False-Positive RIA for Methamphetamine Following Ingestion of an Ephedra-Derived Herbal Product

To the Editor:

Ephedra species contain a mixture of naturally occurring alkaloids, including ephedrine and pseudoephedrine, norephedrine (phenylpropanolamine), and norpseudoephedrine (cathine or khat) (1,2). None of these species is metabolized to any significant degree by humans (3), and in particular, they are not metabolized to amphetamine and/or methamphetamine. These naturally occurring compounds do, however, share common structural features with synthetic phenylisopropylamines, and their presence can result in false-positive immunoassay results for methamphetamine (4–6).

All currently marketed point-of-care testing devices use immunoassays to detect methamphetamine. Presumably, these devices are subjected to the same sorts of cross-reactions that have already been reported with laboratory-based analyzer systems. Indeed, for clinical use, as opposed to workplace testing, the greater the degree of cross-reactivity, the greater the clinical utility of the testing device.

Anecdotal reports suggest that false-positive screening immunoassay tests, secondary to ephedra ingestion, are increasingly common. This issue has never been systematically studied. The intent of our investigation was to measure the concentration of ephedrine in the urine of users of a popular dietary supplement (Metabolife 356) and to determine whether the urine concentrations observed after ingestion of the recommended doses of Metabolife 356 were sufficient to cause a positive screening test for methamphetamine with a commercially available radioimmunoassay (RIA) screening technique (Diagnostic Products Corp.).

Five healthy subjects interested in weight loss and planning on taking diet supplements participated in the study. All volunteers gave written informed consent prior to participating in the study. Each subject took Metabolife 356 for five days. One subject took one caplet in the early morning and a second caplet at noon. The other subjects took two caplets in the early morning and two more caplets at noon. According to the label, each caplet contained 12 mg of “ephedra group alkaloids.” Independent analysis by gas chromatography–mass spectrometry (GC–MS) confirmed the amount of ephedrine per caplet. Urine specimens were collected early morning, immediately before the first daily dose. A second urine specimen was collected 4 h after the first dose and just before the second dose. A third urine specimen was collected 4 h following the second dose. All specimens were collected without preservative and stored at home under refrigeration during the collection period. After the collection period, all specimens were brought to the laboratory, refrigerated at 4°C, and analyzed within one week. Table I summarizes the physical features, dose rates, and the analytical results.

The Diagnostics Product Corp. DPC Coat-A-Count RIA screening assay with semiquantitation for methamphetamine in urine was performed according to manufacturer’s directions. The actual ephedrine concentration in the urine was determined by GC–MS with application of the stable isotopic dilution technique. Quantitative analyses were performed on a Hewlett-Packard 5995 GC–MS equipped with an HP-Ultra 2 (19091B-012) capillary column (25 m × 0.32-mm i.d., 0.17-µm film thickness). The MS was operated in the SIM mode monitoring the 58 and 61 ions for ephedrine-d0 and ephedrine-d3, respectively. The 44 and 91 ions were also monitored to screen for amphetamine and methamphetamine.

Vital signs were not monitored during the studies, and there were no significant medical complaints, although Subject 2 reported being “jittery” after the second dose on the first day and “neck stiffness” during the second day. Even with these side reactions, Subject 2 continued with the five-day protocol, as did all the other participants.

Following the completion of the dosing regimen, the urine specimens, which were collected 4 h after the last dose on the last day for each subject, were screened for methamphetamine by RIA at the 1000-ng/mL cutoff. Three of the five subjects had positive screening test results for methamphetamine, when in fact, no methamphetamine had been taken. The GC–MS analyses were negative for amphetamine and methamphetamine in all of the positive RIA urine specimens.

The extent of cross-reactivity of ephedrine to the methamphetamine antibody in each of the specimens was determined and
reported as methamphetamine equivalents. The concentration of ephedrine in each of the urines was determined by GC–MS. All of the urine specimens for Subject 5 were screened for methamphetamine, and, in addition, those urines close to the positive cutoff value were analyzed for ephedrine content. Table II summarizes the results.

The manufacturer of Metabolife 356 recommends using 1–2 caplets 2–3 times daily, not exceeding 8 caplets per day. Subject 1, taking one caplet twice a day, had a final urine ephedrine concentration of approximately 8000 ng/mL, whereas Subject 3, taking two caplets twice daily, had a final concentration of greater than 120,000 ng/mL. Urine ephedrine concentrations of approximately 40,000 ng/mL (approximately 1000 ng/mL methamphetamine equivalents) are sufficient to produce a positive RIA screen.

There were striking differences in ephedrine concentrations between subjects on the same dosage regimen. Humans do not metabolize ephedrine to any significant degree (1), suggesting that the observed differences in ephedrine concentrations have nothing to do with genetic polymorphism, but rather are a reflection of each individual's states of hydration/dehydration or urinary pH. Excretion is faster in an acidic urine than in an alkaline urine.

Unregulated workplace testing programs, and even hospital emergency rooms, rely on unconfirmed immunoassays, which are often incorporated into point-of-care testing devices and leave no audit trail. There is a very real possibility that innocent herbal product users may be falsely accused of drug abuse.

Results with the immunoassay tested here may or may not be similar to results achieved with other immunoassy kits. In the absence of specific tests, there is no way to know. Pre-market studies of immunoassay cross-reactivity are almost always done with spiked urine samples. Though the concentrations used for such testing may seem high (100,000 ng/mL or even higher), the results of our study suggest that they may not be high enough and do not predict results in clinical practice.

Consumption of less than 50 mg of ephedra alkaloids per day by an individual with unnoticed mild dehydration (Subject 3, who had no medical complaints and who continued to work throughout the study) resulted in urine concentrations of > 100,000 ng/mL. This observation suggests that false-positive screening test results, especially by DPC methamphetamine assays following use of ephedra alkaloids including Metabolife 356, may be more common than had previously been appreciated.

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References