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# Noninvasive Detection of Cocaine and Heroin Use with Single Fingerprints: Determination of an Environmental Cutoff

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**BACKGROUND:** Recent publications have explored the possibility of using fingerprints to confirm drug use, but none has yet dealt with environmental contamination from fingertips. Here we explored the possibility of establishing an environmental cutoff for drug testing from a single fingerprint.

**METHODS:** Fingerprint samples (n = 100) were collected from the hands of 50 nondrug users before and after handwashing to establish separate environmental cutoff values and testing protocols for cocaine, benzoylecgonine, heroin, and 6-monoacetylmorphine. The cutoff was challenged by testing the fingerprints of drug-free volunteers after shaking hands with drug users. Fingerprints from patients who testified to taking cocaine (n = 32) and heroin (n = 24) were also collected and analyzed.

**RESULTS:** A different cutoff value needed to be applied, depending on whether the fingerprints were collected as presented or after handwashing. Applying these cutoffs gave a 0% false-positive rate from the drug-free volunteers. After application of the cutoff, the detection rate (compared to patient testimony) for washed hands of patients was 87.5% for cocaine use and 100% for heroin use.

**CONCLUSIONS:** Fingerprints show enhanced levels of cocaine, heroin, and their respective metabolites in patients who testified to taking the substances, compared with the population of naïve drug users surveyed, and a cutoff (decision level) can be established. The cutoff is robust enough to account for small increases in analyte observed after secondary transfer.

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The possibility of drug testing from a fingerprint has become the subject of many recent research articles, due to the ease and noninvasive nature of sample collection,

as well as the fact that the donor's identity is embedded within the ridge detail of the fingerprint itself (1, 2). This provides, in prospect, the possibility of rapidly and noninvasively carrying out drug testing in a way that is difficult to falsify. Several methods have been proposed for detection of drugs in fingerprints, mostly focused on contact residues or standards (3–9). A few reports have dealt with excreted drug metabolites (10–12) by use of direct or surface mass spectrometry approaches. While these approaches are attractive from the point of view of a fast turnaround (in some cases <2 min per sample), their quantitative capabilities are still limited. In contrast, LC-MS is the technique of choice among toxicologists for drug testing in other matrices owing to its superior selectivity and quantitative power, afforded by the chromatographic separation of analytes before mass spectrometric analysis (13–15). Analysis of fingerprint drug residues by LC-MS has been demonstrated previously (16–18). The limitation of LC-MS for fingerprint residue analysis is that the fingerprint must first be extracted from the deposition substrate, which reduces sample throughput compared with direct mass spectrometry methods. Nonetheless, attempts have been made to explore the detection window of both lorazepam and caffeine in fingerprints (16, 17) and to relate the fingerprint level of caffeine to a blood or oral fluid sample (19).

Despite the interest in testing for drugs from a fingerprint, to our knowledge, no studies have explored the robustness of fingerprint testing itself. Cocaine is an especially common environmental contaminant (20), and this deserves attention before fingerprints could be considered a credible testing matrix. In hair analysis, cutoff levels (21, 22) are used to ensure that environmental exposure can be eliminated as a possible source, but this has never been considered for a fingerprint test, probably because fingerprint testing is far less mature.

Here we report on a new LC-MS protocol that determines the relative mass of heroin, cocaine, and the

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respective metabolites, 6-monoacetylmorphine and benzoylecgonine, in fingerprint samples. Benzoylecgonine rather than ecgonine methyl ester was monitored owing to its longer half-life in urine and for compatibility with current drug testing regimes (23, 24). The method has been applied to the fingerprints ( $n = 99$ ) from 50 individuals who testified to be nondrug users to establish an environmental cutoff value. The fingerprint samples of 13 cocaine users and 12 heroin users were then measured against these cutoffs to determine drug use over the environmental level. The cutoff was challenged by testing nondrug users after shaking hands with drug users.

## Materials and Methods

### SAMPLE COLLECTION

A favorable ethical opinion for collection and analysis of samples was received from the National Research Ethics Service (NRES-REC reference: 14/LO/0346).

Fingerprints were collected on  $2 \times 2$ -cm squares of Whatman 1-Chr-grade chromatography paper, with a single fingerprint collected per sample. Kitchen scales (Sainsbury's Color) were used to measure the pressure applied during collection (800–1200 g for 10 s). Fingerprint samples from the right thumb and right index finger were collected (a) as presented and (b) after handwashing from 50 participants who testified not to be drug users.

