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**TECHNICAL NOTE**

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# Evaluation of Ephedrine, Pseudoephedrine and Phenylpropanolamine Concentrations in Human Urine Samples and a Comparison of the Specificity of DRI® Amphetamines and Abuscreen® Online (KIMS) Amphetamines Screening Immunoassays*

**ABSTRACT:** The purpose of this study was to evaluate the ability of two amphetamine class screening reagents to exclude ephedrine (EPH), pseudoephedrine (PSEPH), and phenylpropanolamine (PPA) from falsely producing positive immunoassay screening results. The study also sought to characterize the prevalence and concentration distributions of EPH, PSEPH, and PPA in samples that produced positive amphetamine screening results. Approximately 27,400 randomly collected human urine samples from Navy and Marine Corps members were simultaneously screened for amphetamines using the DRI® and Abuscreen® online immunoassays at a cutoff concentration of 500 ng/mL. All samples that screened positive were confirmed for amphetamine (AMP), methamphetamine (MTH), 3,4-Methylenedioxyamphetamine (MDA), 3,4-Methylenedioxymethamphetamine (MDMA), EPH, PSEPH, and PPA by gas chromatography/mass spectrometry (GC/MS). The DRI AMP immunoassay identified 1,104 presumptive amphetamine positive samples, of which only 1.99% confirmed positive for the presence of AMP, MTH, MDA, or MDMA. In contrast, the online AMP reagent identified 317 presumptive amphetamine positives with a confirmation rate for AMP, MTH, MDA, or MDMA of 7.94%. The presence of EPH, PSEPH, or PPA was confirmed in 833 of the 1,104 samples that failed to confirm positive for AMP, MTH, MDA, or MDMA; all of the 833 samples contained PSEPH. When compared to the entire screened sample set, PSEPH was present in approximately 3%, EPH in 0.9%, and PPA in 0.8% of the samples. The results indicate that cross reactivities for EPH, PSEPH, and PPA are greater than reported by the manufacturer of these reagents. The distribution of concentrations indicates that very large concentrations of EPH, PSEPH, and PPA are common.

**KEYWORDS:** forensic science, ephedrine, forensic urine drug testing, immunoassay

Resurgence in the popularity of amphetamine (AMP) methamphetamine (MTH), and ecstasy (3,4-Methylenedioxymethamphetamine, MDMA) usage (1) has necessitated that drug-screening laboratories continue to improve and refine their ability to reliably and efficiently detect this class of drug. Several published studies have evaluated aspects of screening reagents that are commonly used in urine testing for drugs of abuse. These studies include the evaluation of Abbott TDx AMP/MTH II (2–4), Abuscreen® ONLINE (2–5), Syva EMIT (2–4,6), Microgenics CEDIA (2), Diagnostic Products Corporation RIA (4–5) and DRI® (6). All of the studies have reported good agreement between screening results and confirmatory methods. However, all of the studies have utilized small sample groups with as few as 20 human urine samples (3) and a maximum of 2,964 samples (4). While these results provide a good indication of the performance of the assays, they do not give an indication of the likely performance of the reagents over tens of thousands of human urine samples.

This type of performance estimation is of particular importance given that all of the reagents have some cross-reactivity to over-the-counter (OTC) compounds such as ephedrine (EPH), pseudoephedrine (PSEPH), phenylpropanolamine (PPA) and other interfering compounds that could produce positive screening results but will not confirm positive for AMP, MTH, 3,4-Methylenedioxyamphetamine (MDA), or MDMA (2). In large-production workplace drug-testing laboratories, relatively small cross-reactive false positive screen rates can produce efficiency problems in the confirmation process leading to an increase in overall sample testing costs and leading to unnecessary longer reporting times of test results.

