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Opiate DAU Screening Using Dried Urine Specimens

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The HIV Prevention Trials Network (HPTN) is a clinical trials network focused on non-vaccine interventions to prevent the transmission of Human Immunodeficiency Virus (HIV). Our laboratory serves as the Toxicology Core for the HPTN Network Laboratory, and one of the challenges we face is to provide support for laboratory needs in remote areas or developing countries. One item of particular interest is storage and shipping of urine specimens for drugs of abuse testing. Storage of urine specimens on filter paper to be tested later at specialized laboratories may offer an attractive way to overcome this problem, because it alleviates the need to ship liquid urine by special preservation and transportation methods and reduces shipping and storage cost.

Although use of filter paper for storage of urine has been previously reported for some clinical applications including screening for purine and pyrimidine disorders by LC-MS/MS [1], urea cycle disorders by MS [2], and methylmalonic aciduria by GC-MS [3], it is not commonly used, as opposed to dried blood. One challenge for using dried specimens (blood or urine) is that elution of analytes collected on the filter paper generally dilutes the specimen, and as a result, analytical methods used for quantitation of analytes in specimens collected by this approach must have very high sensitivity and specificity [4,5]. An additional challenge for toxicology immunoassay screening from these types of specimens is that the results from drug of abuse tests are qualitative rather than quantitative, so applying dilution factors to calculate these analytes' concentrations in original specimens - a strategy used for analysis of dried blood spots - is not feasible for dried urine specimens. In this study, we address this problem by developing a system that does not significantly dilute urine specimens in the elution process.

In order to develop this approach, we first determined the volume of the urine dried onto filter paper using urine enriched with ^{125}I -L-thyroxine [6]. Filter paper strips (20 × 50 mm) with three circles (12.7-mm in radius) printed on them were dipped into 50 mL of urine spiked with ~4 μCi ^{125}I -L-thyroxine (PerkinElmer, Cat#NEX111, Waltham, MA) [6] until completely saturated (~ 5 s). After drying overnight at room temperature on a vinyl sheet, a 6.0-mm punch was taken from the center of every circle and the radioactivity (count per minute) of 10 punches was measured. Using linear regression of a calibration curve established by the radioactivity from 0, 20, 40, 60, and 80 μL of ^{125}I -L-thyroxine spiked urine, we determined that a 6.0-mm spot contains $16.5 \pm 1.0 \mu\text{L}$ urine ($n=10$ and $\text{CV}=6.0\%$). Furthermore, the absorption capability of filter paper was calculated as $58.4 \pm 3.5 \mu\text{L}/\text{cm}^2$ by dividing the volume of urine stored in a 6.0-mm punch by its surface area. Based on these calculations, a filter paper strip of 12.7 × 88.9 mm was determined to store approximately 660 μL of urine. To ensure enough storage capacity, two such strips were used for every urine specimen in subsequent investigations.

Next, we determined the solvent volume needed to achieve a full recovery of drug in the reconstituted specimens (using morphine as a test molecule) by establishing a recovery curve using three different volumes of water (1100, 1200, and 1300 μL). Water was selected as the

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reconstitution solvent for the fact that water-reconstituted specimens have a similar matrix as the original urine specimens, therefore there is little concern for interference with the CEDIA® opiate assay. For the recovery studies, two filter paper strips (12.7 × 88.9 mm) were soaked in urine specimens spiked with morphine for 5 seconds. Excess urine was wiped off and the strips were completely dried overnight. To reconstitute the urine, the strips were rolled longitudinally into a cylinder and placed into a 5 mL syringe. Water (1.25 mL) was then added into the syringe with its bottom capped with a tip cap. After mixing the contents in the syringe for 60 minutes at room temperature using VX-2500 multi-tube vortexer (VWR Scientific Products, West Chester, PA), the syringe plunger was used to squeeze the reconstituted urine into a 1.5 mL eppendorf tube; this process resulted in a reconstituted specimen of approximately 750 µL. Recovery was calculated as the percentage ratio of morphine peak height in the reconstituted specimens, detected by the BioRad REMEDi HS Drug Profiling System REMEDi, to that of the original liquid urine. Table 1 shows the mean, SD, %CV of the morphine peak height in the reconstituted urine at each volume (n=3) as well as the calculated recovery. Linear regression analysis of the recovery curve using the software GraphPad Prism® (version 5) results in the equation:

$$y = (-0.10 \pm 0.01)x + (221 \pm 11)$$

($R^2=0.99$, y is the percentage recovery and x is the volume of water used for reconstitution; slope and intercept are expressed in mean ± SD). Based on this equation, 1180 µL of water was chosen to give 100 ± 16% (mean ± SD) recovery of morphine compared to the parent sample.

