ELSEVIER

Forensic Science International 133 (2003) 63–78

Forensic Science International

www.elsevier.com/locate/forsciint

Comparison of daily urine, sweat, and skin swabs among cocaine users

D.A. Kidwell[a,*], J.D. Kidwell[a], F. Shinohara[a], C. Harper[b], K. Roarty[b], K. Bernadt[b], R.A. McCaulley[b], F.P. Smith[b]

[a]*Chemistry Division, Naval Research Laboratory, Washington, DC 20375, USA*
[b]*Department of Justice Sciences, The University of Alabama at Birmingham, Birmingham, AL 35294, USA*

Received 16 October 2002; received in revised form 15 January 2003; accepted 17 January 2003

Abstract

This study (1) compares urine, skin swabs, and PharmChek™ sweat patches for monitoring drug use; (2) measures possible environmental contamination in recent cocaine (COC) users; and (3) evaluates various immunoassays (IA) for screening COC in diverse matrices. Unique aspects include *daily urine monitoring* of 10 participants for 4 weeks, multiple monitoring methods, analysis for all specimens by IA and gas chromatography (GC)/mass spectrometry (MS), and the potential for continued illicit drug use by participants. Urine served as the ''gold standard'' specimen for determining drug use. Only cocaine and related substances were detected.

Trace amounts of drugs were found on the skin (<50 ng per swab) of urine-negative participants' hands or forehead. In contrast, larger quantities of COC were found on the skin of individuals with BE-positive urines or individuals living with drug users (up to 20 μg per swab). Patch COC amounts among the three regular users (250–9000, 0–240, 160–22,000 ng per patch) exceeded BE (50–950, none, 30–2200 ng per patch). Pre-swabs, valuable for interpreting the source or time frame of positive patch results, contained substantial COC (38–1160, 0–152, 34–762 ng per swab) prior to patch application; therefore, patch results may represent current use, prior use, contamination, or a combination. In three individuals with no indication of cocaine use, false positives (defined as sweat patch positive when urine specimens were <300 ng BE/ml) occurred at a 7% rate. Proposed cut-off concentrations of 75 ng cocaine per patch and 300 ng BE/ml urine curtail the incidence of false positives in this limited population.

Three immunoassays were compared to screen specimens for cocaine: a modified, manual Microgenics CEDIA; a Cozart ELISA; and an OraSure ELISA. CEDIA's limit of detection (LOD) was 81 ng/ml, compared with LODs of 4 ng/ml for the Cozart ELISA and 1.5 ng/ml for the OraSure ELISA. Cozart correlated with OraSure results for COC concentrations <2000 ng per swab ($n = 117$), $r^2 = 0.79$.

Keywords: Environmental contamination; Sweat; Urine; Cocaine

1. Introduction

Historically, drug testing programs have relied on urinalysis as the gold standard for establishing illicit drug use. Due to the pharmacokinetics of cocaine (COC) and the normally applied immunoassay (IA) cut-off concentration (300 ng BE/ml urine), drug testing programs generally require urine collection every 2–3 days to determine an individual's use pattern [1,2]. Lower cut-off concentrations extend the window of detection at the expense of possible false positives from inadvertent environmental exposure, including such sources as gross external contamination (for example, crime lab workers handling cocaine) and trace ingestion. Trace ingestion, considered to be 1–5% (approximately 1–5 mg) of street use dose, may not result in a noticeable euphoric effect, but would be capable of producing a substantial urine positive. For example, in consuming Inca Tea, an individual ingests 2–3 mg of

* Corresponding author. Tel.: +1-202-767-3575; fax: +1-202-767-3321.
E-mail address: kidwell@ccf.nrl.navy.mil (D.A. Kidwell).

0379-0738/03/$ – see front matter
doi:10.1016/S0379-0738(03)00051-3

cocaine. This has been shown to cause positive urine results for 21–26 h [3]. Consuming larger amounts (25 mg) of cocaine produces positive urine results for up to 36 h [4]. Even at this dosage level, only a slight numbing of the mouth was observed during the consumption.

External continuous monitors, such as the PharmChem™ sweat patch, offer substantial advantages over frequent urine testing, such as convenience and cost [5]. Continual sampling of an individual's sweat for up to 7 days using the sweat patch creates a wider time window for detecting drug use, with the potential to trap and accumulate drugs and their metabolites excreted in sweat. The polyurethane covering of the patch is claimed to protect the collection pad from external contamination and to allow water vapor to escape, which permits long-term wear. However, this claim has come under increased scrutiny.

Based on previous laboratory experiments, two sources of contamination can occur [6,7]. First, drug contamination on the external patch membrane cause what we have termed "contamination from without" (CFWO). Under these circumstances, rapid diffusion of drugs through the membrane and into the moistened interior of the patch, within 1 min, resulted in patch drug amounts above the suggested manufacturer's cut-off concentration for determining a positive. Proof of patch penetration by other molecules further substantiates our findings. For example, certain dyes, with molecular weights (molecular weight influencing diffusion) that exceed those of most illicit drugs, penetrate the patch membrane. In the uncharged state, they readily diffuse through the patch membrane and deposit in the patch test pad when the interior pad is moist [6,7]. When the interior pad is dry, penetration was not observed [8,9]. In another example, Uemura et al. [10] reported finding d-5 cocaine in 50% of the sweat patches applied on top of sweat patches spiked with d-5 cocaine and worn by cocaine-naive volunteers. Uemura's results suggest that cocaine penetrates that patch from the inside outward, also confirming our findings [6,7] that the patch membrane is permeable to cocaine.

The second source of contamination, "contamination from within" (CFWI), results from the presence of drugs on the skin before application of the patch. CFWI can occur from at least two sources: (1) an individual's own previous drug use or (2) an individual's being "around drugs" unrelated to intentional use by the individual in question [11]. Drugs persist on skin, even though the skin is "cleaned" before the patch is applied, and are difficult to remove [6,7,12–14]. We have shown that, after an initial application of only 10 μg of drugs to the skin (an amount equivalent to 0.1–0.01% of a dose), 6 days of regular hygiene followed by "cleaning" with isopropanol wipes (as recommended by the manufacturer) does not prevent positive patch results [6,7]. Unfortunately, courts and drug treatment programs rely on the patch to distinguish between current and prior drug use. No known studies have been performed that address the interval during which patches will remain positive after binge drug use cessation, a critical question for future research.

Patch positives accompanied by urine negatives present a legally challenging question (some examples of legal cases are given in Appendix A) [15,16]. Often where urine and patch results are obtained, the drug testing programs fail to collect sufficiently frequent urine samples to demonstrate cocaine abstinence. Thus, the trier of fact must decide whether to punish an individual based on conflicting and potentially erroneous interpretations of drug test results. The positive patch and negative urine results often are explained as due to the longer window of detection of the patch.

A unique aspect of the present research relates to our goal to expand previous studies to "real life" conditions where illicit drug use occurs. In this regard, participants were current or recent cocaine users. For these outpatient participants, their self-prescribed use of street drugs was not limited by approved research protocols (such as hospital ward settings) where the dosage of dangerous drugs is appropriately restricted by ethical research oversight. Separately, no effort was made to remove external contamination in their living environments, including contamination from previous or ongoing cocaine use. Furthermore, outpatient participants were not prohibited from intimate contact with possible cocaine-using spouses. Casual contacts with those who may be cocaine-contaminated included others in their environment (such as domestic cohabitants) as well as fellow patients in the drug treatment program.

Broadly, our research seeks to improve drug use monitoring by: (1) making it more convenient to both those who administer drug testing programs and those who are subject to drug testing; (2) making drug testing less invasive/more dignified; and (3) extending the window of detection and lowering costs, while maintaining the high reliability currently associated with forensic urine drug testing as performed under the federal workplace drug testing guidelines. Immediate research goals include: (1) examining the issue of environmental contamination as it applies to urine, skin wipe, and sweat patch testing, (2) revisiting the issue of cut-off concentrations and their effect on drug testing reliability, and (3) evaluating several immunoassays for their use as pre-screens in diverse matrices.

2. Materials and methods

2.1. Volunteers

Research participants were recruited from adult patients in cocaine dependence treatment and gave informed consent in accordance with an IRB-approved protocol. Ages of the 10 participants ranged from 21 to 50, with 3 male and 7 female participants. The ethnic distribution roughly approximates the local (Alabama) population: seven Caucasian, two African-American, and one Hispanic. To qualify, participants must have tested positive by urinalysis for cocaine within 90 days, indicating the potential for recent use. We predicted that this criterion of recent use might provide some

"real life" participants who were either: (1) currently cocaine abstinent yet living in an environment contaminated by their previous cocaine use, (2) currently cocaine abstinent yet living in an environment contaminated by their spouse's (or other cohabitant's) cocaine use, (3) currently using cocaine occasionally, (4) chronically using cocaine, or (5) currently living in relatively uncontaminated environments.

2.2. Specimens

Urine specimens and skin swabs of fingertips and forehead were collected daily (except Sundays and holidays). Participants opened individually packaged (ordinary, commercially available) isopropyl alcohol (70%) swabs, wiped their normally favored hand, including fingertips (right-handed people swabbed their right hand), replaced the swab in the torn package, and then placed the package in a plastic, zip-lock bag. Participants swabbed their foreheads in a similar fashion. Urine was obtained in standard 60 ml screw-cap cups, supplied with temperature strips as an added safeguard.