We have previously reported on the control performance and AMP/MTH/MDA/MDMA results from screening and confirming a very large human urine sample set (approximately 27,500 samples) with a variety of screening reagents (7). The purpose of

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this study was to compare the specificity of DRI® Amphetamines and Abuscreen® Online (KIMS) Amphetamines screening immunoassays and to characterize the distribution of EPH, PSEPH, and PPA concentrations in a large sampling of randomly collected human urine samples.

## Materials and Methods

Urine samples (27,500) were randomly collected under forensic conditions and submitted to the laboratory as part of the Department of Defense (DoD) directed drug-testing program. These samples are collected under DoD instructions maintaining chain of custody and security of the samples. Random collections are determined by computer software that randomizes both the collection date and individuals collected. Samples were collected in February 2001 from Navy and Marine personnel east of the Mississippi, including the Atlantic fleet and personnel in the European theater. These samples were analyzed on the D modules of a Roche Hitachi modular DDP clinical analyzer system (Indianapolis, IN) using the laboratory's current AMP/MTH screening assay (Roche Abuscreen® ONLINE immunoassay (Indianapolis, IN)). In parallel, these samples were also assayed with the Microgenics DRI® AMP/MTH (Fremont, CA) immunoassay. Testing conducted on eight consecutive production days was performed in accordance with the manufacturer's instructions for the analysis of urine samples.

The qualitative immunoassays were calibrated daily prior to running samples using a single point blank calibration per manufacturer specifications and the resulting absorbance normalized to equal 100 arbitrary units. The production ONLINE assay calibration standard, purchased from Biopool (Ventura, CA), contained d-AMP at 500 ng/mL. The calibrator for the DRI® reagent was manufactured in-house with d-MTH at 500 ng/mL using standard materials purchased from Cerilliant (Austin, TX) and Roche (Indianapolis, IN) certified drug-free urine.

All screening analyses included an above-threshold open quality control (high) and a sub-threshold open quality control (low) of the appropriate drug for every 50 samples to demonstrate the assay's ability to properly differentiate a sample as positive or negative. For the ONLINE assay, the low control (350 ng/mL of d-amphetamine) was manufactured in-house using standard material purchased from Cerilliant and Roche certified drug-free urine. The high control (675 ng/mL of d-amphetamine) was purchased from Biopool (Ventura, CA). The low and high controls for the DRI assay were manufactured in-house from standard materials purchased from Cerilliant and Roche-certified drug-free urine using d-MTH at 350 and 675 ng/mL, respectively. Blind quality controls were inserted within each analytical batch of 100 samples for the production processing of samples. The blind positive control was manufactured in-house with d-amphetamine at 1000 ng/mL using Cerilliant stock and Roche-certified drug-free urine. The blind negative was Roche-certified negative urine.

All AMP/MTH/MDA/MDMA presumptive positive samples were extracted using a solid phase extraction method after pretreatment with sodium periodate (8) and analyzed by GC/MS as described in Stout et al. (7). The GC/MS cutoff calibrator was manufactured in-house using 500 ng/mL of d-MTH, d-AMP, MDMA, and MDA from Cerilliant- and Roche-certified drug-free urine. Negative-, low-, and high-quality controls prepared at 0, 250, and 625 ng/mL, respectively, were manufactured in-house using the same materials. All confirmation batches included a cutoff calibrator, a negative control, a low control, and a high control.

All EPH/PSEPH/PPA analyses were conducted using the same extraction and GC/MS procedure (8) with the following exception to the extraction portion of the method. The samples were not pretreated with periodate, and the internal standard was *n*-ethyl benzylamine (Aldrich, St Louis, MO). All samples screening positive by either immunoassay were diluted ten-fold prior to extraction. A calibrator at 50,000 ng/mL EPH/PSEPH/PPA was manufactured from Cerilliant stock material and Roche-certified drug-free urine. A positive control at 50,000 ng/mL EPH/PSEPH/PPA was manufactured using Roche negative urine and separate stock materials obtained from Cerilliant. Each confirmation batch included a calibrator, a negative control, and a positive control.

