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Toxicological screening - Experience from University Hospital Centre Zagreb Emergency Medical Service

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ABSTRACT

The aim of toxicological screening is to detect drugs of abuse and other drugs, or to demonstrate that they have not been taken. It is performed with a variety of tests capable to detect certain substance or a group of substances. Urine is a sample of choice for toxicological screening because of less invasive sampling and the prolonged detection time of substances in urine. The Department of Laboratory Diagnostics at the University Hospital Centre Zagreb used to perform screening with thin-layer chromatography and confirm results with GC-MS analysis. Since September 2014, the Department has been using GC-MS for toxicological screening 24 hours a day. Compared to the previous period, the experience acquired so far has shown that there are a significantly lower number of samples with unknown substances that cannot be confirmed with certainty.

Key words: gas chromatography, mass spectrometry, toxicological screening

INTRODUCTION

The aim of toxicological screening is to detect drugs of abuse and other drugs, or to demonstrate that they have not been taken. In clinical toxicology, drug screening is performed when a patient exposed to drug shows signs of intoxication or overdose, with symptoms such as excitement, respiratory or central nervous system depression or organ specific reactions: liver failure, cardiac arrhythmia or severe metabolic acidosis.
In practice, it is not possible or necessary to test for all of the hundreds or thousands of clinical toxins that may be encountered. In reality, up to 24 drugs or agents account for 80% or more of intoxication cases treated in most emergency departments. (1, 2) The scope of clinical toxicology testing provided by the laboratory will depend on the pattern of local drug use and on available resources of the institution, and it should be developed in consultation with the appropriate clinical staff. (2)
Laboratory testing for a single drug of abuse can help in antidote selection (N-acetyl-cysteine for acetaminophen of naloxone for opiates). Drug toxicity is often recognizable based on history or clinical signs and symptoms, and treatment is general and supportive and therefore not influenced by drug screening results. Drug screening results are frequently not available soon enough to represent valuable information. However, clinical history is also not always available or reliable; symptoms are not clearly recognizable as drug or substance intoxication. Symptoms may be particularly confusing in case of multiple drug intoxication. It is very important to rule in or rule out intoxication as a possible reason in patients with altered mental status or coma. (2)

Urine as a sample in toxicological screening
Urine is a sample of choice in emergency departments because of less invasive sampling (unconscious patients demand catheter use), as it is possible to obtain a large sample volume and the detection time of substances is usually a few days. Most drugs and drug metabolites are present in the urine in relatively high concentrations. For example, THC metabolites are liposoluble and can be detected several days or even several weeks after consumption. (3) Detection times for some substances are shown in Table 1. The actual detection time depends on dose, frequency of use and individual metabolism. (4)
The patient's degree of hydration has significant influence on substance concentration in urine. Concentration of a substance in urine also depends on the amount of the substance used, on the volume of urine in the bladder, and later on the quantity of urine excreted.

METHODS AVAILABLE FOR TOXICOLOGICAL SCREENING

The proper selection of analytical methods requires knowledge of pharmacology and of pharmacokinetics of the substance of interest (for example, potential hepatotoxicity of acetaminophen is related to the concentration of unmetabolized drug; an analytical test should measure only the parent drug and not inactive metabolites). Quantitative determination in serum is important only for some substances (for example acetaminophen, ethylene glycol, teophyline, digoxin); as regarding a large number of other drugs, their serum concentration and clinical picture do not correlate. In these cases, qualitative identification in urine is generally sufficient.
Screening methods are rapid, usually qualitative and they have adequate sensitivity, but they are not highly specific. A negative result obtained by a screening test rules out the presence of clinically significant concentrations of a particular analyte. Every positive result must be confirmed by a procedure of greater specificity. Screening procedures are designed to detect one drug or a group of drugs and involve the following:
- Simple visual colour tests (spot tests) which are qualitative and noninstrumental; every positive result must be confirmed with a more specific method.
- Serum osmol gap, which is the difference between the actual osmolality; measured

by freezing-point depression and the calculated osmolality. When present in significant concentrations, alcohols, acetone and ethylene glycol increase serum osmolality for >10 mOsm/kg.

