Cross-Reactivity of Phentermine with an Immunoassay Designed to Detect Amphetamine in a Meconium Specimen

To the Editor:

Neonates exposed to drugs of abuse in utero can experience prenatal drug dependence leading to withdrawal symptoms and a number of other health problems (1). Early detection of exposure is critical to guide necessary treatment and improve outcomes for these children. Meconium begins to form in the digestive tract at 12–16 weeks gestation. Drugs and metabolites collect in meconium beginning at about 5 months gestation. Thus, meconium testing can identify exposure to drugs during the last 4 months of a full-term pregnancy (2).

Our laboratory uses ELISA reagents (Immunalysis) to detect drugs of abuse in meconium. Poor specificity of immunoassay reagents for amphetamines is well characterized and as a result, specimens that test positive for amphetamines by immunoassay are routinely tested by a second analytical method to prevent false-positive results. Our ELISA screen for meconium has separate detection antibodies for amphetamine and methamphetamine. The ELISA cutoff for these drugs is 20 ng/g. All positive screen results are confirmed by GC-MS.

We report the investigation of an unconfirmed positive amphetamine result. ELISA assay of the meconium specimen in question was positive for amphetamine but negative for methamphetamine. The confirmation assay failed to detect amphetamine, methamphetamine, 3,4-methylenedioxymethamphetamine, or 3,4-methylenedioxy-N-methylamphetamine. The specimen was then analyzed with a more comprehensive liquid-chromatography–tandem mass-spectrometry method to scan for possible candidates that could elicit a positive screen result.

Briefly, 0.25 g of meconium was extracted with 1 mL of 25 mmol/L phosphate buffer, pH 7.3, and centrifuged. Solid-phase extraction on a Zorbax Eclipse XDB C18 column was performed with a mixed-mode column. Deuterated analogs were added as internal standards. The drugs were eluted with 2 mL of methanol: ammonium hydroxide (98:2 vol:vol), evaporated to dryness under nitrogen, and reconstituted in 50 μL methanol. Analyses were performed with an Agilent Technologies 1200 series liquid chromatograph coupled to a 6410 triple quadrupole mass spectrometer operated in the positive electrospray mode and equipped with a Zorbax Eclipse XDB C18 column (4.6 × 50 mm, 1.8 μm) at 40 °C and an ammonium-acetate:methanol mobile phase. The specimen was found to contain 191 ng/g phentermine, an anorexiant stimulant recommended for short-term use in the treatment of obesity.

We quantified phentermine by using d5-methamphetamine as the internal standard. Because the molecular weight of methamphetamine and phentermine are the same, the transitions for liquid chromatography–tandem mass spectrometry were also the same, so the compounds were separated on the basis of retention time (methamphetamine 5.9 min; phentermine 6.6 min). Two transitions were selected: The quantifying transition was m/z 150.2–91.1 and the qualifying transition was m/z 150.2–65.1. Ion ratios were within ±20% to meet the criterion for a positive result. The monitored transition for the d5-methamphetamine was m/z 155.2–92.1.

The ELISA kit insert indicates that phentermine has 89% cross-reactivity at 25 ng/g with the amphetamine antibody, but does not cross-react with the methamphetamine antibody. Phentermine was fortified at 3 concentrations (100, 200, and 400 ng/g) and run as unknowns in the amphetamine and methamphetamine ELISA. The phentermine-fortified samples showed changes in binding (B/B0) of 44%, 29%, and 16% in the amphetamine assay, whereas the methamphetamine assay showed negligible change in binding (B/B0) at 88%, 86%, and 85%. B/B0 is defined as the absorbance reading of the sample (B) divided by the absorbance reading of the zero-dose standard (B0). The change in B/B0 for phentermine obtained by using both kits is shown in Fig. 1. Although phentermine and methamphetamine have the same molecular weight of 149 Da, they differ in the position of the additional methyl group. Therefore, coupling of the antibody through the aromatic ring of amphetamine, rather than the nitrogen, minimizes cross-reactivity with methamphetamine but still allows cross-reactivity with the amphetamine antibody.

To the best of our knowledge, this is the only reported incidence of the confirmation and quantification of phentermine in a meconium specimen. Phentermine is a pregnancy category C drug, indicating it should not be administered during pregnancy unless clearly needed because no data are available to determine if it adversely affects the fetus. Although no evidence of toxicity or
lethality was observed in rat pups from administering of phentermine to dams (3), 5 adverse outcomes (stillbirths) occurred in a previous study of 118 pregnant women taking phentermine (4). This case demonstrates the importance of performing confirmatory testing for drugs of abuse and supports a need to include phentermine in confirmatory testing to distinguish its use or abuse from that of amphetamine or other related drugs.

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New Highly Sensitivity Assay Used to Measure Cardiac Troponin T Concentration Changes During a Continuous 216-km Marathon

To the Editor:

We recently measured cardiac troponin T (cTnT)1 concentrations serially in blood from 10 participants of the Badwater ultramarathon, a continuous 216-km race that takes place under extreme environmental conditions. Details on the Badwater ultramarathon itself, and on the training experience and health condition of the athletes have been reported previously (1). Briefly, the athletes had completed a mean of 43.4 (median 20, range 5–130) marathons and 20.4 (median 15, range 6–80) ultramarathons, and the mean finishing times for the Badwater race were 51.24 h (median 45.2 h, range 43.5–61.4 h). cTnT was measured with a new highly sensitive assay for cardiac troponin T (Hs-TnT) (Roche Diagnostics) on an ELECSYS 2010 automated analyzer that uses chemiluminescence technology. As described previously (2), the interassay CV of this assay is 8% at 10 ng/L and 2.5% at 100 ng/L, and the intraassay CV is 5% at 10 ng/L and 1% at 100 ng/L. The diagnostic range of this assay is 2 to 10000 ng/L.

Blood samples (EDTA plasma) were drawn at baseline, after the first half marathon, after each full marathon distance, and at the finish. In a previous study we tested the third-generation cTnT assay in the same study population and were unable to detect cTnT concentrations above the lower limit of detection in any blood sample before or during the

1 Nonstandard abbreviations: cTnT, cardiac troponin T; Hs-TnT, highly sensitive assay for cardiac troponin T; BNP, brain natriuretic peptide.