High Amphetamine/Methamphetamine Concentrations in Urine Can Cause Error Codes on the Ortho Vitros Fusion 5,1 FS Automated Chemistry Analyzer

To the Editor:

Automated chemistry analyzers are often used to support the drug testing needs of the emergency department. Although this methodology can provide excellent turnaround time, it is limited in its sensitivity and specificity. The purpose of this study was to evaluate the amphetamine immunoassay (VITROS Chemistry AMPH reagents), on the Ortho Vitros Fusion 5,1 FS instrument, to provide support for the University of Utah Hospital emergency department. While evaluating this system, we discovered that patient urine samples that contained high amphetamine and methamphetamine concentrations generated an error code on the instrument and no result (NR) was reported. To mimic the error code, we analyzed urine samples spiked with a range of amphetamine and methamphetamine concentrations in order to characterize this phenomenon.

The amphetamine assay on the Vitros Fusion instrument is a competitive immunoassay that utilizes antibodies and drug-labeled enzyme (glucose-6-phosphate dehydrogenase) to measure the change in absorbance at 340 nm, when the enzyme converts oxidized NAD+ to NADH (1). The assay is based on competition between amphetamines in the positive sample and the d-amphetamine or d-methamphetamine labeled-enzyme for antibody binding sites. The concentration of amphetamine/methamphetamine in the urine sample is inversely proportional to measured enzyme activity (1). The Vitros Fusion instrument can provide semi-quantitative or qualitative measurement of amphetamine and methamphetamine in urine using a cut-off of 1000 ng/mL. This assay also detects l-amphetamine, l-methamphetamine, methylenedioxymethamphetamine (MDA), and, to a lesser degree, methylenedioxymethamphetamine (MDMA) and methylenedioxymethylamine (MDEA) (1). The assay has 100% cross-reactivity to d-methamphetamine when the concentration is equivalent or greater than 1000 ng/mL. d-Amphetamine has 90.9% cross-reactivity when the concentration is ≥ 1100 ng/mL (1). The assay has 23.8% cross-reactivity with MDA when the concentration is ≥ 4200 ng/mL (1). The assay cross-reacts with MDMA at 4.9% when the concentration is ≥ 20,500 ng/mL (1). Of note, MDA, MDMA, and MDEA were not evaluated in this study.

The amphetamine assay performed on the Vitros Fusion requires two absorbance readings at 340 nm before the sample is analyzed. The urine specimen is first treated with diluent prior to the addition of Reagent 1, which contains the antibodies. The specimen incubates for about 5 min, then Reagent 2, which contains the drug-labeled enzyme, is added. The specimen incubates for about 2 min before the first absorbance reading. The first absorbance reading will determine if the sample was adulterated or if the substrate for the reaction was depleted. If the first absorbance reading is too high due to sample adulteration, the error code U91-193 will occur, and the test will be cancelled. If the first absorbance reading is within the limits of the assay, then the specimen incubates for about 2 min before the second absorbance reading is performed on the specimen. The second absorbance reading will determine if the specimen has an absorbance reading within the range of the assay, in order to quantitify the result. If the second absorbance reading is above the maximum absorbance limit, the cuvette blank (CB) error code U91-200 will be generated, the test is cancelled, and NR is generated. The error code U91-181, outside spline (OS), will occur if the absorbance reading from the specimen is too high to report a semi-quantitative result, but is within the range of the calibration response curve to generate a positive qualitative result (1). The error code U91-186, will occur if the absorbance reading from the specimen is too low to report out a semi-quantitative result, but is within the range of the calibration response curve to generate a negative qualitative result (1). The error code U91-186 may occur if the drug concentration in the specimen is below the analytical measurement range for this assay (100–1450 ng/mL).

Our laboratory selected 41 urine patient samples that were confirmed either positive or negative (< 1000 ng/mL cut-off) by gas chromatography–mass spectrometry (GC–MS). We also collected 30 patient urine samples that generated the CB error code on the Vitros Fusion instrument and were confirmed positive by GC–MS. Specimens were not tested for adulteration prior to analysis. In all, 52 patient urine specimens were positive, and 19 specimens were negative. Positive specimens contained...
amphetamine and methamphetamine at concentrations ranging from 800 to 50,000 ng/mL. For all of the 30 specimens that generated a CB error code on the Vitros Fusion instrument, the CB error code was resolved when the samples were diluted prior to repeat analysis. Samples were diluted with blank urine and dilutions ranged from 1:2 to 1:20. As a result, a standard dilution of 1:10 was implemented for samples that generate a CB error code. Qualitative analysis of 71 specimens on the Ortho Vitros 5.1 FS instrument generated 50 positives, 18 negatives, and 3 false negatives. The false negatives occurred in samples that contained only amphetamine, between 1000 and 1200 ng/mL.

To characterize the “CB” error code phenomenon, an in vitro spiking study was performed on 12 blank urine specimens: 10,000 ng/mL of amphetamine stock (Cerilliant) was spiked into each specimen, followed by methamphetamine (Cerilliant) spikes ranging from 250 to 500,000 ng/mL. Spiked samples with a final amphetamine concentration of 5000 ng/mL and methamphetamine concentrations > 12,500 ng/mL generated the CB error code (Table I). Samples spiked with a final amphetamine concentration of 5000 ng/mL and methamphetamine concentrations < 2500 ng/mL did not generate this error code (Table I). Surprisingly, samples spiked with methamphetamine alone, up to 500,000 ng/mL, did not generate this error code. It is still unclear why high concentrations of methamphetamine do not cause an error code. The CB error code occurred when samples were spiked with high concentrations of amphetamine alone or in combination with methamphetamine. Moreover, we evaluated the VITROS Chemistry opiate and cocaine reagents and did not observe this same error code phenomenon in urine samples or spikes that had high concentrations of benzoylecgonine (up to 500,000 ng/mL) or morphine (up to 500,000 ng/mL).

High amphetamine and methamphetamine concentrations in urine can cause high absorbance readings beyond the absorbance limits of the instrument and may generate error codes on the Ortho Vitros Fusion 5.1 analyzer. This phenomenon was observed independent of reagent lot and also occurred at another hospital laboratory in Utah. The CB error code was resolved for each of the 30 specimens when the samples were diluted prior to repeat analysis. If sample dilution, prior to repeat analysis, does not resolve the error, the specimen should undergo confirmatory testing by an alternate method.

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Reference

1. Amphetamine package insert, Vitros Chemistry Products AMPH Reagents, version 4.0.