- Immunoassays can detect a single drug (e.g., cocaine, methadone) or a group of drugs (e.g., amphetamines, opiates). They are very simple to use, automated, semi-quantitative and rapid with a low detection limit (0.02-1.0 μg/mL). The main disadvantage of immunoassays is that they provide false-positive results and require a second test for confirmation. (3 - 6)
- Chromatographic methods are able to detect a wide range of drugs and metabolites (thin-layer chromatography-TLC, gas chromatography- GC; high performance liquid chromatography-HPLC).

TLC is operationally relatively simple and inexpensive. After extraction and specimen spotting, the TLC plates are developed with appropriate solvents to achieve chromatographic resolution. Drugs and metabolites are then visualised as spots by their fluorescence or ultraviolet absorbance and by their colour development with a combination of dip solutions. Identification is made by co-chromatographing reference compounds with the unknown substances, followed by comparison of their relative migration distances (Rf values) and detection characteristics with those of the unknown substances. Identification requires considerable experience, skill and various detection colour hues.

HPLC is often used for toxicological screening. The advantage of HPLC over GC is the ability to analyse polar compounds without derivatization (e.g., morphine) and thermally labile drugs (e.g., chlordiazepoxide).

GC is relatively rapid and capable of resolving a broad spectrum of drugs and is widely used for qualitative and quantitative drug analysis. Common detectors for drug detection by GC are flame ionisation detectors and mass spectrometers. GC is a chromatographic technique based on repeated partition and adsorption between the mobile phase and a stationary phase. The mobile phase is always gas. A sample must be introduced into the analysis system in the form of vapour, which is achieved by heating the sample before it reaches the column. In GC-MS analysis, a substance eluted from the column reaches mass spectrometer where it is ionized and fragmented. After data processing, the result is mass spectrum; a graphic display of abundance in function of mass and charge. A mass spectrum of molecular fragments is characteristic of the molecule (similar to a finger print) and allows molecule identification by comparing created ions and their intensity with the spectrum of substances from the commercially available library. (7)

It is important to highlight that LC-MS (-MS) analysis is capable of analysing a much wider range of compounds (including polar and thermally labile without derivatization) with shorter time analysis and greater selectivity and sensitivity. (8) Due to these above mentioned advantages, LC-MS-MS has certainly found a place in the analysis of new drugs such as synthetic cannabinoides.

CHANGES IN OUR LABORATORY

Until September 2014, immunoassays for targeted screening, TLC for general screening (24 hours a day) and GC-MS for confirmation in the event of a positive sample during regular working hours were used in our laboratory. Confirmations on GC-MS were conducted by an analytical toxicology specialist. In the spring of 2014, the manufacturer discontinued production of TLC plates and the laboratory was in a situation to perform general screening only with immunoassays for methadone, cocaine, THC, benzodiazepines, amphetamines, and opiates, and to rely on GC-MS analysis only during regular working hours. The laboratory did not have enough analytical toxicology specialists to cover GC-MS screening 24 hours a day, and it was also financially unjustifiable. There were, however, a sufficient number of laboratory medicine specialists who were willing to learn how to handle GC-MS, so it was decided to try to introduce GC-MS screening into our laboratory for emergency service needs. The main problem was how to create our internal database that would automatically notify the analyst which substance/s is/are present in the screened sample. Therefore, one colleague started hard-labouring and embarked on a long-term job: to create our internal database. It was difficult work, as she physically screened every sample containing a known substance and then recorded its retention time and three or more mass ions that are characteristic for the substance. Today our internal database contains information on 240 substances and is still growing. It is always possible that a substance of interest is not in the internal database, but laboratory medicine specialists also learned how to search commercially available libraries. Library searching is not always easy and requires a lot of experience to learn all the "tricks" (e.g., spectrum cleaning) to be 100% sure of the substance found. A result of toxicological screening always says that the presence of a substance is suspected. If a clinician wants, they can ask for screening confirmation on the next working day.