Fingerprints were collected from individuals seeking treatment at drug rehabilitation clinics who testified to taking either cocaine ( $n = 13$ ) or heroin ( $n = 12$ ) in the past 24 h. A fingerprint from each finger of the right hand ( $n = 5$ ) was collected as described above. To investigate different sampling strategies, 8 of the participants were instructed to wash their hands thoroughly with soap and water followed by wearing nitrile gloves for 10 min to induce sweating, followed by removal of the gloves and finally depositing fingerprint samples.

Corresponding oral fluid samples were collected with a Quantisal<sup>TM</sup> (Alere<sup>TM</sup>) collection device. Oral fluid samples were analyzed at Claritest. Claritest screening uses immunoassay testing followed by LC-MS/MS quantification if screening is positive.

To test the possibility of secondary transfer of parent drug or metabolites, fingerprints ( $n = 5$ , right hand) were collected from drug-free volunteers. Hands were shaken with a drug user for approximately 2 s. Fingerprints ( $n = 5$ , right hand) were then collected from the drug-free volunteers after contact with the drug user.

### MATERIALS

Drug standards (cocaine, benzoylecgonine, heroin, 6-monoacetylmorphine, cocaine- $d_3$ , benzoylecgonine- $d_3$ , heroin- $d_5$ , and 6-monoacetylmorphine- $d_3$ ) were prepared from certified reference materials (Cerilliant). Optima LC-MS grade solvents [methanol, dichloromethane, ace-

tonitrile (ACN),<sup>5</sup> and water] were used to prepare all solutions and solvent mixtures (Fischer Scientific). Formic acid (Fischer Scientific) was added to the mobile phase at 0.1% v/v. Artificial eccrine perspiration was purchased from Pickering Laboratories.

### SAMPLE EXTRACTION AND PREPARATION

The paper samples were placed in a 2-mL Eppendorf microcentrifuge tube, following which the extraction solution (1.5 mL of 10% dichloromethane in methanol) was added. The tube was then centrifuged for 2 min (at 9500g centrifugal force). The paper was removed from the microcentrifuge tube and discarded. The solvent extract was evaporated to dryness under a stream of nitrogen at room temperature (20 °C) and reconstituted in 100- $\mu$ L mobile phase solution [50 ng/mL cocaine- $d_3$ , benzoylecgonine- $d_3$ , heroin- $d_5$ , and 6-monoacetylmorphine- $d_3$  in 5% (v/v) ACN in water + 0.1% formic acid] before being vortex-mixed and transferred to a 300- $\mu$ L glass microinsert vial, with 5  $\mu$ L being injected onto the LC-MS/MS system.

Chromatographic separation was performed on a Thermo Scientific<sup>TM</sup> Ultimate3000 UHPLC system equipped with a binary solvent manager, column manager, and autosampler. Separation was performed on a Kinetex XB-C<sub>18</sub> column (100  $\times$  2.1 mm, 5  $\mu$ m) operated at 30 °C at a flow rate of 0.25 mL/min. Gradient analysis was performed with an initial mobile phase comprising 95% water (0.1% formic acid) and 5% ACN (0.1% formic acid) increased to 80% ACN (0.1% formic acid) and 20% water (0.1% formic acid) over 2 min and kept constant for 0.5 min before returning to the initial mobile phase composition (see Table 1 in the Data Supplement that accompanies the online version of this article at <http://www.clinchem.org/content/vol64/issue6>). The samples were introduced to a Thermo Orbitrap Q-Exactive Plus mass spectrometer by the standard electrospray ionization interface with a capillary temperature of 320 °C and spray voltage of 3 kV (see Table 2 in the online Data Supplement). Positive mass spectra were acquired in full scan mode within a range of  $m/z$  50–500 at a mass resolution of 70 000 at  $m/z$  200.

### METHOD VALIDATION

Extracted ion chromatograms for  $m/z$  304.15 (assigned to cocaine),  $m/z$  290.14 (assigned to benzoylecgonine),  $m/z$  370.16 (assigned to heroin), and  $m/z$  328.15 (assigned to 6-monoacetylmorphine) for supplemented (10  $\mu$ L at 600 ng/mL) samples extracted from chromatography paper are shown in Fig. 1 in the online Data Supplement.

