K2 Evaluation of the Immunalysis Tapentadol Enzyme Immunoassay Kit

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After attending the presentation, attendees will understand the performance of a new tapentadol enzyme immunoassay (EIA) screening kit, using a chemistry immune analyzer with a UPLC-TQD for confirmation of all urine samples.

This presentation will impact the forensic science community by demonstrating the applicability of the Immunalysis Tapentadol EIA screening kit for the consistent detection of tapentadol in urine samples.

Tapentadol (Nucynta®) is a schedule II synthetic opiate that is often used as an analgesic for moderate to severe pain. It is excreted in urine as unchanged drug (3%) and as metabolites: N-desmethyltapentadol (13%), tapentadol glucuronide (55%) and tapentadol sulfate (15%).[1] Urine drug testing of pain patients plays a significant role in monitoring their prescribed medication. Tapentadol's availability as a prescription drug in the United States and its potential for abuse similar to other opioid agonists has lead to the development of a homogenous enzyme immunoassay to screen for this drug in urine.[2] Currently, Immunalysis has developed an immunoassay kit for tapentadol. This study aims to evaluate and validate the Immunalysis Tapentadol EIA screening kit, at a cutoff concentration of 200 ng/mL.

The Tapentadol EIA was validated by determining the linearity, precision, accuracy and carryover of tapentadol standards and controls using an chemistry immune analyzer. Linearity was assessed in the range of 200-20,000 ng/mL (n=5). The precision and accuracy of the screening kit were evaluated at the proposed cutoff concentration (200 ng/mL) and at 25% above and below cut-off concentrations (250 ng/mL and 150 ng/mL, respectively) for tapentadol. Controls (n=10) were tested on three separate runs on three consecutive days. Carryover was assessed by screening certified negative urine (n=5) following the injection of urine fortified with tapentadol up to a concentration of 40,000 ng/mL. For the parallel study, de-identified urine specimens (n=300) from pain management patients that were previously confirmed positive or negative for tapentadol were obtained. All 300 patient samples were tested with the Immunalysis Tapentadol EIA kit at the cut-off concentration for tapentadol. All urine samples were then analyzed on a Waters Acquity UPLC-TQD for tapentadol and its metabolites (N-Desmethyltapentadol, Tapentadol Glucuronide and Tapentadol Sulfate) at a confirmation cut-off concentration of 100 ng/mL.

The immunoassay kit was found to be linear up to 1,000 ng/mL. The inter-assay and intra-assay precision of the Immunalysis Tapentadol EIA screening did not exceed a coefficient of variation of 10%. Accuracy was determined to be within ± 25% of each target concentration tested. No carryover was observed in the negative urine samples preceded by 40000 ng/mL of tapentadol. A total of 125 true positives and 167 true negatives were confirmed, by UPLC-MS/MS, based on free tapentadol concentrations only. No false negatives were demonstrated in the parallel study and only eight samples produced a false positive result. The immunoassay exhibits a cross reactivity with N-Desmethyltapentadol (2%) and tapentadol glucuronide (25%).[2] Similarly, it was found that the Tapentadol EIA was also cross reactive with tapentadol sulfate at 25%. The accuracy of the kit improved when examining total tapentadol concentrations versus free tapentadol (128 true positives, 167 true negatives, 0 false negatives, 5 false positives). The sensitivity and specificity results obtained for the parallel study (sensitivity=100% and specificity = 95.4%) compare to the expectations per the package insert.[2]

The results of this study demonstrated that the specificity of the tapentadol immunoassay kit is based off of not only tapentadol, but its metabolites as well. The assay demonstrated good agreement with the UPLC-TQD confirmation results (97.3%). The Immunalysis tapentadol EIA appears to be a reliable homogenous enzyme immunoassay for the detection of tapentadol in urine.

References:

1. Bourland J, Collins A, Chester S, Ramachandran S, Backer C. Determination of Tapentadol (Nucynta®) and N-Desmethyltapentadol in Authentic Urine Specimens by Ultra- Performance Liquid Chromatography- Tandem Mass Spectrometry. J Anal Toxicology. 2010; 34: 450-457.
2. Immunalysis Corporation. Tapentadol Enzyme Immunoassay Package Insert. Revision A: April 2011.

Tapentadol, Homogeneous Enzyme Immunoassay, Validation

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