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Workplace drug testing on urine samples: evidence for improving efficacy of a first-level screening programme

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Key words
Workplace drug testing; drugs of abuse; diluted urine sample; urine creatinine normalization; public safety; prevention policies

Parole chiave
Analisi tossicologiche nei luoghi di lavoro; sostanze di abuso; campione di urina diluito; normalizzazione per creatinina urinaria; sicurezza pubblica; politiche di prevenzione

Summary
Background: *Previous reports revealed poor performance in identifying drugs of abuse users through first-level workplace drug testing (WDT), based on urine samples. In a cross-sectional study, we evaluated: (i) the effect of creatinine normalization of drug values from diluted urine samples (creatinine levels ≤20 mg/dL) on the prevalence of drug users; (ii) the independent procedure-related predictors of positivity and dilution.* **Methods:** *Workers' urine samples were collected at the workplace or at our certified laboratory between 2008 and 2012. All samples were analysed for drugs of abuse by immuno-enzymatic method in our laboratory, according to the Italian WDT law. Detectable drugs of abuse concentrations lower than the positive cutoff values were normalized based on mean levels of urinary creatinine. Detectable concentrations of drugs were confirmed by GC/MS. Multivariate logistic regression was used to detect independent procedure-related predictors of positive and diluted urine samples.* **Results:** *Of the 3080 urine samples screened, 51 (1.7%) were found positive for some drugs of abuse (26 cannabinoids and 16 cocaine) and 116 (3.8%) were diluted. Seventeen out of 23 diluted urine samples with detectable concentrations of cannabinoids or cocaine were found positive after urine creatinine normalization and GC/MS confirmed both negative and positive results. This increased the percentage of positivity for cannabinoids and cocaine from 1.35% to 2.09% (+55%, p=0.0005), which is closer to the expected prevalence of drug users based on Italian self-reported surveys. Collection of samples in the laboratory was an independent predictor of positivity (OR=2.33, 95%CI 1.27-4.28) and diluted urine sample (OR=1.65, 95%CI 1.04-2.61).* **Conclusions:** *Efficacy of first-level WDT could be improved by well-controlled pre-analytical procedures and urine creatinine normalization of detected concentrations of drugs of abuse.*

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A preliminary analysis of some of these data, was presented at the 2010 Congress of the Italian Society of Occupational Medicine and Industrial Hygiene and published as an extended abstract (G Ital Med Lav Ergon. 2010;32: 196-8).

Riassunto

«Test antidroga su campioni di urina nei luoghi di lavoro: evidenze per migliorare l'efficacia di un programma di screening di primo livello». **Introduzione:** *Le analisi tossicologiche di primo livello su campioni di urina hanno mostrato una scarsa performance nell'individuare lavoratori che utilizzano sostanze d'abuso. In questo studio sono stati valutati: (i) la prevalenza della positività alle sostanze d'abuso prima e dopo normalizzazione per creatinina urinaria media; (ii) i fattori associati al riscontro di campioni positivi o diluiti (creatinina ≤20 mg/dL).* **Metodi:** *I campioni di urina dei lavoratori sono stati raccolti tra il 2008 e il 2012 sul luogo di lavoro o nel nostro laboratorio accreditato. Tutti i campioni sono stati analizzati con metodo immunoenzimatico nel nostro laboratorio. Le concentrazioni di sostanze d'abuso rilevabili ma inferiori ai cutoff di positività sono state normalizzate e confermate in GC/MS. L'analisi multivariata è stata utilizzata per identificare i predittori indipendenti di positività e di diluizione.* **Risultati:** *Dei 3080 campioni di urine esaminati, 51 (1.7%) erano positivi (cannabinoidi:26; cocaina:16) e 116 (3.8%) diluiti. Diciassette su 23 campioni diluiti e con concentrazioni rilevabili di cannabinoidi o cocaina sono diventati positivi dopo normalizzazione; la GC/MS ha confermato sia i risultati negativi sia i positivi. La percentuale di positività per cannabinoidi e cocaina è aumentata dall'1.35% al 2.09% (+ 55%, p=0.0005), con una prevalenza più vicina a quella attesa sulla base dei dati italiani (prevalenza riferita dai lavoratori). Il luogo di raccolta dei campioni è risultato un predittore indipendente di positività (laboratorio vs azienda; OR = 2.33, 95% CI 1.27-4.28) e di diluizione (OR = 1.65, 95% CI 1.04-2.61).* **Conclusion:** *L'efficacia dell'indagine di primo livello potrebbe essere migliorata con procedure pre-analitiche ben controllate e attraverso la normalizzazione dei campioni con concentrazioni rilevabili di sostanze d'abuso.*

