Egyptian Journal of Forensic Sciences (2014) 4, 129–136

HOSTED BY

Contents lists available at ScienceDirect

Egyptian Journal of Forensic Sciences

journal homepage: http://www.journals.elsevier.com/egyptian-journal-of-forensic-sciences

ORIGINAL ARTICLE

Cocaine contamination in pubic hair: Analysis of the decontamination method

Guido Romano, Francesca Indorato *, Giorgio Spadaro, Salvatore Barbera, Nunziata Barbera

Department "G.F. Ingrassia", Laboratory of Forensic Toxicology, University of Catania, Italy

Received 17 April 2014; revised 20 August 2014; accepted 27 September 2014
Available online 28 November 2014

KEYWORDS

Pubic hair;
Cocaine contamination;
Decontamination method;
False positive

Abstract Even if pubic hair represents a reliable and widely accepted alternative hair matrix to identify drug abusers, it might produce false positive results due to external contamination.
The aim of this study was to verify whether the external contamination of pubic hair with cocaine could influence the discrimination between active users and false positive subjects. The analysis was performed on *in vivo* and *in vitro* samples; the contamination was carried out by rubbing pubic hair with cocaine hydrochloride contaminated hands for three consecutive days. Five days after the beginning of the contamination, the pubic hair was collected and analysed at different times for two months.
Data from our studies show that all *in vivo* samples yielded false positives; the *in vitro* samples were negative only for 10 days and then yielded false positives.

1. Introduction

Hair testing is widely employed in many forensic laboratories to identify drug abusers.[1–16] Nevertheless, the methods used for hair testing are limited by the risk of false positives due to external contamination.[17–21] The search for a method that can discriminate between passive exposure and active drug consumption has long been debated. However, two important aspects should be considered in order to correctly study this problem: the experimental models of contamination and the decontamination procedures.

Experimental models of contamination should be as similar as possible to reality. Humans habitually touch themselves, especially on the head. Thus, hair is one of the most frequently touched areas. Therefore, persons who are professionally or illegally in a close contact with the drug, such as narcotic officers and subjects involved in illicit cocaine manufacturing or distribution, can contaminate themselves by touching their own hair. Similarly, the hair of a non-addict subject could be contaminated if a pusher or addict subject handles his head: in these cases, a hair test for drug abuse is extremely likely to give a false positive result.

* Corresponding author at: Department of Anatomy, Biology and Genetics, Legal Medicine, Neurosciences, Diagnostic Pathology, Hygiene and Public Health, "G. F. Ingrassia", Section of Legal Medicine, Laboratory of Forensic Toxicology, University of Catania, Via S. Sofia, 87, Comparto 10, 95123 Catania, Italy. Tel.: +39 095 378 21 64; fax: +39 095 378 21 65.
E-mail address: fra.indorato@gmail.com (F. Indorato).
Peer review under responsibility of The International Association of Law and Forensic Sciences (IALFS).

http://dx.doi.org/10.1016/j.ejfs.2014.09.004

Often, people who require a hair test have very short hair; in these cases, the sample could be collected from the pubic area. Pubic hair represents a reliable and widely accepted alternative matrix, often employed to ascertain or exclude drug abuse.[14,23,24] This matrix could also be contaminated, such as by an addict partner or, in case of non-addict pushers, by "doses" often hidden inside underwear.

Regarding this issue, Cairns et al.[22] propose three contamination models: (1) soaking, (2) coating with drug followed by sweat conditions for 6 h, and (3) soaking in a very high concentration of cocaine (10,000 ng/ml for 10 min), followed by storage and multiple shampoo treatments. The latter experimental model was carried out to verify contamination effects reported in a previous study about the external contamination of cocaine and a risk of false positive results.[17] The protocol published by Cairns and colleagues[22] proposes a "wash criterion": this decontamination method is based on repeated aqueous washes, analysis of the wash solutions and the application of an empirical "wash criterion" formula.

In the present study, we applied this decontamination method, which should allow externally contaminated samples and positive samples due to active consumption to be distinguished.

The present study was performed using the pubic hair of non-addict subjects contaminated with cocaine to verify that this "wash criterion" can discriminate between active users and false positives due to exogenous contamination. Moreover, our study was conducted for a longer period in order to investigate the real confidence of the method.

The endogenous formation of cocaine metabolites in contaminated pubic hair will be the aim of a successive study.

2. Materials and methods

The study was conducted on ten drug-free volunteers that gave informed consent. Before the start of the study, urine and pubic hair samples were analysed to exclude the presence of

Table 1 *In vivo* contamination followed by 5, 10, 20, 30, 40, 50 and 60 days of exposure and washing: cocaine (ng/mg) in washes and pubic hair.

