Hair Analysis for Cocaine: The Requirement for Effective Wash Procedures and Effects of Drug Concentration and Hair Porosity in Contamination and Decontamination

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Abstract

It has been proposed that issues of external contamination of hair can be resolved by the presence of metabolites. This is true for some drugs in some circumstances. However, even if some metabolites such as cocaethylene are sufficiently definitive to indicate certainty of use, others such as benzoylecgonine can occur as contaminants. In addition, for phencyclidine, only the parent compound is determined. Therefore, effective washing procedures are needed to rule out contamination as the source of the parent or metabolite. The protection provided by cutoffs is also dependent on washing to remove surface contamination, which can range from very little to greater than 20 times the amount in the hair. In cocaine-positive head hair samples from 67 subjects with cocaine-positive urine tests, contamination of positive hair samples ranged from 0.7 to 8009 ng/10 mg hair, demonstrating that analyses of such samples to determine use is meaningless without removal of contamination. Wash procedures have been a focus of this laboratory for many years. In this paper, the effectiveness of our wash procedure was further challenged by application to negative hair (blonde, auburn, brown, and black) contaminated by soaking for 1 h in 1000, 10,000, and 50,000 ng cocaine/mL water. The uptake of the cocaine was approximately linear with increasing concentrations of cocaine in the soaking solutions. By application of the wash criterion, all samples were clearly identified as contaminated (i.e., negative). The effects of hair porosity on uptake of cocaine were also studied with 10 hair samples of all colors. Permed and unpermed samples were soaked in 10,000 ng cocaine/mL for 1 hour. All hair responded to the perm with increased uptake, and all were decontaminated or identified as contaminated (i.e., negative for cocaine use). Porosity, not hair color, determined the rate of penetration of hair by cocaine in solution, and, likewise, washing characteristics were unrelated to hair color.

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Introduction

Although there is widespread agreement that drugs deposit in hair from the bloodstream, there is also general awareness that drug from sources such as exogenous sweat and environmental contamination may deposit on hair (1,2). Yet, the role of washing as an integral part of hair analysis for drugs is still surprisingly unappreciated among many practitioners (3).

An obvious and important external drug source includes contamination from the environment, whether during use of a drug, by being in the vicinity of drug users, or from residues left in an environment by various means. Most emphasis has been given to removing or identifying such contamination in order to protect against a hair sample containing drug due to contamination from being interpreted as being due to drug use (4–12), and this important concern will be addressed further in this paper. However, what seem to be underappreciated are the reasons to remove or identify such contamination from a drug user's hair. One reason is to allow the valid use of cutoffs, both for parent drugs and metabolites, which can only be meaningful if applied to hair that is largely cleansed of external contamination. Secondly, the use of metabolites to distinguish use from contamination is valid only in combination with washing. Two metabolites that are definitive indicators of use even without washing because they are formed in vivo are cocaethylene in the case of ingestion of ethanol along with cocaine (4) and carboxy-THC from use of cannabinoids (13). Other metabolites, however, such as benzoylecgonine (BE) and 6-monoacteylmorphine can form on the hair from parent drug by nonmetabolic processes (11,12). The policy of requiring the presence of BE at a certain percent of the parent cocaine as an indicator of use (3) can only be meaningful if the sample has been adequately washed. Finally, the ability of hair analysis to provide information about the amount of drug ingested over a period of time requires the exclusion from the quantitation any drug that is not due to use (i.e., contamination). And this,

of course, applies equally to segmental analysis for the purpose of following the pattern of use over a period of time.

In addition to removal of environmental contamination, there are further considerations of the role of washing in quantitative analysis of hair for meaningful interpretation. Contamination by drugs from sweat on the surface of the skin is an acknowledged route for deposition of drugs on hair (1,2). Because a nonuser's sweat would not contain drug, sweat is not a realistic concern in terms of false-positive results. In the case of drug users, however, drug in sweat on the surface of the skin is a variable that needs to be minimized for quantitative drug testing. Variabilities in perspiration rates due to differences among subjects in physiology, activities, and environmental conditions cause large variations in exposure of the hair to drug-containing sweat. Furthermore, widely varying cosmetic and hygiene practices among subjects create variation both in the amount of surface drug that can penetrate the hair as well as the amount of drug left on or in the hair when it reaches the laboratory. This externally derived drug, although coming from the subject, needs to be largely removed if the result is to be interpreted as reflecting the amount of ingested drug. For example, consider the different amounts of drug-containing sweat to which hair is exposed for a drug user with porous hair, in a hot climate performing manual labor outdoors, compared with a person with nonporous hair rarely venturing out of air-conditioned environments. Without washing, the two hair samples (even if the subjects' doses were similar) may appear to contain enormously different concentrations of drug. Studies of drug binding in hair following administration of controlled doses have frequently discounted the need for washing, and have then drawn conclusions regarding hair color and drug uptake that may be totally invalid because this variable of perspiration, combined with differences in condition of hair and subsequent hygiene, has not been accounted for (14–16). Even though there has been acknowledgment that anomalies such as appearance of drug in hair within hours after administration (instead of the days required for growing hair to emerge from the skin surface), or appearance of drug dose in multiple segments along a hair strand after injection of only a single injected dose could be artifacts caused by sweat (17), this recognition has not motivated investigators to apply extensive washing in order to eliminate or minimize these effects. The range and extent of drug removed by this laboratory's washing procedure from cocaine-positive hair samples of urine-positive cocaine users, provided in the present paper, demonstrate the requirement for validated washing procedures to be an integral part of quantitative analysis of drugs in hair.