Introduction

Since the 1980s, some European and North American countries have introduced workplace drug testing (WDT) (7, 25) aimed at improving employees' safety, health and productivity. Users of drugs of abuse showed impaired short-time decision-making ability, inhibitory response and behaviour control, including post-error corrections, altered perceptions of visual stimuli, and reduced psychomotor speed (1, 10, 11, 16, 21), all likely to reduce job performance. However, evidence of effectiveness of WDT programmes to deter workers abuse of drugs or improve workplace safety is weak, basically due to the poor quality of available studies (26).

In Italy, WDT was introduced in 1990 (8) and came into force in 2007 (29, 30). All workers performing work tasks at high risk of injury and potentially involving third parties, must undergo periodic assessments for drugs of abuse, consisting in a first-level screening on urine followed, in case of positivity, by a second-level assessment on urine and/or hair (18). Briefly, the key elements of the first-level screening are: workers should not be notified earlier than 24 hours before urine sampling; urine samples can be collected at the workplace, with supervision to avoid sample adulterations; the collected sample can be analysed on-site or by certified laboratories (screening sample); positive results must be confirmed by certified laboratories with a mass spectrometry technique on a second independently stored aliquot (confirmation sample).

A recent study observed that in Italy the proportion of urine samples positive for drugs of abuse showed a decreasing trend after approval of the WDT law (36). However, increasing evidence suggests that current first-level procedures underestimate the prevalence of drugs of abuse users (20, 36, 37), mainly due to pitfalls in urine sample collection and analysis procedures. Urine is the biological matrix reference for drugs of abuse determinations since the urine test is non-invasive, low-cost, sufficiently reliable at some cutoff points and able to detect previous use of drugs of abuse for a more extensive period than oral fluids (35). Nevertheless, an excess of fluid intake dilutes concentrations of drugs up to values below the threshold limits for positive results. In addition, such threshold limits

for screening methods (cutoffs) were set by law at higher levels to allow the use of on-site tests (23). Urinary creatinine ≤20 mg/dl is widely accepted as an indicator of in vivo dilution (4,5). A normalization formula based on creatinine levels was proposed for diluted samples, showing good performance when applied in athletic drug testing and pain treatment programmes (6). The same approach was recently proposed for WDT programmes (27, 28, 34), however few studies quantified the improvement in efficacy. Price (28) reported a 100% increase in the prevalence of positive urine samples after urine creatinine normalization. Moreover, some authors showed that improving the standardization of pre-analytical procedures may reduce the probability of false negative results (20, 27, 31).

Adopting a cross-sectional study, we aimed to assess the improvement in the efficacy of the first-level of WDT programme after urine creatinine normalization of diluted and non-diluted urine samples, analysed with the immuno-enzymatic method in a toxicology laboratory in Northern Italy. In particular, we re-analysed all creatinine normalized samples in GC/MS, quantified the change as a proportion of positive samples, and compared them with expected prevalence of users of drugs of abuse based on data provided by concurrent Italian self-reported surveys. Furthermore, we analysed the process-related predictors of diluted as well as of positive urine samples.

Materials and methods

Population and sample

Our population consisted of male workers, aged between 15 and 64 years, employed in various industries in Lombardy (Northern Italy), who underwent first-level WDT screening for the identification of drugs of abuse users, between September 2008 and June 2012.