<sup>5</sup> Nonstandard abbreviations: ACN, acetonitrile; IS, internal standard; A/IS, analyte to internal standard.

ment. Peak assignment was confirmed by verifying that the corresponding *m/z* peak was within 5 ppm of the expected value and by MS/MS fragmentation. The run time for each sample was 3 min, and the retention times for cocaine, benzoylecgonine, heroin, and 6-monoacetylmorphine were 2.12, 2.00, 1.85, and 2.06 min, respectively.

A linear calibration curve of added drugs in ACN was prepared with a working range of 500 pg/sample to 10 ng/sample. Each calibrator was prepared from a stock solution containing cocaine, benzoylecgonine, heroin, and 6-monoacetylmorphine in ACN at 5000 ng/mL. The stock solution was prepared from the certified reference material of the individual analyte at 1 g/L. Calibrators then were prepared at 50, 100, 200, 400, 600, 800, and 1000 ng/mL in ACN by dilution of the stock solution. Ten microliters of the calibrator were added to the sample substrate (Whatman 1-Chr-grade chromatography paper, 2 × 2 cm) and allowed to dry overnight in the fume hood before being extracted and analyzed as detailed above. Each calibrator was reinjected 5 times. The mean peak area of the 5 repeated measurements was used to calculate the ratio analyte/internal standard (A/IS) and is shown in Fig. 2 in the online Data Supplement. The  $R^2$  value was >0.9995 for all analytes, and the precision was greater than ±1% (n = 25).

To determine limits of detection, 10 μL of solutions of the drug standard at 1, 2, 3, 4, and 5 ng/mL was pipetted onto paper substrates (2 × 2 cm) and allowed to dry in the fume hood. The subsequent sample was then extracted by using the developed extraction and analysis procedure. The limit of detection was determined as the mass of standard below which the analyte signal was no longer observed. The limits of detection, (provided in Table 3 in the online Data Supplement) were 10, 30, 40, and 40 pg for cocaine, benzoylecgonine, heroin, and 6-monoacetylmorphine, respectively.

#### MATRIX EFFECTS

To test the matrix effects, samples were prepared as follows: 10 μL of supplemented drug standard (500 ng/mL) was deposited on a paper substrate in (a) ACN, (b) artificial eccrine sweat, (c) ACN after deposition of a fingerprint from each of 4 participants after washing hands with soap, and (d) ACN after deposition of a fingerprint from each of 4 participants after wiping hands. In each case, extraction was performed as described earlier. Four replicate samples for each of the above sample were prepared and 5 injections of each were performed.

## Results and Discussion

#### MATRIX EFFECTS

Fig. 3 in the online Data Supplement shows the ratio A/IS obtained in each sample type, described in the previous section. For cocaine and benzoylecgonine, no sub-

