Letter to the Editor

Enhanced Detection of Benzodiazepines by Immunoassay

To the Editor:

Fraser and Meatherall (1) recently reported their results on the ability of five immunoassay systems to detect alprazolam and triazolam metabolites. One of the methods evaluated in the study was the CEDIA DAU benzodiazepine assay from Boehringer Mannheim Corporation (Concord, CA). Subsequent to this study, this product was replaced worldwide with a new product which has substantially different sensitivity to some benzodiazepine compounds. The crossreactivity to triazolam was increased from 18 to 198%, and the crossreactivity to α-OH-triazolam was increased from 12 to 193%.

This substantial increase in recognition of triazolam and one of its major metabolites significantly improves the probability of detecting a triazolam positive sample, particularly if a low cutoff calibrator is used when screening samples. Because of the low dose of triazolam typically taken and its extensive metabolism, even these high crossreactivities do not guarantee a long window of detection of triazolam in urine.

In their conclusion, Fraser and Meatherall suggested that, "It would be very helpful to laboratories testing for triazolobenzodiazepines when the major diagnostic companies provide enzymatic hydrolysis as an integral part of their product." Given the facts that many benzodiazepine metabolites are conjugated with glucuronic acid and that most commercially available diagnostic kits do not react with these metabolites, it is obvious that the incorporation of an enzymatic hydrolysis step would enhance sensitivity of the immunoassays substantially.

At Boehringer Mannheim Corporation, we have demonstrated that incorporating β-glucuronidase in the CEDIA DAU benzodiazepine assay reagents is possible and results in increased sensitivity to samples containing the glucuronide metabolites of several benzodiazepines. This product is currently being reviewed by the Food and Drug Administration, and it will be available commercially when cleared.

Neal Bellet
Manager, Assay Development
Boehringer Mannheim Corporation
2400 Bisso Lane
Concord, California

Reference

1. A.D. Fraser and R. Meatherall. Comparative evaluation of five immunoassays for the analysis of alprazolam and triazolam metabolites in urine: Effect of lowering the screening and GC–MS cut-off values. *J. Anal. Toxicol.* **20:** 217–23 (1996).