Although some workers were selected for the WDT programme many times during this period, we restricted our data to the first determinations only, to avoid the effect of the more frequently repeated tests made on the same positive worker on our estimates. We excluded urine samples from female workers due to their low number (n=64), thus avoiding the effects of gender-specific prevalence of drugs of abuse and creatinine levels. The final sample consisted of 3080 subjects.

Pre-analytical phase

Urine samples were collected either at the workplace or at our Laboratory. Samples collected at the workplace by occupational physicians were sent to the laboratory, where the chain-of-custody which certifies the integrity and authenticity of urine samples was controlled by laboratory staff. At collection, each sample was split into three separate vials, as required by Italian law, one used for screening analysis, the second stored for confirmatory analysis and the third stored at -20°C for any legal dispute. All urine samples were stored at the laboratory and were identified by a unique barcode for each worker. Samples collected at the workplace were stored at +4°C if transported to the laboratory within 24 hours or frozen to -20°C when transportation occurred later. For urine samples gathered at the laboratory, the list of selected workers was available to the laboratory staff, participation was carefully checked and inspection during urine collection was guaranteed.

Age, self-reported information of job title and positive history of drug use as well as the main characteristics of the enterprise were also collected by the laboratory staff. All enrolled workers read the purpose statement of the drug-screening and gave a written informed consent.

Analytical phase

Determinations of drugs of abuse in urine were performed at the Toxicology Laboratory of the Work and Preventive Medicine of the Varese University Hospital. Based on regional guidelines for the application of Italian law, urine samples were tested for cocaine (COC), cannabinoids (CANs), opiates (OPI), methadone (MET), amphetamine (AMPH), metamphetamine (MAMPH), MDMA and buprenorphine (BUP).

All drug determinations were carried out using an immuno-enzymatic method (EMIT II plus, Siemens Healthcare Diagnostics, Milan, Italy). Urine creatinine levels were determined with the Jaffè method. In this study, the urine samples were categorised as negative ([drugs of abuse)< *LLOQ*), non-negative (*LLOQ*< [drugs of abuse)< cutoffs) and positive ([drugs of abuse)> cutoffs). Non-negative drug concentrations were normalized using the formula proposed by Cone (5): $[\text{drugs of abuse}]_{\text{normalized}} = [\text{drugs of abuse}]_{\text{sample}} \times [\text{urine creatinine}]_{\text{reference}} / [\text{urine creatinine}]_{\text{sample}}$, where $[\text{urine creatinine}]_{\text{reference}}$ is the urine creatinine mean level determined in our WDT population (126.72 mg/dL). The non-negative samples underwent GC/MS confirmatory analysis independently of the creatinine levels.

Non-negative urine sample determinations were confirmed using a GC/MS method (5975C, *Agilent Technologies Italia*, Milan, Italy), including solid-phase extraction, tri-methylsilylation and instrumental analysis in SIM mode. GC/MS is the accepted standard in confirmatory analytical technologies for drugs of abuse in urine, with a large number of validation studies proving its accuracy and precision (25,22). The main analytical characteristics of the methods are reported in Table 1.

Table 1 - Analytical characteristics of screening and confirmation methods for drugs of abuse in urine established by Italian law

	Immunoenzymatic method		GC/MS method	
Drugs of abuse classes	LLOQ ng/mL	Cutoff ng/mL	LLOQ ng/mL	Cutoff ng/mL
CANs	25	50	5	15
COC	100	300	10	100
OPI	100	300	10	100
MET	100	300	10	100
AMPH	300	500	50	250
MAMPH	300	500	50	250
MDMA	150	500	50	250
BUP	3	10	2	5

LLOQ: lower limit of quantification for immunoenzymatic and GC/MS methods.
Cutoff: threshold values to determine the positivity recommended for immunoenzymatic and GC/MS methods.