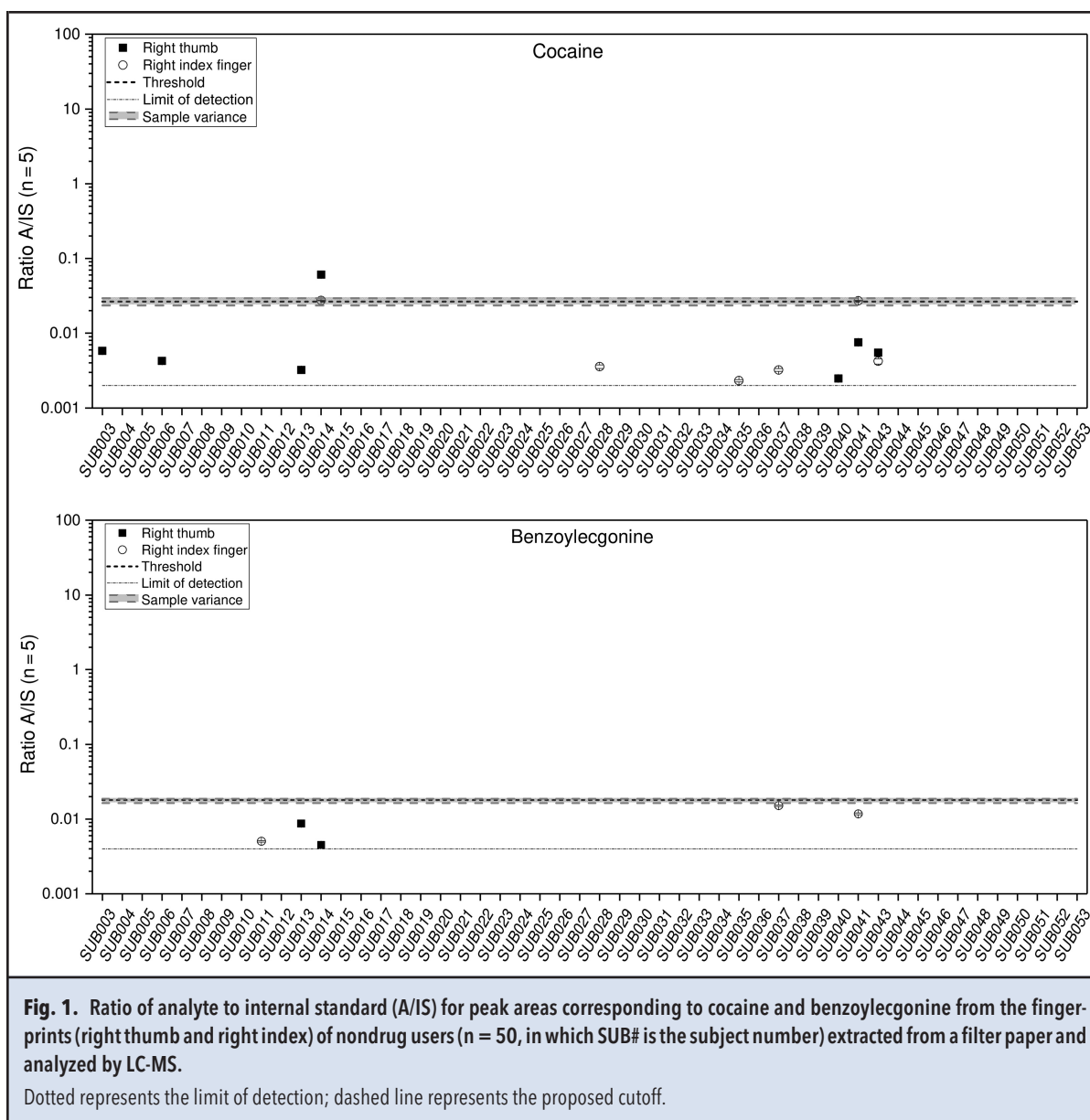
stantial difference was observed between the standards in the presence of ACN only and the standards in the presence of a fingerprint, and therefore no matrix effects were observed. For heroin and 6-monoacetylmorphine, however, the presence of a fingerprint increased the ratio A/IS by 29% and 17%, respectively, showing that the matrix effects needed to be considered for absolute measurements (i.e., mass per fingerprint). However, the close distribution of values (<5% variability) between the 4 participants studied here implied that there was no donor-dependent matrix effect. Additionally, given the small number of participants studied for matrix effects, a more thorough evaluation of participant dependence and fingerprint aging on matrix effects should be completed in the future. The presence of artificial eccrine sweat increased the ratio A/IS by a factor of 3, showing that this standard was not representative of a fingerprint matrix. Therefore, in the absence of a validated standard, we took a previously used approach (16, 18, 19) and opted to not use a matrix-matched calibration curve. Subsequent results are therefore stated in terms of A/IS rather than ng/fingerprint.

#### COCAINE AND BENZOYLECGONINE DETECTION IN FINGERPRINTS FROM THE BACKGROUND POPULATION

Fingerprint samples (right thumb and right index) were taken from 50 participants who testified not to be drug users. These were extracted and analyzed with the LC-MS method described earlier. Fig. 1 displays the ratio A/IS peak areas (5 replicate injections) corresponding to cocaine and benzoylecgonine. The limit of detection of the method (dashed line) shows detection of cocaine in 13 out of the 99 fingerprint samples and that of benzoylecgonine in 5 of the 99 fingerprint samples analyzed using this method. Note that for 1 sample, the LC-MS run failed—SUB016 (right index finger). There was clearly a requirement to impose an environmental cutoff on the data, particularly in the case of cocaine, as the method readily detected cocaine even in the fingerprints of nondrug users. We propose an A/IS cutoff of 0.026 for cocaine and 0.018 for benzoylecgonine on the basis of a 99% confidence limit calculated from the background samples, as indicated by the dotted line in Fig. 1.

