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TECHNICAL NOTE

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Proficiency testing for psychoactive substances in Italy

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Abstract This paper describes the general design and main results of the Italian proficiency testing program for the analysis of psychoactive substances in urine, a long-term initiative created in 1995 on an educational basis and characterized by an innovative internet-based service for data exchange between laboratories and the organizing body. Batches of six urine samples, validated by reference laboratories, are sent every 3 months to participating laboratories, which may choose which classes of substances to test from those planned by the program panel and, within those classes, which type of analytical commitment to work on: identification of just one class (Option 1), identification of single substances (Option 2), or identification and quantification of single substances (Option 3). Comprehensive periodical reports and annual reports are provided to participants with evaluation of their performance and an annual workshop is organized to discuss technical-scientific topics related to clinical, forensic and analytical toxicology. About 200 laboratories currently participate in the program and a total of 67,059 analyses have been carried out since 1995. The mean percentage of correct results was 96.8%, with a yearly improvement of about 0.4%. The best average false positive and false negative rates were obtained for methadone (0.2% and 2.1% respectively) and cocaine (0.3% and 2.2%). The worst average false positive rates were obtained for amphetamines and opiates (3.2% and 5.0%) and worst average false negative rates for amphetamines, barbiturates and cannabinoids (17.4%, 30.7% and 19.9%).

Key words Proficiency testing · Quality control · Analytical toxicology · Psychoactive substances

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Introduction

Laboratories performing analyses in the field of clinical and forensic toxicology produce data which may have great social and juridical importance and analytical errors may therefore have a great impact on an individual's livelihood, freedom and civil rights [1–3]. In Europe, only about 50% of laboratories working in the field of clinical and forensic toxicology take part in some sort of proficiency testing program (PTP) [4, 5].

In Italy, after 15 years of experience in quality control programs for drugs of abuse [6–10], the Centre of Behavioural and Forensic Toxicology (CBFT) of the University of Padova started a national PTP in 1995, characterized by an educational internet-based service. This paper describes the general design and main results of the first 3 years of this PTP.

Materials and methods

The program was first set up as a continual, real-time, educational service to allow laboratories to participate with options tailored to fit their special needs. In particular, the program involves:

1. A 3-monthly shipment of six samples of sterile urine, spiked with analytes (parent substances, metabolites and interfering substances [11,12]), in concentrations less than, equivalent to, or higher than the cut-offs established by the organizing body, as listed in Table 1. These cut-offs allow each participant laboratory to evaluate its own analytical results against given reference concentrations and, consequently, to report on the presence or absence of analytes in the above PTP samples.

2. Special educational trials.

3. A 3-monthly and annual processing of analytical results from each laboratory according to the option chosen, i.e., identification of one or more classes of substances (Option 1), one or more single substances (Option 2), or quantification of one or more substances (Option 3).

4. A 3-monthly and annual processing of general results, techniques and analytical procedures reported by all laboratories and by the reference laboratories in Finland, Germany, the United Kingdom and Italy.

Table 1 Substances added to PTP control samples, their concentration limits (cut-offs) set by the organizing body, and concentration ranges for every single substance added