For the immuno-enzymatic method the coefficients of variations range from 3.7% for OPI to 10.4% for BUP at the cut-off values and from 4.6% for methadone to 14.9% for CANs at the LLOQ (lowest concentration level in the calibration curve). The LLOQ for GC/MS method was set at the lowest concentration level in the calibration curve (r^2> 0.998) for all analytes (range: 5-200 ng/mL for CANs, 10-1000 ng/mL for COC, OPI and MET, 50-1000 ng/mL for AMPH, MAMPH and MDMA, 2-40 ng/mL for BUP). For both the immunoenzymatic and the GC/MS methods, the laboratory participates in external quality assessment, through the EQC Programme of Lombardy Region and used internal quality standards provided by the Liquichek Urine Toxicology Control (Bio-Rad Laboratories, Segrate, Italy). Based on this performance, the laboratory is certified for drugs of abuse testing by the Regional Accreditation Office (2).

Statistical analysis

The prevalence of drug of abuse positivity was estimated by age group (15-24, 25-34, 35-44, 45-54, 55-64 years) and on the overall sample (32). The null hypothesis of prevalence of positive samples for either CANs or COC after normalization equal to the estimated prevalence before normalization was tested through a Z test. The expected number of workers positive for CANs or COC was calculated based on age- and gender-specific self-reported use of drugs of abuse in the last month, according to the 2010-2011 IPSAD-Italy report (19) on 15-64 years old men.

Finally, through multivariate logistic regression models we explored the contributions of selected variables to the probability of an urine sample being *i)* a diluted sample or *ii)* a positive sample for at least one drug of abuse, the latter based on urine creatinine normalized values. The covariates of interest were: worker's age; place of urine collection (categorized as on-site at the company or at the laboratory); the presence of at least one positive sample in the same company/year of sample collection; the presence of at least one diluted urine sample in the same company/year; the number of workers tested from the same company/year (cate-

gorized as 1, 2-5, 6 or more). For these analyses, we excluded n=87 samples (n=4 diluted, n=4 positive) with information missing on the predictors of interest. All analyses were conducted using SAS 9.3.1 release.

Results

Prevalence of users before urine creatinine normalization

Of the 3080 urine samples screened, 116 (3.8% of total samples) were diluted (urine creatinine mean±SD: 14.18±4.33 mg/dL). Of the 2964 non-diluted samples, 51 were positive for at least one drug of abuse, corresponding to an overall prevalence of 1.7% (Table 2). Of these, CANs and COC were the two most frequently observed drugs. Three samples were positive for both COC and CANs or OPI, and one urine sample for COC, MET and OPI. The prevalence of positive samples decreased with age from 4% among the youngest to 0.8% among workers aged 55-64 (Table 2).

Prevalence of users after urine creatinine normalization and GC/MS confirmation

In Table 3 we report the drug determinations before and after normalization, as well as the confirmatory GC/MS assessment, for all the non-negative samples for CANs (n=21, part A) and for COC (n=9, part B).

CANs values were above the screening threshold limits in n=18 samples (86%; 14 non-diluted, 4 diluted) (Table 3.A). The corresponding samples for COC (Table 3.B) were 6 (67%; 3 non-diluted and 3 diluted). All the normalized-positive and the normalized-negative samples were confirmed by GC/MS analysis. After normalization, the prevalence of positive samples for either CANs or COC increased from 1.35% (n=40/2971) to 2.09% (n=62/2971), a statistically significant increase of 55% (Z-test p-value: 0.0005).

Observed vs expected CANs and COC positivity

Table 4 shows the age-specific and overall observed numbers of positive workers for CANs and

Table 2 - Number of positive urine samples and prevalence of drugs of abuse on screened urine samples, overall and by age group. Crespi et al: Improving effectiveness of first-level WDT