#### COCAINE AND BENZOYLECGONINE DETECTION IN FINGERPRINTS FROM INDIVIDUALS SEEKING TREATMENT FOR DRUG DEPENDENCY

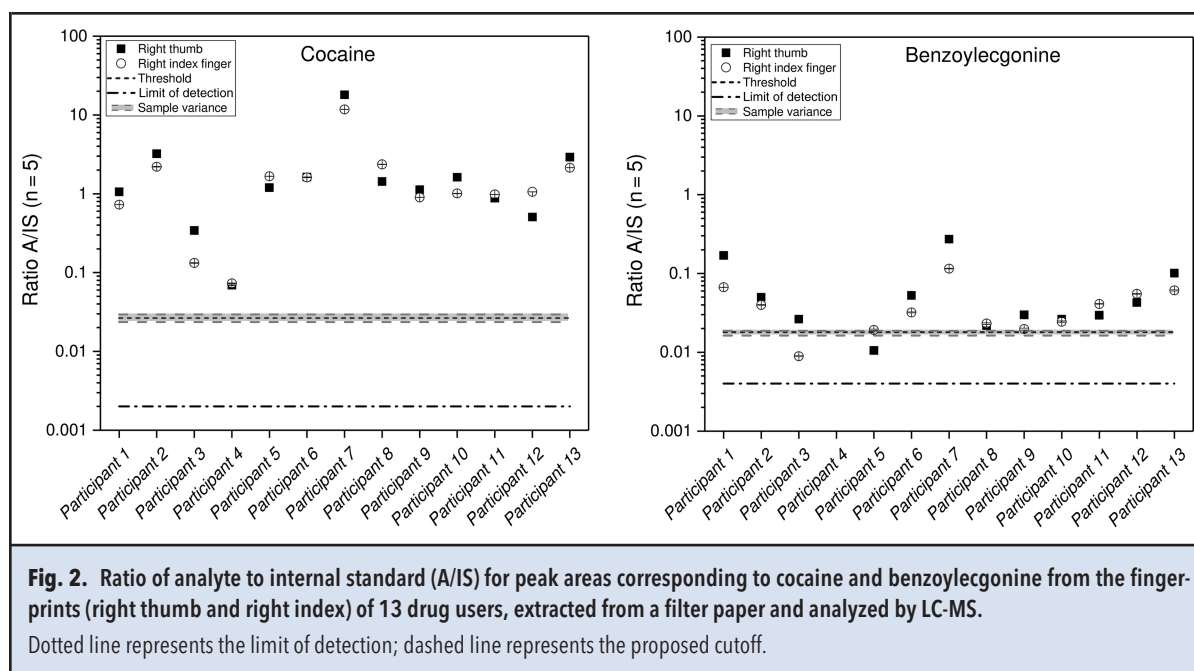
Fingerprint samples (all fingers of the right hand) were taken from 13 participants who testified to taking cocaine in the past 24 h. These samples were extracted and analyzed with the same LC-MS method. Fig. 4 in the online Data Supplement shows the ratio A/IS (5 replicate injections) corresponding to cocaine and benzoylecgonine for all 5 fingerprint samples collected. The data show the considerable variability between the fingerprint samples collected from the same participant, which could



be expected owing to various factors that include the difference in surface area of the different fingers. Fig. 2 displays the same data, but for the same 2 fingers (right thumb and right index) as those presented for the background study. There was, in general, good agreement with patient testimony and the detection of cocaine and benzoylcegonine, with these compounds being detected above the cutoff in 100% and 85% of the fingerprint samples, respectively. There are of course obvious limitations of patient testimony—the patient may not have known what they were taking, may have forgotten when they had taken a substance, or deliberately falsified their information. This could be the case for participant 4,

