall agreement with HPLC/MS/MS results ≥300ng/ml of 97.5%. The intra-run precision of EDDPI as determined from the absorbance rates of the negative and positive controls yielded CVs of ≤ 2% (n=8); while inter-run precision of the controls yielded CVs of ≤ 6% (n=21). EDDPI demonstrated no cross reactivity with drugs of abuse or popular prescription drugs added to urine at 100mg/ml. The Lin-Zhi EDDPI provides a precise, reliable method for the routine detection of methadone metabolite in urine specimens.

Enzyme Immunoassay, EDDP, HPLC/MS/MS

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