Age group	Positive urine samples																	
	CANs		COC		OPI		MET		AMPH		MAMPH		MDMA		BUP		At least one drug of abuse^	
	n	%	n	%	n	%	n	%	N	%	n	%	n	%	n	%	n	%
15-24 y	4	3.2	1	0.8	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	5	4.0
25-34 y	10	1.6	10	1.6	3	0.5	1	0.2	0	0.0	0	0.0	0	0.0	0	0.0	21	3.5
35-44 y	11	1.0	5	0.5	3	0.3	1	0.1	0	0.0	0	0.0	0	0.0	1	0.1	19	1.7
45-54 y	0	0.0	0	0.0	2	0.2	1	0.1	0	0.0	0	0.0	0	0.0	1	0.1	4	0.5
55-64 y	1	0.4	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	1	0.5	2	0.8
Total	26	0.9	16	0.5	8	0.3	3	0.1	0	0.0	0	0.0	0	0.0	3	0.1	51	1.7

All urine samples regard men aged 15-64 years at first screening and with urine creatinine values > 20 mg/dL.
Threshold values to determine the positivity recommended for screening analysis (cutoff): Cannabinoids (CANs) 50 ng/mL, cocaine (COC) 300 ng/mL, opiates (OPI) 300 ng/mL, methadone (MET) 300 ng/mL, buprenorphine (BUP) 5 ng/mL, amphetamines, metamphetamines and MDMA 500 ng/mL;
^ Three urine samples were positive to 2 drugs of abuse (COC and either CANs or OPI), one to 3 drugs (COC, MET and OPI).

Table 3 - Screening, urine samples after normalization and GC/MS confirmed values of non-negative urine samples.

Part A. Non-negative* urine samples for cannabinoids (CANs), ranked by descending screening values. Crespi et al: Improving effectiveness of first-level WDT.

Workers	Screening values (ng/mL)	Urine creatinine values (mg/dL)	Normalized to CRref values (ng/mL)^	GC/MS confirmation values (ng/mL)^^
n. 1	48	120,0	50,7	26,8
n. 2	45	94,5	60,3	19,0
n. 3	43	104,0	52,4	19,9
n. 4	42	86,0	61,9	24,7
n. 5	39	98,0	50,4	22,9
n. 6	39	80,9	61,1	24,9
n. 7	35	86,0	51,6	22,2
n. 8	35	78,0	56,9	24,7
n. 9	32	64,1	63,3	26,7
n. 10	31	66,3	59,3	26,8
n. 11	31	65,0	60,4	28,3
n. 12	30	168,0	22,6[a]	11,4[b]
n. 13	30	154,0	24,7[a]	14,4[b]
n. 14	29	161,0	22,8[a]	13,2[b]
n. 15	29	70,0	52,5	29,6
n. 16	28	70,1	50,6	15,9
n. 17	27	54,4	62,9	26,9
n. 18	44	19,0[c]	293,5	19,5
n. 19	37	11,0[c]	426,2	29,9
n. 20	35	19,0[c]	233,4	16,8
n. 21	26	8,6[c]	383,1	28,3

* Non-negative urine samples: CAN concentrations lower than the threshold value to determine the positivity recommended for screening analysis (cutoff= 50 ng/mL) but higher than lower limit of quantification (*LLOQ*= 25 ng/mL);
^ Reference urine creatinine ($[\text{urine creatinine}]_{ref}$= 126.72 mg/dL) and $[\text{drug}]_{normalized}= [\text{drug}]_{sample} \times [\text{urine creatinine}]_{ref} / [\text{urine creatinine}]_{sample}$;
^^ Threshold value to determine the positivity recommended for GC/MS confirmation analysis (cutoff): 15 ng/mL; lower limit of quantification (*LLOQ*): 5 ng/mL;
[a] Negative urine samples after normalization;
[b] Negative urine samples after GC/MS confirmation analysis;
[c] Diluted (urine creatinine ≤20 mg/dL) and non-negative urine samples.

Part B. Non-negative* urine samples for cocaine (COC), ranked by descending screening values. Crespi et al: Improving efficacy of first-level WDT.