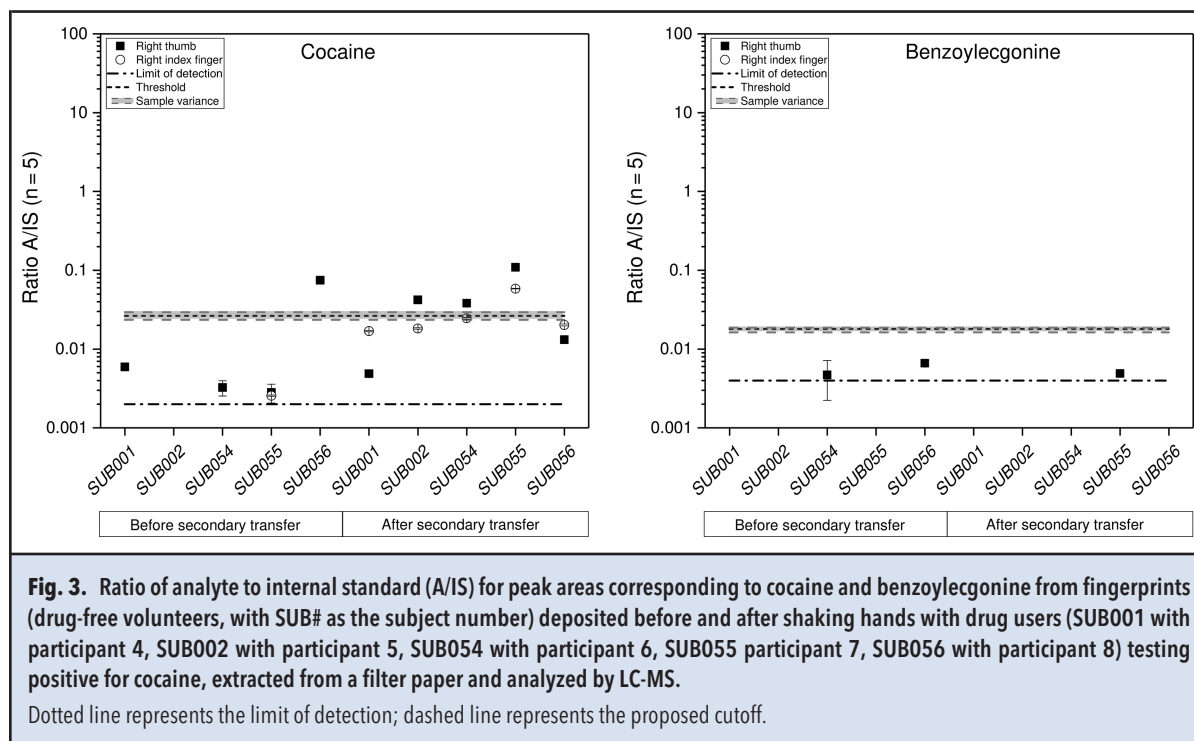
who tested negative in oral fluid and benzoylcegonine in the fingerprint samples. Benzoylcegonine was detected above the proposed cutoff only in 1 out of 2 fingerprints for participants 3 and 5, despite a positive oral fluid test result and cocaine detection in both fingerprints. This therefore shows an inevitable limitation of imposing an environmental cutoff, as benzoylcegonine was above the limit of detection in both fingerprints.

Table 4 in the online Data Supplement compares the oral fluid testing results to the fingerprint test results (based on the presence of a signal above the environmental cutoff). For participants 1 and 13, both cocaine and benzoylcegonine were detected at levels considerably greater



than the environmental cutoff, despite the negative oral fluid test result. Sweat has a longer detection window than oral fluid (25), and therefore, we provide this as an explanation for the discrepancy in fingerprint and oral fluid results observed here. This is consistent with previous observations with paper spray mass spectrometry (12).

The model was challenged by the collection of fingerprints from 5 nondrug users working at the clinic before and then directly after shaking hands with 5 different drug users (Fig. 3). The levels of cocaine exceeded the proposed cutoff for SUB002 (right thumb), SUB054 (right thumb), and SUB055 (right thumb and right in-





**Table 1. Comparison of fingerprint and oral fluid screening for cocaine and benzoylecgonine (BZE) in samples collected after handwashing from individuals seeking treatment for drug dependency.**

Participant	Fingerprint screening results (with LOD as cutoff)		Oral fluid screening results		Patient testimony
	Cocaine	BZE	Cocaine	BZE	
1	2/2	2/2	Negative	Negative	Cocaine, morphine
2	2/2	2/2	64 ng/mL	64 ng/mL	Cocaine, heroin
3	2/2	2/2	Negative	64 ng/mL	Cocaine, heroin
4	2/2	0/2	Negative	Negative	Cocaine
5	2/2	2/2	>64 ng/mL	>64 ng/mL	Cocaine
6	2/2	2/2	>64 ng/mL	>64 ng/mL	Cocaine, heroin
7	2/2	2/2	>64 ng/mL	>64 ng/mL	Cocaine, heroin
8	2/2	2/2	>64 ng/mL	>64 ng/mL	Cocaine, heroin

dex) after shaking hands (Fig. 3). Benzoylecgonine was never observed to exceed the cutoff. This is important given that cocaine can chemically convert to benzoylecgonine (26, 27). This was even true after shaking hands with participant 7, who also had the highest levels of drug present in their fingerprints (Fig. 2). Therefore, if the testing regime requires benzoylecgonine to be present in a fingerprint sample for a positive test result, the test is robust enough that secondary transfer presented here would return a negative result. This would result in a reduced detection rate (of 85%), but no false positives, from the data presented here.