Sweat patches were applied and removed according to the PharmChek™ sweat patch package insert and training video. For example, isopropyl alcohol (70%) swabs were used by study personnel who wore new (clean), latex gloves to prepare the patch application sites on the upper arms. In addition to the manufacturer's instructions, swabs were saved and analyzed in the same manner as the sweat patch pads. Two sweat patches were worn at all times, removed and replaced on alternating arms every 3–4 days on a weekly basis. Due to this alternating pattern, a patch was worn less than 7 days during the first and last week of the 4-week study. Patches were checked frequently; those that were not adhering well were removed for testing and replaced. Any compromised patches were not included in the data analysis. Participants were discouraged from tampering with patches by loss of their weekly financial incentive (US$ 50 merchandise gift card) when samples were not collected as scheduled, including premature removal or compromised patches. Participants who successfully completed the 4-week study received one US$ 50 gift card at the end of each week, plus a bonus US$ 50 gift card at the end of the study, for a total of US$ 250 in gift card incentives. Specimens were coded in a manner designed to prevent linking specimen results to individual participants.

Even with the monetary incentive and anonymity, some patches were compromised. Of the 97 patch results reported in this study, two patches had an interior anomaly, such as movement or tearing of the absorbent pad, but because the outer membrane was intact and firmly attached to the skin, these patches were considered uncompromised and their results were considered valid. Five patches were removed and replaced early because the patch was peeling back. Of these five exposed patches, four were considered compromised. We also observed tearing of the inner pad in our previous laboratory evaluations [6] involving heavy sweating and exercise. In some drug use monitoring programs, authorities accuse the wearer of violating a condition of their supervised release when a patch is compromised. Based on the results of this research, previous research, and the random nature of the patch failure, it appears that such a position is untenable.

2.3. Extraction and analysis

Dried skin swabs or patches were placed in a 15 ml plastic test tube held in place mechanically by a permeable divider at the upper third of the test tube. (Patches were dried because those still moist with isopropanol showed higher background.) The swabs and patches were washed with two 1 ml portions of 0.1 M HCl separated by brief centrifugation after each addition. Aliquots of either the swab or patch extracts or the urine samples, varying from 10 to 1000 μl (depending on the immunoassay results) were taken, diluted with 2 ml of 0.1 M HCl, and then spiked with a deuterated internal standard in ethanol. The aqueous extracts were applied to DAU solid phase extraction (SPE) columns (Ansys, Inc.) using a Zymark Rapid Trace automated SPE extractor. The columns were conditioned with methanol, 0.1 M hydrochloric acid, and 20% aqueous acetone. The columns were dried under positive pressure for 1 min, and the drugs were then eluted with 40:10:1 methylene chloride:isopropanol:ammonium hydroxide. The eluate was then concentrated to dryness under a stream of nitrogen and derivatized using 70 μl 1% triethylamine in methylene chloride, 50 μl acetic anhydride, and 20 μl pentafluoropropanol at 70 °C for 30 min. The excess derivatization reagents were evaporated under a stream of nitrogen. The drugs were reconstituted in 20 μl of ethyl acetate. Aliquots (2 μl) were injected into a Varian 4 GC/MS with the following parameters: 30 m DB-5MS column (J&W Scientific), initial temperature100 °C (20 s) ramped at 18 °C/min to 280 °C then 5 °C/min to 300 °C for 2.9 min for a total run time of 17.1 min. Samples were ionized using isobutane chemical ionization. Quantitation was performed by ratioing the peak areas of the protonated molecular ions to their respective deuterated internal standards. The limit of detections (LODs) for the CI-GC/MS assay (calculated from the blanks, which comprised 10% of all samples analyzed) were ca. 4 ng/ml for cocaine and 2 ng/ml for BE ($n = 41$ blanks). Alternatively, the LODs from "blank" urines were 6 ng/ml for cocaine and 9 ng/ml for BE ($n = 73$ from three individuals). LODs determined from a series of different specimens/individuals (rather than pooled specimens) are considered to be more representative of the true LOD [17].

2.4. Immunoassays

Three immunoassays were investigated. Because the manufacturers' procedures were substantially modified to accommodate the diverse matrices, the complete procedure for each immunoassay is given. All were calibrated with cocaine solutions in 0.1 M HCl at 5, 10, 50, 100, 500, and 1000 ng/ml. All assays were performed manually.

2.5. Cozart cocaine metabolite microplate enzyme immunoassay (EIA)

In an antibody-coated plate provided by the manufacturer was added 75 μl of a 0.1 M phosphate buffer, pH 8.8, followed by 25 μl of the acid specimen extract or urine sample. The plate was incubated at room temperature for 15 min shaking, 100 μl of enzyme conjugate added, and the plate shaken for 30 min. The plate was then washed at least six times with a wash buffer, provided by the company. After washing, 100 μl of substrate solution was added and the plate shaken at room temperature for 30 min. During this time, the plate may be read at 620 nm to confirm the absorbances are within range (0–2 o.d.) by noting that the reading at 620 nm is ca. 1/3 of the final reading at 450 nm. After the last incubation period, 100 μl of an acid stop solution is added and the plate read.

2.6. Microgenics cocaine CEDIA DAU immunoassay

This assay was greatly modified from the manufacturer's procedure to increase the sensitivity. The solutions come in three packages, two of which are to be mixed together in the normal assay. In this modified assay, they were kept separate and mixed in the well at the appropriate times. A microtiter plate was used as the optical wells. To each well, 10 μl of sample was added, followed by 50 μl of antibody solution. The plate was incubated at 37 °C for 20 min while shaking. After 20 min, 50 μl of enzyme acceptor, dissolved in 0.1 M PIPES buffer with calcium chloride, was added. The plate was incubated at 37 °C for an additional 20 min. Enzyme donor (50 μl) was added, the plate incubated at 37 °C while shaking, and the plate read at 492 nm every 5 min. Normally, a 20–30 min incubation provided an acceptable calibration curve.

2.7. OraSure technologies intercept cocaine metabolite microplate enzyme immunoassay (EIA)

All manufacturer's solutions were prepared as directed. In an antibody-coated plate provided by the manufacturer was added 100 μl of a 0.1 M phosphate buffer, pH 8.8, followed by 50 μl of sample. The plate was incubated at room temperate for 15 min shaking. Then, 50 μl of enzyme conjugate was added, the plate shaken for 30 min, and the plate then washed at least six times with a wash buffer (phosphate buffered saline + 0.05% Tween®-20). After washing, 100 μl of substrate solution was added and the plate treated as for the Cozart cocaine ELISA.

2.8. Data analysis

Plotting the log[cocaine] vs. log[(absorbance of standard/absorbance of blanks) × 100/((100 − absorbance of standard/absorbance of blanks) × 100)] produced linear logit plots. For the Cozart assay, the correlation coefficients ranged from 0.8 to 1 (average = 0.96, $n = 24$). For the OraSure assay, the correlation coefficients ranged from 0.91 to 1.0 (average = 0.96, $n = 11$). Several blanks (3–6) were run for each plate with the LOD determined by the scatter in the blank results as transformed by the logit plot. For the modified, manual Microgenics CEDIA assay the best LOD was 81 ng/ml, compared with LODs of 4 ng/ml for the Cozart ELISA and 1.5 ng/ml for the OraSure ELISA.

3. Results and discussion

The drug use pattern can be estimated for cocaine by monitoring daily urine levels of BE [18,19]. Briefly, a negative urine specimen, followed by a positive specimen of a significant amount, can be interpreted to indicate cocaine use sometime after the last negative specimen and before the positive specimen. For example, Participant #10 (Fig. 1b) whose urine tested negative Friday morning but positive on Saturday morning then having decreasing concentrations the following Monday, Tuesday, probably ingested cocaine on Friday afternoon/night. Participant #10 would be positive through Tuesday morning (4 days), at occasionally used 100 ng BE/ml cut-off. In another example, Participant #4 (discussed below and shown in Fig. 3), whose urine tested negative Saturday morning, then decreasingly positive the following Monday, Tuesday, and (depending on which cut-off concentration is employed) Wednesday mornings, probably ingested cocaine Saturday or Sunday. Without extensive knowledge of the participant's metabolism of cocaine and an estimate of the physiological dilution of the urine, the analysis of urine BE levels cannot tell how much cocaine was ingested during the use period, which may comprise a single use or a binge use over a short period of time. Although not a perfect measure of dilution of the urine, the creatinine levels were tested in all specimens. None showed the abnormally low creatinine concentrations that would be consistent with large fluid intake (''flushing''). Flushing (and urine adulteration) shortens the window of detection for single dosages, affecting the reliability of estimating the post-drug use interval.