Classes and single substances	Concentratgion range		
	cut-off (ng/mL)	min (ng/mL)	max (ng/mL)
Amphetamine and analogues			
– Amphetamine	1000	102	1490
– Methamphetamine	1000	510	1510
– 3,4-Methylendioxyamphetamine (MDA)	1000	270	1890
– 3,4-Methylendioxyethylamphetamine (MDEA)	1000	1410	1450
– 3,4-Methylendioxymethamphetamine (MDMA)	1000	1010	1480
Barbiturates			
– Amobarbital	500	750	905
– Butalbital	500	645	970
– Butabarbital	500	–	–
– Phenobarbital	500	680	1980
– Secobarbital	500	–	–
Benzodiazepines			
– Diazepam	500	700	700
– Nordiazepam	500	370	700
– Oxazepam	500	640	930
– Nitrazepam	500	855	855
– 7-Aminonitrazepam	500	800	800
– Flunitrazepam	500	–	–
– 7-Aminoflunitrazepam	500	800	1390
– Flurazepam	500	480	910
– Desalkylflurazepam	500	870	980
– N-hydroxyethylflurazepam	500	95	95
– Lorazepam	500	1420	1420
– Triazolam	500	750	1440
– α-Hydroxytriazolam	500	390	390
Cannabinoids			
– 11-nor-9-COOH-Δ^9-THC	50	81	156
* 11-nor-9-COOH-Δ^9-THC-glucuronide			
Cocaine			
– Benzoylecgonine	150	340	970
* Cocaine		190	200
* Ecgonine methylester		280	505
Methadone			
– Methadone	300	350	880
* 2-Ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine		290	700
Opiates			
– Codeine	300	90	890
– Morphine	300	95	1515
* 6-Monoacetylmorphine		115	540
* Morphine-3-O-glucuronide		400	1105
* Morphine-3-O-sulphate			
** Interfering substances			
– Phentermine		965	965
– Phenylpropanolamine		1980	7850
– Ephedrine		1920	5800
– Selegiline		830	830
– Chlorpromazine		9850	9850
– N-methyl-3,4-methylendoxyphenyl-2-butanamine		3800	4800
– Dantoine		9500	9500
– Dihydrocodeine		400	500
– Ethylmorphine		480	950

* Metabolites
** Interfering substances added with the function of mimicking real samples

5. An internet-based service, called 'PTP on line', which may be consulted by each laboratory for real-time reporting of its results (by means of a specific password), global statistics regarding each shipment, for the year in progress and for preceding years, and for a question-and-answer service (Forum) regarding topics related to the PTP.

6. An annual workshop with the participation of all laboratories devoted to discussion of topics of analytical, clinical and forensic toxicology within the PTP.

Results and discussion

Type of participation

Since 1995, 200 laboratories have participated in the PTP, of which 173 participated from the beginning. Participating laboratories belong to the following organizations: hospitals (85.2%), universities (4.8%), armed forces (8.3%), private institutions (1.7%).

The average participation percentages were 70.1% Option 1, 15.8% Option 2 and 14.1% Option 3, with a steady increase for Option 3 (3%) over this time period. For the type of substance participation was 99.4% for cocaine, 98.8% for opiates, 97.5% for cannabinoids, 85.5% for amphetamines, 84.0% for methadone, 80.9% for benzodiazepines and 46.9% for barbiturates. Of the laboratories 55.7% used screening procedures only and 44.3% also adopted confirmation procedures. In the case of Option 1, confirmation was carried out in 13.5% of total analyses, 96.3% for Option 2 and 88.0% for Option 3.

Analytical results

Since 1995, a total of 67,059 analyses have been carried out, with a mean for correct results of 96.8% and a yearly improvement in the global percentage of correct results of 0.4%.

Table 2 lists false negative (FN) and false positive (FP) rates obtained during the program according to classes of substances and options with respect to the number of analyses carried out on samples containing and not containing analytes, respectively.

From a general viewpoint, laboratories choosing Option 3 performed better than the others, demonstrating that well-equipped and experienced laboratories challenged the most difficult option and the results reflect the greater skills. The results obtained by laboratories performing the two qualitative options were similar with regard to total results but differed with respect to the various classes of substances planned by the program.

The best results, in terms of average FP and FN rates, were obtained for the classes of methadone (0.2% and 2.1%, respectively) and cocaine (0.3% and 2.2%). The worst average FP rates were obtained for amphetamines and opiates (3.2% and 5.0%) and worst average FN for amphetamines, barbiturates and cannabinoids (17.4%, 30.7% and 19.9% respectively).

For opiates relatively high FN rates were found for Options 2 and 3, due to misinterpretation of morphine for codeine and vice versa. For benzodiazepines and barbiturates, high FN rates were due to control samples containing difficult analytes for immunochemical techniques, such as 7-aminoflunitrazepam and phenobarbital. Cannabinoids gave the second highest FN rates for Option 1, due to the use of kits with different analytical cut-offs. The low cross-reactivity of some immunochemical techniques for amphetamine analogues was probably the main reason for the high FN rates obtained for this class.