Subjects	Day of collection	Isopropanol wash 15′	1st PO_4 wash 30′	2nd PO_4 wash 30′	3rd PO_4 wash 30′	4th PO_4 wash 60′	5th PO_4 wash 60′ (LW)	Pubic hair after wash	Pubic hair minus (5 × LW)	Cocaine wash and pubic hair values	Result
Subject 1	5	1.191	78.241	33.049	20.446	24.009	11.655	103.764	45.489	272.355	Positive
	10	0.635	26.433	12.679	8.418	12.093	5.674	79.810	51.440	145.742	Positive
	20	0.260	7.666	3.974	2.756	4.352	2.143	44.105	33.390	65.256	Positive
	30	0.151	3.600	2.255	1.130	1.881	0.978	23.331	18.441	33.326	Positive
	40	n.d.	1.387	0.753	0.474	1.004	0.552	17.039	14.279	21.209	Positive
	50	n.d.	0.678	0.433	0.311	0.415	0.297	10.549	9.064	12.683	Positive
	60	n.d.	0.413	0.202	0.154	0.279	0.205	7.195	6.170	8.448	Positive
Subject 2	5	1.094	67.582	21.437	11.439	15.573	7.265	79.681	43.356	204.071	Positive
	10	0.534	23.245	10.711	5.748	6.471	3.187	55.933	39.998	105.829	Positive
	20	0.223	9.366	3.963	1.814	2.572	1.410	39.406	32.356	58.754	Positive
	30	0.144	3.746	1.978	1.213	2.415	1.017	23.371	18.286	33.884	Positive
	40	0.105	1.344	0.773	0.395	1.213	0.706	15.711	12.181	20.247	Positive
	50	n.d.	0.873	0.281	0.195	0.437	0.287	10.805	9.370	12.878	Positive
	60	n.d.	0.441	0.207	0.148	0.361	0.280	7.792	6.392	9.229	Positive
Subject 3	5	0.839	53.653	20.569	11.682	15.052	6.837	65.839	31.654	174.471	Positive
	10	0.474	22.099	8.515	5.062	8.270	1.863	49.573	40.258	95.856	Positive
	20	0.195	7.519	3.396	2.079	3.789	1.583	33.449	25.534	52.010	Positive
	30	0.541	3.210	1.875	1.056	2.001	0.929	21.247	16.602	30.859	Positive
	40	0.085	1.123	0.790	0.474	1.064	0.569	14.274	11.429	18.379	Positive
	50	n.d.	0.710	0.380	0.251	0.447	0.315	9.571	7.996	11.674	Positive
	60	n.d.	0.412	0.170	0.119	0.365	0.216	6.514	5.434	7.796	Positive
Subject 4	5	1.742	113.648	49.721	35.667	39.273	19.818	154.107	55.017	413.976	Positive
	10	0.876	34.125	21.843	14.692	18.317	10.144	127.815	77.095	227.812	Positive
	20	0.407	8.291	6.203	3.859	5.348	3.497	61.811	44.326	89.416	Positive
	30	0.215	4.119	2.917	1.305	2.573	1.381	25.419	18.514	37.929	Positive
	40	0.137	1.203	0.755	0.397	0.904	0.615	21.763	18.688	25.774	Positive
	50	n.d.	0.743	0.471	0.394	0.422	0.275	13.182	11.807	15.487	Positive
	60	n.d.	0.450	0.216	0.186	0.211	0.193	8.475	7.510	9.731	Positive
Subject 5	5	0.811	48.367	20.927	10.403	17.673	7.415	48.756	11.681	154.352	Positive
	10	0.453	21.798	8.785	5.116	10.491	3.529	37.453	19.808	87.625	Positive
	20	0.187	7.684	3.418	2.144	4.533	1.770	25.517	16.667	45.253	Positive
	30	0.880	3.148	1.537	0.983	2.131	0.918	18.416	13.826	28.013	Positive
	40	n.d.	1.245	0.776	0.516	1.215	0.632	12.117	8.957	16.501	Positive
	50	n.d.	0.764	0.511	0.305	0.583	0.348	8.752	7.012	11.263	Positive
	60	n.d.	0.367	0.203	0.108	0.290	0.176	5.611	4.731	6.755	Positive

[a]LW: Last Wash; n.d.: not detected.

drugs or substances that could interfere with the test. The powdered cocaine hydrochloride was purchased from S.A.L.A.R.S. S.p.A. (Como, Italy). Urine samples from each subject were analysed everyday (for 5 days) during the contamination phase to exclude the possibility of cocaine transcutaneous absorption, and each week (for the entire duration of the study) to exclude drug the use of screened subjects. Each subject could wash their pubic hair following their own normal routine.

Contamination was carried out by subdividing subjects in two experimental groups (five subjects for each group). The study was realised using two different modalities: *in vivo* and *in vitro*.

2.1. In vivo study

Each subject of this group was asked to contaminate his or her own pubic hair *in situ*. Specifically, each subject was invited to apply 10 mg of powdered cocaine hydrochloride to the hands and to rub the hands for two minutes until the cocaine powder particles were dissolved by the sebaceous and sweat layer of the skin on the hands. The subjects were then adequately instructed to contaminate their own pubic hair by hand for 2 min as uniformly as possible in order to contaminate the pubic hair with a small amount of cocaine rubbed on hands. Eight hours after contamination, each subject could follow his or her usual personal hygiene routines; therefore, most of the contamination on the surface of the pubic hair was removed. This procedure of contamination was repeated over the following two days.

