

Importance of Creatinine Analyses of Urine When Screening for Abused Drugs

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We report here a simple method involving urine creatinine measurements for testing authenticity and reducing false-negative results in urine testing for drugs of abuse. Urinary creatinine in consecutive patient samples ($n = 176$) ranged between 0.1 and 31.9 mmol/L (mean $9.8 \pm$ SD 6.2) and the osmolality in these urines ranged between 49 and 1183 mOsm/kg (mean $595 \pm$ SD 276). With other consecutive samples in which creatinine was (arbitrarily chosen) <4.3 mmol/L ($n = 85$), the correlation with osmolality was lower. In 10 randomly selected urine samples from different patients, all "clean" for all drugs of abuse in initial immunological drug testing with approved methodology (in which creatinine was <4.3 mmol/L and osmolality was <200 mOsm/kg), five patients turned out to be drug positive after a simple concentration by volume. In a formerly heavy smoker of cannabis, the excretion of cannabinoids and creatinine was monitored for 93 days. The substances showed very good correlation throughout this period ($r = 0.93$, $P < 0.001$), whereas simple measurements of cannabinoid concentrations would have falsely indicated several relapses of cannabis abuse. Urine samples used in drug-abuse testing should be tested for creatinine; if creatinine is <4.0 mmol/L, negative results for drugs may not be valid.

Toxicological screening of urine based on immunological assays has been introduced in many drug-abuse programs during the last decade. The commercially available assays often used are EMIT™ (Syva Co., Palo Alto, CA) and fluorescence polarization immunoassay (Abbott Labs., Abbott Park, IL). Reagents from Syva may be used with instruments manufactured by Syva or by other manufacturers, whereas Abbott reagents are only used with Abbott equipment (TDx™ or ADx™). Both assays are based on immunological recognition of the analyte, e.g., cannabinoids (1, 2).

Although the degree of urine dilution may influence testing for abused drugs, few reports deal with this type of sample adulteration (3-6). With regard to diurnal fluctuations and the effects of fluid intake, data on the normal variability of urine creatinine concentrations and urine osmolality are outdated and are relevant only

in part for determining urine quality in abused-drug testing (7-9). The normal range of creatinine excretion in urine is 14-26 mg/kg per day (men) and 11-20 mg/kg per day (women) with a variability of 15-20%, depending on the dietary intake of meat (10).

Both false-positive and false-negative results may undermine a drug-abuse program relying on urine screening. Chromatographic verification, which reveals false-positive results, is therefore a recommended procedure (11). However, high cutoff values in immunological assays may lead to false-negative results, and the providing of diluted urine reduces detection. The control of urine quality is, however, not an established procedure. An easy way to detect probable false-negative results in the laboratory is needed.

Cannabinoids may be found in urine long after intake if a low cutoff value (e.g., 20 μ g/L, corresponding to 0.06 μ mol/L equivalents of Δ^9 -tetrahydrocannabinol-11-carboxylic acid) is used (12, 13). To evaluate the dose and time of intake, quantitative analyses of cannabinoids in urine may be performed; however, this requires attention to renal function, because excretion may be influenced by both drug concentrations in blood and urine production. On hypothetical grounds, measuring creatinine in urine has been proposed for distinguishing true and false increases in cannabinoid concentrations in urine to verify resumption of marijuana smoking (14). A case report has been presented in which this idea was tested during a few weeks' follow-up (12). Continual use of creatinine-corrected cannabinoid excretion to monitor adherence to abstinence has, however, not been tested.

We report here that measurements of creatinine and, in some cases, osmolality in urine improve the quality in testing for drugs of abuse. Creatinine measurement detects samples that may be falsely negative. Determination of the ratio of cannabinoids to creatinine appears to be of considerable value when testing for adherence to abstinence from this drug.

Materials and Methods

Patients. The patients consisted of former heroin abusers participating in a structured outpatient care program based on methadone maintenance treatment. All patients provided urine samples daily. The urine was cooled immediately and if not analyzed within 24 h, stored at -16 °C. All patients joined their respective program voluntarily and agreed to be subjected to repeated compliance testing for abused drugs as part of the rehabilitation program.

One patient with a history of heavy cannabis smoking was monitored for 93 consecutive days. He was

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treated in a high-security ward with continual supervision. No clinical signs of relapse occurred during this treatment.

Healthy volunteers. Also participating in this study were 23 staff members. They were instructed to provide urine samples in the morning, without prior intake of fluids, and were requested to collect the urine for 5 h in 1-h portions after intake of different amounts of water. In the first test, the volunteers drank 0.5 L during 15 min; the next week (the second test), the water intake was 1.0 L during 15 min. During the sampling period, they were allowed free intake of food, but not of liquids.