Relatively high FP rates for amphetamines were due to interfering substances (Option 1) or amphetamine analogues (Option 2). Similarly, opiate analysis resulted in high FP rates, mainly due to interfering substances such as dihydrocodeine and ethylmorphine.

Special trials

The results of three emblematic trials carried out in the 3-year period 1995–1998 were:

1. With the aim of evaluating performance time-trends of participating laboratories, two batches, the first of 1995 (1/95) and the last of 1996 (4/96), were prepared with a very similar qualitative-quantitative composition of ana-

Table 2 False negative and false positive rates by class of substances and option with respect to analyses on samples containing or not containing analytes

Classes	Option 1				Option 2				Option 3			
	N1	FNr	N2	FPr	N1	FNr	N2	FPr	N1	FNr	N2	FPr
Amphetamines	2069	16.4	3859	2.9	466	22.1	6284	3.7	55	12.7	575	0.5
Barbiturates	718	32.2	2960	1.5	73	20.5	1877	0.2	10	0.0	230	0.4
Benzodiazepines	1891	8.3	4889	0.3	125	31.2	4747	0.7	11	27.3	457	0.4
Cannabinoids	1070	23.7	5944	0.5	159	5.0	885	0.2	119	5.0	631	1.1
Cocaine	1136	1.7	6070	0.4	173	5.2	941	0.1	86	2.3	423	0.0
Methadone	937	2.5	4685	0.2	180	1.1	900	0.2	106	1.0	530	0.4
Opiates	2724	1.0	4284	6.9	547	6.0	2117	2.7	253	2.8	863	1.2
Total	10545	10.0	32691	1.6	1723	12.1	17751	1.9	640	4.1	3709	0.7

N1: Number of analyses on samples containing analytes
N2: Number of analyses on samples not containing analytes
FNr: False negative rate (%)
FPr: False positive rate (%)

lytes. For the second batch, a higher percentage of correct results (+0.9%) was found due to a homogeneous decrease in the number of FPs (–0.5%) and FNs (–0.4%). In particular, FNs for cannabinoids (103 ng/mL and 105 ng/mL of delta-9-tetrahydrocannabinol-9-carboxylic acid, for batches 1/95 and 4/96, respectively) fell by 19.1% for Option 1, FNs for amphetamines (1110 ng/mL and 1150 ng/mL of MDMA for batches 1/95 and 4/96) by 5.1% for Option 1 and FPs for dihydrocodeine (500 ng/mL and 480 ng/mL for batches 1/95 and 4/96) by 27.3% for Option 2.

2. With the aim of alerting laboratories to the existence of new sustances on the illicit market at the beginning of 1996, some urine samples were spiked with N-methyl-3,4-methylendioxyphenyl-2-butanamine (MBDB). The first analytical results were disastrous, with very high numbers of FPs (62.0%), due to erroneous identification of MBDB with other amphetamines included in the program. A fast feedback by the organizing body and subsequent proper countermeasures by participants led to a rapid improvement in performance on later batches (21.1% and 5.2%, respectively).

3. In order to verify possible variations of FN results in relation to variation in analyte concentrations, amphetamine alone (with no other substances or metabolites belonging to the same class) was added at concentrations of 1150, 1380 and 1490 ng/mL to control samples in three subsequent batches. The resulting FN rates (10.2%, 8.6% and 5.3%, respectively) were inversely proportional to the amphetamine concentrations, although all control samples had analyte concentrations well above the cut-off value of 1000 ng/mL, thus highlighting the extensive use of immunochemical techniques not accurately calibrated for amphetamines.

Comparison of techniques

The performance of techniques in terms of sensitivity (percentage of correct results with respect to samples containing analytes) and specificity (percentage of correct results with respect to samples not containing analytes) [13] was as follows:

Immunochemical techniques were, in general, characterized by homogeneous analytical behaviour (with average sensitivity and specificity of 88.3% and 98.5%), with respect to both the psychoactive substances planned by the program and the particular analytical difficulties inserted during the trials.