Our results show that the time window for detecting cocaine use from urinalysis, sweat, and skin swabs depends, in part, on use pattern. Fig. 1 contrasts two participants—a chronic cocaine user and an individual with an apparent single use. Among active, chronic cocaine users, urine results seldom if ever decrease to negative (below cut-off concentration). For example, urine BE concentrations in one participant (Fig. 1a), ranged from approximately 900–160,000 ng/ml urine, remaining positive throughout the study. Although the cocaine use by Participant #1 would be evident from any urine sample during this 28-day monitoring period, the single use by Participant #10 would not. A random urinalysis program of one sample/month would have only a 10% (3 days per month) chance of detecting the use of cocaine by Participant #1. Even a once-per-week monitoring

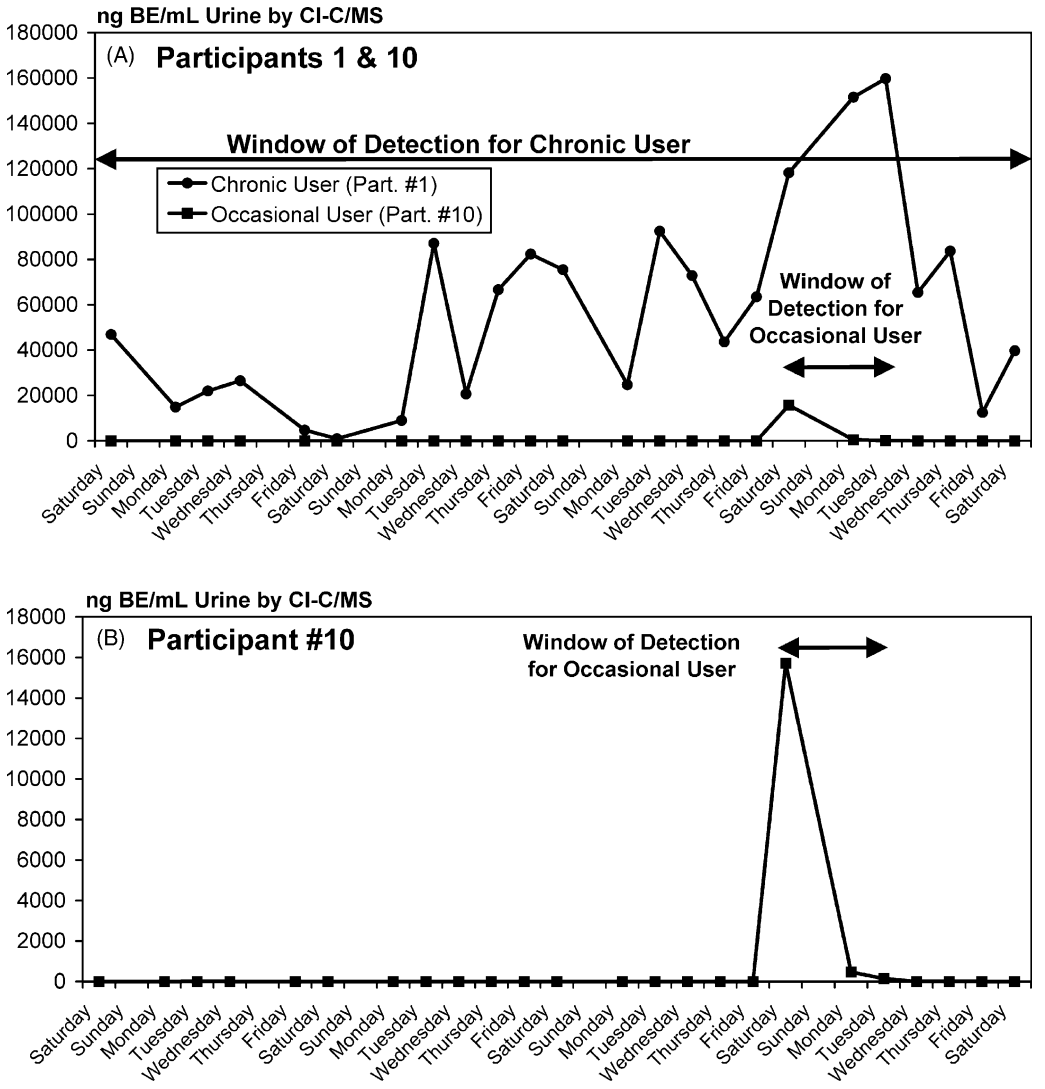

Fig. 1. Comparison of chronic (A) vs. occasional use (B). Consistently high-positive urine results are characteristic of chronic cocaine use. Urine positives spanning 3–4 days and returning to negative are characteristic of occasional use. Participant #10, in Fig. 1B, is also plotted in Fig. 1A to emphasize the different BE levels. Because of the high BE values, the window of detection of cocaine use in Participant #1, a chronic user, is much longer than that of Participant #10, an occasional user.

program might not detect drug use in Participant #10 if the specimens were taken only during weekdays and moderate, physiological dilution of the urine had occurred. Because high BE levels may not be reached for the occasional user (Participant #10, plotted in Fig. 1a on the same scale as Participant #1) physiological dilution of the urine would more easily evade detection of drug use.

A device that can continuously monitor drug use, such as the patch, would not have the window of detection problem apparent in Fig. 1a. Additionally with detection of drugs in sweat, physiological dilution that occurs with urine would not be a concern. In the case of chronic cocaine users, the patch worked quite well. The patch results for the three chronic cocaine users, followed in this study, are shown in Fig. 2. The patches of two participants were positives at quite high levels (340–10,000 ng cocaine per patch for Participant #1 and 160–21,000 ng cocaine per patch for Participant #9). The patches of Participant #2 were lower (40–131 ng cocaine per patch) as was the frequency of cocaine use, according to spikes in the urine BE concentrations. Although all these urine samples were positive at the LOD of GC/MS, two specimens contained less than 300 ng but more than 100 ng BE/ml urine for Participant #9. Two specimens contained 77 ng BE/ml and 271 ng BE/ml for Participant #2.

Of great concern to the forensic analysis is a false positive. Two such instances of false patch positives are

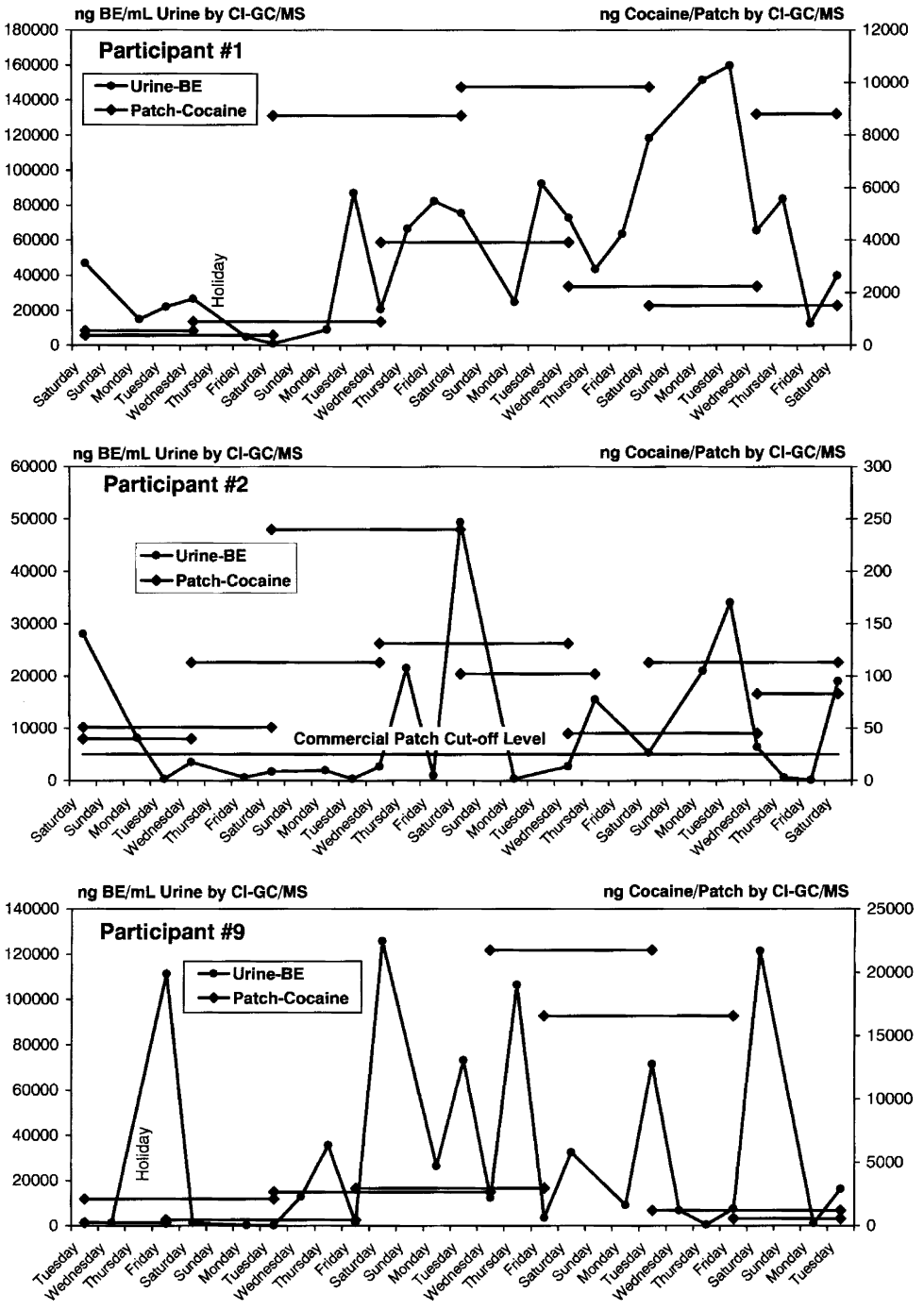

Fig. 2. Urine BE levels and patch cocaine levels of chronic users. Chronic cocaine use patterns may include short periods of urine specimens testing below cut-off concentrations. For Participant #1, no urine samples had a BE concentration <900 ng/ml. For Participant #9, 22/24 urine samples were >300 ng BE/ml and 24/24 were >100 ng BE/ml. For Participant #2, 20/22 urine samples were >300 ng BE/ml and 21/22 were >100 ng BE/ml. Sweat patches tested positive throughout the study at high levels (compared to other participants) showing a range of 164–22,000 ng per patch for Participant #1, 390–10,000 ng per patch for Participant #9, and 40–240 ng per patch for Participant #9.