Analyses. At our laboratories, a rigorous chain of custody, including voiding under mirror supervision, according to the criteria of the National Institute on Drug Abuse (NIDA) (11), was performed. All patients' samples were examined with immunological methods for benzodiazepines, opiates, methadone, cannabinoids, and amphetamines. Cannabinoid and benzodiazepine screening was performed with the automated Abbott ADx instrument. Positive results were confirmed by thin-layer chromatography (15). Opiates, amphetamines, and methadone were screened with the Monarch Chemistry System 1000 (Instrumentation Labs., Lexington, MA) with EMIT reagents. Positive results from screening for opiates and amphetamines were confirmed by gas chromatography.

All positive results were confirmed by other techniques. Detection of amphetamines and related amines was verified by a gas-chromatographic method (modified after the method of Budd, 16), based on a solvent-extraction procedure with mexiletine as internal standard. Aliquots of the final extracts were injected with a moving needle device into a fused-silica capillary column (CP-Sil-8-CB, 25 m, 0.25 mm ϕ ; Nova Kemi, Stockholm, Sweden) installed in a Model 427 gas chromatograph (Packard Instrument Co., Downers Grove, IL) and equipped with a nitrogen-phosphorus detector. Verification of opiate alkaloids was also made by gas chromatography. Opiate alkaloids were isolated by solid-phase extraction (Bond Elut; Analytichem International, Palo Alto, CA) and derivatized by treatment with *N*-methyl-*N*-trimethylsilyl trifluoroacetamide (MSTFA) with nalorphine as internal standard. Aliquots of the final extracts were injected with a moving needle device into a fused-silica capillary column (BP1, 25 m, 0.2 mm ϕ ; Scantec, Stockholm, Sweden) installed in a Model GC-9AM gas chromatograph (Shimadzu GmbH, Duisberg, F.R.G.) equipped with a flame ionization detector. Confirmation of Δ^9 -tetrahydrocannabinol-11-carboxylic acid in long-term follow-up patients was by high-performance liquid chromatography, as previously reported (17).

Osmolality was measured by the decrease in freezing point (Roebing osmometer; Svenska Labex AB, Helsingborg, Sweden). Creatinine in urine was analyzed by using standard reagents (Jaffé reaction) from Abbott in the automated TDx system.

Sampling schemes. We collected 176 consecutive samples from different patients and analyzed them for creatinine and osmolality. We also analyzed 85 other samples consecutively collected for osmolality, for which creatinine was <4.3 mmol/L (arbitrarily chosen limit). The formerly heavy smoker of cannabis provided a urine sample daily, of which only the sample from every third day was analyzed.

Without conscious bias, we selected from initially negative urine samples 10 samples from different patients in which creatinine was <4.3 mmol/L and osmolality was <200 mOsm/kg (arbitrarily chosen limit). These samples were concentrated ~ 6.5 times (range 5–8) (by vol) by evaporation at 37 °C overnight and then reconstituted in water.

Statistics. We used linear-regression analysis to examine linear associations. Student's paired *t*-test was used for hypothesis testing. Data are presented as the mean \pm SD, with ranges in some cases. In the serial measurements of the healthy volunteers, the lowest concentration achieved (C_{\min}) in each subject was tested vs the individual baseline according to Matthews et al. (18), but data are presented as the mean with respect to the time course to illustrate the variance over time.

Results

Patients. In the first trial evaluation of urine creatinine and osmolality in consecutive samples ($n = 176$), both creatinine and osmolality were symmetrically distributed and ranged between 0.1 and 31.9 mmol/L (mean 9.8 ± 6.2) and 49 and 1183 mOsm/kg (mean 595 ± 276), respectively. Linear-regression analysis between creatinine and osmolality showed a correlation coefficient (r) of 0.76 ($P < 0.001$). In the second trial with urine samples from patients, all samples in which creatinine was <4.3 mmol/L were further checked for osmolality. Osmolality in these samples ranged between 27 and 816 mOsm/kg (mean 233 ± 120), and creatinine ranged between 0.2 and 4.3 mmol/L (mean 2.8 ± 1.0). Between the two variables in this lower range, $r = 0.52$ ($P < 0.001$).

The formerly heavy smoker of cannabis showed variable excretion of cannabinoids, which were still detectable after 93 days since cessation of smoking. The excretion of creatinine mirrored the excretion of cannabinoids ($r = 0.93$, $P < 0.001$) and the ratio between cannabinoids and creatinine showed a rather steady decrease with an elimination half-life of 32 days (Figures 1 and 2).

In the dilute urine samples, five of 10 initially negative samples were found to be positive for either cannabinoids, opiates, or benzodiazepines after the concentration procedure (not shown).