Some authors have been reluctant to wash hair aggressively because of concerns that drug from ingestion will also be removed (14). Although it is possible that some ingested drug may be removed by extensive aqueous washing, Baumgartner et al. (10) showed in 1993 that drug users' samples, after a first wash with alcohol and 3 half-hour washes with dilute phosphate buffer, still contained 10 times or more as much drug as the last wash. Further experiments with decontamination of soaked and sweat-contaminated samples, as well as hair from users, have shown that an additional 2 h of washing in dilute phosphate buffer does not remove siginificant drug from the hair of users but does remove much of any contaminating drug. Measurement of the drug in the last wash and use of that value to simulate (but with a deliberate overestimation) the effect of another 5 h of washing provides a wash criterion to identify a sample as contaminated (12,18–20). Even in the case where a light drug user might be interpreted as negative due to aggressive washing, this outcome is greatly preferred to false positives and erroneous quantitations that must result from inadequate washing methods.

A problem that some laboratories may have experienced is the use of ineffective methods for extracting the drug from intact hair; this gives the appearance of having lost the drug to washing, when in actuality the drug that was removed by washing was indeed contamination and the drug in the hair has simply not been recovered (25,26). In the authors' laboratory, the washing procedure is followed by enzymatic digestion of the hair sample. Extraction of the drugs from the liquid supernatant of the digested sample is then carried out in preparation for confirmation by mass spectrometry (MS). Ability to interpret results obtained under conditions of incomplete extraction is further confounded by such differences among hair samples as porosity, thickness, and texture (fine vs. coarse).

That exogenous drug from sweat or contamination enters hair by diffusion-like mechanisms has been proposed by other authors (21). It is reasonable that removal of this drug requires long exposure to aqueous solutions to allow diffusion of the drug out of the hair. Various washing approaches offered in the literature have included methods that are little more than rinsing, have used organic instead of aqueous solvents, or do not include a method to estimate the effectiveness of the washing (22–24). Measurement of the effectiveness of washing (i.e., determining the amount of drug in the washes relative to that remaining in hair) has demonstrated that 100% removal of heavy contamination from the hair is not a reliable expectation (even though more than 99% may be removed). For this reason, an additional criterion to distinguish use from contamination (12) is needed. The method of washing and applying a wash criterion based on the drug in the last wash and in the washed hair of a given sample used in this laboratory and in the present paper has been developed from analyses of thousands of positive samples and hundreds of samples contaminated by soaking or exposed to drug under simulated sweat conditions (9–12).

Porosity among hair samples, most often increased by cosmetic treatments but also varying naturally among different hair types, has contributed greatly to confusion about binding of drugs to hair, as well as about contamination and washing issues. (27). Various theories and explanations of melanin binding of drugs have been proffered based on simple soaking experiments of a few hair samples of assorted colors from subjects of different ethnicities (28,29). Results from such studies vary widely and are at times conflicting, probably because the actual determining variable in these cases has likely been porosity rather than color. Also, the suggestion has been made that soaking in higher concentrations of drug would create samples that could not be detected as contaminated by the washing procedure and criterion proposed and utilized in this paper (27). One author suggests, for example, that a highly porous black hair of an African-American subject would soak up more

drug than an equivalent light-colored hair, that it would retain that drug differently than the light-colored hair, and soaking in higher concentrations of drug would enhance these effects. Additionally, the same author asserts not only that the wash procedures and wash criterion would fail to identify samples, especially the black porous hair, as contaminated, but that such failure constitutes a "cultural bias", insofar as people of some cultures tend to use different hair cosmetic treatments causing variable porosities (27). Data presented in this paper will show that none of these concerns are valid: all hair types of all colors take up drug in proportion to the concentration of drug in the solution, all take up more drug when made porous, all release the drug similarly during the washing procedure, and all are equally readily identified as contaminated.