The aims of this method were to reproduce a truthful scenario and simultaneously contaminate the public hair as uniformly as possible.

Once the period of contamination was complete after the fourth day, each participant could follow his or her personal hygiene habits. The frequency of washing was variable among

Table 2 *In vitro* contamination followed by 5, 10, 20, 30, 40, 50 and 60 days of exposure and washing: cocaine (ng/mg) in washes and pubic hair.

Subjects	Day of collection	Isopropanol wash 15′	1st PO_4 wash 30′	2nd PO_4 wash 30′	3rd PO_4 wash 30′	4th PO_4 wash 60′	5th PO_4 wash 60′ (LW)	Pubic hair after wash	Pubic hair minus (5 × LW)	Cocaine wash and pubic hair values	Result
Subject 1	5	0.398	25.180	11.517	6.026	8.286	4.776	17.187	−6.693	73.370	Negative
	10	0.262	11.368	6.739	3.840	5.787	3.518	16.372	−1.218	47.886	Negative
	20	0.151	5.371	3.610	2.319	3.193	1.966	15.491	5.661	32.101	Positive
	30	0.103	3.949	2.751	1.624	3.065	1.643	14.514	6.299	27.649	Positive
	40	n.d.	2.668	2.299	1.499	2.767	1.467	14.210	6.875	24.910	Positive
	50	n.d.	2.462	1.501	1.016	1.502	0.845	13.362	9.137	20.688	Positive
	60	n.d.	1.946	1.164	0.915	1.085	0.636	11.589	8.409	17.335	Positive
Subject 2	5	0.473	32.641	11.378	5.118	6.372	4.831	16.384	−7.771	77.197	Negative
	10	0.238	7.782	6.481	4.512	6.973	4.315	17.642	−3.933	47.943	Negative
	20	0.167	5.047	4.570	3.117	5.326	2.875	16.438	2.063	37.540	Positive
	30	0.101	3.648	3.883	2.311	4.381	1.948	14.861	5.121	31.133	Positive
	40	n.d.	3.736	2.496	1.983	3.118	1.643	14.511	6.296	27.487	Positive
	50	n.d.	3.152	2.185	1.497	1.870	1.007	13.721	8.686	23.432	Positive
	60	n.d.	2.673	1.135	0.972	1.801	0.914	11.830	7.260	19.325	Positive
Subject 3	5	0.394	14.316	6.931	2.817	5.311	3.615	14.803	−3.272	48.187	Negative
	10	0.249	6.571	3.915	2.149	3.791	2.308	10.038	−1.502	29.021	Negative
	20	0.136	2.694	1.611	1.037	1.955	1.078	9.516	4.126	18.027	Positive
	30	0.113	2.455	1.483	0.844	1.733	1.182	8.937	3.027	16.747	Positive
	40	n.d.	1.279	1.095	0.736	1.648	1.074	8.994	3.624	14.826	Positive
	50	n.d.	0.931	0.711	0.493	0.878	0.521	7.753	5.148	11.287	Positive
	60	n.d.	0.752	0.497	0.364	0.829	0.462	6.733	4.423	9.637	Positive
Subject 4	5	0.411	31.688	13.577	7.188	9.711	5.214	18.927	−7.143	86.716	Negative
	10	0.280	13.699	8.057	4.614	6.841	4.201	18.855	−2.150	56.547	Negative
	20	0.164	6.672	4.636	2.898	4.086	2.350	18.645	6.895	39.451	Positive
	30	0.101	4.738	3.342	2.051	3.677	1.857	17.308	8.023	33.074	Positive
	40	n.d.	3.451	2.760	1.869	3.285	1.651	16.742	8.487	29.758	Positive
	50	n.d.	3.231	1.922	1.294	1.906	1.063	16.054	10.739	25.470	Positive
	60	n.d.	2.494	1.458	1.176	1.271	0.766	14.147	10.317	21.312	Positive
Subject 5	5	0.387	28.927	13.748	7.611	13.116	5.960	22.144	−7.656	91.893	Negative
	10	0.276	17.648	8.226	3.694	5.983	4.149	20.642	−0.103	60.618	Negative
	20	0.169	8.633	3.915	2.237	2.981	1.918	20.722	11.132	40.575	Positive
	30	0.128	5.836	3.215	1.662	3.327	1.885	19.241	9.816	35.294	Positive
	40	n.d.	3.317	1.982	1.744	3.270	1.744	17.409	8.689	29.466	Positive
	50	n.d.	2.811	1.616	1.095	2.149	1.260	17.949	11.649	26.880	Positive
	60	n.d.	2.113	1.168	0.835	1.237	0.749	16.422	12.677	22.524	Positive

[a]LW: Last Wash; n.d.: not detected.

the subjects. Moreover, further washings were added to the personal hygiene habits during the last month of experimentation as a consequence of sea bathing because it was the beginning of the summer season.