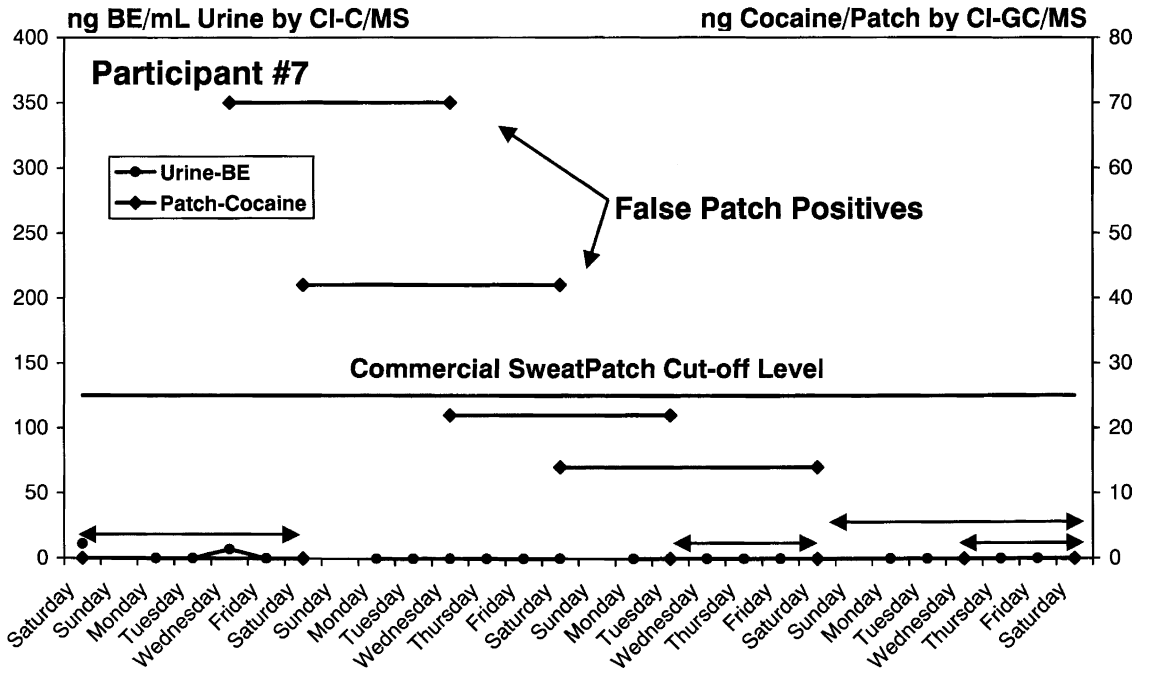

Fig. 3. Participant #7 showing false positive sweat patches with negative urine BE levels. Consistently blank urine specimens accompanied by above cut-off positive sweat patches are consistent with environmental contamination and inconsistent with cocaine ingestion. The four patches, with no cocaine present, are indicated by double arrows and offset from the *X*-axis for clarity.

shown in Fig. 3; Participant #7's urine specimens tested consistently negative, yet two patches were above the recommended 25 ng per patch commercial cut-off. The positive patches followed several negative patches, indicating that carry-over of drugs in the skin from prior use is probably not the explanation for these two positive sweat patch results. Additionally, since the skin swabs prior to patch application were consistently blank, there is no evidence that CFWI (cocaine on the skin prior to patch application) caused these positive patch results. Since prior research shows that CFWO (cocaine penetrating the protective membrane while patches are worn) can occur [6,7] and does occur with patches applied to research volunteers [10], it is reasonable to conclude that these positive patch results are consistent with cocaine contamination of the external patch membrane. Furthermore, the pattern in this example of decreasing cocaine in patches over time suggests decreasing exposure to environmental contamination over time. Traces of BE also were detected in the cocaine positive patches from this participant illustrating that BE can result from sources other than ingestion. In vitro degradation of cocaine to BE has been demonstrated for cocaine applied to hair (another protein matrix) and human skin [6,7,20].

Other than Participant #7, only two other participants had completely negative urines during the study period. All 18 patches from these other two participants tested negative for cocaine at a 25 ng per patch cut-off level. Taken as a group, the results from the three participants, where cocaine abstinence is evident throughout the study, show that the patch correctly reported cocaine use 25/27 times (93%) and incorrectly reported cocaine use 2/27 times (7%). Both incorrect/false positive patches from Participant #7 appear to indicate that this volunteer was environmentally exposed to cocaine.

An analysis of the commercial cut-off level for the patch as applied to the participants in this study is given in Table 1. Raising the cut-off level from 25 to 75 ng per patch increased the specificity of the assay to 100% without substantially decreasing the efficiency of the patch. Nevertheless, it is unlikely that a simple cut-off level for the patch would be definitive of use because of the difficulty in cleaning the skin of prior drug residues and the potential to contaminate an individual with arbitrarily high concentrations of drugs.

Besides false positives, false negatives also occur with the patch. Fig. 4 shows the urine and patch results from Participant #4; based on urine results, this participant likely used cocaine twice during the study period. The second use occurred after the urine sample was taken on Saturday and was detected by urinalysis Monday. The patch correctly detected cocaine use during the early part of the study but both patches missed the second use. False negatives occur frequently with the patch due to poor transfer of drugs from the skin to the patch [10,13,21]. We have suggested the use of glycerol to increase transfer [13], and a full report will be published later. Increasing the commercial cut-off to 75 ng per patch to reduce false positives would not have changed

Table 1
Statistical parameters for the patch from all 10 participants

	Patch/urine cut-off (ng per patch or ml)					
	25/100	50/100	75/100	25/300	50/300	75/300
True positive	33	30	26	30	28	26
False positive	4	1	0	7	3	0
False negative	9	12	16	8	10	12
True negative	51	54	55	52	56	59
Sensitivity (%)	78.6	71.4	61.9	78.9	73.7	68.4
Specificity (%)	92.7	98.2	100.0	88.1	94.9	100.0
Efficiency (%)	86.6	86.6	83.5	84.5	86.6	87.6

Sensitivity = (TP)/(TP + FN); specificity = (TN)/(TN + FP); efficiency = (TN + TP)/total number of patches.

the analysis for this individual (a substantially lower cut-off level would be necessary to detect the second, apparently single use).

Interpretations of the second possible use (or inadvertent ingestion) of cocaine by Participant #4, depicted in Figs. 4 and 6, are ambiguous. In support of use during the weekend is that the concentration of BE in urine on Monday (2200 ng/ml) is consistent with 1–2 days after cocaine use. Additionally, both the skin swabs and patches recorded some cocaine use or exposure during this time. Alternatively, inadvertent ingestion may have occurred (as discussed below). The low BE levels present in the urine are consistent with oral ingestion of 25 mg of cocaine 1 day prior to testing or as little as 1–2 mg a few hours before testing [3,4]. Monitoring of low to moderate level (above 1–2 mg but below the 50–100 mg required for a substantial physiological effect) of cocaine ingestion through the use of skin swabs or patches has not been investigated in the published literature and needs of further research.