The use of confirmatory techniques did not greatly improve the average specificity of the analytical process (98.3%). While single techniques failed almost exclusively when interfering substances were present, FP results due to coupled techniques were mainly due to misinterpretation in the identification of one analyte in the presence of more than one substance belonging to the same class in the same control sample. For amphetamines and opiates, a very low degree of specificity was noted in the presence of substances such as MBDB (batches 4/95, conc. 3800 ng/mL; 2/96, conc. 4800 ng/mL; 1/97, conc. 4750 ng/mL), often reported as MDA, MDEA, MDMA, or ethylmorphine (batch 2/95, conc. 950 ng/mL) or dihydrocodeine (batches 1/95, conc. 500 ng/mL; 4/96, conc. 480 ng/mL), often reported as morphine or codeine. Table 3 lists the FP results for amphetamines and opiates and shows how this kind of error was quite common even when sophisticated confirmatory techniques such as GC/MS were used.

In conclusions the main sources of errors for participating laboratories were found to be:

Table 3 False positive results obtained for amphetamines and opiates by coupled techniques

Analyte present	Analytes reported by participating laboratories using				
	IC-GC/MS	IC-HPLC	IC-TOXILAB	IC-GC	IC-REMEDi
Amphetamine	MDMA				
Methamphetamine	Amphetamine MDA MDMA	MDA			
MDEA		MDMA			MDMA (6)
MDMA	Amphetamine Methamphetam.				MDEA
MBDB	MDEA (3) Amphetamine	MDEA (2) Methamphetam. MDMA	MDEA MDMA (2)		MDMA (7) MDA MDEA
Ephedrine	Methamphetam.	Methamphetam. MDEA			
Codeine	Morphine	Morphine		Morphine	Morphine (2)
Morphine	Codeine	Codeine			
Dihydrocodeine	Codeine (2)	Codeine	Morphine	Morphine	Codeine (4)
Ethylmorphine	Morphine (2) Codeine	Codeine (4)	Codeine		Codeine (5)

IC: Immunochemical techniques
( ): Numbers between brackets indicate frequency of report

– Difficulty in evaluating the presence or absence of analytes if the concentrations had to be compared with the cut-offs planned by the program and different from those usually adopted by the participating laboratories.

– The presence of analytes in control samples not listed in the program's panel (and not to be reported on the analysis form), which have been frequent causes of FP results.

– The presence in control samples of deliberately added interfering substances, which caused a great number of FPs, since about half the participating laboratories did not use confirmatory techniques.

– The presence in the same sample of two or more substances belonging to the same class for those laboratories participating for the identification of single substances. Although analyses were carried out using sometimes sophisticated confirmatory techniques, analytes were often confused with one another or only one was reported. This appeared to be quite widespread and not restricted to any specific laboratory.

In general, comparison of the percentages of correct results for the various options of the program clearly shows that better results are obtained by the small group of laboratories choosing the more demanding analytical challenges (Options 2 and 3), due to the greater experience in this kind of analysis. However, the constant improvement in performance noted for the easier Option 1 over the past 3 years is also an encouraging sign of the effectiveness of activities within the program.

Both periodical reports and annual workshops were used to stress the importance of using confirmatory techniques and to avoid routine analytical methodology habits. Furthermore, the improvement in participating laboratory performance was helped by means of a technical-scientific service, upon direct request, providing analysis of samples, scientific information on analytical methodology for particular analytes or alternative matrices, bibliographic references, etc.

Some focal points have emerged from this 3-year experience:

– It is desirable for laboratories involved in toxicological analyses to participate in some kind of accreditation program.

– If the accreditation program cannot be achieved, an effective system of exchange of regulatory, technical and scientific information should be set up among those laboratories which appear to work without common rules, which should not happen in the forensic environment. Due to its inherent speed and low cost, it was found that an internet-based facility is a really useful tool to this end.

– Performance may be improved if laboratories are supplied with standard solutions of drugs or metabolites and if training courses on confirmatory techniques are planned on a regular basis.

– Lastly, many of the considerations expressed here are probably applicable to most European countries and the possible interaction of this program with other international initiatives in this field should therefore be carefully considered.

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