



Removing and identifying drug contamination in the analysis of human hair

Thomas Cairns, Virginia Hill^{*}, Michael Schaffer, William Thistle

Psychomedics Corporation, 5832 Uplander Way, Culver City, CA 90230, USA

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Abstract

The procedure used in this laboratory for removing and identifying contamination of hair specimens with drugs is demonstrated by its application to hair contaminated by various experimental models. The models include soaking; coating with drug followed by sweat conditions for 6 h; and soaking in a very high concentration of cocaine followed by storage and multiple shampoo treatments. A multi-part wash procedure along with a wash criterion is applied to all samples containing drug above the cutoff. The failure of the wash criterion is a signal that the sample may be positive due to contamination rather than use, and in the absence of other over-riding evidence, the sample would be considered to be negative for drug use. This Wash Criterion has also been tested with hair from subjects demonstrated to be drug users by one or more drug-positive urines; in these studies, all hair samples from demonstrated users passed the Wash Criterion test.

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1. Introduction

For over 16 years, this laboratory has been examining characteristics of external contamination of hair with drugs. Development of washing methods and analytical tools to identify hair samples exhibiting drug presence due to contamination from external sources rather than from use is chronicled in a number of publications [1–5]. The earlier procedures have been extended and interpretation of washing results has been reformulated over these years. The present paper describes the method currently in use in this laboratory and demonstrates its successful application to samples contaminated by soaking, samples contaminated with drug and then subjected to simulated sweat conditions, and samples contaminated with cocaine and subjected to extended storage and multiple shampoo treatments. These latter storage and shampoo effects were studied in order to examine contamination effects reported by Romano et al. [6]. The wash procedure and application of the wash criterion (which essentially provides the additional safeguard of mathematically estimating the effect of further washing)

identified all contaminated samples correctly. This wash methodology has also been tested with hair from subjects demonstrated to be drug users by one or more drug-positive urines [7–9].

Various wash methods have been applied in other laboratories. For example, Koren et al. [10] used extended washes with ethanol and was able to remove all contamination from hair due to cocaine free-base vapors. Wang and Cone [11] were unable to remove cocaine contamination from hair using three 1 min methanol washes. Marsh and Evans [12] compared three methods for removal of contaminating methadone on hair: four 15 min methanol washes at 37 °C, two 15 min acetone washes followed by two water washes, and three 15 min washes with 1% dodecyl sulphate at 37 °C followed by two water washes. These latter authors found in most cases greater than 90% removal of contaminating drug. While adequate washing procedures can usually remove contaminating drug, as shown by the Koren et al. and Marsh et al. studies as well as by this laboratory [2–5], what is critical is to also identify those samples that may still exceed the cutoff due to the small percent of contaminating drug that may not have been removed by the wash procedures. Therefore, in order not to rely entirely on removal of contamination, this laboratory has presented

^{*} Corresponding author. Tel.: +1 310 2167776.

E-mail address: virginiah@psychomedics.com (V. Hill).

methods of washing combined with analysis of the washes themselves, and comparison of the wash values with the drug found in hair after washing, as the basis for criteria by which contamination can be identified. The studies presented here demonstrate the effectiveness of repeated aqueous washes and application of a newly formulated wash criterion, which allows the laboratory to distinguish between samples externally exposed to drugs and those samples positive through ingestion. For the purposes of this publication, the data are presented in the units "pg/mg hair," although this laboratory routinely reports its results in the units "ng of drug/10 mg of hair."

2. Methods

2.1. Soaking experiments

Twelve to 15 mg of each of the negative hair samples of varying color and porosity were weighed. The hair samples were placed in 12 mm × 75 mm polystyrene tubes to which 2 mL of 1000 ng/mL drug (cocaine, morphine, PCP, methamphetamine) in water was added. The hair samples were soaked for 1 h; after soaking, they were rinsed three times by addition, mixing, and removal of 2 mL of water. After rinsing, the hair samples were transferred to clean tubes and allowed to dry. They were stored dry in these tubes until the next day when they were washed according to the procedure described below.

2.2. Sweat experiments

Approximately 200 mg of negative hair, 4–6 cm in length, was placed in a solution of chloroform containing 10,000 ng/mL of each drug (in one experiment, morphine and 6-MAM; in another experiment, morphine only; and in another, cocaine, PCP, and methamphetamine). After 15 min, the hair was gently removed, placed on blotting paper, and allowed to dry. Chloroform was used as solvent expressly to deposit drug only on the surface of the hair strands with minimal penetration of the hair such as occurs with soaking in aqueous solution. The intent was to minimize penetration of the hair by the drug except for that occurring due to sweat.

After being coated with drug by the chloroform procedure, hair was mounted with tape on supports such that the strands were thinly spread out to ensure wetting each hair with the synthetic sweat solution. The simulated sweat solution consisted of 65 mM NaCl, 5 mM KCl, 9 mM sodium lactate, and 22 mM urea. [13,14].

The sweat solution was applied from a spray bottle as a very fine mist. The mounted hair was held over a large beaker at about a 45° angle for misting every 20 min. Between mistings, the hair was suspended at ambient temperature by hanging the wire frame on a ring-stand clamp. The hair remained in a damp state during the intervals between

mistings, with tiny droplets of the sweat solution readily discernible on the hair.