Workers	Screening values (ng/mL)	Urine creatinine values (mg/dL)	Normalized to CRref values (ng/mL)^	GC/MS confirmation values (ng/mL)^^
n. 1	212	85,4	314,6	101,5
n. 2	195	66,3	372,7	117,6
n. 3	180	104,0	219,3[a]	54,5[b]
n. 4	169	65,3	328,0	111,8
n. 5	152	201,0	95,83[a]	72,19[b]
n. 6	132	191,0	87,6[a]	89,93[b]
n. 7	131	18,2[c]	912,1	123,7
n. 8	123	16,3[c]	956,2	131,6
n. 9	107	17,5[c]	774,8	108,1

(table 3 continued)

* Non-negative urine samples: COC concentrations lower than the threshold value to determine positivity recommended for screening analysis (cutoff= 300 ng/mL) but higher than lower limit of quantification (*LLOQ*= 100 ng/mL)];
^ Reference urine creatinine ([urine creatinine]$_{ref}$ = 126.72 mg/dL) and [drug]$_{normalized}$= [drug]$_{sample}$ x [urine creatinine]$_{ref}$/ [urine creatinine]$_{sample}$;
^^ Threshold value to determine the positivity recommended for GC/MS confirmation analysis (cutoff): 100 ng/mL; lower limit of quantification (*LLOQ*): 10 ng/mL;
[a] Negative urine samples after normalization;
[b] Negative urine samples after GC/MS confirmation analysis;
[c] Diluted (urine creatinine ≤20 mg/dL) and non-negative urine samples.

Table 4 - Total number of urine samples screened and positive urine samples (observed before and after urine creatinine normalization) for cannabinoids (CANs) and cocaine (COC) in relation to expected number, calculated based on age- and gender-specific self-reported use of drugs of abuse in the last month according to the IPSAD-Italy 2010/2011 report (19). Crespi et al: Improving effectiveness of first-level WDT

		CANs Positive				COC Positive			
Age group	Samples#	Observed^	After Normalization	Total	Expected^^	Observed^	After Normalization	Total	Expected^^
15-24 y	126	4	3	7	13.9	1	0	1	1.3
25-34 y	612	10	5	15	56.9	10	3	13	6.1
35-44 y	1097	11	6	17	34.0	5	1	6	4.4
45-54 y	889	0	3	3	11.6	0	2	2	3.6
55-64 y	247	1	1	2	1.5	0	0	0	0.2
Total	2971	26	18	44	117.8	16	6	22	15.6

[#] Total number of urine samples screened including also 7 non-negative samples with urine creatinine ≤20 mg/dL (diluted samples);
^ Two urine samples were positive to 2 drugs (CANs and COC);
^^ Based on IPSAD-Italy 2010/2011 age- and gender- specific prevalence, applied to the number of urine samples screened.

COC, before and after urine creatinine normalization, and the corresponding expected numbers based on self-reported use of drugs of abuse in the last month derived from IPSAD-Italy data.

The expected number of positive samples for CANs was 117.8, 4.5 times greater than the observed number (n=22). Creatinine normalization slightly mitigated the discrepancy by increasing the observed positivity to 44. Conversely, the expected number of positive samples to COC was 15.6, lower than the observed positivity after normalization (n=22).

Predictors of diluted and positive urine samples

In the multivariate logistic model, increasing worker age was associated with a decreasing probability of diluted sample (Table 5, endpoint 1). In addition, the presence of another diluted sample from the same workplace in the same period (OR: 3.3) and sample collection at the laboratory (OR: 1.7) were independently associated with an increased probability of sample dilution (Table 5, Endpoint 1).

These last two findings supported the hypothesis that the low probability of finding urine sample dilution in urine samples collected at the workplace may be attributed to poor organisation at company level. Conversely, dilution, is the only remaining chance to falsify urine samples when collection took place at the laboratory, where a careful inspection during urine collection and compliance with chain-of-custody can be easily achieved.

In the multivariate logistic model, worker's age

Table 5 - Multivariate analysis: odds ratio (OR) and 95% confidence interval (95%CI) of major predictors. Analysis performed with separate models for diluted samples (n=112, **Endpoint 1**) and samples positive for drugs of abuse (n=69, **Endpoint 2**). Crespi et al: Improving effectiveness of first-level WDT.

