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
respectively 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- × 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™ (Alere™) collection device. Oral fluid samples were analyzed at Claritest. Claritest screening uses immunoassay testing followed by LC-MS/MS analysis in water + 0.1% formic acid before being vortex-mixed and transferred to a 300-μL glass microinsert vial, with 5 μL being injected onto the LC-MS/MS system.

Chromatographic separation was performed on a Thermo Scientific™ Ultimate3000 UHPLC system equipped with a binary solvent manager, column manager, and autosampler. Separation was performed on a Kinetex XB-C18 column (100 × 2.1 mm, 5 μm) 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 μL at 600 ng/mL) samples extracted from chromatography paper are shown in Fig. 1 in the online Data Supplement.

5 Nonstandard abbreviations: ACN, acetonitrile; IS, internal standard; A/IS, analyte to internal standard.
Results and Discussion

pared and 5 injections of each were performed. Each replicate sample for each of the above sample was prepared as described earlier. Four fingerprints from each of 4 participants after wiping hands. In each case, extraction was performed as described earlier. Four fingerprints were prepared at 500 pg/sample to 10 ng/sample. Each fingerprint 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 R² 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.

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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 benzoylecgonine, 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 benzoylecgonine in the fingerprint samples. Benzoylecgonine 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 benzoylecgonine 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 benzoylecgonine 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 exceed the proposed cutoff for SUB002 (right thumb), SUB054 (right thumb), and SUB055 (right thumb and right in-
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 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.

**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

<table>
<thead>
<tr>
<th>Participant</th>
<th>Cocaine Fingerprint screening results (with LOD as cutoff)</th>
<th>BZE Fingerprint screening results</th>
<th>Oral fluid screening results</th>
<th>Patient testimony</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2/2</td>
<td>2/2</td>
<td>Negative</td>
<td>Cocaine, morphine</td>
</tr>
<tr>
<td>2</td>
<td>2/2</td>
<td>2/2</td>
<td>64 ng/mL</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>3</td>
<td>2/2</td>
<td>2/2</td>
<td>Negative</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>4</td>
<td>2/2</td>
<td>0/2</td>
<td>Negative</td>
<td>Cocaine</td>
</tr>
<tr>
<td>5</td>
<td>2/2</td>
<td>2/2</td>
<td>&gt;64 ng/mL</td>
<td>Cocaine</td>
</tr>
<tr>
<td>6</td>
<td>2/2</td>
<td>2/2</td>
<td>&gt;64 ng/mL</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>7</td>
<td>2/2</td>
<td>2/2</td>
<td>&gt;64 ng/mL</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>8</td>
<td>2/2</td>
<td>2/2</td>
<td>&gt;64 ng/mL</td>
<td>Cocaine, heroin</td>
</tr>
</tbody>
</table>
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 population, 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.

<table>
<thead>
<tr>
<th>Participant #</th>
<th>Fingerprint screening results</th>
<th>Oral fluid screening results</th>
<th>Patient testimony</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heroin 6-MAM</td>
<td>Morphine 6-MAM</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2/2 2/2</td>
<td>Negative 22.6 ng/mL 32 ng/mL</td>
<td>Cocaine, morphine</td>
</tr>
<tr>
<td>2</td>
<td>2/2 2/2</td>
<td>Negative</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>3</td>
<td>1/2 2/2</td>
<td>Negative</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>6</td>
<td>2/2 2/2</td>
<td>Negative 22.6 ng/mL 32 ng/mL</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>7</td>
<td>2/2 2/2</td>
<td>90 ng/mL</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>8</td>
<td>2/2 2/2</td>
<td>Negative</td>
<td>Cocaine, heroin</td>
</tr>
<tr>
<td>14</td>
<td>2/2 2/2</td>
<td>&gt;240 ng/mL</td>
<td>Heroin</td>
</tr>
<tr>
<td>15</td>
<td>2/2 2/2</td>
<td>138 ng/mL</td>
<td>32 ng/mL</td>
</tr>
</tbody>
</table>
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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