Before misting was started, a sample of the hair before "sweat" was saved as a 0-time control. Following the last misting, the samples were allowed to air dry for about an hour, after which the portions of the hair between the tapings onto the support were cut out; 12 mg of this hair from between the taped ends was weighed for the washing procedure.

2.3. Storage and shampoo effects

These experiments were intended to examine the results of experiments of Romano et al [6]. In that work, four subjects contaminated hair by first rubbing cocaine-HCl on their hands and then rubbing onto their hair. The subjects then proceeded to perform their normal cosmetic and hygiene habits for a number of weeks. Hair samples were collected at intervals during that time. It is not clear from the publication of Romano et al. how uniform contamination was accomplished by this method—for example, from the root to the distal ends, with hair lengths of 65, 40, 23, and 5 cm long among the four individuals. And without knowing the nature of the transfer of the cocaine to the hair, it would be difficult to know where to sample the hair at subsequent intervals. Since in our decontamination experiments, hair from the same donor was to be sampled on days 1, 3, 8 and 16 and samples compared with one another, it was important to ensure that the hair was uniformly contaminated. We contaminated by a method which would produce similar if not more severe, but uniform, contamination of the hair. Instead of rubbing dry cocaine hydrochloride on the hair surface, we contaminated hair from six different donors by soaking 0.5 g of hair at room temperature for 10 min in 20 mL of an aqueous solution of cocaine hydrochloride at a concentration of 10,000 ng/mL. This is equivalent to exposure to 0.4 mg cocaine per gram of hair, or 40 mg/100 g of hair. For comparison, in the experiment by Romano et al, 10 mg of cocaine-HCl was applied to the hands and then rubbed on an unknown amount of hair. After soaking, hair was removed from the solution, rinsed quickly with water, blotted dry and then allowed to air dry to expel the approximately 30% of water by weight that is still contained in towel-dried hair. The water is lost by evaporation within 15–30 min. The day of soaking is considered day 0; aliquots of the soaked samples were washed by the procedure described below, and the hair digested and analyzed on days 1, 3, 8, and 16. The soaked hair samples were subjected to shampoo on days 3, 6, 8, 10, 14, and 15. Shampoo treatments were done by lathering for 3 min with a warm 1:1 water:shampoo solution of Nexus Hydruss moisturizing shampoo. The hair was placed on a fine gauze cloth for lathering, after which the cloth was wrapped around the hair and the wrapped hair rinsed copiously with warm tap water. The dripping wrapped hair was then blotted with paper towels, after which the wrap was opened to expose the hair to air for drying. On days 1, 3, 8 and 16, aliquots of the dried hair were weighed, placed in

tubes and washed by the method described in this paper, and the hair digested and analyzed by MS.

2.4. The wash procedure and wash criterion

The wash procedure that we challenged with the contamination models presented here is as follows. First, dry isopropanol (2 mL) was added to about 12 mg of hair in 12 mm × 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 0.01 M phosphate buffer/0.01% 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 a 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 (2) prior to RIA analysis or confirmation by GC/MS or LC/MS/MS.

For these experiments, all washes were saved and analyzed; routinely, however, only the last phosphate buffer-BSA wash is saved and analyzed. The amount of drug per mg hair in the last wash is multiplied by 5 (or 3.5 for methamphetamine), and this result is subtracted from the amount of drug per 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 estimate of the amount of drug that would remain in the hair if further washing were to be applied—five 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 the subtraction is less than the cutoff for the parent drug, the result is considered negative in indicating drug use. The parent-drug cutoff values for the drugs cocaine, opiates, PCP, and methamphetamine were 500, 200, 300, and 500 pg/mg hair, respectively.

2.5. Analytic procedures

2.5.1. Radioimmunoassay (RIA)

The digests and washes of the soaking experiments were analyzed by in-house quantitative radioimmunoassays or LC/MS/MS. The RIA methods used, with the exception of the cocaine wash RIA, were the FDA-cleared screening procedures except for the use of standard curves over the useful ranges of the assays rather than cutoff standards. In the case of cocaine, the assay was also the same as the FDA-cleared screening assay except that the first antibody for the quantitative RIA used was at a different concentration than used for the screening assay.

2.5.2. MS procedures

The washed hair was enzymatically digested at low pH as previously described [2]. MS analyses of cocaine, opiates, and amphetamines were done by liquid chromatographic-

tandem mass spectrometric (LC-MS/MS) analysis. Analysis was performed on a triple quadrupole API 2000 Perkin-Elmer Sciex (Thornhill, Ont., Canada) MS equipped with an atmospheric pressure ionization source via an ionspray interface. For LC, a binary pump with an autosampler ISS 200 Perkin-Elmer was used. The high-performance (HPLC) column was a keystone Scientific BETASIL C8. The mobile phase was a mixture of water and acetonitrile containing 0.1% HCOOH. The proportion of water/acetonitrile was 80:20. Ionization of analytes was obtained in positive mode. Fragmentation was obtained using nitrogen as the collision gas. The MS was operating in the multiple reaction monitoring mode (MRM).

Samples for cocaine analysis were extracted using Isolute SPE columns. The target ions for cocaine and its internal standard cocaine-d3 were m/z 182 and 185, respectively. Ions m/z 210 and 213 were monitored for derivatized benzoylecgonine and its d3-internal standard. Ions m/z 196 and 299 were monitored for cocaethylene and its d3-internal standard. Ions m/z 168 and 171 were monitored for norcocaine and its d3-internal standard. The instrument was operating using unit resolution on both Q1 and Q3.