		Endpoint 1: diluted sample (n=112, N=2930)				Endpoint 2: positive sample (n=69, N=2887)			
Variables		OR	95%CI		p-val	OR	95%CI		p-val
Worker's age		0.97	0.95	0.99	0.007	0.94	0.91	0.97	<.0001
Presence of another positive test in the same company/year		0.75	0.44	1.28	0.3	7.25	3.56	14.7	<.0001
Presence of at least one diluted test in the same company/year		3.34	1.96	5.68	<.0001	0.56	0.28	1.13	0.1
Urine samples collected at:	Company	ref	-	-	0.03	ref	-	-	0.01
	Laboratory	1.65	1.04	2.61		2.33	1.27	4.28	
N° of workers tested in the same company/year									
1		ref	-	-	0.3	ref	-	-	0.2
2-5		1.42	0.58	3.48		2.20	0.64	7.51	
6 or more		0.90	0.36	2.23		1.24	0.34	4.46	

In this logistic model N=2993 urine samples were included;
N= 87 records with missing information on the firm were excluded, of which 4 were positive and 4 diluted;
OR: odds ratio from multivariate logistic regression models.

was negatively associated with drugs of abuse positivity (OR=0.94; Table 5, Endpoint 2). The presence of another positive sample from the same firm in the same period (OR= 7.3) and sample collection at the laboratory (OR= 2.3) increased the probability of a positive sample.

A possible explanation of these findings is that the combination of well conducted recruitment at company level and accurate urine sample collection at the laboratory are strong positive predictors of positive urine samples. Moreover, the observed clusters of positive subjects within the same companies also suggest that a drugs abuser could have induced his workmates to share his habit. It is also important to point out that this part of our results was obtained after having included the urine creatinine normalized results in the positive endpoint, allowing a more precise estimate of the predictors' effects.

Discussion

In our study, urine samples with drugs of abuse concentrations between the sensitivity limits of the immuno-enzymatic method and the threshold limits established by law were normalized for urine creatinine and confirmed with GC/MS. Adopting this method, we found an increase of 55% in the prevalence of CANs and COC positive urine samples, and an observed number of workers tested positive for COC higher than the expected number based on prevalence data from the Italian population. Nevertheless, the observed number of workers tested positive for CANs remained low, almost 3 times lower than expected. Finally, a younger age, urine sample collected directly at the laboratory and the presence of clusters of diluted or positive samples in the same firm were independent predictors of diluted and positive samples, respectively.

CANs and COC were the drug classes most frequently detected in urine of workers (51% and 31%, respectively) and these percentages were in line with the results of other studies carried out at national and regional levels in Italy (20,24,36). The higher prevalence of CANs with respect to COC can be attributed to their more widespread use in Italy, but also to their high lipophilic characteristics which determine a large volume distribution and a slow release from stored tissues back into the blood, allowing CANs to be detected in urine for days or weeks after last use (17,33,35). However, the large difference between the observed prevalence of CANs and the national self-reported data (IPSAD-Italy 2010/2011) persisted even after normalization. Possible explanations include the dilution attempt to mask positivity made mainly by CANs users, due to the higher probability of detecting CANs in urine even after several weeks.

In fact, it is known that a large intake of fluids before the urine analysis reduces drugs of abuse levels, as well as urine creatinine levels, which allow possible counterfeit samples to be identified (5,14,39). In our population, the percentage of diluted samples was 3.8%, and positive/non-negative samples had lower creatinine than the overall average (97.6 vs.126.72 mg/dL; p-value=0.0002), thus suggesting the intentionality of urine dilution.

Our data suggest that urine creatinine normalization of urinary drugs of abuse concentrations is an efficient method to deal with diluted urine samples, increasing the positive results rate up to 55%. Our study then confirmed an improvement in performance of first-level WDT screening, as reported by Price, but in a small proportion (28): 18 out of the 21 CANs false negative samples and 6 out of the 9 COC false negative samples turned out to be positive.

