

High Sensitivity Homogeneous Enzyme Immunoassay for Benzodiazepines and Metabolites

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Background: Benzodiazepines are widely prescribed for treating various therapeutic conditions; however, concerns persist regarding their potential for misuse, dependence, and addiction. Urine drug screening immunoassays serve as an important tool in monitoring benzodiazepine usage, facilitating necessary medical interventions. Current limitations of benzodiazepine immunoassays are well-documented in literature, with issues surrounding their lack of sensitivity to glucuronidated and 7-amino metabolites, inability to detect newer benzodiazepines, and poor cross-reactivity. ARK Diagnostics has developed a highly sensitive homogeneous enzyme immunoassay capable of detecting both classic and designer benzodiazepines, in addition to their glucuronide and 7-amino metabolites at a cutoff concentration 200 ng/mL, without the need for glucuronidase or sample pretreatment.

Methods: The ARK™ Benzodiazepine Plus Assay is a liquid-stable homogeneous enzyme immunoassay consisting of two reagents. The assay uses temazepam as the 200 ng/mL cutoff calibrator. The performance characteristics of this assay, including precision, spiked recovery, specificity, and method comparison to LC-MS/MS, were evaluated on the Beckman Coulter AU680 automated clinical analyzer.

Results: The ARK™ Benzodiazepine Plus demonstrated acceptable precision, with no overlap between cutoff (200 ng/mL) and $\pm 25\%$ control levels (150 ng/mL and 250 ng/mL) in a histogram overlap analysis, and $\leq 3.6\%$ CV in semi-quantitative mode. Spiked temazepam samples spanning the semi-quantitative assay range of 1000 ng/mL were recovered between 95.0% and 103.7% of the spiked levels. Thirty-eight benzodiazepines produced cross-reactivities $> 80\%$, including glucuronide and 7-amino metabolites, oxazepam glucuronide, lorazepam glucuronide, temazepam glucuronide, 7-aminclonazepam and 7-aminoflunitrazepam. In method comparison studies, a total of 103 urine samples containing benzodiazepines with LC-MS/MS values ranging from 1.3 ng/mL to 3257.6 ng/mL were tested with the ARK™ Benzodiazepine Plus Assay. Eighty samples tested positive with values ≥ 200 ng/mL and 23 samples tested negative with values < 200 ng/mL by the Benzo Plus assay. Of the 23 samples that tested negative, 21 had LC-MS/MS values < 200 ng/mL and 2 samples were identified as midazolam samples with metabolite α -OH-midazolam levels > 200 ng/mL.

Conclusion: The ARK™ Benzodiazepine Plus performs a sensitive, rapid, and reliable measurement of benzodiazepines and their metabolites in human urine, without the need for hydrolysis or pretreatment, applicable to a wide range of clinical chemistry analyzers.