All samples for amphetamine MS analysis were extracted using liquid-liquid extraction. The target ions for methamphetamine and its internal standard methamphetamine-d11 were m/z 91 and 96, respectively. Ions m/z 91 and 96 were also used to monitor for amphetamine and its d8-internal standard. Ions m/z 135 and 136 were used to monitor for MDMA, and m/z 135 and 137 were used to monitor for MDA. The instrument was operating using unit resolution on both Q1 and Q3.

For analysis of opiates by MS, samples were extracted using Cerex SPE columns. The target ions for codeine and its internal standard codeine-d3 were m/z 165 and 165, respectively. Ions m/z 165 was also used to monitor for morphine, 6-acetylmorphine, and its d3-internal standard. The run time was approximately 10 min. The instrument was operating using unit resolution on both Q1 and Q3.

For analysis of PCP by mass spectrometry, approximately 10 mg of hair was extracted using a liquid-liquid extraction procedure. GC-MS analysis was performed on a Hewlett-Packard (HP) 6890 GC interfaced with a HP 5972 MSD operating in the EI mode (70 eV) with SIM monitoring. The GC was equipped with a 30 M DB5-MS capillary column (J & W) × 0.25 mm i.d. 0.25 μm film thickness) at a flow of 1 mL of helium/min. The transfer line was held constant at 280 °C. Ions monitored for PCP were m/z 200, 242, and 243 and m/z 205 and 246 for the PCP-D₅ internal standard. A single point calibration curve was used based upon one standard at the cutoff concentration. The curve fit is linear and it is forced through the origin.

3. Results and discussion

All of the hair samples from the contaminations described in the following discussion were washed with the Wash

Procedure described in Section 2—i.e., a short isopropanol wash, three 30 min and two 60 min phosphate buffer/B SA washes. After analyses of the washes and the hair digest, the wash criterion was calculated and applied in order to determine its effectiveness in identifying contaminated samples. The wash criterion is determined by multiplying by 5 (or 3.5 for methamphetamine) the amount of drug per 10 mg hair in the last wash and subtracting that from the amount of drug per mg hair in the hair digest. If the result is less than the cutoff for the drug in question, the hair is considered to be contaminated. Cutoff levels for cocaine, morphine, PCP and methamphetamine are 500, 200, 300, and 500 pg/mg hair, respectively.

3.1. Soaked hair samples

Twenty-eight hair samples, ranging in color from blonde to black and showing large differences in porosity, were

soaked for an hour in 1000 ng/mL of cocaine, morphine, PCP, and methamphetamine. The rinsed and dried samples were then washed by the method described above. All of the samples soaked in all 4 drugs failed the wash criterion—i.e., they were identified as contaminated samples. The analytical results of all washes of 10 of the samples are shown in Table 1. The samples in the table are representative in that 2 or 3 of each of the hair colors brown, blonde, grey–white, and black are shown, as well as samples showing the ranges of drug uptake that occurred with each hair color. Although the amount of drug entering the hair samples varies as much as two orders of magnitude, depending on the porosity of the hair, this does not hamper the effectiveness of the wash method and wash criterion. For example, samples 3 and 5 in Table 1 contain, before washing, tens of thousands of pg/mg hair of all of the drugs, whereas samples 6 and 8 contain about only hundreds of pg/mg hair of cocaine, morphine, and methampheta-

Table 1
Application of wash procedure and wash criterion to 28 hair samples soaked in 1000 ng/mL cocaine, morphine, PCP, and methamphetamine*

Sample #, color	Wash fraction or hair digest	Cocaine (pg/mg hair)		Morphine (pg/mg hair)		PCP (pg/mg hair)		Methamphetamine (pg/mg hair)	
		Wash and hair values	Hair minus (5 × LW)	Wash and hair values	Hair minus (5 × LW)	Wash and hair values	Hair minus (5 × LW)	Wash and hair values	Hair minus (3.5 × LW)
1, brown	Isopropanol	56		29		188		30	
	1st PO ₄	847		1,153		2,459		929	
	2nd PO ₄	159		226		382		179	
	3rd PO ₄	32		59		88		62	
	4th PO ₄	20		26		56		50	
	5th PO ₄	71		15		32		41	
	Hair digest	39	−316	77	2	100	−60	104	−39
2, brown	Isopropanol	550		477		846		281	
	1st PO ₄	7,138		6,923		11,538		9,846	
	2nd PO ₄	1,442		1,846		2,538		1,923	
	3rd PO ₄	377		700		769		662	
	4th PO ₄	300		515		542		396	
	5th PO ₄	177		308		323		250	
	Hair digest	467	−418	821	−719	520	−1,095	439	−436
3, brown	Isopropanol	100		033		218		61	
	1st PO ₄	38,909		52,727		12,097		49,939	
	2nd PO ₄	11,758		15,515		3,164		11,394	
	3rd PO ₄	2,955		4,364		2,942		3,152	
	4th PO ₄	1,452		2,667		2,812		1,333	
	5th PO ₄	745		767		2,309		612	
	Hair digest	790	−2,935	2,180	1,655	4,880	−6,665	358	−1784
4, blonde	Isopropanol	277		135		610		123	
	1st PO ₄	5,277		8,387		9,381		7,484	
	2nd PO ₄	577		781		1,423		4,903	
	3rd PO ₄	339		477		1,142		345	
	4th PO ₄	219		268		719		219	
	5th PO ₄	106		126		377		116	
	Hair digest	226	−304	468	−162	505	−1,380	193	−213
5, blonde	Isopropanol	305		123		559		127	
	1st PO ₄	33,636		48,000		18,836		48,727	
	2nd PO ₄	8,000		9,818		4,618		9,091	