TABLE 1—*Ions (m/z) monitored in SIM mode for the method. The quantitation and identity ratios are also listed.*

| Compound | Ions | Ratios |
|---|---|---|
| AMP | 240, 118, 192 | *Q* 240/261, 118/240, 192/240 |
| D14 MAMP | 261, 128 | 128/261 (ISTD) |
| MAMP | 254, 210, 118 | *Q* 254/261, 210/254, 118/254 |
| MDA | 240, 162, 375 | *Q* 240/258 162/240, 375/240 |
| D5-MDMA | 258, 394 | 394/258 (ISTD) |
| MDMA | 254, 210, 389 | *Q* 254/ 258, 210/254, 389/254 |
| MDEA | 268, 240, 403 | *Q* 268/258, 240/268, 403/268 |
| N-ethylbenzylamine | 331, 302 | 302/331 (ISTD) |
| PPA | 240, 169, 330 | *Q* 240/331 169/240 330/240 |
| EPH | 254, 210, 169 | *Q* 254/331 210/254 169/254 |
| PSEPH | 254, 210, 169 | *Q* 254/331 210/254 169/254 |

NOTE: $Q$ = quantitation ratio.

All GC/MS analyses were performed in selected ion monitoring (SIM) mode using the ions, quantitation, and identity ratios indicated in Table 1. EPH/PSEPH/PPA concentrations for controls were determined by single-point calibration against the 50,000 ng/mL standard. Identification of the target analyte was considered acceptable if the specimens and controls exhibited retention times within ±1% and identity ion abundance ratios within ±20% of the calibration standard. Additionally, all open and blind-quality controls within each analytical set were required to quantitate within ±20% of the expected theoretical concentration.

## Results and Discussion

Of the 27,500 human urine samples, 1,104 (4%) screened positive by the DRI AMP kit. In contrast, the Abuscreen® Online amphetamines immunoassay reagent produced 317 screened positives. Figure 1*A* represents the distribution of PSEPH concentrations detected in the screened positive samples. Consistent with the manufacturer's reported cross-reactivities (9,10), the mean concentration of PSEPH in samples producing a positive screening result for DRI was less than that for Online (83,600 ng/mL compared to 218,000 ng/mL). When these data were compared using a student two-tailed *t*-test (Excel, Microsoft, Seattle WA), the difference was significant at the $p < 0.05$ level.

PSEPH was present in 3% of the samples from the overall population of 27,500 samples tested. Eight hundred and thirty-three samples confirmed positive for PSEPH. While the majority of samples contained less than 100,000 ng/mL of PSEPH (440 samples), twelve samples contained PSEPH at concentrations ranging from 1,000,000 to 2,500,000 ng/mL. These results are not consistent with the manufacturer's reported cross-reactivities for PSEPH and EPH. PSEPH concentrations of less than 250,000 ng/mL for the online assay, and 125,000 ng/mL for DRI should not have produced a positive response above the 500-ng/mL cutoff. Of the 833 samples containing PSEPH, 30% of the samples contained less than