Methods

The wash procedure and wash criterion

The wash procedure used for the cocaine-positive samples as well as for the soaking and porosity experiments is as follows. First, dry isopropanol (2 mL) was added to about 12 mg of hair in 12- × 75-mm tubes; the tubes were shaken vigorously at 37°C for 15 min; after 15 min, the isopropanol was removed to a separate tube and saved for later analysis. Then 2 mL of 0.01M phosphate buffer/0.1% BSA (pH 6) was added to the hair samples in the tubes and the tubes shaken vigorously for 30 min at 37°C, after which the buffer was removed and saved to another tube for later analysis. This 30-min wash was repeated twice more, followed by two 60-min washes using the same conditions. After the final (5th) phosphate buffer wash and removal of the buffer, the hair sample was enzymatically digested (10) prior to confirmation by liquid chromatography (LC)–MS–MS. The washes were assayed by quantitative radioimmunoassay (RIA).

For the cocaine-using clinical subjects and the soaking contamination experiments presented here, all phosphate buffer washes were saved and analyzed by RIA; routinely, however, only the last phosphate buffer-BSA wash is saved and analyzed in routine commercial testing processes. The amount of drug per 10 mg hair in the last wash is multiplied by 5 and this result is subtracted from the amount of drug per 10 mg hair in the hair digest. The result of subtracting the indicated multiple of the last wash drug value from the digest value is termed the wash criterion, and is an underestimate of the amount of drug that would remain in the hair if further washing were to be applied (5 additional 1-h washes in the cases of cocaine, morphine, and PCP, and 3.5 additional hours of washing in the case of methamphetamine). If the result after subtraction is less than the cutoff for the parent drug, the result is considered negative for drug use. The parent-drug cutoff value for cocaine was 5 ng/10 mg hair.

Cocaine-positive hair samples from clinical setting

The results of hair analysis of the chronic cocaine users study have been presented previously (18); however, the wash data reported there included only the last wash, which is utilized routinely in this laboratory for calculating the wash criterion. For the purposes of that report, we did not present the additional information of the total contaminating cocaine of these samples relative to the remaining drug in the samples after washing.

In brief, the study collected hair from drug-using subjects at four rehabilitation clinics in the Southern California area. The protocols were reviewed and monitored by Quorum Review, Inc. Urine specimens were collected from volunteer subjects prior to hair being collected. A urine specimen and brief qualitative self-report were collected at first encounter with the subject. If the specimen tested positive for any of the SAMHSA-5 drugs (cocaine, opiates, PCP, amphetamines, and marijuana), a second urine (at 7–10 days following the 1st urine) and a hair specimen (at 14–21 days following 1st urine) were requested. Hair sample collectors were trained to cut the hair from the vertex region of the head, near the scalp, and to place the hair sample in a collection container that identifies the root end of the sample.

Of 73 samples that screened positive in the RIA screening assay, three samples, after washing, confirmed below the cutoff of 5 ng cocaine/10 mg hair; these will be discussed further in the Results section. For an additional three samples, although they were washed normally and confirmed positive, analysis of only the last wash was performed, rather than all the washes, and therefore these three samples could not be included in this report. Thus the total wash data of 67 of the original 73 screen-positive samples is presented.

Urines were screened by EMIT using a cocaine screening cutoff of 300 ng/mL, and confirmation by gas chromatography–MS at a SAMHSA-certified laboratory using a cutoff for cocaine of 150 ng/mL

For confirmation of samples identified as positive in the RIA screening assay, after washing (described previously), hair was enzymatically digested as previously described (10) at pH 5.5. The samples for cocaine analysis were extracted using Isolute SPE columns. A triple-quadrupole API 2000 PerkinElmer (PE) Sciex (Thornhill, ON, Canada) MS equipped with an atmospheric pressure ionization source via a TurboIon Spray ion source was used in all measurements. For LC, a model 200 binary micropump with a PE series 200 autosampler was used. The HPLC mobile phase consisted of water and acetonitrile (80:20) containing 0.1% HCOOH. The MS was optimized for cocaine to give optimum ion yields. Ionization of analytes was obtained in positive chemical ionization (PCI) mode. Positive ion precursors for cocaine (*m/z* 304) and cocaine-d_3 (*m/z* 307) were selected as the target analytes through the first quadrupole (Q1). Nitrogen was used as the collision gas in the second quadrupole (Q2). The product ions monitored in the third quadrupole (Q3) were *m/z* 182 and 185 for cocaine and cocaine-d_3, respectively. The instrument was operating using unit resolution on both Q1 and Q3.

Hair perming and soaking experiments

Drug-negative hair for soaking and porosity experiments were from ponytail lengths of hair obtained from haircuts by professional hair dressers. For handling during permanent-wave treatments, the hair was placed on a polyester fabric screen (McMaster Carr, catalog # 92255T31) for application of the product. For submerging the hair in the perming or neu-

tralizer solutions, the screen was folded over the hair so as to form a sort of envelope, and the enclosed hair was placed in the solution; for rinsing the hair, the screen was also folded over the sample and the envelope placed under a stream of water.