To verify the penetration of the drug, the pubic hair of each participant was cut. After the first day of contamination, 50 mg of each sample were collected and analysed at the following times: on the 5th day, 10th day, 20th day, 30th day, 40th day, 50th day and 60th day.

2.2. In vitro study

Each subject of this group was asked to cut all of his or her own pubic hair as close as possible to the skin. The staff of our laboratory then contaminated the samples of pubic hair of this group after collection by rubbing 10 mg of powdered cocaine hydrochloride on their hands for two minutes until the cocaine powder particles were no longer visible. Each sample was then contaminated by hand for two minutes as uniformly as possible. Eight hours after contamination, each sample was washed using liquid soap for personal hygiene. This type of contamination was also repeated over the following two days.

After the contamination period, the samples of pubic hair were washed every day with liquid soap (for 2 min) and rinsed with water (for others 2 min); every sample of pubic hair was dried with blotting paper and then maintained at room temperature, wrapped in blotting paper and stored until the next washing.

After the first day of contamination, 50 mg samples were collected and analysed from each sample of pubic hair of each subject at the following times: on the 5th day, 10th day, 20th day, 30th day,40th day, 50th day and 60th day.

2.3. Decontamination and extraction procedures

Both the *in vivo* and *in vitro* samples were decontaminated using the procedure previously described by Cairns.[21] Each pubic hair sample was washed with 3 ml of dry isopropanol for 15 min in a vial. The vials were shaken vigorously at

Table 3 *In vivo* contamination followed by 5, 10, 20, 30, 40, 50 and 60 days of exposure and washing: benzoylecgonine (BE) (ng/mg) in washes and pubic hair.

Subjects	Day of collection	Isopropanol wash 15′	1st PO_4 wash 30′	2nd PO_4 wash 30′	3rd PO_4 wash 30′	4th PO_4 wash 60′	5th PO_4 wash 60′ (LW)	Pubic hair after wash	Pubic hair minus (5 × LW)	BE wash and pubic hair values
Subject 1	5	n.d.	1.217	0.547	0.471	0.594	0.383	4.604	2.689	7.816
	10	n.d.	0.685	0.356	0.305	0.462	0.311	5.378	3.823	7.497
	20	n.d.	0.503	0.291	0.217	0.288	0.242	5.552	4.342	7.093
	30	n.d.	0.380	0.202	0.145	0.265	0.167	4.621	3.786	5.780
	40	n.d.	0.282	0.141	0.110	0.184	0.126	4.188	3.558	5.031
	50	n.d.	0.137	0.091	0.075	0.120	0.087	4.046	3.611	4.556
	60	n.d.	0.102	0.071	0.055	0.093	0.069	3.582	3.237	3.972
Subject 2	5	n.d.	1.387	0.774	0.548	0.936	0.573	5.933	3.068	10.151
	10	n.d.	0.761	0.402	0.359	0.751	0.446	7.741	5.511	10.460
	20	n.d.	0.473	0.335	0.271	0.527	0.388	7.556	5.616	9.550
	30	n.d.	0.394	0.227	0.172	0.389	0.236	5.823	4.643	7.241
	40	n.d.	0.264	0.153	0.118	0.235	0.183	3.980	3.065	4.933
	50	n.d.	0.158	0.136	0.085	0.182	0.128	3.522	2.882	4.211
	60	n.d.	0.117	0.097	0.063	0.125	0.102	2.830	2.320	3.334
Subject 3	5	n.d.	0.895	0.415	0.326	0.433	0.258	3.106	1.816	5.433
	10	n.d.	0.571	0.283	0.211	0.287	0.203	3.477	2.462	5.032
	20	n.d.	0.408	0.216	0.183	0.255	0.188	3.854	2.914	5.104
	30	n.d.	0.380	0.193	0.175	0.259	0.175	4.103	3.228	5.285
	40	n.d.	0.291	0.160	0.129	0.207	0.158	3.117	2.327	4.062
	50	n.d.	0.170	0.115	0.103	0.185	0.132	2.936	2.276	3.641
	60	n.d.	0.137	0.085	0.057	0.139	0.095	2.711	2.236	3.224
Subject 4	5	n.d.	1.343	0.602	0.550	0.681	0.443	5.850	3.635	9.469
	10	n.d.	0.714	0.332	0.294	0.511	0.350	6.122	4.372	8.323
	20	n.d.	0.404	0.257	0.209	0.367	0.245	6.350	5.125	7.832
	30	n.d.	0.352	0.173	0.141	0.279	0.159	4.771	3.976	5.875
	40	n.d.	0.265	0.133	0.101	0.185	0.132	4.075	3.415	4.891
	50	n.d.	0.137	0.090	0.081	0.109	0.085	3.891	3.466	4.393
	60	n.d.	0.097	0.068	0.054	0.093	0.067	3.530	3.195	3.909
Subject 5	5	n.d.	1.083	0.594	0.402	0.580	0.357	2.381	0.596	5.397
	10	n.d.	0.774	0.472	0.371	0.422	0.318	3.785	2.195	6.142
	20	n.d.	0.806	0.389	0.231	0.417	0.283	3.840	2.425	5.966
	30	n.d.	0.426	0.280	0.178	0.320	0.217	4.416	3.331	5.837
	40	n.d.	0.317	0.171	0.139	0.194	0.135	4.301	3.626	5.257
	50	n.d.	0.175	0.133	0.085	0.158	0.141	3.975	3.270	4.667
	60	n.d.	0.126	0.083	0.068	0.132	0.086	3.209	2.779	3.704

[a]LW: Last Wash; n.d.: not detected.