Healthy volunteers. There was a significant decrease in both creatinine and osmolality after intake of 0.5 L of water. This decrease was significant up to 5 h after intake (Table 1). Of these samples, 95% showed creatinine <3.8 mmol/L. After intake of 1.0 L of water, the decrease was also significant for at least 5 h, with 95% of

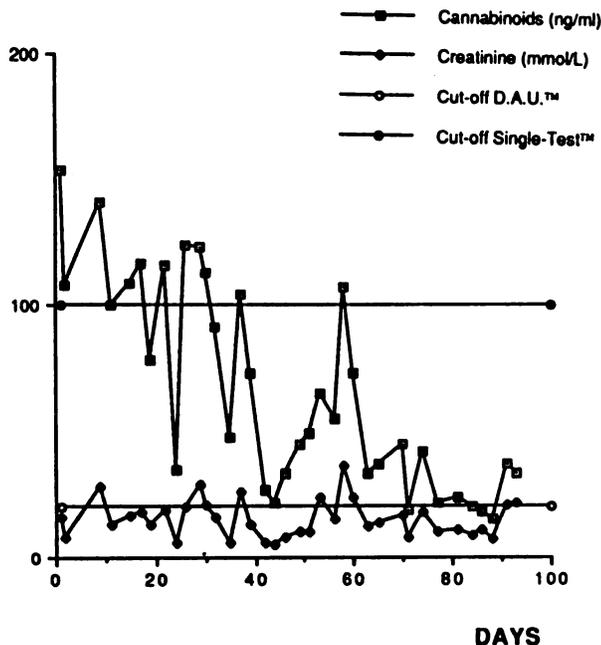


Fig. 1. Cannabinoid and creatinine concentrations in urine during a long-term (93 days) follow-up of a formerly heavy smoker of cannabis

Cutoff values are shown for 100 $\mu\text{g/L}$ (corresponding to 0.3 $\mu\text{mol/L}$ equivalents of Δ^9 -tetrahydrocannabinol-carboxylic acid and suggestive of recent intake) and 20 $\mu\text{g/L}$ (corresponding to 0.06 $\mu\text{mol/L}$ cannabinoid equivalents). The values suggestive of new drug intake after a period of low values coincide with high concentrations of creatinine; $r = 0.93$, $P < 0.001$. The CV for creatinine determinations is 53.6%

nmol cannabinoid
equivalents/
mmol creatinine

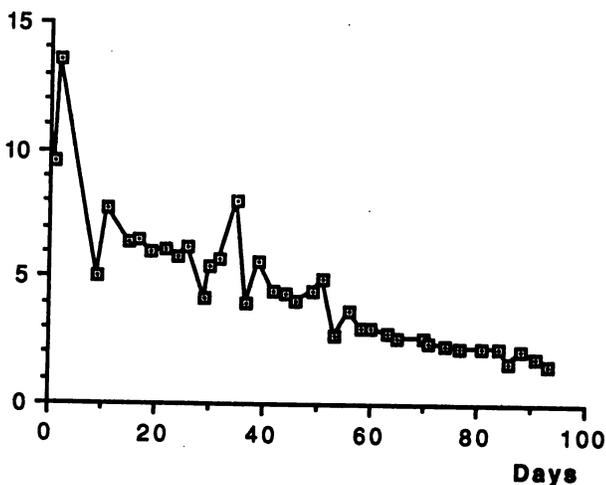


Fig. 2. The ratio of cannabinoids and creatinine (nanomoles of Δ^9 -tetrahydrocannabinol-carboxylic acid per millimole of creatinine) revealed a steady decline, with a half-life of 32 days; the equation for the line obtained by linear regression is $y = 8.3150 - 0.0905x$

the samples being < 0.7 mmol/L during this time (Table 1).

Discussion

Urine used in abused-drug testing may be adulterated directly (3–6) or more physiologically by intake of

Table 1. Effects on Creatinine and Osmolality in Urine in Healthy Subjects after Intake of 0.5 or 1.0 L of Water

	Creatinine, mmol/L	Osmolality, mOsm/kg
	Mean (range)	
0.5 L		
Before	16.5 (4.0–30.0)	857 (380–1187)
1 h	4.0 (0.7–19.1)	291 (72–835)
2 h	3.0 (0.7–10.6)	262 (69–725)
3 h	7.3 (0.8–17.7)	472 (69–975)
4 h	10.1 (0.9–21.8)	651 (69–980)
5 h	11.8 (2.8–22.7) ^a	734 (278–1075) ^a
1.0 L		
Before	15.6 (4.4–23.9)	850 (179–1199)
1 h	2.2 (0.7–4.3)	190 (46–996)
2 h	1.4 (0.6–4.8)	110 (45–305)
3 h	3.8 (0.8–11.6)	266 (73–640)
4 h	8.6 (1.9–17.7)	552 (168–824)
5 h	10.7 (1.5–22.7) ^a	661 (147–964) ^a

^a $P < 0.01$. All others, $P < 0.001$ in comparison with values before intake of water.

n = 23.

fluids, thus diluting the sample. Regardless of the reason, dilution may explain some false-negative results. False-negative results may undermine public confidence in a qualitative rehabilitation program and should be avoided. False-positive results may also create therapeutic problems, but this is solved by mandatory chromatographic verification of positive screening results. In this report, we found that screening of urine samples by measurements of creatinine or osmolality evaluates the urine and the validity of drug-testing results. Because results from testing for drugs of abuse in urine depend on the amount of drug (or metabolite) excreted in the sample and the sample volume, at least one of these variables (creatinine or osmolality) should be assessed for a correct determination and interpretation of the drug content in the sample.