In the last 10 years, several studies have investigated the analytical sensitivity of immuno-enzymatic and chromatographic assays for detecting drugs of abuse, concluding that drugs and metabolites can be detectable at concentrations much lower than the cutoffs established by law (12,23,38). The 6 non-negative urine samples found to be negative also after normalization deserve particular consideration, since normalized concentrations of CANs and COC, confirmed by the GC/MS values, were very close to the respective cutoffs for positive results. Consequently, this study suggests reflecting on the possibility of lowering the cutoffs in order to also include in the positive rate those samples with drugs of abuse concentrations below the cutoffs in force but within the limits of quantification of the analytic method.

We should acknowledge that the large majority of diluted urine samples - 109 out of 116 samples in our working population - showed non-detectable levels of drugs of abuse. All these subjects can then be considered potentially positive and consequently submitted to new enlistments to repeat the analysis, as this procedure is useful to further deter their use (9,13). At present, Italian WDT law does not recommend this specific action in the case of diluted urine samples (with a few exceptions in some Regions).

The number of positive samples for COC after urine creatinine normalization was higher than expected based on IPSAD-Italy 2010/2011 data (22 observed *vs* 16 expected) indicating a slightly higher use of COC in our population of workers, and the excess was concentrated in the age-group 25-34 years (13 observed *vs* 6.1 expected). This more than double number of observed *vs* expected COC users supports the belief that among young adult workers, in particular among truck drivers (36% in our worker population), COC is frequently assumed to increase work performance (15). At the same time, self-reported IPSAD data may underestimate the real prevalence of COC users, given the negative social connotation of COC.

As regards WDT procedural aspects that may increase first-level screening efficacy, we observed that the probability of finding a diluted or a positive urine sample was higher among workers from companies where other diluted or positive samples were identified in the same screening. The presence of these clusters confirms the significant role played by some procedures of the pre-analytical phase of screening, in particular the compliance with the requirement of the 24-hour term for notification (35). Moreover, a careful inspection and stricter compliance with the chain-of-custody can both be better achieved when collection is made at

the laboratory (31,34), where the staff is trained and able to apply correctly the procedures. The observed increase in the percentage of diluted urine samples when collection took place at the laboratory can be explained as a rebound effect of a poorly controlled planning phase at company level, inducing drugs of abuse users to dilute their urine samples, as this is the only remaining chance to bias positive results when samples are collected under strict control at the laboratory.

Strengths and limitations

One strength of our study is the confirmation analysis by GC/MS for all urine samples found non-negative even after normalization. The concordance between urine creatinine normalization and GC/MS confirmation was 100% both for positive and negative samples. As regards the limitation of this study, several variables associated with unintentional dilution, such as the percentage of lean body mass and renal function (3) were not collected. Another study limit lies in the relatively small sample size available for the analysis, with limited statistical power to test the interaction between detected operational aspects which can influence each other; for instance there was not sufficient power for testing the interaction between company- and laboratory-related factors in determining the probability of diluted urine samples. A third shortcoming is the absence of follow-up data, so we do not know how many positive urine samples are really habitual or occasional users of drugs of abuse.

Conclusion

Our results indicate that urine creatinine normalization increased the prevalence of positive tests by about 55%, with total concordance with the GC/MS method. Furthermore, better planning at company level and urine sample collection may improve WDT efficacy. Based on our findings some recommendations can be made to improve current WDT programmes and their impact on occupational injury prevention: 1. adopting creatinine normalization followed by GC/MS method to all non-negative urine samples; 2. collecting urine samples at a laboratory, where staff is adequately trained to follow the standardized WDT protocol fixed by law. Furthermore, based on the higher sensitivity of laboratory assays, screening thresholds for positivity can be lowered. Finally, the suggested improvements in WDT first-level screening and the randomization of workers to be tested will make a better use of the nowadays very limited available economic resources.

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