#### EFFECTS OF HANDWASHING ON THE DETECTION OF COCAINE AND BENZOYLECGONINE

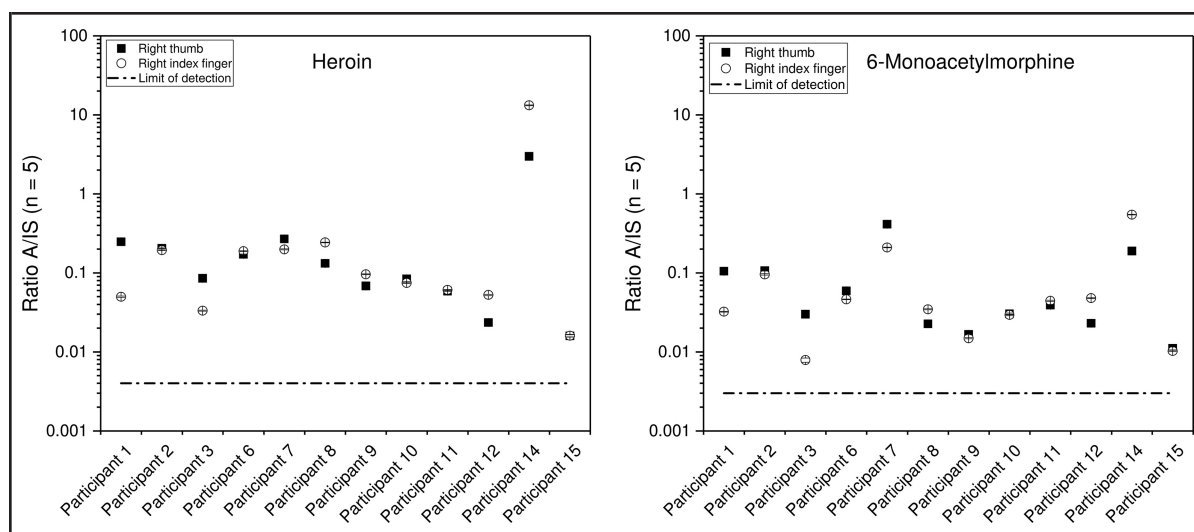
The results showed good agreement with patient testimony for cocaine use but have so far only considered unwashed hands. Discrimination of contact residue from excreted drugs and metabolites would be essential for any quantitative test. Also, any test from a fingerprint must be robust enough to have a good detection rate even if an individual has washed their hands before being tested, otherwise the test would be easily falsified. Therefore, 8 patients who testified to taking cocaine were asked to wash their hands with soap and water after initial deposition of fingerprints. The signals corresponding to cocaine and benzoylecgonine are plotted in Fig. 5 in the online Data Supplement. Cocaine was present in all fingerprints even after handwashing (Table 1), and benzoylecgonine was present in all but in the fingerprints of participant 4, consistent with the data for unwashed hands for this participant. It is noteworthy that for participant 1, whose oral fluid tested negative, both benzoylecgonine and cocaine were still detected in the fingerprints, even after handwashing. Imposing the same environmental cutoff conditions as for the unwashed

hands on these data is of course not appropriate here, because the background population had not washed their hands. Therefore, a cutoff based on the fingerprints of the background population after handwashing was imposed. The limit of detection was used here as the cutoff, because cocaine and benzoylecgonine were detected in only 1 out of 100 and 0 out of 100 fingerprint samples, respectively. The detection rate was therefore 87.5% and the false-positive rate was 0%, based on the detection of benzoylecgonine.

#### HEROIN AND 6-MONOACETYLMORPHINE DETECTION FROM FINGERPRINTS

Fingerprint samples (right thumb and right index) were taken from 50 participants who testified to not being drug users and extracted and analyzed by the same LC-MS method described in previous sections. No signals corresponding to heroin were observed in any sample, and a signal above the limit of detection (0.007 compared with 0.003) was observed for 6-monoacetylmorphine in only 1 of the 99 fingerprints tested.