Table 1 (Continued)

Sample #, color	Wash fraction or hair digest	Cocaine (pg/mg hair)		Morphine (pg/mg hair)		PCP (pg/mg hair)		Methamphetamine (pg/mg hair)	
		Wash and hair values	Hair minus (5 × LW)	Wash and hair values	Hair minus (5 × LW)	Wash and hair values	Hair minus (5 × LW)	Wash and hair values	Hair minus (3.5 × LW)
6, grey-white	3rd PO ₄	1,850		3,091		3,741		1,682	
	4th PO ₄	1,291		1,295		3,059		573	
	5th PO ₄	482		727		1,732		177	
	Hair digest	538	-1,872	2,001	-1,634	2,440	-6,220	201	-419
	Isopropanol	26		19		110		19	
	1st PO ₄	142		142		761		219	
	2nd PO ₄	23		26		77		35	
	3rd PO ₄	0		6		26		13	
	4th PO ₄	0		6		19		13	
	5th PO ₄	0		3		13		13	
Hair digest	18	18	16	1	29	-36	7	-42	
7, grey-white	Isopropanol	093		83		515		83	
	1st PO ₄	970		1,860		4,010		2,020	
	2nd PO ₄	233		155		713		343	
	3rd PO ₄	80		153		223		100	
	4th PO ₄	58		95		145		68	
	5th PO ₄	28		60		95		48	
	Hair digest	72	-68	198	-102	167	308	91	-77
	Isopropanol	38		14		341		38	
8, black	1st PO ₄	119		97		690		207	
	2nd PO ₄	31		31		128		52	
	3rd PO ₄	11		7		34		21	
	4th PO ₄	0		3		28		17	
	5th PO ₄	0		0		21		12	
	Hair digest	9	9	4	4	53	-425	75	33
	Isopropanol	166		87		566		71	
	1st PO ₄	1,200		2,168		4,432		2,232	
9, black	2nd PO ₄	239		376		705		403	
	3rd PO ₄	45		76		126		124	
	4th PO ₄	32		50		92		116	
	5th PO ₄	21		24		55		84	
	Hair digest	140	35	197	77	164	-111	419	125
	Isopropanol	434		272		1,197		306	
	1st PO ₄	2,750		3,563		6,313		5,000	
	2nd PO ₄	478		709		1,169		781	
10, black	3rd PO ₄	153		197		369		334	
	4th PO ₄	106		119		253		272	
	5th PO ₄	056		81		134		206	
	Hair digest	315	35	391	-14	376	-294	700	-21

mine, and about 1000 pg PCP/mg hair. Among the hair samples in the study, the blonde and brown hair samples included porous specimens that absorbed drug in high amounts; the grey hair samples resisted absorbing drug, and there were no black specimens that absorbed high amounts of drug. In general, however, we have observed that increases in the amount of drug a hair absorbs is mainly related to increasing porosity. The high amounts of drug in very porous specimens, however, washed out as readily as it

entered the hair, and the samples were readily identified as negative (contaminated) by the wash criterion. Table 2 shows the drug values of just the last washes and the digests of the remaining 18 samples, and the calculation of the wash criterion. In spite of the variability in uptake of drug, all samples are less than the cutoff for the drug in question after application of the "wash criterion,"—i.e., the subtraction of five times the last wash drug value (or 3.5 × the last wash for methamphetamine) from the hair digest value.

Table 2

Application of wash procedure and wash criterion to 28 hair samples soaked in 1000 ng/mL cocaine, morphine, PCP, and methamphetamine

Sample #, color	Wash fraction or hair digest	Cocaine (pg/mg hair)		Morphine (pg/mg hair)		PCP (pg/mg hair)		Methamphetamine (pg/mg hair)	
		Wash and hair values	Hair minus ($5 \times LW$)	Wash and hair values	Hair minus ($5 \times LW$)	Wash and hair values	Hair minus ($5 \times LW$)	Wash and hair values	Hair minus ($3.5 \times LW$)
1, brown	Last wash	388		512		712		582	
	Hair digest	1008	-932	1706	-854	240	-3,320	535	-1502
2, light brown	Last wash	463		580		847		893	
	Hair digest	1260	-1055	1910	-990	3060	-1,175	946	-2180
3, brown	Last wash	58		92		146		100	
	Hair digest	248	-42	316	-144	292	-438	326	-24
4, light brown	Last wash	100		156		232		138	
	Hair digest	273	-227	245	-534	398	-385	128	-355
5, brown	Last wash	21		27		67		61	
	Hair digest	119	14	176	41	169	-166	175	-39
6, light brown	Last wash	1014		1114		3019		214	
	Hair digest	1308	-3762	1968	-3602	3700	-11,395	102	-647
7, blonde	Last wash	194		221		553		271	
	Hair digest	815	-155	1248	143	2240	-525	482	-467
8, grey	Last wash	6		8		36		53	
	Hair digest	70	40	90	50	165	-15	251	65
9, black	Last wash	35		59		100		129	
	Hair digest	11	36	384	89	298	-118	391	-61
10, white-grey	Last wash	0		0		3		0	
	Hair digest	8	8	14	14	6	-9	4	4
11, black	Last wash	16		23		39		65	
	Hair digest	114	34	129	14	121	-74	236	8
12, black	Last wash	0		0		0		3	
	Hair digest	10	10	0	0	15	15	26	15
13, black	Last wash	94		169		175		147	
	Hair digest	445	25	768	1485	466	-409	408	-107
14, brown	Last wash	47		63		172		150	
	Hair digest	300	65	345	30	417	-443	424	-101
15, black	Last wash	8		0		24		23	
	Hair digest	40	0	47	47	93	-27	201	120
16, black	Last wash	9		6		35		62	
	Hair digest	78	33	81	51	161	-14	460	243
17, blonde	Last wash	267		333		500		279	
	Hair digest	654	-681	980	-685	1282	-1,218	321	-656
18, brown	Last wash	1132		471		1139		126	
	Hair digest	410	-680	870	-1485	2000	-3,695	120	-321