GC-MS as a screening method has its own limitations:

- Thermolabile and polar compounds can not be screened (e.g., opiates must be derivatized before GC-MS analysis)
- We cannot screen for substances that are not in the library (e.g., synthetic cannabinoides, new synthetic drugs…)
- Time-consuming (it lasts longer than TLC, approx. 1.5 hour from sample reception to results).

Because of the above mentioned first two limitations, it is always emphasised in the laboratory report that the analysis does not cover all psychoactive substances.

In drug screening, analysts do not rely only on GC-MS analysis for opiates, THC, benzodiazepines and amphetamines. These substances can not always be seen on GC-MS. Because analysts do not want to miss any substances, immunoassays for these are also performed.

From September 2014 to May 2015, a total of 233 toxicological screenings were performed on GC-MS. In these samples, 55 different substances were found. As shown in table 2, the most frequent among detected drugs were: antipsyhotics (39 samples), anxiolytic sedative hypnotics - benzodiazepines (32 samples), antidepressants (27 samples), antiepileptics (26 samples). Local anesthetic (lidocaine) was present in 53 samples due to urine sampling with catheter containing lidocaine.

During the same period one year earlier (September 2013 - May 2014), 343 toxicological screenings were performed with TLC and only 32 different substances were found. Some details of toxicological

screening results are shown in table 2. On TLC, analysts were not able to see which benzodiazepine, phenotiazine or tricyclic antidepressant was present in the sample; they were just able to see them as a group. On GC-MS, the exact substance can be seen, which is very important for benzodiazepines as the therapeutic range for this drugs very much varies from benzodiazepine to benzodiazepine. This information can point to a toxic concentration of a certain benzodiazepine in a patient.

It is important to point out that TLC screening detected 25.1% samples with unknown substances that are impossible to confirm with certainty, and only 2.6% of such samples were detected with GC-MS screening (mostly due to the fact that we did not have some of the substances in the internal database yet).

Only a few requests for screening confirmation were received since clinicians are provided with more information from GC-MS screening than from TLC screening, especially when a sample contains several drugs.

From September 2014 to May 2015, 436 targeted screenings were performed: for benzodiazepines (59.9% were positive), 147 for opiates (4.8% were positive), 53 for methadone (24.5% were positive), 54 for cocaine (1.9% were positive), 77 for amphetamines (8.5% were positive), and 150 for THC (18.1% were positive).

Although the number of samples for general toxicological screening decreased by 32%, the number of samples without any substance found still remained similar and relatively high (38.6%). The same phenomenon was observed for targeted screening. These data point at the importance of communication between clinicians and laboratory in order to reduce the number of unnecessary screening tests. It is also very important that the analyst receive information regarding symptoms, substances taken (if the clinician has that information from the patients themselves or from someone else) or substances administered during emergency medical services. Such information can reduce the number of unnecessary general screenings and can direct targeted screening, which is both cheaper and faster. In case of, for example, synthetic cannabinoides, it can rule out screening because it is not possible to detect these substances with current equipment.

CONCLUSION

Although GC-MS analysis is time-consuming, more expensive and a demanding method, it provides clinicians with more information than TLC. The Department of Laboratory Diagnostics at the University Hospital Centre Zagreb demonstrated that GC-MS can be successfully used for toxicological screening 24 hours a day. GC-MS screening significantly lowered the number of samples with unknown substances that are impossible to prove with certainty. The Department of Laboratory Diagnostics at the University Hospital Centre Zagreb is the only laboratory in Croatia that performs toxicological screening 24 hours a day during the whole year on GC-MS. This method introduction was a very brave and demanding step. Currently there are 15 specialists performing toxicological screenings. They continue to learn since every new toxicological case is material for discussion. It is very important to be aware of the limitations of the methods used for screening and to introduce them to clinicians. On the other hand, it is also very important for the laboratory to receive information regarding the patient status, symptoms, drugs a patient is taking or has received during emergency medical services. This valuable information can guide toxicological analysis in the right direction.