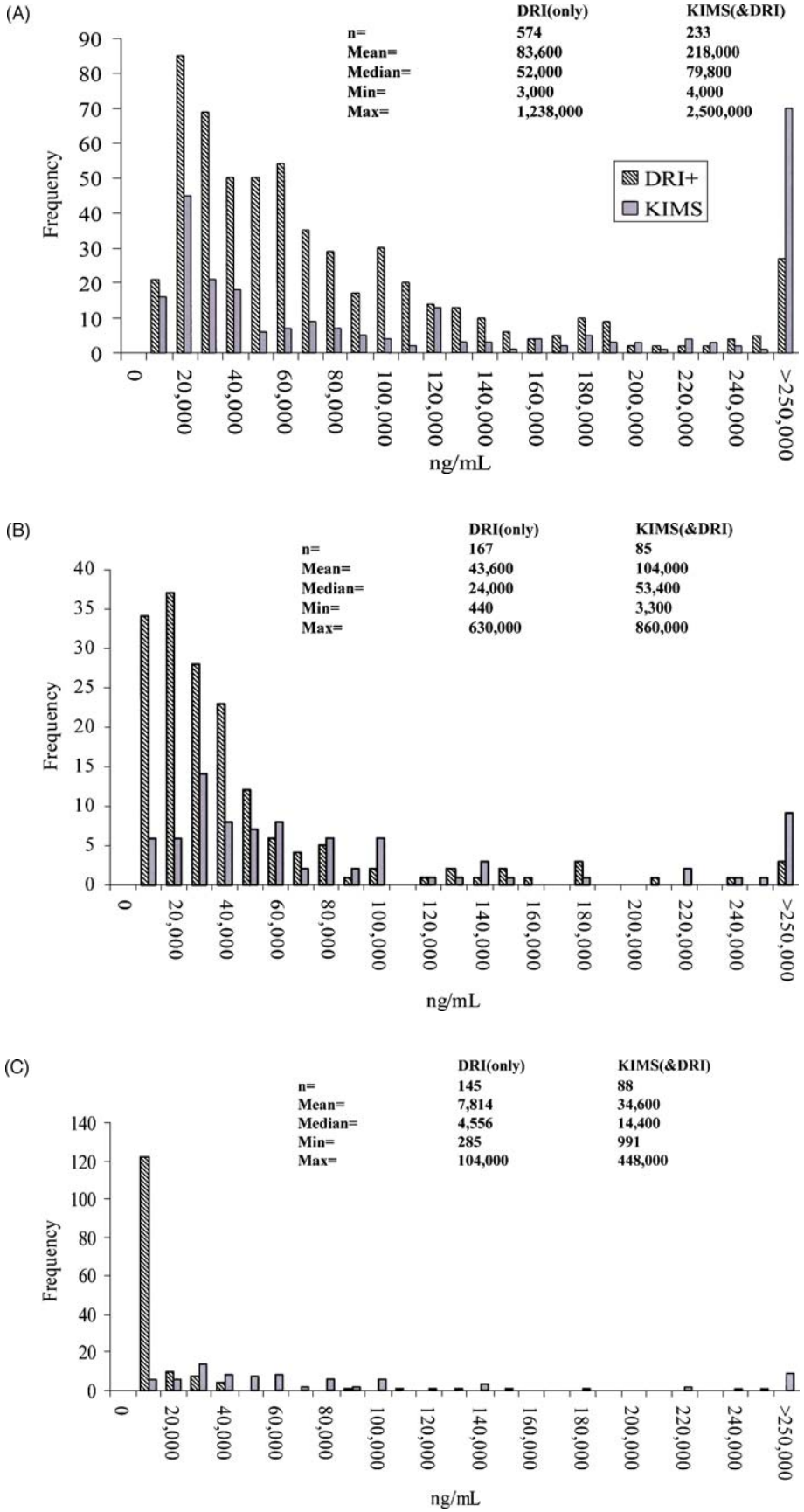


FIG. 1—*Distribution of pseudoephedrine (A), ephedrine (B), and PPA (C) concentrations in samples. Of the 1,104 samples screening positive, 833 had PSEPH present, 252 had EPH present, and 233 had PPA present. The distribution indicated a broad range of concentrations ranging to some exceptionally high concentrations. Note also that all compounds had concentrations producing positive screening results well below the reported cross-reactivities for these compounds.*

TABLE 2—*Compounds present in samples positive by each screening reagent. The majority of samples screening positive by either reagent contained PSEPH/EPH/PPA with very few samples confirming positive for AMP/MTH/MDA/MDMA. DRI produced more samples that did not indicate the presence of any analyte than did Online.*

| Sample Result | DRI Positive | Online Positive |
|---|---|---|
| Confirmed AMP/MTH/MDA/MDMA | 2% | 8% |
| AMP/MTH/MDA/MDMA present at <500 ng/mL | 1% | 4% |
| PSEPH/EPH/PPA present | 75% | 81% |
| Negative for all compounds | 22% | 7% |

30,000 ng/mL of PSEPH, indicating that in in-vitro samples, with other potential metabolites, the cross-reactivity of the reagents to PSEPH is greater than previously considered. These results are also indicative that PSEPH use is widespread and in some cases used excessively.