37 °C for 15 min; and the isopropanol was subsequently collected and analysed by gas chromatography coupled with a mass spectrometry detector (GC/MS). Three millilitres of 0.01 M phosphate buffer/0.01% BSA at pH 6 was then added to the pubic hair samples remaining in the vials and shaken vigorously for 30 min at 37 °C; the buffer was collected in another vial to be analysed. This wash of 30 min was repeated twice more, followed by two washes of 60 min using the same conditions.

Cocaine-d3 and d3-benzoylecgonine were added to the washes as internal standards and extracted using Bond Elut Certify™ columns on a Vac-Elut SPS 24™ vacuum manifold following the procedure recommended by the manufacturer.

2.4. Pubic hair analysis

After decontamination, each sample was finely cut, spiked with 1 ng/mg of cocaine-d3 and d3-benzoylecgonine as internal standards and incubated at 45 °C for 24 h in 1 ml of HCl 0.1 N.

The aqueous acid solutions were collected and added to 1 ml of phosphate buffer 0.1 M (pH 7), adjusted to pH 6, and then extracted using Bond Elut Certify™ columns. The samples were derivatised and analysed using GC/MS. Each extract was derivatised with 50 ml N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) + 1% trimethylchlorosilane (TMCS), sealed and heated at 70 °C for 30 min.

The analysis was performed with an Agilent Technologies (AT) 6890N gas chromatograph coupled to an AT 5973 *Inert* Mass Selective Detector (MSD) equipped with AT 7683 *Series* autosampler, operating in the EI mode (70 eV) with SIM monitoring.

An HP5 cross-linked fused-silica capillary column (30 m, 0.25 mm i.d.) with a 0.25 μm film thickness (Agilent) was linked to the Mass Selective Detector (MSD) through a direct capillary interface.

Table 4 *In vitro* contamination followed by 5, 10, 20, 30, 40, 50 and 60 days of exposure and washing: benzoylecgonine (BE) (ng/mg) in washes and pubic hair.

Subjects	Day of collection	Isopropanol wash 15′	1st PO_4 wash 30′	2nd PO_4 wash 30′	3rd PO_4 wash 30′	4th PO_4 wash 60′	5th PO_4 wash 60′ (LW)	Pubic hair after wash	Pubic hair minus (5 × LW)	BE wash and pubic hair values
Subject 1	5	n.d.	0.140	0.099	0.068	0.070	0.044	0.321	0.101	0.742
	10	n.d.	0.148	0.106	0.073	0.104	0.065	0.383	0.058	0.879
	20	n.d.	0.146	0.101	0.083	0.129	0.079	0.528	0.133	1.066
	30	n.d.	0.137	0.108	0.085	0.149	0.095	0.678	0.203	1.252
	40	n.d.	0.146	0.120	0.087	0.189	0.111	0.772	0.217	1.425
	50	n.d.	0.083	0.070	0.057	0.069	0.079	0.789	0.394	1.147
	60	n.d.	0.067	0.051	n.d.	0.092	0.054	0.698	0.428	0.962
Subject 2	5	n.d.	0.174	0.118	0.068	0.125	0.073	0.437	0.072	0.995
	10	n.d.	0.193	0.146	0.083	0.133	0.058	0.481	0.191	1.094
	20	n.d.	0.234	0.154	0.105	0.187	0.094	0.759	0.289	1.533
	30	n.d.	0.218	0.132	0.091	0.205	0.107	1.022	0.487	1.775
	40	n.d.	0.170	0.118	0.078	0.253	0.135	1.217	0.542	1.971
	50	n.d.	0.105	0.089	0.054	0.176	0.113	1.093	0.528	1.630
	60	n.d.	0.096	0.064	0.050	0.152	0.072	0.825	0.465	1.259
Subject 3	5	n.d.	0.087	0.058	0.040	0.075	0.051	0.296	0.041	0.607
	10	n.d.	0.108	0.063	0.048	0.091	0.075	0.378	0.003	0.763
	20	n.d.	0.127	0.053	0.056	0.103	0.081	0.451	0.046	0.871
	30	n.d.	0.088	0.061	0.051	0.116	0.090	0.513	0.063	0.919
	40	n.d.	0.092	0.070	0.066	0.172	0.109	0.625	0.080	1.134
	50	n.d.	0.058	0.051	n.d.	0.089	0.075	0.512	0.137	0.785
	60	n.d.	0.050	n.d.	n.d.	0.073	0.067	0.473	0.138	0.663
Subject 4	5	n.d.	0.125	0.085	0.069	0.058	0.040	0.294	0.094	0.671
	10	n.d.	0.138	0.093	0.070	0.091	0.057	0.371	0.086	0.820
	20	n.d.	0.142	0.103	0.085	0.142	0.080	0.646	0.246	1.198
	30	n.d.	0.155	0.111	0.087	0.156	0.099	0.785	0.290	1.393
	40	n.d.	0.154	0.122	0.094	0.222	0.128	0.905	0.265	1.625
	50	n.d.	0.092	0.080	0.053	0.061	0.082	0.921	0.511	1.289
	60	n.d.	0.071	0.063	0.046	0.117	0.094	0.791	0.321	1.182
Subject 5	5	n.d.	0.192	0.143	0.096	0.105	0.073	0.392	0.027	1.001
	10	n.d.	0.163	0.127	0.078	0.133	0.084	0.455	0.035	1.040
	20	n.d.	0.151	0.093	0.075	0.126	0.078	0.489	0.099	1.012
	30	n.d.	0.122	0.104	0.062	0.115	0.061	0.520	0.215	0.984
	40	n.d.	0.128	0.094	0.071	0.128	0.075	0.535	0.160	1.031
	50	n.d.	0.069	0.054	0.048	0.105	0.061	0.487	0.182	0.824
	60	n.d.	0.071	n.d.	n.d.	0.085	0.054	0.431	0.161	0.641