Previous use is a concern if an individual abruptly stops cocaine use and the patch may be positive for an unknown period after cocaine cessation. In a legal setting, the issue of *when* drug use occurs is crucial. For example, judges require drug abstinence as a standard condition of probationary release. Prior drug use may be irrelevant to meeting this condition. The probationer is required to remain drug-free from the time of sentencing or release onward. If the sweat patch is used as a stand-alone test for determining drug use

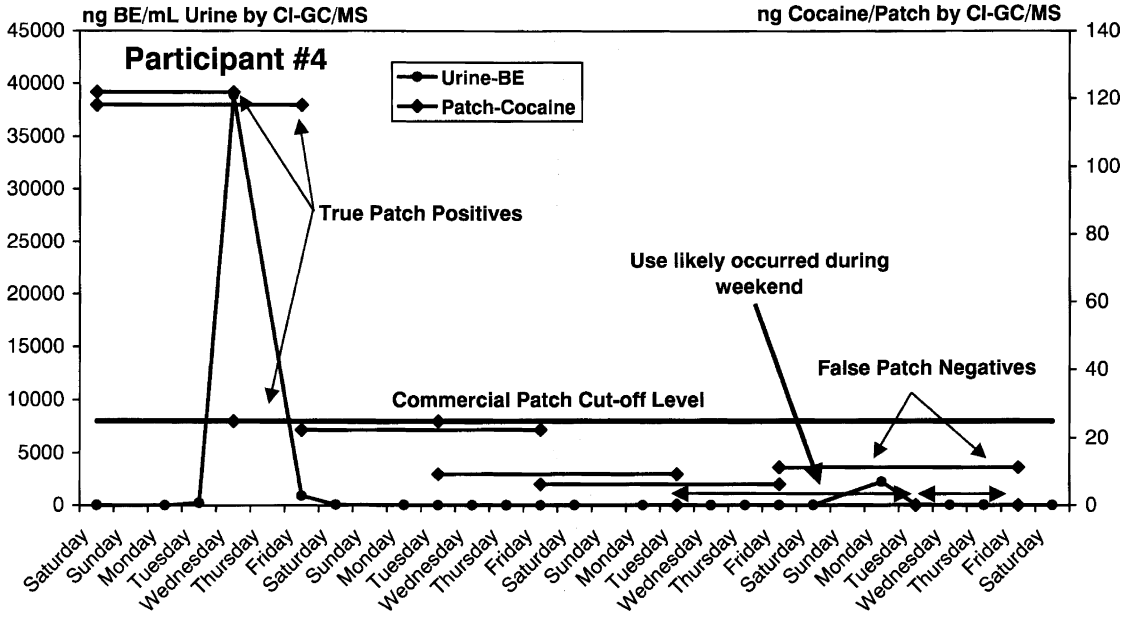

Fig. 4. Occasional use was easily detected by frequent urinalysis in Participant #4. Sweat patches were positive when worn during the first documented use period, but not during the second use period. Meanwhile, one positive sweat patch was worn during a period of consistently negative urine results. The two patches, with no cocaine present, are indicated by double arrows and offset from the *X*-axis for clarity.

status during the period that the sweat patch is worn, *then it is essential that the patch does not falsely report previous drug use* as ''*current drug use*''. In Fig. 4, it appears that previous cocaine use by Participant #4, including that documented by urinalysis during the first week of this study, may have caused or contributed to some cocaine in the sweat patch (barely negative at the 25 ng per patch cut-off) result during the second week, when urine results were consistently blank.

For chronic users, as shown in Fig. 2, it is not clear whether cocaine appearing in the patches came from current drug ingestion, previous drug ingestion, previous drug contamination, current drug contamination, or a combination of the above. From previous research, drugs were shown to bind to skin and persist for many days [7,12]. We proposed to save and analyze the ''cleaning'' swabs to help distinguish the source of the cocaine [6,7]. Fig. 5 shows the results for selected ''cleaning'' swabs from these three chronic cocaine users. The coloration on the clean swab is a combination of dirt and dead skin cells. Because melanin containing skin cells are easier to observe, some individuals may have more cleaning applied than others. In this study, the skin was cleaned until the cleaning pads appeared visually clean (note variable number of swabs used for cleaning in Fig. 5), according to the sweat patch instructions. Even with four swabbings (Participant #9), cocaine is still present. Based on previous research [6,7] this contamination on the cleaning pads likely will be accompanied by cocaine persistence on skin sufficient to cause positive patch results even if this individual had ceased cocaine use. Although the sequential patches were placed in similar locations to those removed, the skin contamination (removed by the swabs) may originate from use rather than from exposure during use or from the environment. Alternatively, drug may move on the skin during the swabbing process, allowing drug in skin areas not covered by one patch to contaminate subsequent patches.

Given the high levels of cocaine found in the patches of these two users (up to 21,000 ng cocaine per patch), prior skin contamination likely contributed some of the cocaine in the patches. For Participant #1, the cocaine present in the cleaning swabs is a substantial fraction of that found in the patch (Fig. 5). In prior publications, we proposed an arbitrary 10:1 ratio for patch:swab drug concentration to distinguish CFWI from use [7,13]. If this criterion were applied to Participant #1, four of the seven patches would have been positive and three negative (indicated by asterisks in Fig. 5). If this criterion were applied to Participant #9, three of the four patches would have been positive and the fourth would have been negative. However, this patch was on the volunteer for only 3 days compared to the 5–7 days for most other patches. Also, based on the urine data, Participant #9 used cocaine only once (or during 1 day as the frequency and quantity within the time frame cannot be estimated) during the time represented by the wearing of this patch. In addition, based on the excretion curve and the presence of cocaine in the urine, the use was likely to have occurred over a holiday and a mere 1 day before the patch was removed. Either reason could account for the lower levels of cocaine (237 ng) in the patch. Likewise for Participant #1, the patches that would fail the 10:1 criterion are those worn during lower cocaine use periods. For Participant #2 a similar number of patches fail this criterion. Of the three chronic cocaine users, Participant #2 used cocaine the least often and had lower amounts of cocaine in the patches.

Skin swabs measure both exposure and use of cocaine. Besides swabbing the area of patch placement, daily skin swabs of the hands and foreheads of the participants were also obtained. Fig. 6 shows the skin swab cocaine results from two participants as compared to their urinalysis results. For Participant #1, a chronic cocaine user, the skin swabs were also consistently positive and at high levels (micrograms of cocaine per swab). Likewise, skin swabs for Participant #4 are consistent with cocaine use on the day before the holiday. The skin swabs on the following day also were positive at fairly high levels. Additionally, the skin swab for the second likely use by Participant #4, indicated by an arrow in Fig. 6, appears positive but far below that of the earlier use. Although the timing of cocaine use may be speculative, if the use occurred on the Saturday, 2 days would have intervened between use and skin swabs. However, as expected for a matrix that measures environmental exposure, there are several cases where the skin swabs are positive but the urine samples are negative. No correlation could be found for the quantities of cocaine on the skin and the BE levels in urine. This is reasonable because the skin swabs measure environmental contamination AND removal rate by hygiene. While environmental contamination that affects sweat patch results should be correlated with the incident of use, it should not be correlated with the amount of cocaine used.

For most of the non-using participants in this study, skin contamination was low, while continual users had substantial skin contamination. Frequent cocaine users contaminate themselves with high levels of drug (Figs. 5 and 6). It is conceivable that individuals that live with drug users but who are not users themselves could also become contaminated [11]. Therefore, skin swabs should only be used as an indicator of the potential for cocaine use and as basis to obtain other biological specimens. For example, an individual with no cocaine on their skin is an unlikely user of cocaine and need not be subjected to additional testing with an alternative testing procedure such as urinalysis. Alternatively, an individual who has cocaine present may be a cocaine user and urinalysis should be the deciding factor. One such individual may be Participant #7. This volunteer claims to have a spouse who is a cocaine user. Some of Participant #7's skin swabs (Fig. 7a) reach substantial values (2117 ng cocaine per swab for fingertips and 344 ng per swab for forehead) whereas his/her urine BE levels are low or negative.

Table 2 gives the statistical parameters for the fingertip and forehead swabs for all the participants in this study.