Ten hair samples from non-drug users, ranging in color from blonde to black, were divided into two aliquots. One aliquot of each was subjected to a permanent waving treatment, according to product instructions. The permed samples were exposed to the waving solution (Ogilvie brand permanent wave kit) for 20 min, rinsed, neutralized with the kit neutralizer, and rinsed again. Dry permed and unpermed aliquots of the 10 hair samples were then soaked in 10 µg/mL cocaine as described for our other soaking experiments. The dried soaked samples were then weighed and washed according to the wash procedure; the washes and digested hair were analyzed.

For all soaking experiments, approximately 100 mg of hair was placed in 50-mL beakers containing 20 mL of cocaine solution at 1, 10, or 50 µg cocaine/mL water. Hair was stirred about in the cocaine solution, and strands separated, to ensure uniform contact of the hair with the solution. Soaking was allowed to proceed at room temperature for 1 h, with gentle swirling of the hair in the solutions at 5–10 min intervals. Using a forceps, the hair was removed from the cocaine solution and blotted, then rinsed twice by sloshing in 2 changes of about 100 mL of water. The rinsed hair was again well-blotted and left open to ambient air for drying, about 1 h. Approximately 12 mg of dried hair was weighed and placed in tubes for the wash procedure

Results and Discussion

Prevalence of contamination of cocaine-positive hair samples

We have presented in a previous publication the results of analyses of cocaine from hair in a large group of subjects in a rehabilitation clinic who had contributed cocaine-positive urines during the growth period of the hair sample (18). For the purposes of that report, the last wash and hair digest results were presented, but not the total drug content of all five phosphate buffer washes. In order to demonstrate the amounts of contamination commonly occurring on cocaine users' samples, we are showing here the total cocaine in all 5 of the phosphate buffer washes of each sample and in the hair after washing. The cocaine content after washing of 67 cocaine-positive hair samples ranged from 6.5 to 2270 ng/10 mg hair, and all samples passed the wash criterion. The cocaine in the five buffer washes of the samples ranged from 0.7 to 8009 ng/10 mg hair. A demonstration of the range and variability of the total contamination removed by the wash procedure applied to these samples is provided in Figures 1–3, which show the total cocaine removed in the 5 buffer washes, and the cocaine remaining in the hair, for the 67 cocaine-positive hair samples. The data are arranged in order of increasing total combined cocaine in washes and hair.

The results are arranged in order of total cocaine in both washes and hair. The results in Figure 1, with the lowest values of the group, do not show extremely large proportions of drug in the washes, but rather in the order of 10% (Subject 2) up to 40%. Figure 2 shows samples with greater contamination relative to the amount in the hair, the worst case being Subject 26, with about 86% of the drug on the hair being contamination. The 40 ng/10 mg hair reflects low to moderate cocaine use (11); without washing, the 240 ng cocaine in the washes would make the subject appear to be a heavy user. A similar case is Subject #53 in Figure 3, where the wash contained 1307 ng cocaine/10 mg hair, and the hair only 64 ng/10 mg hair. After washing, Samples 26 and 53 are in a use category similar to the subjects in Figure 1, whereas without washing they would appear to be very heavy users. Consequently, without washing

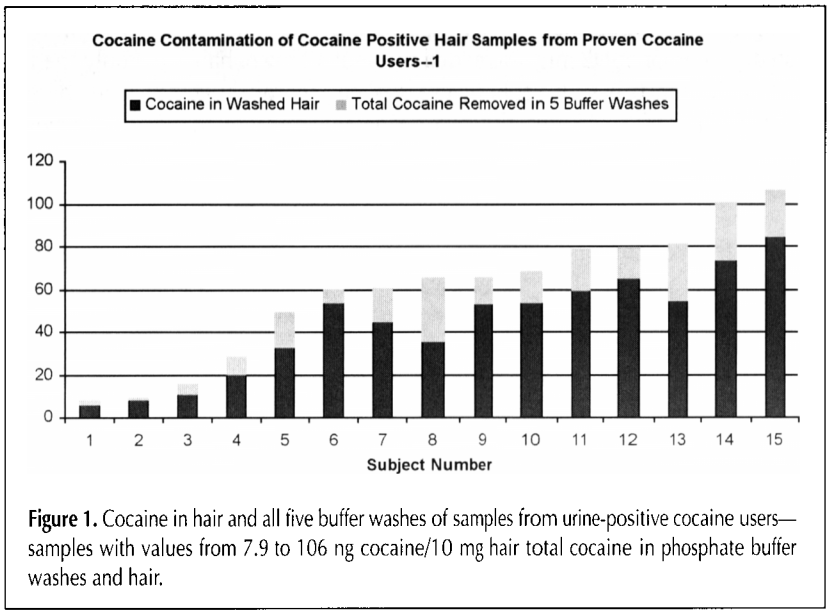

Figure 1. Cocaine in hair and all five buffer washes of samples from urine-positive cocaine users—samples with values from 7.9 to 106 ng cocaine/10 mg hair total cocaine in phosphate buffer washes and hair.