The result of subtracting the indicated multiple of the last wash is an estimate of the amount of drug that would remain in the hair if further washing were to be applied—five additional 1 h washes in the cases of cocaine, morphine and PCP, and 3.5 additional hours of washing in the case of methamphetamine.

3.2. Effects of sweat in contamination

3.2.1. Morphine

Washes and hair digests of six samples exposed to morphine and then followed by sweat-like conditions were analyzed by RIA. These results are shown in Table 3, which

Table 3
Application of wash procedure and wash criterion to hair samples exposed to morphine followed by 6 h sweat conditions

Sample, color	Wash fraction or hair digest	Morphine (pg/mg hair)			
		Contamination but no sweat		Contamination and 6 h sweat	
		Wash and hair values	Hair minus ($5 \times LW$)	Wash and hair values	Hair minus ($5 \times LW$)
A, brown	Isopropanol	7,640		240	
	1st PO ₄	21,820		2,032	
	2nd PO ₄	4,000		3,920	
	3rd PO ₄	470		750	
	4th PO ₄	180		350	
	5th PO ₄	70		180	
	Hair digest	160	-190	440	-460
B, blonde	Isopropanol	4,050		190	
	1st PO ₄	18,450		7,840	
	2nd PO ₄	1,490		1,350	
	3rd PO ₄	150		250	
	4th PO ₄	30		200	
	5th PO ₄	20		130	
	Hair digest	80	-20	330	-320
C, reddish-brown	Isopropanol	3,080		100	
	1st PO ₄	17,360		18,702	
	2nd PO ₄	3,170		2,840	
	3rd PO ₄	680		780	
	4th PO ₄	300		440	
	5th PO ₄	180		220	
	Hair digest	320	-580	180	-920
D, brown	Isopropanol	4,360		70	
	1st PO ₄	17,570		8,180	
	2nd PO ₄	150		1,580	
	3rd PO ₄	30		270	
	4th PO ₄	50		250	
	5th PO ₄	20		160	
	Hair digest	80	-20	430	-370
E, brown	Isopropanol	1,962		261	
	1st PO ₄	9,333		10,286	
	2nd PO ₄	1,314		1,878	
	3rd PO ₄	248		359	
	4th PO ₄	76		212	
	5th PO ₄	19		131	
	Hair digest	10	-85	110	-545
F, grey	Isopropanol	533		560	
	1st PO ₄	8,000		4,380	
	2nd PO ₄	444		360	
	3rd PO ₄	53		140	
	4th PO ₄	18		140	
	5th PO ₄	18		60	
	Hair digest	10	-80	280	-20

shows the entire wash profiles of these samples before and after sweat exposure. The high concentration of morphine in the first wash with organic solvent, isopropanol, reflects the presence of morphine on the surface of the hair in those samples not exposed to sweat. The differences in the isopropanol fraction before and after sweat exposure can be attributed to movement into the hair under the aqueous sweat

environment, and also possibly to mechanical loss from the surface of the hair during handling. In five of the six cases, the non-sweat samples have less morphine remaining in the hair after all washes. Increases in morphine content of washes after sweat exposure are most pronounced in the 3rd–5th phosphate buffer washes, demonstrating the penetration of morphine into the hair during the 6 h of sweat. All

Table 4
Application of wash procedure and wash criterion to hair samples exposed to morphine and 6-MAM followed by sweat conditions