[a]LW: Last Wash; n.d.: not detected.

The injector and interface temperatures were 250 °C and 280 °C, respectively. The oven temperature was maintained at 100 °C for 2.25 min, then programmed to 200 °C at 40 °C/min, to 260 °C at 5 °C/min, to 290 °C at 20 °C/min, and maintained at 290 °C for 2 min. The source temperature was 230 °C; the quadrupole temperature was 150 °C; the carrier gas was helium with a flow of 1.2 ml/min.

Analysis was performed by monitoring the following ions: *m/z* 182, 303 (cocaine); *m/z* 240, 361 (benzoylecgonine TMS); *m/z* 185, 306 (internal standard cocaine-d3); and *m/z* 243, 364 (internal standard benzoylecgonine-d3).

3. Results

Urinalysis for cocaine and its metabolites was negative for each subject for the entire duration of the present study.

After analyses of the wash solution and pubic hair extracts, the wash criterion was applied[22] in order to determine its effectiveness in identifying the contaminated samples.

Specifically, the wash criterion was determined by multiplying the amount of drug per mg of pubic hair in the last wash (LW) by 5 and subtracting the result from the amount of drug per mg of pubic hair in the pubic hair extract. As assessed by Cairns,[22] if the result was less than the cut-off for cocaine (0.5 ng/mg hair), the hair should be considered contaminated.

The data from our studies are shown in Tables 1–4. The decontamination procedure could not remove benzoylecgonine from pubic hair, as shown in Tables 3 and 4.

All subjects yielded false positives in the *in vivo* experiment (Table 1). The *in vitro* samples only remained negative until the 10th day (Table 2).

4. Discussion

The drug analysis of hair has expanded in forensic and clinical toxicology, such as in illegal drug abuse cases, *post-mortem* cases, drug-facilitated crimes, workplace drug testing and drug monitoring during treatment programs, etc.[25,26] When scalp hair is not available, axillary and/or pubic hair is analysed. The aim of this study was to verify the ability of external pubic hair contamination to influence the discrimination between active users and false positive subjects. The analysis was performed on *in vivo* and *in vitro* samples.

In *in vivo* contamination, both the hair and skin are soiled by sebum; the hair not touched directly by the drug becomes contaminated by means of sebum diffusion. The sweat is mixed with fatty sebum, especially in hairy zones. As reported in the literature,[27] sebaceous glands could represent a route of penetration for the substances applied externally on the hair. Because cocaine is both water-soluble (as a hydrochloride) and fat-soluble (as a free base), it is soluble both in aqueous (such as sweat) and lipid media (such as sebum) and can penetrate into the hair matrix.

The data obtained from *in vitro* experiments are less realistic than those obtained *in vivo,* the multiple variables due to the individual pharmacokinetic characteristics cannot be verified. Our studies indicate that the data obtained from *in vitro* samples are more uniform.

Although many authors[19,27–30] have used several experimental models of hair contamination (*i.e.,* soaking in aqueous drug solutions, soaking in organic solvent drug solutions and exposing to drugs vapour or smoke), we have contaminated the pubic hair by rubbing drugs on the samples by hand. This choice was made because the other contamination procedures, such as soaking the hair in an aqueous drug solution or treating the hair with drug vapours, do not reproduce a real scenario.

5. Conclusions

We believe that the contamination method represents a fundamental matter. Exogenous contamination occurs without a planned scientific method; in real life, hair contamination occurs randomly, and the drug does not uniformly contaminate hair.