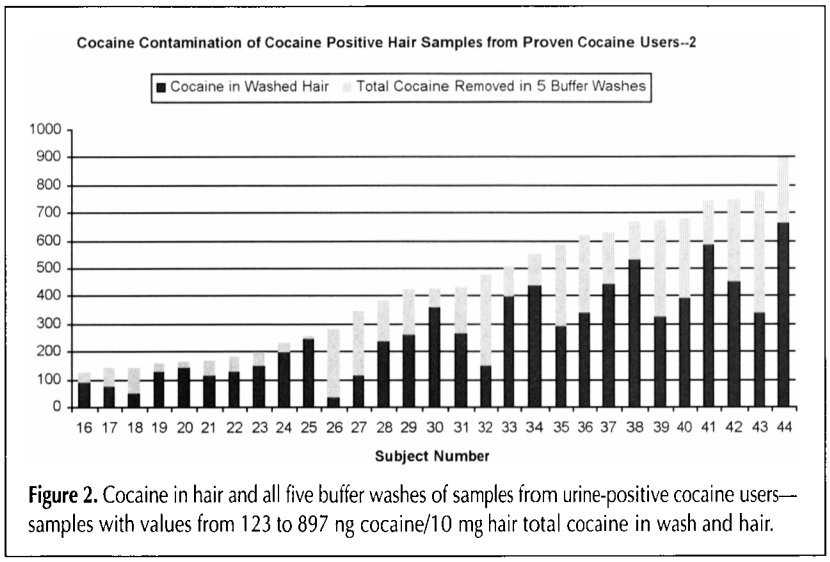

Figure 2. Cocaine in hair and all five buffer washes of samples from urine-positive cocaine users—samples with values from 123 to 897 ng cocaine/10 mg hair total cocaine in wash and hair.

there is no way of estimating the subject's use.

Hair cocaine levels from a clinical population, as shown in Figure 3, are likely higher than normally seen in workplace samples. To approximate workplace results, in Figure 4 we show results of samples with values from Figures 1–3 arranged in order of increasing hair cocaine content rather than total drug in hair and washes, but this only up to 200 ng/10 mg hair. Figure 4 illustrates the severe distortions in hair drug results in workplace testing that would be obtained if hair is not adequately washed. Because these are all positive samples as proven by concurrent urine analyses, there are no cases where the contamination would have caused a false-positive result relative to the cutoff (i.e., not considering other criteria such as metabolites and metabolite ratios). But this could also occur, of course, in testing unknown workplace samples, if the samples were assayed without washing procedures similar to that used in this laboratory for these samples.

Apropos of the concern that aggressive washing can remove drug due to ingestion, resulting in false negatives, three samples in the full set of 73 screen-positive samples confirmed below the cutoff of 5 ng cocaine/10 mg hair. One of these had 3.7 ng cocaine/10 mg hair, and 0.75 ng cocaine in all 5 buffer washes; this sample would therefore have been negative even without washing. Two other samples contained 4 and 2.9 ng cocaine/10 mg hair, with 3.0 and 5.3 ng cocaine in all five washes, respectively; these would have been above the cutoff without washing. Two of these three subjects, those with 3.7 and 2.9 ng cocaine/10 mg hair, did not claim cocaine use in the self-reports; the third subject admitted to use, but gave no estimate of dose. Heroin was the primary drug used by all three. For each of the three subjects, one of two urines was positive, with urine BE values of 395, 370, and 1910 ng BE/mL corresponding, respectively, to the subjects with hair values of 3.7, 2.9, and 4.0 ng cocaine/10 mg hair. It would seem that all three subjects were light users of cocaine and that they were false negatives if the goal is to identify all such low use. However, in workplace testing there is an emphasis on safety to protect nonusers from false positives. For such purposes as clinical, rehabilitation, epidemiological, or historical use, low hair results like these can be reported to the caretakers or investigators for informational use.