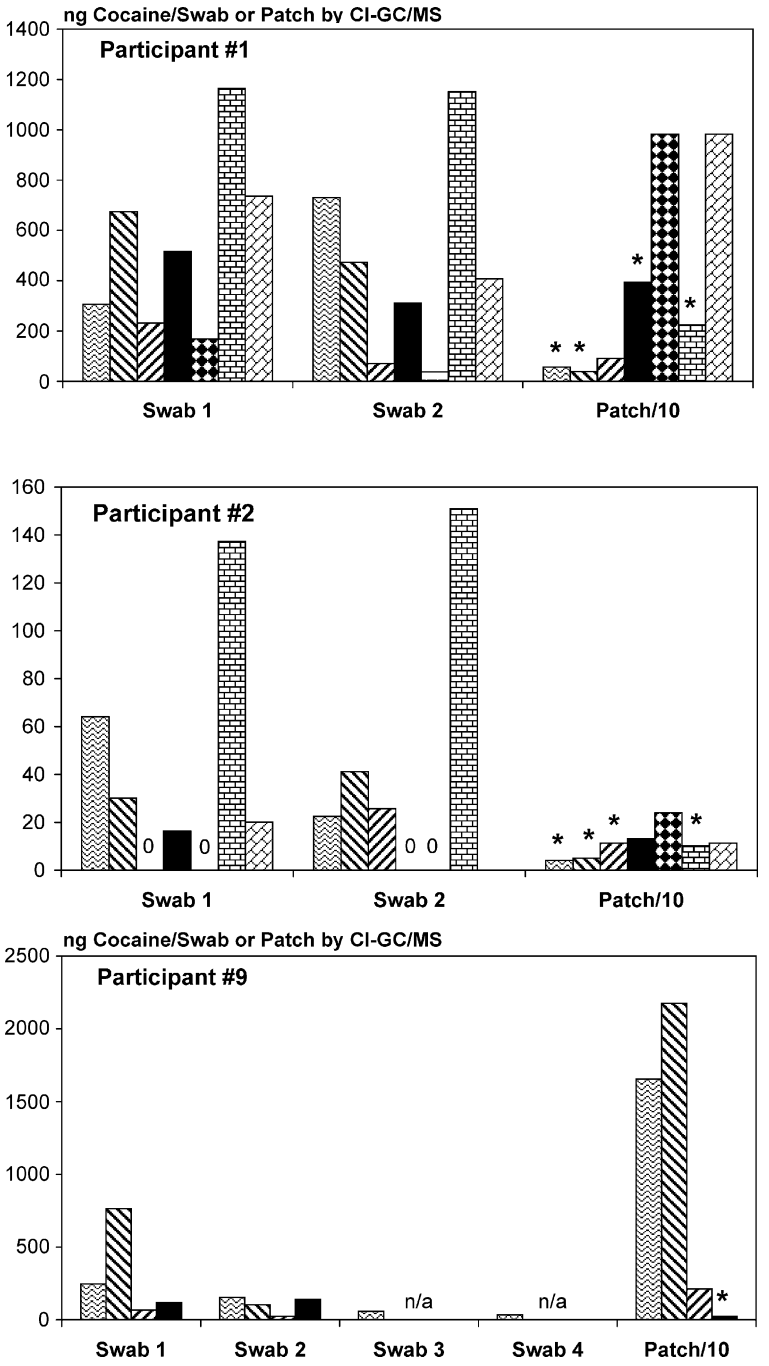

Fig. 5. Selected, matched swab and patch levels of chronic users. Each bar series represents swabs used to clean the skin prior to applying the patches and the subsequent patch results. The patch results are divided by 10 to keep the data on scale and make visual comparison to the swabs easier. Asterisks indicate series that failed the 10:1 criterion and n/a (not available) indicate missing data because swabs were not taken. Positive arm swabs from the cleaning prior to patch placement often accompany positive sweat patches.

The efficiency of swabs is high for detecting cocaine users (86–88% at a cut-off of 100 ng cocaine per swab). By excluding Participant #8 from the statistics, the false positive rate drops by about 50% and the efficiency increases 1–2%. Clearly, skin swabs are not definitive of drug use for individuals living in a drug-using environment yet abstaining from drug use. Also, Participant #8 is responsible for three of the seven false positive patches for the total cohort shown in

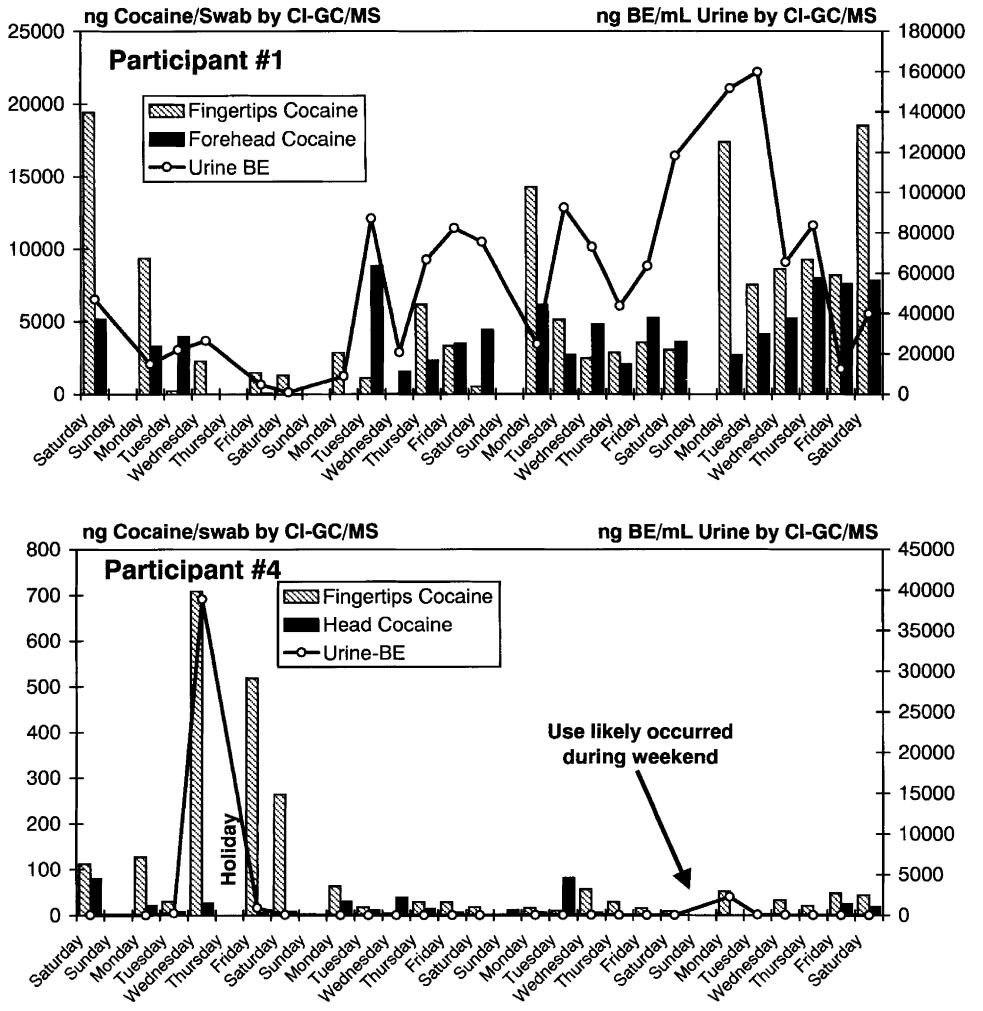

Fig. 6. Skin swabs of hands and forehead with comparison to urine levels for Participants #1 and #4. As expected, almost no linear correlation was observed for Participant #1 of hands:urine ($r^2 = 0.03$) and forehead:urine ($r^2 = 0.07$).

Table 1, indicating that sweat patches should be used with caution in such an environment. Interestingly, all these patches would have been considered contaminated using the 10:1 patch:swab criteria, discussed above.

Incorrect interpretations of positive results can occur with urine testing as well as patch testing, if too low a cut-off were applied. Low urine positives have occurred from the casual handling of drugs [22]. In this research, several participants

Table 2
Statistical parameters for the for skin swabs using urine BE >100 ng/ml

	Swab cut-off (ng per swab)					
	Fingertips 50	Fingertips 100	Fingertips 250	Forehead 50	Forehead 100	Forehead 250
True positive	69	61	48	57	48	38
False positive	28	11	4	10	6	3
False negative	9	17	30	14	23	33
True negative	125	142	149	124	128	131
Sensitivity (%)	88.5	78.2	61.5	80.3	67.6	53.5
Specificity (%)	81.7	92.8	97.4	92.5	95.5	97.8
Efficiency (%)	84.0	87.9	85.3	88.3	85.9	82.4

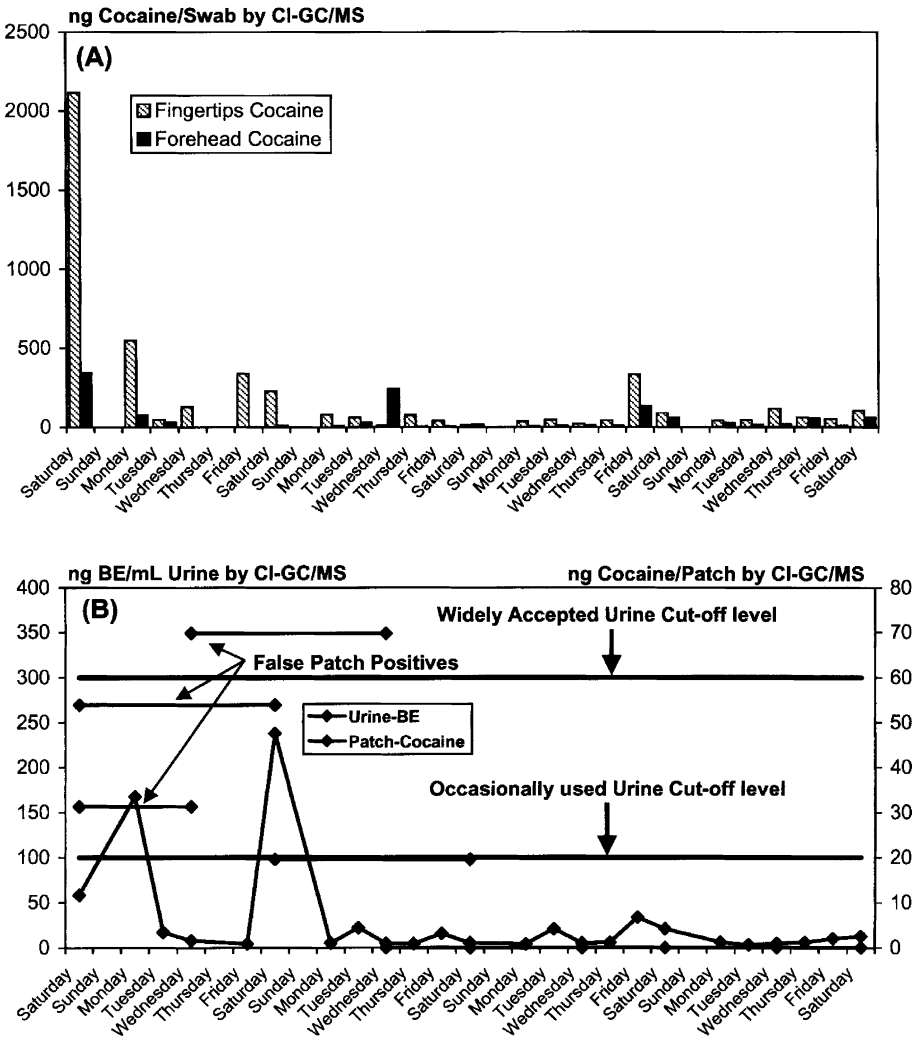

Fig. 7. Swab, Urine and patch data for Participant #8 claiming inadvertent ingestion. Two patches were positive (at 25 ng per patch) during this period. Forehead and fingertip swabs often showed the presence of cocaine.

