K5 Validation and Comparison of the Microgenics and Immunalysis Buprenorphine EIA Kits

MacKenzie L. Willis, BS, 1202 NW 15th Street, Andrews, TX 79714; Kayla N. Ellefsen, BSc, 3123 7th Line, Innisfil, ON L9S 4G8, CANADA; Ayodele A. Collins, MSc, and James A. Bourland, PhD, Ameritox Limited, 9930 West Highway 80, Midland, TX 79706; and Ronald C. Backer, PhD, 502 Solomon Lane, Midland, TX 79705*

After attending this presentation, attendees will gain knowledge of the validation and performance comparison of two buprenorphine EIA screening kits, at a cut-off of 5ng/ml using a chemistry immune analyzer and a UPLC-TQD for confirmation.

This presentation will impact the forensic science community by demonstrating the applicability and performance of the Microgenics and Immunalysis buprenorphine screening kits using a cut-off of 5ng/mL in authentic urine samples.

Buprenorphine is a semi-synthetic opioid that is closely related to morphine. It has recently been prescribed for treating opioid-dependence.[1] Around 10-30% is excreted in the urine primarily as conjugated metabolites. There is a need for specific assays with low detection limits.[1] A sensitive and rapid immunoassay is critical for drug screening situations. This study aims to validate and compare two buprenorphine EIA kits at a cut-off of 5ng/mL.

Both kits were validated based on linearity, precision, accuracy and carryover. The linearity study was completed by running five replicates at nine concentrations ranging from 5-500 ng/mL. Precision and accuracy were tested on controls at concentrations of 3.75, 5 (cut-off) and 6.25 ng/mL. Ten replicates of each were tested on three separate runs on three separate days. The carryover study was performed by injecting certified negative urine (n=3) following the injection of urine fortified with buprenorphine up to 1000 ng/mL and observing the response of the samples. For the parallel study, de-identified patient urine specimens (n=400) that were previously confirmed positive or negative were obtained. Each sample was screened by both kits. All 400 samples were confirmed on a UPLC-TQD for buprenorphine, buprenorphine-glucuronide, norbuprenorphine and norbuprenorphine-glucuronide.

The Microgenics kit displayed linearity up to 100ng/mL, while the Immunalysis kit was linear up to 15ng/mL. Inter-assay and intra-assay precision for the Immunalysis kit demonstrated a lower coefficient of variation (<8%) for all concentrations compared to the Microgenics kit (<13%). The inter-assay and intra-assay accuracy was determined to be within ± 25% for Microgenics and ± 20% for Immunalysis from the target concentrations. No carryover was observed for either kit. The 200 previously confirmed positive samples all screened positive with the exception of one negative by the Immunalysis kit. From the 200 previously confirmed negative samples, five screened positive by Immunalysis and 16 by Microgenics. The confirmation results demonstrated the accuracy of both kits. A total of 203 true positives, 193 true negatives, one false positive, and three false negatives were observed for Immunalysis. Microgenics displayed the following results: 205 true positives, 183 true negatives, 11 false positives and one false negative. The sensitivity and specificity results obtained for the parallel study were 98.5% and 99.5%, respectively for Immunalysis and 99.5% and 94.3%, respectively for Microgenics. Both sets of sensitivity and specificity results compared well with the predictions of the respective package inserts.

The results of this study imply that both kits are reliable yet vary in screening ability due to cross reactivity with certain metabolites. Microgenics and Immunalysis were both in good agreement with the confirmation results at 97% and 99% respectively. Norbuprenorphine was present by UPLC-TQD confirmation in 201 of the confirmed positive samples out of 206 total positives by Immunalysis and Microgenics. The Immunalysis kit, that has significant cross reactivity with this metabolite, is likely to display a more ideal performance when norbuprenorphine is present. The Immunalysis kit proved to be more specific yielding less false positives while the Microgenics kit proved slightly more sensitive with less false negatives.

References:

1. Kacinko S, Jones H, Johnson R, Choo R, Concheiro-Guisan M, Huestis M. Urinary Excretion of Buprenorphine, Norbuprenorphine, Buprenorphine-Glucuronide, and Norbuprenorphine-Glucuronide in Pregnant Women Receiving Buprenorphine Maintenance Treatment. Clinical Chemistry 2009; 55(6):1177-1187.

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 ** Presenting Author*