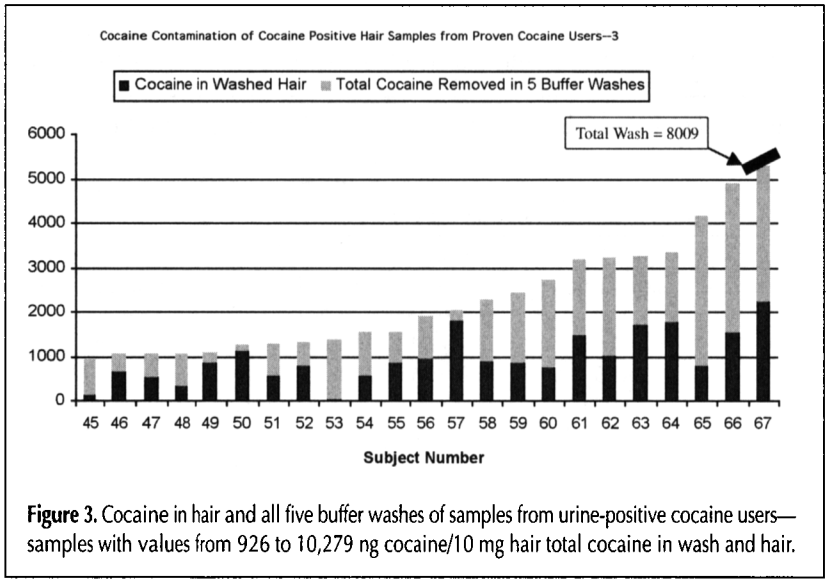

Figure 3. Cocaine in hair and all five buffer washes of samples from urine-positive cocaine users—samples with values from 926 to 10,279 ng cocaine/10 mg hair total cocaine in wash and hair.

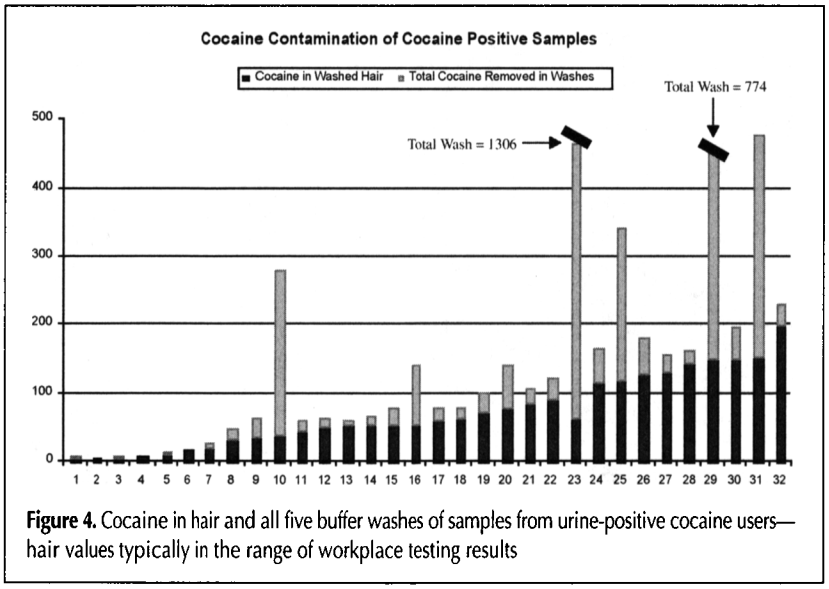

Figure 4. Cocaine in hair and all five buffer washes of samples from urine-positive cocaine users—hair values typically in the range of workplace testing results

Effects of cocaine concentration on drug uptake during in vitro soaking, and on decontamination and identification of contamination by the wash procedure and wash criterion

We have previously published the results of challenging the wash procedure by observing whether it decontaminated, or detected as contaminated, samples soaked in 1 µg cocaine/mL water, and also samples exposed to sweat conditions (12). In this paper, we have increased the challenge to the procedure by contaminating negative hair (including blonde, auburn, brown and black samples) by soaking for 1 h in 1, 10, and 50 µg/mL cocaine. After drying, the samples were washed and digested by the procedures described, and the digest supernatants extracted for confirmation by LC–MS–MS.

These higher concentrations of cocaine in solution far exceed any likely real-life contamination scenarios. For example, the soaking conditions of 20 mL of 50 µg/mL constitute uniform exposure of the 100 mg of hair to a total of 1 mg of cocaine, in a condition where the 20 mL of solution is not greatly in excess but is sufficient to surround all the hair. As a frame of reference, if an entire head of hair is about 100 g, the above is equivalent to soaking the head in a solution containing a gram of cocaine.

As another reference for estimating realistic contamination levels, Preston et al. published results of cocaine analysis of sweat patches, wherein the levels of cocaine detected were 985 (± 1005.8) ng/mL of extraction buffer, which (when multiplied by 2.5 because the total

extraction buffer is 2.5 mL) is equivalent to 2.4 µg of cocaine collected in an unknown volume of sweat over a one-week period (30), far below the soaking conditions used in these experiments.

Table I shows the results of soaking five different hair samples ranging in color from blonde to black in increasing cocaine concentrations from 1 µg/mL to 50 µg/mL in water. Regardless of the color of the hair or the concentration of cocaine soaking solution, all hair samples were readily identified as contaminated, and interpreted as negative for cocaine use by the wash criterion. For all hair, the higher the concentration of cocaine in the soaking solution, the higher the overestimate of remaining contamination provided by the wash criterion. This overestimate of the amount of drug that would be removed if washing continued for five more 1-h washes is a critical feature of the procedure, as it provides the protection from erroneously considering the remaining drug residues in hair due to contamination as being from use. Regarding the prediction raised by some authors that soaking at higher concentrations of drug would defeat the wash procedure (8), this is not only false, but the opposite actually occurs in that the wash criterion's overestimate of remaining contamination is further exaggerated with more severe conditions of soaking.