volunteered information that their spouse or significant other continued cocaine use during their abstinence. In one such volunteer, two urine specimens contained detectable amounts of BE, above the 100 ng BE/ml cut-off and below the 300 ng BE/ml cut-off (Fig. 7b). Based on the urine BE levels and no sign of dilute urine (creatinine normal), this participant is unlikely to have used physiologically active amounts of cocaine. Three sweat patches during these periods were positive (above 25 ng cocaine per patch) but below 75 ng cocaine per patch. In addition, the swabs used in "cleaning" for these three patches had trace amounts of cocaine present, indicating prior exposure and casting suspicion on the patch results. These data suggest that the higher cut-offs (300 ng BE/ml urine and 75 ng cocaine per patch) would provide greater protection to individuals from allegations of intentional drug use.

Other derivatives of cocaine (BE, cocaethylene, and methyl ecgonine) also appeared frequently in the skin swabs of Participant #1 and have been attributed to human metabolism and excretion in the sweat [23]. These substances tended to be most prevalent when the cocaine concentration was high. Although their source is unknown, they may originate from placement in the environment during prior cocaine use followed by replacement on the skin. For Participant #4, the BE concentration was not useful in distinguishing use from exposure as it paralleled that of the cocaine concentration. The other two cocaine derivatives were not measurable for this individual. Thus, the usefulness of metabolites to determine the source of the drugs in the patch is not clear in this population.

Immunoassays are often used to screen specimens and any positive samples submitted to GC/MS confirmation.

Table 3
Statistical parameters for the swab ELISA assays using GC/MS cocaine >10 ng/ml

	ELISA Cut-off (ng/ml)					
	Cozart-25	Cozart-50	Cozart-75	OraSure-25	OraSure-50	OraSure-75
True positive	19	16	14	7	4	2
False positive	32	15	9	5	1	1
False negative	3	6	8	0	3	5
True negative	101	118	124	20	24	24
Sensitivity (%)	86.4	72.7	63.6	100.0	57.1	28.6
Specificity (%)	75.9	88.7	93.2	80.0	96.0	96.0
Efficiency (%)	77.4	86.5	89.0	84.4	87.5	81.3

Table 4
Statistical parameters for the patch ELISA assays using GC/MS cocaine >10 ng/ml

	ELISA Cut-off (ng/ml)					
	Cozart-25	Cozart-50	Cozart-75	OraSure-25	OraSure-50	OraSure-75
True positive	15	15	11	8	8	7
False positive	5	1	0	1	1	0
False negative	1	1	5	0	0	1
True negative	45	49	50	9	9	10
Sensitivity (%)	93.8	93.8	68.8	100.0	100.0	87.5
Specificity (%)	90.0	98.0	100.0	90.0	90.0	100.0
Efficiency (%)	90.9	97.0	92.4	94.4	94.4	94.4

Thus, an immunoassay that has few false negatives (to avoid discarding positive samples) and one that has few false positives (to avoid a heavy workload on the more time-consuming and expensive GC/MS) would be desirable.

This study allowed evaluation of immunoassays on matrices not commonly tested (skin swabs and patches). For cost considerations, a modification of the Microgenics CEDIA cocaine assay for urine was attempted to adapt it to these

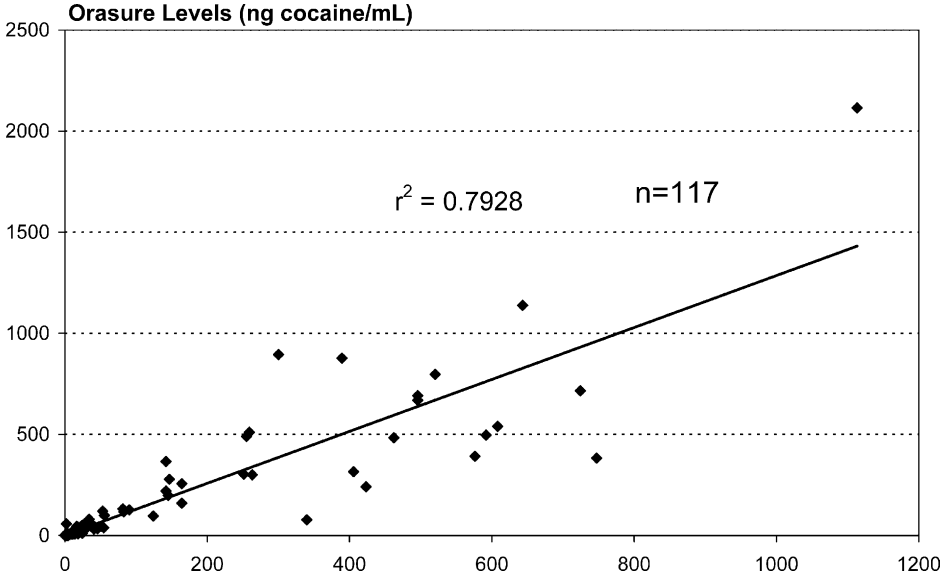

Fig. 8. Correlation of Cozart and OraSure ELISAs for skin wipes. Only those samples whose quantitations were in the linear range of the assays were compared.

other matrices and increase its sensitivity. Although it was capable of detecting 1 ng/ml of cocaine under certain circumstances, precise timing requirements made the precision poor when it was run in the manual mode. Two ELISA cocaine assays were evaluated. Both ELISAs gave similar results, with the OraSure ELISA having slightly better efficiency than the Cozart ELISA (Tables 3 and 4). The intraday %CVs for the Cozart ELISA was 22% (at 10 ng/ml, $n = 29$) and 13% (at 50 ng/ml, $n = 25$). The intraday %CVs for the OraSure ELISA was 28% (at 10 ng/ml, $n = 12$) and 24% (at 50 ng/ml, $n = 12$). The linear correlation for the Cozart ELISA versus the OraSure ELISA showed an r^2 of 0.79 (Fig. 8). This is consistent with similar cross-reactivities with cocaine-related substances and immunologically similar materials. The slope of 1.3 also indicates similar cross-reactivities.

4. Conclusions

Three patterns of drug use (chronic, occasional, and no-use) were identified readily by daily urinalysis; however, patch results were less clear. The patch identified some of the occasional cocaine use episodes and virtually all of the frequent, chronic uses. Some positive patches from participants could not be attributed to cocaine use while the patch was worn. This confirms, in a real life environment, previous experiments regarding external contamination and false patch positives. In some of these positive patch/negative urines instances, skin swabs identify cocaine and BE on the skin prior to the application of patches, which later tested positive. Prior skin contamination (CFWI) is the only identifiable source of these positive patch results. In other positive patch/negative urine results, skin swabs did not reveal cocaine-related substances, suggesting that external contamination of the patch (CFWO) may also play a role. Furthermore, in cases of concurrent cocaine use, it is not known how much of the cocaine and BE in patches originated from CFWI, CFWO, or within the body (from ingestion), as many of the ''cleaning'' swabs from frequent cocaine users had substantial amounts of cocaine and BE present.

In prior publications [6,7] we had suggested saving the ''cleaning'' swabs and testing them for the presence of cocaine if exposure was suspected. A ratio of >10:1, patch: swab, was proposed to screen for active use. In the cases found here, that ratio appears reasonable and warrants further study and corroboration. Additionally, it is proposed to increase the sweat patch cut-off concentration. Based on this research with a limited number of participants, a cut-off of 75 ng cocaine per patch would reduce the false positive rate. Both criteria must be considered because of it is difficult to clean the skin of prior drug residues and arbitrarily high levels of cocaine may be reached in contaminated environments.

Both the Cozart and OraSure cocaine immunoassays performed similarly and showed a reasonably strong correlation with each other. In contrast, although the modified Microgenics assay showed the requisite sensitivity for the matrices examined, it had poor precision when run in a manual mode. Unfortunately, automation was unavailable to allow more complete evaluation.

The most reliable method for detecting drug use appears to be daily urinalysis, followed by frequent skin wipes, which are approximately equivalent to sweat patch results. Intermittently, both skin wipes and sweat patches will miss drug use as well as wrongly indicate drug use. Where external contamination is an issue, urinalysis will provide more reliable proof of drug use.