Once the method was established to recover approximately 100% of morphine from dried urine specimens, we evaluated it using replicate measurements of low and high levels of quality controls for the CEDIA® opiate assay (Microgenics Fremont, CA) containing 225 and 375 ng/mL of morphine, respectively. The reconstituted urine specimens (n=5) were measured by the qualitative CEDIA® opiate assay using 300 ng/mL of morphine as the cutoff for a positive test. The results showed that the replicate results for reconstituted urine specimens from low and high controls were 100% negative and 100% positive, respectively (n = 5). After developing and evaluating the system in a controlled environment using morphine-spiked urine specimens and morphine-containing quality control material, we applied this system to clinical specimens submitted for opiate screening that potentially contained other opiates such as codeine and heroin in a patient correlation study. This study used 100 clinical specimens that were previously characterized as 50 negative and 50 positive by the CEDIA® opiate assay in our clinical laboratory. The results demonstrated 100% concordance with the reconstituted specimens with the clinical specimens, which suggests that our system recovered other opiates in the clinical specimens in a similar way as morphine and therefore could be applied clinically.

In conclusion, we have developed a system that stores urine on filter paper and obtains a reconstituted urine specimen similar to the original one without significant dilution afterward. We demonstrated the validity of this system for opiate screening by its 100% concordance with established results when applied to (i) quality controls for the CEDIA® opiate assay and (ii) 100 clinical urine specimens. In the future we will evaluate long-term stability of urine specimens on filter paper strips and establish the feasibility of this approach in screening for other drugs of abuse. In addition, optimization of the system such as the minimum reconstitution time for the highest recovery of morphine from dried urine specimens will be considered in future studies.

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REFERENCES

- [1]. Ito T, van Kuilenburg AB, Bootsma AH, et al. Rapid screening of high-risk patients for disorders of purine and pyrimidine metabolism using HPLC-electrospray tandem mass spectrometry of liquid urine or urine-soaked filter paper strips. *Clin Chem* 2000;46:445–52. [PubMed: 10759467]
- [2]. McCann MT, Thompson MM, Gueron IC, et al. Quantification of orotic acid in dried filter-paper urine samples by stable isotope dilution. *Clin Chem* 1995;41:739–43. [PubMed: 7729053]
- [3]. McCann MT, Thompson MM, Gueron IC, et al. Methylmalonic acid quantification by stable isotope dilution gas chromatography-mass spectrometry from filter paper urine samples. *Clin Chem* 1996;42:910–4. [PubMed: 8665683]
- [4]. McDade TW, Burhop J, Dohnal J. High-sensitivity enzyme immunoassay for C-reactive protein in dried blood spots. *Clin Chem* 2004;50:652–4. [PubMed: 14981035]
- [5]. Chace DH, Adam BW, Smith SJ, et al. Validation of accuracy-based amino acid reference materials in dried-blood spots by tandem mass spectrometry for newborn screening assays. *Clin Chem* 1999;45:1269–77. [PubMed: 10430794]
- [6]. Adam BW, Alexander JR, Smith SJ, et al. Recoveries of phenylalanine from two sets of dried-blood-spot reference materials: prediction from hematocrit, spot volume, and paper matrix. *Clin Chem* 2000;46:126–8. [PubMed: 10620584]

Table 1

Recovery of morphine from urine dried onto filter paper using 3 different volumes of water (n=3)

	Original Urine	Dried Urine Reconstituted Using Different Volumes of Water		
		1100 uL	1200 uL	1300 uL
Average Morphine Peak Height	176783	189964	174678	153558
SD	1401	12728	3375	6529
%CV	0.79	6.70	1.93	4.25
Recovery (%)	100.00	107.46	98.81	86.86