Sample#, color	Wash fraction or hair digest	Contamination with no sweat				Contamination followed by 6 h sweat			
		Morphine (pg/mg hair)		6-MAM (pg/mg hair)		Morphine (pg/mg hair)		6-MAM (pg/mg hair)	
		Wash and hair values	Hair minus (5 × LW)	Wash and hair values	Hair minus (5 × LW)	Wash and hair values	Hair minus (5 × LW)	Wash and hair values	Hair minus (5 × LW)
1, grey	1st PO ₄ wash	5,644		2667		3,320		1620	
	5th PO ₄ wash	16		14		40		18	
	Hair digest	22	-58	0	-70	237	37	22	-68
2, brown	1st PO ₄ wash	7,038		6143		7,453		6041	
	5th PO ₄ wash	0		16		64		56	
	Hair digest	22	22	4	-076	104	-216	18	-262
3, brown	1st PO ₄ wash	11,396		4988		6,347		1938	
	5th PO ₄ wash	0		5		102		22	
	Hair digest	25	25	4	-21	213	-297	13	-97
4, reddish-brown	1st PO ₄ wash	10,468		6604		10,426		6077	
	5th PO ₄ wash	76		78		103		93	
	Hair digest	101	-279	35	-355	85	-430	28	-437
5, light-brown	1st PO ₄ wash	11,478		6490		5,851		3702	
	5th PO ₄ wash	0		11		82		35	
	Hair digest	30	30	0	-55	236	-174	23	-153
6, brown	1st PO ₄ wash	16,054		5982		16,528		5288	
	5th PO ₄ wash	45		29		98		52	
	Hair digest	53	-172	14	-131	178	-312	34	-226
7, black curly	1st PO ₄ wash	35,477		8348		17,060		6001	
	5th PO ₄ wash	276		111		883		360	
	Hair digest	1,386	6	187	-368	3,511	-904	866	-934
8, blonde	1st PO ₄ wash	7,428		5121		5,177		5966	
	5th PO ₄ wash	0		5		190		180	
	Hair digest	41	41	3	-22	1,023	73	243	-657
9, black curly	1st PO ₄ wash	2,124		888		1,685		1187	
	5th PO ₄ wash	0		0		53		44	
	Hair digest	30	30	0	0	237	-30	84	-136
10, blonde	1st PO ₄ wash	10,405		9757		5,520		3480	
	5th PO ₄ wash	0		20		32		47	
	Hair digest	43	043	8	-92	72	-88	24	-211

the samples were identified as negative after application of the wash criterion (i.e., a value less than the cutoff of 2000 pg/mg hair was obtained after subtracting five times the morphine value of the last wash from the hair digest morphine value).

3.2.2. Morphine and 6-MAM

Ten samples were contaminated with both morphine and 6-MAM followed by exposure to sweat. The results of morphine and 6-MAM analysis of the first and last phosphate buffer washes and of the hair digest are shown in Table 4. The values of morphine and 6-MAM in the first phosphate buffer washes range from 1187 to 8348 pg/mg hair 6-MAM and from 1685 to 35,477 pg/mg hair morphine. In general, the amounts of morphine and 6-MAM

remaining in the hair after washing are increased relative to the control after sweat treatment. All of the last washes and most of the digests of the samples showed more morphine and 6-MAM after the sweat exposure. Six of the 10 samples were above the cutoff of 200 pg morphine/mg hair before the application of the wash criterion. However, all samples, both with and without sweat, were determined to be negative after application of the wash criterion, demonstrating that the contamination followed by sweat could be removed or identified by the wash procedure and criterion.

3.2.3. Cocaine, PCP, and methamphetamine

Ten negative hair samples exposed to cocaine, PCP, and methamphetamine and then followed by sweat-like

Table 5

Application of wash procedure and wash criterion to hair contaminated with cocaine, PCP and methamphetamine followed by 6 h sweat conditions

Sample and treatment	Wash fraction or hair digest	Cocaine (pg/mg hair)		PCP (pg/mg hair)		Methamphetamine (pg/mg hair)	
		Wash and hair values	Hair minus (5 × LW)	Wash and hair values	Hair minus (5 × LW)	Wash and hair values	Hair minus (3.5 × LW)
1, contaminated, no sweat	Isopropanol	2427		1618		1649	
	1st PO ₄	1565		1435		2366	
	2nd PO ₄	160		176		275	
	3rd PO ₄	23		61		53	
	4th PO ₄	15		46		38	
	5th PO ₄	8		31		23	
	Digest	38	-2	60	-95	37	-44
1, contaminated, 6 h sweat	Isopropanol	42		105		28	
	1st PO ₄	2300		1965		2787	
	2nd PO ₄	300		3.28		460	
	3rd PO ₄	114		160		223	
	4th PO ₄	105		1.53		209	
	5th PO ₄	70		91		1.05	
	Digest	350	0	340	-115	240	-127
2, contaminated, no sweat	Isopropanol	1384		1472		760	
	1st PO ₄	1416		1592		2720	
	2nd PO ₄	192		216		288	
	3rd PO ₄	24		64		48	
	4th PO ₄	16		48		32	
	5th PO ₄	8		32		24	
	Digest	38	-0.02	90	-70	36	-48
2, contaminated, 6 h sweat	Isopropanol	88		160		48	
	1st PO ₄	1912		1312		1896	
	2nd PO ₄	216		248		384	
	3rd PO ₄	64		96		192	
	4th PO ₄	48		96		192	
	5th PO ₄	32		72		128	
	Digest	260	100	280	-80	278	-170
3, contaminated, no sweat	Isopropanol	1938		1650		1774	
	1st PO ₄	1035		988		1868	
	2nd PO ₄	62		101		132	
	3rd PO ₄	16		47		47	
	4th PO ₄	12		39		39	
	5th PO ₄	0		16		16	
	Digest	67	67	100	20	77	21
3, contaminated, 6 h sweat	Isopropanol	582		351		211	
	1st PO ₄	1340		1551		2049	
	2nd PO ₄	189		302		351	
	3rd PO ₄	25		84		133	
	4th PO ₄	21		84		154	
	5th PO ₄	14		63		112	
	Digest	204	134	300	-15	795	403

conditions were analyzed by RIA. Results are shown in Tables 5 and 6. In Table 5, the entire wash profiles of three samples before and after sweat exposure are shown. For the samples coated with drug but not exposed

to sweat, the high concentration of drug in the first wash with organic solvent, isopropanol, reflects the presence of the drugs on the surface of the hair in those samples not exposed to sweat. The differences in the