For each hair, there is a near-linear relationship between total cocaine uptake and cocaine concentration in the soaking solution, with the blonde and one of the black hair samples taking up the most drug. The amount of drug taken up, however, is not correlated with color, but to porosity, which is further investigated and described.

Effects of porosity on cocaine uptake

The effect of hair porosity on penetration of cocaine into samples soaked in cocaine solutions was studied by increasing the porosities of 10 different hair samples by subjecting them to permanent wave treatments, and subsequently soaking the nonporous and porous hair samples in solutions of 10 µg cocaine/mL water. The samples varied in color from blonde to black and included a red-colored hair. Variations in uptakes among the unpermed samples are likely due to varying porosities even before the perm treatments. This is consistent with a smaller increase in uptake of drug after perming, for those samples with higher uptake before perming. However, the purpose of the experiment was to demonstrate whether increasing porosity, which is expected to cause increased uptake by diffusion-like processes, in any way defeats the washing procedure or the effectiveness of the wash criterion in identifying a sample as contaminated. In this experiment, as shown in Table II, all 10

Table I. Effect of Increasinig Concentration on Hair Uptake of Cocaine During Soaking and on Results of Wash Procedure and Wash Criterion

Soaking Dose (Cocaine)	Wash or Hair Fraction	Cocaine (ng/10 mg hair) Sample 1 dark brown-black	Sample 2 medium brown	Sample 3 auburn	Sample 4 dark brown-black	Sample 5 light brown-blonde
1 µg/mL	Isopropanol Wash 15	1.14	1.35	0.34	0.84	1.52
	Buffer wash 1 30 min	15.08	25.59	18.15	51.13	17.61
	Buffer wash 2 30 min	2.15	3.09	2.18	7.22	2.84
	Buffer wash 3 30 min	0.49	1.03	0.50	2.11	0.90
	Buffer wash 4 60 min	0.37	0.68	0.40	1.20	0.57
	Buffer wash 5 60 min	0.18	0.41	0.20	0.81	0.39
	Digested hair	2.37	2.02	0.95	4.05	1.21
	Digested hair minus (5 x 5th wash)	1.45	-0.04	-0.06	-0.01	-0.73
	Result	Negative	Negative	Negative	Negative	Negative
10 µg/mL	Isopropanol wash 15 min	24.42	7.97	2.78	13.45	13.45
	Buffer wash 1 30 min	293.23	146.36	145.28	396.36	249.09
	Buffer wash 2 30 min	25.65	27.58	25.28	100.76	66.36
	Buffer wash 3 30 min	6.61	5.00	4.00	25.45	20.61
	Buffer wash 4 60 min	10.00	3.64	2.88	21.21	15.30
	Buffer wash 5 60 min	5.52	1.97	1.92	10.61	6.97
	Digested hair	24.17	12.68	6.25	36.39	16.57
	Digested hair minus (5 x 5th wash)	-3.41	2.83	-3.35	-16.64	-18.28
	Result	Negative	Negative	Negative	Negative	Negative
50 µg/mL	Isopropanol wash 15 min	133.98	60.46	24.63	89.6	123.28
	Buffer wash 1 30 min	981.65	576.62	590.48	944.64	1027.50
	Buffer wash 2 30 min	206.32	181.85	152.22	451.84	423.13
	Buffer wash 3 30 min	100.3	88.15	48.25	134.72	135.31
	Buffer wash 4 60 min	70.83	62.15	34.76	115.84	113.59
	Buffer wash 5 60 min	47.22	33.23	17.14	81.92	79.22
	Digested hair	147.90	89.40	42.80	212.60	138.00
	Digested Hair minus (5 x 5th wash)	-88.19	-76.75	-42.91	-197.00	-258.09
	Result	Negative	Negative	Negative	Negative	Negative

samples, both before and after perming, were identified as contaminated by the wash criterion, and therefore would be called negative for use of cocaine. The wash criterion, or overestimate of the remaining drug that would be removed by 5 additional 1-h washes (what can be considered the margin of safety) is increased with increased porosity after perming. This is true for all colors of hair and for all hair regardless of the magnitude of the effect of perming. The range of effects of perming is summarized in Table III, which shows the total cocaine in washes and hair for all the samples before and after perming. The effect of perming on increased uptake ranged from 1.9 to 75.1 times more total cocaine uptake after perming, this range being dependent on the condition of the hair before perming and each hair's widely varying susceptibility to the effects of perming. All hair responded to the perm with increased uptake, and all were decontaminated or identified as contaminated, resulting in them being interpreted as negative for cocaine use. These experiments demonstrate that porosity, not hair color, determines the rate of penetration of contaminating drug into hair. Likewise, decontamination characteristics, as shown in the data of the wash procedure and wash criterion for these samples, are determined by porosity, not hair color.