Figure 1*B* documents the distribution of EPH concentrations in screened positive samples. Consistent with the manufacturer's reported relative cross-reactivities, the mean of samples screening positive by DRI that contained EPH was significantly lower than those screened positive by the Online assay ($p < 0.05$ by the two-tailed *t*-test). EPH was present in 0.9% of all the samples tested (i.e., 30% of the samples that screened positive contained EPH). Although the majority of the samples contained EPH concentrations consistent with values available in the literature (11), 34 samples contained EPH at concentrations ranging from 100,000 to 860,000 ng/mL.

Figure 1*C* presents the distribution of PPA concentrations in the samples that screened positive. PPA was present in 28% of the samples or 0.8% of the total population tested. While initially a curious finding to detect PPA in human urine since the U.S. Food and Drug Administration took steps to remove PPA from all OTC drug products in November 2000, several explanations may account for its presence. First, the analytical procedure used for confirmation was not stereo-selective. Thus, both norephedrine and norspeudoephedrine metabolites would have contributed to the PPA peak. The concentrations of PPA typically were greater than 10% of the PSEPH and EPH in 76% of the samples containing PPA. Less than 1% of a PSEPH (12) and 4% of an EPH (13) dose are reported to be excreted as a nor-metabolite. Thus, it is unlikely that simple metabolism accounts for the excessive PPA measured. Reports do indicate that many herbal preparations of ephedra do contain significant concentrations of norephedrine and norpseudoephedrine in the plant material (14,15). This source may account for some of the measured PPA. Additionally, urinary pH has been reported to alter the relative proportions of PSEPH/EPH and PPA excreted in the urine (16,17). Given the poor nature of product QC in many nutritional supplements, it is also possible that PPA may have been present as a contaminant or unlabeled component of supplements that individuals may have consumed. Thus, all of the above factors may have contributed to the presence of PPA in these samples.

Table 2 presents a breakdown of the confirmation results by each immunoassay. The vast majority of samples screening positive by either assay contained PSEPH. PSEPH was found to be present in all samples that contained EPH/PPA. Approximately 250 samples had PPA present. In these 250 samples, all but eleven samples that had PPA present; EPH was also present. For the DRI assay, only 2% of the screened positive samples confirmed positive for the presence of AMP, MTH, MDA, or MDMA. For the KIMS Online assay, 7.9% confirmed positive for AMP, MTH, MDA, or MDMA as previously reported (7). Five percent of the samples (1 and 4%, DRI and KIMS Online, respectively) contained AMP/MTH/MDA or MDMA below the 500-ng/mL confirmation cutoff with concentrations as low as 27 ng/mL of MTH, and 41 of ng/mL MDMA.

The results indicate that cross-reactivities for EPH, PSEPH, and PPA are greater than reported by the manufacturers for these reagents. While these reagents may produce fewer false positives due to PSEPH when using a 1000-ng/mL cutoff, at a 500-ng/mL cutoff a substantial number of falsely positive screening results were obtained. Potential cooperativity of binding in samples may also have accounted for increased apparent immunoassay cross-reactivity. Even in samples where only PSEPH, EPH, or PPA were found, additional metabolites that were not tested may have contributed to cooperative binding effects. A distribution of PSEPH/EPH and PPA concentrations in the population tested revealed that these three compounds are commonplace and very prevalent. Likewise these compounds are commonly present at concentrations that may indicate excessive use of the drugs.

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