Our *in vivo* pubic hair contamination resulted in higher cocaine concentrations than our *in vitro* procedure, even if the same amount of cocaine and the same contamination method were employed. This observation suggests that sweat and sebum might represent the carriers by which a drug penetrates into the hair matrix, as reported above.

Moreover, we observed that organic solvents can remove the sebum and the drug present only on the external surface of the hair; when the drug crossed this first layer of hair, it could be removed by aqueous solutions, such as phosphate buffer. However, aqueous solutions can only partly remove drugs that deeply penetrate the hair matrix.

Our findings seem to be confirmed by the fact that our *in vitro* decontamination values are similar to those obtained by Cairns et al.[22] Indeed, isopropanol washing is less effective than PO_4 buffer washing to remove the drug from hair.

The data obtained from our *in vitro* study indicated that all samples tested negative until the 10th day, as shown in Table 2. Therefore, all *in vitro* samples tested on the 20th day and thereafter yielded false positives.

Our study was performed for a longer period (60 days) than the previous one.[22] Washing most likely removed most of the external contamination when the drug penetrated only the first layer over a short period (until the 10th day), whereas these washings are not effective in removing the drug that had deeply penetrated the hair matrix after the 20th day. In fact, all *in vitro* samples yielded false positive tests twenty days after contamination.

Despite the adoption of the decontamination procedure and wash criterion, all of our *in vivo* samples produced false positive tests (Table 1). Initially, the *in vivo* cocaine concentrations were higher than those detected *in vitro*, likely due to greater drug penetration related to the sebum medium; subsequently, the *in vivo* cocaine concentrations decreased more rapidly than the *in vitro* concentrations. This difference could be explained by the normal hygiene habits of the subjects and by the increased frequency of washings (the study was carried out in spring and summer).

We also observed that benzoylecgonine was present in the hair matrix after Cairns' washing procedure (Table 3 and 4). These data show that this decontamination method cannot remove this cocaine metabolite.

The detection of benzoylecgonine suggests a progressive penetration of cocaine into the hair, where the parent drug is partially transformed into its metabolite. This issue will be the aim of a subsequent manuscript.

A review of the current literature suggests that decontamination procedures are highly variable and not consistently performed by forensic laboratories.[31–33] The debate about the possibility of distinguishing between consumption and external contamination is not new[28,34,35] and remains open. New decision criteria may be necessary to adequately and reliably identify contamination.

In fact, the external contamination of pubic hair with cocaine can lead to the deposition of cocaine at concentrations that cannot be discriminated from the incorporation after drug use, even by extensive decontamination and washing procedures of the specimen.

Funding

None.

Conflict of interest

None declared.

Ethical approval

Necessary ethical approval was obtained from the institute ethics committee.