Conclusions

The amounts of contaminating drug on samples from known cocaine users would seem to demonstrate unequivocally the necessity for aggressive washing of hair samples in workplace testing, both for protection of nonusers from erroneous accusations of use and for valid utilization of such quantitative criteria as parent drug cutoffs, presence of metabolites, use of metabolite ratios, or estimates of ingested dose. The wash method used in this laboratory was demonstrated to protect equally against erroneous interpretation of a contaminated hair as positive whether the hair has been soaked at 1 µg cocaine/mL, 10 µg/mL, or 50 µg/mL, whether the hair is blonde,

Table II Effects of Hair Porosity on Uptake of Cocaine During Soaking at 10 µg Cocaine/mL

	Cocaine (ng/10 mg hair)									
Hair Condition	**Black**	**Black**	**Dark Brown**	**Brown**	**Blonde-Brown**	**Blonde-Brown**	**Blonde**	**Blonde**	**Light Blonde**	**Red**
Hair untreated (nonporous)	112.86	2.09	19.00	22.67	4.43	0.53	6.17	15.36	9.57	21.98
	282.54	507.46	280.00	253.33	18.98	741.09	293.23	68.12	181.16	30.15
	41.90	82.39	43.43	34.37	2.92	119.07	64.96	7.54	27.25	42.31
	8.10	18.96	11.57	6.96	0.65	26.20	19.25	2.75	6.38	9.26
	7.30	14.33	10.43	6.52	0.49	8.06	14.29	2.03	5.80	7.77
	2.89	6.00	3.86	3.00	0.18	1.71	4.87	0.64	2.17	3.14
	7.45	15.35	15.35	16.76	0.64	1.18	10.14	5.33	4.30	7.70
	-7.00	-14.65	-3.95	1.76	-0.28	-7.35	-14.21	2.13	-6.57	-8.00
	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Hair permed (porous)	6.81	1.89	17.01	13.64	6.78	0.50	21.21	25.52	4.81	3.77
	971.01	1555.91	1559.00	1987.88	1580.17	1354.33	1151.52	1053.73	1735.34	2127.54
	234.78	311.18	555.59	1360.61	395.04	275.59	218.18	524.18	372.93	430.43
	61.74	70.55	143.46	600.00	94.55	46.46	42.12	141.79	65.11	66.52
	18.70	15.75	47.56	96.21	30.25	12.76	11.97	142.24	14.89	19.42
	7.54	4.09	13.23	28.48	11.24	2.99	2.58	36.12	3.16	5.22
	3.43	1.23	7.79	20.94	8.46	1.82	0.91	19.03	1.19	1.61
	-34.27	-19.22	-58.36	-121.46	-47.74	-13.14	-11.99	-161.57	-14.60	-24.48
	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table III. Comparison of Total Cocaine Uptake During Soaking at 10 µg/mL Before and After Perm

	Cocaine (ng/10 mg hair)									
Hair Condition	**Black**	**Black**	**Dark Brown**	**Brown**	**Blonde-Brown**	**Blonde-Brown**	**Blonde**	**Blonde**	**Light Blonde**	**Red**
Hair untreated (nonporous)	463.0	646.6	383.6	343.6	28.3	897.8	412.9	101.8	236.6	122.3
Hair permed (porous)	1304.0	1960.6	2343.6	4107.8	2126.5	1694.4	1448.5	1942.6	2197.4	2654.5
Effect of perm on uptake (total drug uptake after perm/drug uptake before perm)	2.8	3.0	6.1	12.0	75.1	1.9	3.5	19.1	9.3	21.7

brown, black, or red, or whether the hair is nonporous or highly porous. Different wash procedures may also be effective; in developing these, however, there are some basic essentials: (1) The washing should include an initial short nonaqueous wash to remove strictly surface contamination. The remainder of the washing must be performed with aqueous wash solution. (2) Multiple changes of the aqueous wash solution and extended washing times are required to facilitate diffusion of the contaminating drug out of the hair. (3) Because washing may not remove 100% of contaminating drug, it is imperative that a laboratory develop a method for determining whether the drug remaining in the hair after washing is indicative of use or incomplete decontamination. (4) Because the development of a wash method is an empirical process highly dependent on available methods and resources, each laboratory will need to test its developed method independently, comparing the results of the method with samples contaminated by various means and with samples from known users.

Variations such as the conditions under which the hair is washed, the method by which the drug in the last wash is measured, and the efficiency of the extraction procedure are just three critical factors of our hair analysis methods. Our results also suggest that interpretations of studies of hair color effects are severely hampered by failure to address the need for aggressive washing methods together with high-recovery extraction methods, and this applies equally to hair positive from drug ingestion and negative hair that is contaminated. These failures in effective washing and extraction could produce results influenced by sweat and environmental contamination further complicated by porosity-related effects. The various confounding variables can produce results that might easily be misinterpreted as hair color differences.

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