Table 6
Application of wash procedure and wash criterion to hair contaminated with cocaine, PCP and methamphetamine followed by 6 h sweat conditions

Sample # and treatment	Wash fraction or hair digest	Cocaine (pg/mg hair)		PCP (pg/mg hair)		Methamphetamine (g/mg hair)	
		Wash and hair values	Hair minus ($5 \times LW$)	Wash and hair values	Hair minus ($5 \times LW$)	Wash and hair values	Hair minus ($3.5 \times LW$)
4, no sweat	5th PO ₄	8		31		31	
	Digest	15	-25	70	-85	88	-21
4, 6 h sweat	5th PO ₄	26		91		0	
	Digest	150	20	260	-195	295	295
5, no sweat	5th PO ₄	8		89		41	
	Digest	0	-40	240	-205	70	-74
5, 6 h sweat	5th PO ₄	45		114		180	
	Digest	130	-95	610	40	151	-479
6, no sweat	5th PO ₄	33		31		98	
	Digest	107	-58	60	-95	164	-179
6, 6 h sweat	5th PO ₄	80		32		201	
	Digest	316	-84	90	-70	185	-519
7, no sweat	5th PO ₄	12		16		49	
	Digest	0	-60	100	20	64	-108
7, 6 h sweat	5th PO ₄	35		31		127	
	Digest	131	44	70	-85	261	-184
8, no sweat	5th PO ₄	24		49		63	
	Digest	47	-73	90	-155	137	-84
8, 6 h sweat	5th PO ₄	62		89		154	
	Digest	320	10	320	-125	194	-345
9, no sweat	5th PO ₄	16		57		24	
	Digest	160	80	110	-175	64	-20
9, 6 h sweat	5th PO ₄	44		95		163	
	Digest	203	-17	240	-235	167	-404
10, no sweat	5th PO ₄	12		33		62	
	Digest	38	-22	90	-75	174	-43
10, 6 h sweat	5th PO ₄	46		69		274	
	Digest	331	100	200	-145	502	-457

isopropanol fraction before and after sweat exposure can again, as for morphine, be attributed to movement into the hair under the aqueous sweat environment, and also to mechanical loss from the surface of the hair during handling.

As expected, with the drugs cocaine, PCP and methamphetamine, the exposure to sweat moves these drugs into the hair such that after sweat exposure more drug remains in the hair after washing, as shown by increases in drug content of the digests and aqueous washes, especially the 3rd–5th phosphate buffer washes. Table 6 shows just the last wash and digest values for the remaining seven of the 10 samples in the sweat experiment. All samples were negative after application of the wash criterion.

3.3. Storage and shampoo effects on contamination

Table 7 contains the data of the decontamination studies of six different cocaine-contaminated hair samples, before and after multiple shampoos over a period of 16 days. As expected, because samples were contaminated by soaking in aqueous cocaine-containing solutions, only a small fraction (range of 1.2–37.7%) was found in the non-hair-swelling isopropanol wash, which removes drug only from the surface of the hair. (Sample #2, a grey hair, was highly resistant to penetration by the aqueous soaking and thus the percentage on the surface appears high but only relative to the small amount that penetrated.) This distribution of the drug in the hair in our experiments contrasts with the results of the unshampooed

Table 7
Contamination followed by 1, 3, 8, and 16 days of exposure and shampooing: cocaine in washes and hair

Sample #	Wash and test hair after	pg cocaine/mg hair								Total cocaine in wash and hair	Report
		15 min isopropanol	1st PO ₄ wash 30 min	2nd PO ₄ wash 30 min	3rd PO ₄ wash 30 min	4th PO ₄ wash 60 min	5th PO ₄ wash 60 min (LW)	Hair after washing ^a	Hair minus 5 × LW ^a		
1	1 day	1258	8,794	646	116	92	57	258	NA	11,221	NEG
	3 days	803	13,559	1178	355	344	133	663	-2	17,035	NEG
	8 days and 3 shampoos	81	1,489	446	248	27	132	771	111	3,437	NEG
	16 days and 6 shampoos	27	715	298	149	171	132	705	45	2,197	NEG
2	1 day	505	715	63	22	17	18	59	NA	1,399	NEG
	3 days	193	361	39	14	12	7	32	NA	658	NEG
	8 days and 3 shampoos	12	91	30	11	15	6	42	NA	207	NEG
	16 days and 6 shampoos	8	53	28	15	12	12	42	NA	170	NEG
3	1 day	938	3,160	327	127	118	70	323	NA	5063	NEG
	3 days	676	4,681	465	170	164	69	394	NA	6619	NEG
	8 days and 3 shampoos	85	854	268	121	174	90	665	215	2257	NEG
	16 days and 6 shampoos	80	477	216	75	118	87	619	184	1672	NEG
4	1 day	1217	9,685	1106	258	192	107	554	19	13,119	NEG
	3 days	292	12,743	1884	619	650	271	978	-377	17,437	NEG
	8 days and 3 shampoos	57	1,354	466	229	250	149	932	187	3437	NEG
	16 days and 6 shampoos	31	713	323	155	189	133	985	320	2,529	NEG
5	1 day	156	10,866	2576	909	693	310	925	-625	16,435	NEG
	3 days	81	12,709	2706	1034	980	417	1070	-1015	18,997	NEG
	8 days and 3 shampoos	34	4,781	1755	961	947	517	1436	-1149	10,431	NEG
	16 days and 6 shampoos	15	2,406	990	468	563	432	1340	-820	6214	NEG
6	1 day	1069	6378	1668	613	580	318	900	-690	11,526	NEG
	3 days	455	4595	239	1305	1415	531	2456	-199	13,147	NEG
	8 days and 3 shampoos	103	2816	1306	742	1000	558	2100	-691	8,725	NEG
	16 days and 6 shampoos	106	1947	896	466	520	420	1887	-213	6,242	NEG