Table 1. Detection time for some substances

SUBSTANCE	DETECTION TIME UP TO
Amphetamine-Type Stimulans	
Amphetamine	3 days
Methamphetamine	3 days
Methylenedioxyamphetamine (MDA)	2 days
Methylenedioxymetamphetamine (MDMA)	2 days
Benzodiazepines	
Long-acting (Diazepam, Nordiazepam)	10 days
Intermediate-acting (Alprazolam, Lorazepam, Oxazepam, Temazepam, Chlordiazepoxide, Clonazepam, Flunitrazepam)	5 days
Short-acting (Triazolam, Flurazepam)	2 days
Cocaine and metabolite	
Cocaine	<1 day
Benzoylecgonine	5 days
THC	
Single use	3 days
Moderate use (4 times per week)	5 days
Heavy use (daily)	10 days

Chronic heavy use	30 days
Methadone, EDDP (methadone metabolite)	7 days
Opiates	
6-MAM	1 day
Morphine	3 days
Codeine	3 days

6-MAM - 6-Monoacetylmorphine
EDDP - 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
THC – tetrahydrocannabinol

Table 2. Substance groups found in toxicology screening

SUBSTANCE GROUP	September 2013-April 2014 TOXICOLOGICAL SCREENING (TLC)	September 2014-April 2015 TOXICOLOGICAL SCREENING (GC-MS)
analgoantipyretic	7	16
antiarrhythmic		4
anticholinergic	1	1
antidepressant	1	27
antiemetic		4
antiepileptic	14	26
antihistaminic	2	1
antihypertensive	2	
antiparkinsonic		2
antiplatelet drug		3
antipsyhotic	11	39
antitussic		2
anxiolytic and sedative hypnotic (benzodiaz-epine)	40	32
barbiturate, anesthetic		1
beta blocker		18
bronchodylatator		6
diuretic		1
H2 blocker	1	
6-monoacethylmorphine (heroin metabolite)		1
hypnotic	1	6
illicit drug	9	4
local anesthetic	11	53
NSAID	2	17
analgesic, antitussic opiate	1	2
opiate analgesic		2
sinthetic opioid analgetic	19	
synthetic opioid narcotic	7	7
tetracyclic antidepressant	13	
tricyclic antidepressant	5	

GC-MS - gas chromatography - mass spectrometry, NSAID - nonsteroidal anti-inflammatory drug, TLC - thin layer chromatography

Table 3. Some details of toxicological screening results

	September 2013-April 2014 ŽTOXICOLOGICAL SCREENING (TLC)	September 2014-April 2015 TOXICOLOGICAL SCREENING (GC-MS)
TOTAL NUMBER OF TOXICOLOGICAL SCREENING SAMPLES	343	233
NUMBER OF SAMPLES WITHOUT ANY SUBSTANCE	125 (36.4%)	90 (38.6%)
NUMBER OF SAMPLES WITH ONLY ONE SUBSTANCE	80 (23.3%)	15 (6.4%)
NUMBER OF SAMPLES WITH MORE THAN ONE SUBSTANCE	52 (15.2%)	120 (51.5%)
NUMBER OF SAMPLES WITH UNKNOWN SUBSTANCE	86 (25.1%)	6 (2.6%)
NUMBER OF SAMPLES WITHOUT ANALYSIS (SHORT SAMPLE VOLUME)		2 (0.9%)
TOTAL NUMBER OF DIFFERENT SUBSTANCES FOUND	32	55

GC-MS - gas chromatography - mass spectrometry
TLC - thin layer chromatography

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