References

1. Kim J, Ji D, Kang S, Park M, Yang W, Kim E, et al. Simultaneous determination of 18 abused opioids and metabolites in human hair using LC–MS/MS and illegal opioids abuse proven by hair analysis. *J Pharm Biomed Anal* 2013;**89C**:99–105.
2. Cooper GA, Kronstrand R, Kintz P, Society of Hair Testing. Society of hair testing guidelines for drug testing in hair. *Forensic Sci Int* 2012;**218**:20–4.
3. Aleksa K, Walasek P, Fulga N, Kapur B, Gareri J, Koren G. Simultaneous detection of seventeen drugs of abuse and metabolites in hair using solid phase micro extraction (SPME) with GC/MS. *Forensic Sci Int* 2012;**218**:31–6.
4. Vignali C, Stramesi C, Vecchio M, Groppi A. Hair testing and self-report of cocaine use. *Forensic Sci Int* 2012;**215**:77–80.
5. Favretto D, Vogliardi S, Stocchero G, Nalesso A, Tucci M, Ferrara SD. High performance liquid chromatography-high resolution mass spectrometry and micropulverized extraction for the quantification of amphetamines, cocaine, opioids, benzodiazepines, antidepressants and hallucinogens in 2.5 mg hair samples. *J Chromatogr A* 2011;**1218**:6583–95.
6. Coulter C, Tuyay J, Taruc M, Moore C. Semi-quantitative analysis of drugs of abuse, including tetrahydrocannabinol in hair using aqueous extraction and immunoassay. *Forensic Sci Int* 2010;**196**:70–3.
7. Baumgartner MR, Guglielmello R, Fanger M, Kraemer T. Analysis of drugs of abuse in hair: evaluation of the immunochemical method VMA-T vs. LC–MS/MS or GC–MS. *Forensic Sci Int* 2012;**215**:56–9.
8. Spiehler V. Hair analysis by immunological methods from the beginning to 2000. *Forensic Sci Int* 2000;**107**:249–59.
9. Ricossa MC, Bernini M, De Ferrari F. Hair analysis for driving licence in cocaine and heroin users. An epidemiological study. *Forensic Sci Int* 2000;**107**:301–8.
10. Montagna M, Stramesi C, Vignali C, Groppi A, Polettini A. Simultaneous hair testing for opiates, cocaine, and metabolites by GC–MS: a survey of applicants for driving licences with a history of drug use. *Forensic Sci Int* 2000;**107**:157–67.
11. Kronstrand R, Grundin R, Jonsson J. Incidence of opiates, amphetamines, and cocaine in hair and blood in fatal cases of heroin overdose. *Forensic Sci Int* 1998;**92**:29–38.
12. Uhl M. Determination of drugs in hair using GC/MS/MS. *Forensic Sci Int* 1997;**84**:281–94.
13. Pepin G, Gaillard Y. Concordance between self-reported drug use and findings in hair about cocaine and heroin. *Forensic Sci Int* 1997;**84**:37–41.
14. Mangin P, Kintz P. Variability of opiates concentrations in human hair according to their anatomical origin: head, axillary and pubic regions. *Forensic Sci Int* 1993;**63**:77–83.
15. DuPont RL, Baumgartner WA. Drug testing by urine and hair analysis: complementary features and scientific issues. *For Sci Int* 1995;**70**:63–76.
16. Bermejo Barrera AM, Strano-Rossi S. Hair and urine analysis: relative distribution of drugs and their metabolites. *Forensic Sci Int* 1995;**70**:203–10.
17. Romano G, Barbera N, Lombardo I. Hair testing for drugs of abuse. Evaluation of external cocaine contamination and risk of false positives. *Forensic Sci Int* 2001;**123**:119–29.
18. Romano G, Barbera N, Spadaro G, Valenti V. Determination of drugs of abuse in hair: evaluation of external heroin contamination and risk of false positives. *Forensic Sci Int* 2003;**131**:98–102.
19. Wang WL, Cone EJ. Testing human hair for drugs of abuse. IV. Environmental cocaine contamination and washing effects. *Forensic Sci Int* 1995;**70**:39–51.
20. Blank DL, Kidwell DA. Decontamination procedures for drugs of abuse in hair: are they sufficient? *Forensic Sci Int* 1995;**70**:13–38.
21. Ropero-Miller JD, Huestis MA, Stout PR. Cocaine analytes in human hair: evaluation of concentration ratios in different cocaine sources, drug-user populations and surface-contaminated specimens. *J Anal Toxicol* 2012;**36**:390–8.
22. Cairns T, Hill V, Schaffer M, Thistle W. Removing and identifying drug contamination in the analysis of human hair. *Forensic Sci Int* 2004;**145**:97–108.
23. Lee S, Han E, In S, Choi H, Chung H, Chung KH. Analysis of pubic hair as an alternative specimen to scalp hair: a contamination issue. *Forensic Sci Int* 2011;**206**:19–21.
24. Offidani C, Strano-Rossi S, Chiarotti M. Drug distribution in the head, axillary and pubic hair of chronic addicts. *Forensic Sci Int* 1993;**63**:105–8.
25. Pragst F, Balikova MA. State of the art in hair analysis for detection of drug and alcohol abuse. *Clin Chim Acta* 2006;**370**:17–49.
26. Paterson S, Cordero R, Stearns E. Chronic drug use confirmed by hair analysis: its role in understanding both the medical cause of death and the circumstances surrounding the death. *J Forensic Leg Med* 2009;**16**:143–7.
27. Henderson GL. Mechanism of drug incorporation into hair. *Forensic Sci Int* 1993;**63**:19–29.
28. Blank DL, Kidwell DA. External contamination of hair by cocaine: an issue in forensic interpretation. *Forensic Sci Int* 1993;**63**:145–56.
29. Kidwell DA, Blank DL. Hair analysis: techniques and potential problems. In: Sunshine I, editor. *Recent developments in therapeutic drug monitoring and clinical toxicology*. New York: Marcel Dekker; 1992. p. 555–63.
30. Kidwell DA, Blank DL. Comments on the paper by Baumgartner WA and Hill VA: sample preparation techniques. *Forensic Sci Int* 1993;**63**:137–43.
31. Ropero-Miller JD, Stout PR. Analysis of cocaine analytes in human hair: evaluation of concentration ratios in different hair types, cocaine sources, drug-user populations, and surface-contaminated specimens. NIJ Final Report. Document No.: 22553;

2009 Final Report. Available from: http://www.ncjrs.gov/pdffiles1/nij/grants/225531.pdf (March 2012).
32. Paterson S, Lee S, Cordero R. Analysis of hair after contamination with blood containing cocaine and blood containing benzoylecgonine. *Forensic Sci Int* 2010;**194**:94–6.
33. Pragst F, Sachs H, Kintz P. Hair analysis for cocaine continues to be a valuable tool in forensic and clinical toxicology. *J Anal Toxicol* 2010;**34**:354–5.
34. Baumgartner WA, Hill VA. Sample preparation techniques. *Forensic Sci Int* 1993;**63**:121–35.
35. LeBeau MA, Montgomery MA. Considerations on the utility of hair analysis for cocaine. *J Anal Toxicol* 2009;**33**:343–4.