Numerous legal challenges have asserted that positive patch results were not the result of current drug ingestion. Most of the individuals in these cases had some number of negative urine results to buttress their legal positions of not using drugs. In cases lacking frequent urine results, an expert could argue against external contamination as the cause of positive patches by claiming the defendant's cocaine use was undetected by urinalysis. This study clearly demonstrates that patch positives can arise under real life conditions from sources other than drug use; therefore, interpretation of sweat patch results must proceed with caution.

Acknowledgements

This research was supported by US Naval Surface Warfare Center grant number N00178-01-1-9002. Opinions expressed are strictly those of the authors and do not necessarily reflect those of the US Department of Defense or the US Government. The authors thank Rachel Gill for technical assistance and helpful comments with manuscript preparation and Lissette Drinkwater, Celina Rodrigues, and Inas Mahdi for assistance with participants.

Appendix A. Some legal cases

November 1999, US v. McLemore, Las Vegas, NV. US District Judge Lloyd George, Franny Forsman, Federal Public Defender, Las Vegas, NV. The US Attorney withdrew their prosecution after PharmChem testified about their in-house research. McLemore's frequent (including every-other-day) urine specimens tested negative while her sweat patches tested positive. She lived in a contaminated apartment with her young child, playing with child on the carpet. The apartment manager testified that previous tenants left behind cocaine paraphernalia sufficient to fill three large trash bags.

January 2000, US v. Self, Kansas City, MO. Federal District Magistrate Judge Rob Larson, Laine Cardarella, Assistant Federal Public Defender. Self's positive sweat patch results conflicted with occasional negative urinalysis results. Self had extensive contamination, sweated on the job, and used lotion for scabies. Judge Larson ruled not to revoke.

March 2000, US v. Gillette, Kansas City, MO. Federal District Magistrate Judge Rob Larson, Laine Cardarella, Assistant Federal Public Defender, Kansas City, MO. After testimony by the expert called by the prosecution, Judge Larson stopped the proceedings before the scheduled testimony of the expert to be called by the defense. Judge Larson ruled not to rely on positive sweat patch results to revoke Gillette's probation.

June 2001, US v. Snyder, Syracuse, NY. Federal District Judge Howard Munson, James Greenwald, Assistant Federal Public Defender, Syracuse, NY. A cohabitant of Snyder's home used crack cocaine during positive patch periods. Snyder sweated on the job and working out; his patch was exposed during both. Judge Munson ruled not to rely on positive sweat patch results to revoke probationary release. Judge Munson's published decision is located at the following web address: http://www.nysd.uscourts.gov/courtweb/pdf/D02NYNC/02-01702.pdf.

December 2001, US v. Redd, Kansas City, MO. US District Judge Gary A. Fenner, Defense Attorney Bruce Houdek, AUSA Marietta Parker. Judge Fenner ruled in favor of patch test results as evidence of intentional drug use during the patch wear period. While a prosecution expert testified, no defense expert testified.

May 2002, State of California v. Ian C., Sonora, CA. Presiding Judge Eric DuTemple, Defense Attorneys Dennis Dunn (Office of the Public Defender, County of Tuolumne) and Julian Gross (Drug Policy Alliance, Oakland, CA). The defendant's apartment was inhabited previously by a methamphetamine user. The defendant sweated on the job. The defendant's patch tested positive for methamphetamine while limited urinalysis was negative. Judge Eric DuTemple ruled that the proceedings would be expanded into a Kelly-Frye hearing to determine the admissibility of the sweat patch. Subsequently, the prosecution withdrew their petition to terminate the defendant's parental child custody rights.

October 2002, US v. Zubeck, Kansas City, MO. US District Judge Dean Whipple, Defense Attorney Elizabeth Carlyle. Zubeck admitted to recent binge methamphetamine use, but not during patch wear period. Zubeck reported heavy sweating. Questions concerning Zubeck's credibility were raised through other testimony. Zubeck's patch tested positive for methamphetamine while limited urinalysis was negative. Issues raised included longer-than-recommended sweat patch wear period and other collection site concerns. Judge Whipple ruled in favor of patch test results as evidence of intentional methamphetamine use during the patch wear period.

References

[1] K.L. Preston, M.A. Huestis, C.J. Wong, A. Umbricht, B.A. Goldberger, E.J. Cone, Monitoring cocaine use in substance-abuse-treatment patients by sweat and urine testing, J. Anal. Toxicol. 23 (1999) 313–322.

[2] J. Ambre, T.T. Ruo, J. Nelson, S. Belknap, Urinary excretion of cocaine, benzoylecgonine, and ecgonine methyl ester in humans, J. Anal. Toxicol. 12 (6) (1988) 301–306.

[3] G.F. Jackson, J.J. Saady, A. Poklis, Urinary excretion of benzoylecgonine following ingestion of Health Inca Tea, Forensic Sci. Int. 49 (1) (1991) 57–64.

[4] R.C. Baselt, R. Chang, Urinary excretion of cocaine and benzoylecgonine following oral ingestion in a single subject, J. Anal. Toxicol. 11 (2) (1987) 81–82.

[5] M. Burns, R.C. Baselt, Monitoring drug use with a sweat patch: an experiment with cocaine, J. Anal. Toxicol. 19 (1) (1994) 41–48.

[6] D.A. Kidwell, F.P. Smith, Susceptibility of PharmChek(tm) drugs of abuse patch to environment contamination, Naval Research Laboratory Memorandum Report #6170-99-8414, 3 November 1999.

[7] F.P. Smith, D.A. Kidwell, Susceptibility of PharmChek™ drugs of abuse patch to environmental contamination, Forensic Sci. Int. 116 (2–3) (2001) 89–116.

[8] G. Skopp, L. Potsch, H.P. Eser, M.R. Moller, Preliminary practical findings on drug monitoring by a transcutaneous collection device, J. Forensic Sci. 41 (6) (1996) 933–937.

[9] D.J. Crouch, C.L. Metcalf, M.H. Slawson, J. Baudys, An assessment of the potential for vapor phase contamination of the PharmChek™ sweat patch, Bull. Int. Assoc. Forensic Toxicol. 32 (3) (2002) 7–10.

[10] N. Uemura, L. Lester, R.P. Nath, J. Mendelson, M. Harkey, G.L. Henderson, Cocaine levels in sweat patches decline over time, in: Abstracts of Papers Presented at the 2001 Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics, Orlando, FL, March 2001, p. 62 (Abstract PII-117).

[11] F.P. Smith, D.A. Kidwell, Cocaine in hair, saliva, skin swabs, and urine of cocaine users' children, Forensic Sci. Int. 83 (1996) 179–189.

[12] D.A. Kidwell, M.A. Blanco, F.P. Smith, Cocaine detection in a university population by hair analysis and skin swab testing, Forensic Sci. Int. 84 (1997) 75–86.

[13] M. Long, D.A. Kidwell, Improving the PharmChek™ sweat patch: reducing false positive from environmental contamination and increasing drug detection, NRL Memorandum Report #6170-01-8597, 19 December 2001.

[14] D.J. Crouch, C.L. Metcalf, M.H. Slawson, An assessment of the effectiveness of the PharmChek™ sweat patch skin cleansing procedures, Bull. Int. Assoc. Forensic Toxicol. 32 (2) (2002) 5–8.

[15] J.A. Levisky, D.L. Bowerman, W.W. Jenkins, D.G. Johnson, J.S. Levisky, S.B. Karch, Comparison of urine to sweat patch test results in court ordered testing, Forensic Sci. Int. 122 (1) (2001) 65–68.

[16] H. Weingarten, Sweating the patch: a case report, in: Proceedings of the Paper Presented at the California Association of Toxicologists Annual Meeting, 3 August 2002, San Jose, CA, vol. 30, no. 3, 2002.

[17] F.P. Smith, S.A. Reuschel, K.C. Jenkins, Online opiate immunoassay evaluation: precision, Sci. Justice 35 (1) (1995) 65–71.

[18] M.A. Huestis, E.J. Cone, Differentiating new marijuana use from residual drug excretion in occasional marijuana users, J. Anal. Toxicol. 22 (6) (1998) 445–454.

[19] B.A. Goldberger, B. Loewenthal, W.D. Darwin, E.J. Cone, Intrasubject variation of creatinine and specific-gravity

measurements in consecutive urine specimens of heroin users, Clin. Chem. 41 (1) (1995) 116–117.

[20] G. Romano, N. Barbera, I. Lombardo, Hair testing for drugs of abuse: evaluation of external cocaine contamination and risk of false positives, Forensic Sci. Int. 123 (2-3) (2001) 119–129.

[21] K.L. Preston, M.A. Huestis, C.J. Wong, A. Umbricht, B.A. Goldberger, E.J. Cone, Monitoring cocaine use in substance-abuse-treatment patients by sweat and urine testing, J. Anal. Toxicol. 23 (1999) 313–322.

[22] M.A. ElSohly, Urinalysis and casual handling of marijuana and cocaine, J. Anal. Toxicol. 15 (1) (1991) 46.

[23] E.J. Cone, M.J. Hillsgrove, A.J. Jenkins, R.M. Keenan, W.D. Darwin, Sweat testing for heroin, cocaine, and metabolites, J. Anal. Toxicol. 18 (1994) 298–305.