^a If the sample is below the cutoff of 500 pg/mg hair, application of the Wash Criterion is not required.

samples of Romano et al., where the dry ethanol-removable fraction represented a much larger fraction (80–90%). The bulk of the surface contamination, however, is not the major contamination problem, since it is largely removed by shampooing. Drug that penetrates beyond the surface region is the greater challenge, and in this region our soaking contamination procedure produced a much higher level of contamination (as much as nearly 19,000 pg/mg hair before shampoo) than did the Romano procedure (subjects A–D, respectively, contained 3060–4950, 3370–5860, 3070–5240, 3530–8140 pg/mg hair), demonstrating that our contamination method was even more severe than Romano's applications of rubbing cocaine on the hair. Our contamination also showed good reproducibility of the contamination process; one grey hair (#2) showed resistance to cocaine uptake, with 890 and 460 pg/mg hair, and the other five hair samples ranged from 4120 to 18,910 ng/mg hair in all fractions excluding the isopropanol. Continued shampoo treatments did not produce a significant increase of cocaine in the washed hair samples. As expected, the total content of cocaine deposited by the contamination is decreased, in some cases drastically, as a result of shampooing.

The main effect of repeated shampoo treatments, or normal hygiene, according to both our study and Romano's,

is simply decontamination of the surface of the hair. All of our contaminated hair samples, including the shampooed ones, are negative after the wash and wash criterion are applied, and seven of the 24 hair samples are negative even without the application of the wash criterion. This result indicates that neither storage nor shampoo treatments produce a significant challenge to our wash procedures.

Neither extended storage nor multiple shampoos caused an obstacle to identification of cocaine contamination by the wash criterion in our experiments. A number of procedural errors may account for the differences in results between the two laboratories. Instead of washing at 37 °C, Romano et al. [6] washed at 45 °C. The ratio of hair to wash solution was another deviation by Romano et al., who used 100 mg of hair in 5 mL of wash buffer, or 20 mg/mL, whereas our method usually uses about 12 mg, in 2 mL of wash buffer. This is important for two reasons: with the higher volume to hair ratio, the solution will become less contaminated by the residual drug, i.e., it will reduce the contaminating tendency of the wash solution. Second, with too much hair in the test tube, hair tends to be physically matted together, with little movement of wash solution at the surface of the hair strands. Finally, the manner of shaking the tubes during the washing is important. Our method uses a high shaking rate of between

110 and 120 oscillations/min. Most importantly, the tubes must be allowed to bump against the rack to cause sudden changes in momentum at each return stroke. Thus, effective washing is assured by the combination of high frequency shaking and bumping. With the excess of hair in the tube, the shaking/bumping rate would become even more critical in moving the wash solution around and through the hair. Since the wash formulas described in our publications are based on empirical data using exact specified conditions, deviations from those conditions preclude application of those same formulas without establishing their applicability under the altered conditions. On the basis of these results, we attribute the differences in the outcomes of our studies and those of Romano to differences from the wash procedures in use in our laboratory and, possibly, to other uncontrolled experimental variables.

4. Conclusion

Two models of contamination, soaking and sweat, were used to test procedures of decontamination and identification of contamination by cocaine, morphine and 6-MAM, PCP, and methamphetamine. The combination of Psychomedics' extensive wash procedure and application of the wash criterion successfully identified all cases of contamination correctly, regardless of hair color or method of contamination.

The studies described here demonstrate successful decontamination and/or identification of samples exposed to environmental drug in a variety of scenarios: soaking, powder followed by sweat, and soaking followed by extended storage and multiple shampooing. No contamination scenario, including sweat-induced movement of cocaine into deeper lying regions, created difficulties for the decontamination procedures in distinguishing contaminated samples from samples positive through drug ingestion. Clearly, however, without washing, essentially all of the samples reported in this paper would be positive by virtue of containing drug above the cutoff levels. After washing, but without the application of the wash criterion, some of the samples would still have been positive above the cutoff levels of the parent drugs. When there is presence of a metabolite that can only be formed physiologically, such as cocaethylene, washing may be less critical. However, metabolites such as benzoylecgonine or 6-MAM, which can also be formed *in vitro*, are only definitive in determining use when samples are effectively washed and contamination identified. Only with a thorough washing procedure and a means of identifying contamination, can hair analysis results be reliably reported as indicating drug use.

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