Measurements of creatinine and osmolality in patients revealed that the correlation between the two variables is lower in samples with creatinine < 4.3 mmol/L. One explanation for this loss of correlation between creatinine and osmolality in the low-creatinine urine samples may be that osmolality depends on the total amount of dissolved molecules in the solution, whereas creatinine is an endogenous substance. Adulteration of the sample through intake of fluids may, therefore, affect creatinine and osmolality differently, depending on what liquid was taken (e.g., glucose-containing lemonade may dilute urine without affecting osmolality). Furthermore, creatinine is easily measured by many laboratories with existing methods, whereas measuring osmolality requires specific instruments (e.g., freezing-point reduction). Given this theoretical background and the results in the present work, we recommend determination of creatinine rather than osmolality.

In the healthy volunteers, 95% of the morning urine

samples showed creatinine >10.0 mmol/L and osmolality >648 mOsm/kg. Thus, urine is normally concentrated in healthy adults even when it is not provided as a 24-h sample (to which many clinical ranges refer, 7–10). However, creatinine concentrations in 95% of the urine samples in healthy volunteers after intake of 0.5 L of water were <3.8 mmol/L. In these samples, 95% also showed an osmolality <292 mOsm/kg. If not taken into account, this marked effect on the urine concentration of a small water intake may create considerable problems with false-negative results in a drug-abuse screening program.

False-negative results due to dilution may be difficult to identify, and an easy way of determining the dilution rate for incoming samples is needed. We tested a simple evaporation procedure at ambient temperature overnight in 10 randomly selected urine samples with signs of dilution (e.g., creatinine <4.3 mmol/L and osmolality <200 mOsm/kg). Among these samples, five developed positive drug results after a new immunological screening followed by confirmation. Although it may be questioned whether it is ethically justifiable to concentrate urine, this test showed a high frequency of positive drug results in urine samples that were thought to be "clean." The problem of physiological or nonphysiological dilution of urine to mask drug intake may thus be overcome by simply concentrating the sample. This concentration procedure cannot be recommended as part of the routine control, on ethical and legal grounds, but it constitutes an easy way for determining the rate of false-negative results. One should, however, be aware that different drugs may react differently in such concentration procedures; e.g., there may be better detectability of opiates than of amphetamines due to different chemical properties of the molecules. We have not investigated this idea further.

During the long-term follow-up of formerly heavy cannabis smokers, we found that the excretion of cannabinoids closely followed that of creatinine. Simple measurements of cannabinoid concentration gave highly variable results, sometimes positive and indicating new intake if not related to creatinine. The elimination of cannabinoids in relation to creatinine excretion in one subject followed a steadily declining course, with an elimination half-life of 32 days. These findings indicate the benefit of repeated quantitative measurements of creatinine when evaluating adherence to abstinence from cannabinoids by urinalysis.

In conclusion, we recommend measurements of creatinine or osmolality in urine for detection of presumably false-negative samples when testing for drugs of abuse. Because creatinine is an endogenous substance not affected by intake of highly osmolar liquids and is measured easily, we recommend creatinine before osmolality. We suggest that a creatinine value <4.0 mmol/L be used as a cutoff to evaluate if the sample has been diluted, physiologically or nonphysiologically. We base

this cutoff limit on the following: (a) the correlation between creatinine and osmolality was lower in samples with a creatinine value <4.3 mmol/L, (b) 95% of the samples in healthy volunteers had creatinine concentrations <3.8 mmol/L after intake of 0.5 L of water during 5 h, and (c) five of 10 samples with creatinine <4.3 mmol/L were falsely negative. The cutoff limit is the mean of these data. Negative results in such diluted samples may be false and are not valid, requiring another sample. To elucidate the rate of false-negative results, we suggest a simple concentration of the sample. Our preliminary findings concerning the relationship between creatinine and cannabinoid excretion in urine suggest that creatinine excretion should also be taken into account in these cases. Quantitative measurements of creatinine and cannabinoids make it possible to calculate a ratio that eliminates falsely increased cannabinoids in urine due to metabolism rather than resumption of marijuana smoking. However, the routine use of creatinine determinations to evaluate quantitative cannabinoid excretion requires further documentation.

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