As detailed in the previous section, fingerprint samples (all fingers of the right hand) were taken from 12 participants who testified to taking heroin in the past 24 h. These samples were extracted and analyzed by the LC-MS method described in the previous sections. Fig. 6 in the online Data Supplement shows the ratio A/IS for peak areas (5 replicate injections per sample) corresponding to heroin and 6-monoacetylmorphine for all 5 fingerprint samples collected. The data again show the considerable variability between the fingerprint samples collected from the same participant. It is perhaps surprising to see the parent drug together with the metabolite in the fingerprint samples, as heroin is quickly metabolized by the body (28). It is possible that the detection of heroin and its metabolite in these fingerprint samples



**Fig. 4.** Ratio of analyte to internal standard (A/IS) for peak areas corresponding to heroin and 6-monoacetylmorphine from the fingerprints (right thumb and right index) of 12 drug users, extracted from a filter paper and analyzed by LC-MS.

Dotted line represents limit of detection.

therefore arose from a combination of drug contact and excretion of metabolites, as the fingerprints were taken without handwashing before deposition. Fig. 4 displays the same data, but for the 2 fingers (right thumb and right index) corresponding to those used for the background study. Here, there was excellent agreement with patient testimony, with heroin and 6-monoacetylmorphine detected at levels above the limit of detection for all samples.

Table 5 in the online Data Supplement compares the oral fluid test results to the fingerprint testing results. The fingerprints of participants 1, 2, 3, and 8 tested positive for both heroin and 6-monoacetylmorphine at levels considerably greater than the background popula-

tion, despite negative oral fluid test results for these participants. This can be explained by either a longer detection window for heroin in sweat than in oral fluid or the prevalence of contact residue on the patients, as fingerprints were deposited without handwashing.

To investigate the potential for contact residue and secondary transfer, fingerprints from researchers working at a clinic session were taken before and directly after shaking hands with 3 different heroin users (participants 6–8). The level of heroin observed exceeded the limit of detection in only 1 case after working at the clinic and after contact with a drug user (see Fig. 7 in the online Data Supplement). It is therefore likely that

**Table 2.** Comparison of fingerprint and oral fluid screening for heroin and 6-monoacetylmorphine (6-MAM) in samples collected from individuals seeking treatment for drug dependency.

Participant #	Fingerprint screening results		Oral fluid screening results		Patient testimony
	Heroin	6-MAM	Morphine	6-MAM	
1	2/2	2/2	Negative	Negative	Cocaine, morphine
2	2/2	2/2	Negative	Negative	Cocaine, heroin
3	1/2	2/2	Negative	Negative	Cocaine, heroin
6	2/2	2/2	Negative	22.6 ng/mL	Cocaine, heroin
7	2/2	2/2	90 ng/mL	Negative	Cocaine, heroin
8	2/2	2/2	Negative	Negative	Cocaine, heroin
14	2/2	2/2	>240 ng/mL	>32 ng/mL	Heroin
15	2/2	2/2	138 ng/mL	32 ng/mL	Heroin



the heroin present in the fingerprints collected from the patient population would have come from a source other than contact with other users or surfaces within the clinic.

#### EFFECTS OF HANDWASHING ON THE DETECTION OF HEROIN AND 6-MONOACETYLMORPHINE

The eight patients who testified to taking heroin were asked to wash their hands with soap and water after initial deposition of fingerprints. The signals corresponding to heroin and 6-monoacetylmorphine are plotted in Fig. 8 in the online Data Supplement. 6-Monoacetylmorphine was present in all fingerprints, and heroin was present in all fingerprints except those from participant 3, even after handwashing, as shown in Table 2. Therefore, a testing protocol that requires 6-monoacetylmorphine to be present in a fingerprint sample for a positive test would give a 100% detection rate with 0% false positives.

In summary, we have developed an LC-MS method for testing both cocaine and heroin use from a single fingerprint. Testing from a fingerprint is rapid and affords the opportunity for biometric identification directly from the sample, ensuring traceability. Although this is not explored here, the development of a fingerprint ridge detail before mass spectrometry analysis has been demonstrated (12) and could in theory be applied to fingerprint testing with the method presented here.

This is, we believe, the first study to explore the significance of testing for drugs from a fingerprint, and therefore, the first effort dedicated to establishing an environmental cutoff. By testing the fingerprints from 50 nondrug users, and fingerprints from nondrug users after shaking hands with patients, we have constructed and

tested an environmental cutoff for cocaine and heroin use from a fingerprint. The cutoff used here cannot be applied universally but it serves to illustrate the distinction between the fingerprints of drug users and nondrug users of cocaine and heroin.

**Author Contributions:** All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

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