SOHAG MEDICAL JOURNAL
Vol. 22 No.1 Jan 2018

Review on Collection , Preservation and Fowarding of Biological Samples for Toxicological Analysis

Prof. Dr. Maha A. Hilal [1], Dr . Essam M. Abdullah[2] , Dr. Reda M. El-Sayed[2], Hend M. A. Salman[3]

1. Prof. of Forensic Medicine and Clinical Toxicoogy, Faculty of Medicine - Sohag University.
2. Lecturer of Forensic Medicine & Clinical Toxicoogy, Faculty of Medicine - Sohag University.
3. Demonstrator in the Department of Forensic Medicine & Clinical Toxicoogy, Faculty of Medicine - Sohag University.

Abstract

Forensic Toxicology is the study and practice of the application of toxicology to the purposes of the law. The relevance of any finding is determined, in the first instance, by the nature and integrity of the specimen(s) submitted for analysis. This means that there are several specific challenges to select and collect specimens for *ante-mortem*
and *post-mortem* toxicology investigation. *Post-mortem* specimens may be numerous and can endow some special difficulties compared to clinical specimens, namely those resulting from autolytic and putrefactive changes. Storage stability is also an important issue to be considered during the pre-analytic phase, since its consideration should facilitate the assessment of sample quality and the analytical result obtained from that sample. The knowledge on degradation mechanisms and methods to increase storage stability may enable the forensic toxicologist to circumvent possible difficulties. Therefore, advantages and limitations of specimen preservation procedures are thoroughly discussed in this review. Presently, harmonized protocols for sampling in suspected intoxications would have obvious utility. In the present review an overview is given on sampling procedures for routinely collected specimens as well as on alternative specimens that may provide additional information on the route and timing of exposure to a specific xenobiotic. Last, but not least, how to forward a correct report and how to interpret a toxicological results is provided. This comprehensive review article is intented as a significant help for forensic toxicologists to accomplish their frequently overwhelming mission.

Key words: sampling , collection, Preservation, toxicological analysis.

Introduction

Forensic toxicology is the study and practice of toxicology to the purposes of the law. The relevance of any finding is determined, in the first instance, by the nature and integrity of the specimen(s) submitted for analysis. This means that there are several specific challenges to select and collect specimens for ante-mortem and post-mortem toxicology investigations. Post-mortem specimens may be numerous and can endow some special difficulties compared to clinical specimens, namely those resulting from autolytic and putrefactive changes. Storage stability is also an important issue to be considered during the pre-analytic phase, since its consideration should facilitate the assessment of sample

quality and the analytical result obtained from that sample ***(Dinis-Oliveira et al., 2010) .***

and postmortem blood samples are notably different, and the site of the Antemortem postmortem blood draw (central (25ml) or peripheral (10-20ml)) can be of critical importance. . Antemortem blood is collected by venepuncture, typically from the antecubital region of the arm (10-20ml) ***(Karinen et al., 2010).***

In most postmortem cases, blood remains the single most important specimen to analyze. ***(SOFT / AAFS, 2006 and Flanagan et al., 2005).***

Aim of the work

The present study aims at:

1. Discussing specimen selection considerations.
2. The guidelines for the collection of samples for toxicological analysis.
3. Interpretation of forensic toxicological investigations.

Principles for sample dealing

The first step in the specimen collection process (including evidence collection) is to ensure that the specimen containers are labeled appropriately. It is essential that the sample and any intermediate containers used to carry it are labeled in sufficient detail to eliminate any doubt about the sample origin ***(Moffat et al., 2013).***

self-adhesive tamper-resistant stickers should be placed over both the specimen container and transport container lids to document specimen integrity ***(Helper and Isenschmid , 2008).***

The tape seal shall bear collector's initials and the collection date. A unique identification number that accompanies the sample at all stages is a valuable safeguard ***(Moffat et al., 2013).***

The best container to utilize when collecting and storing biological fluids or tissue specimens is glass in appropriate storage racks, since glass is inert and does not contain any plasticizer contaminants ***(Moffat et al. 2013; Jickells and Negrusz 2008).***

Sample Collection and Preservation

There are many types of ioxicological samples including: blood, urine,gastric contents, vitreous humor, bile, C.S.F., sweat, saliva, tissues (liver, lungs, kidneys, spleen, brain, muscles, bone, bone marrow and keratinized- tissues).

Blood is one of the most important specimens of toxicological interest as it provides unique advantages over other matrices in terms of the wide variety of analytical methodologies available ***(Kerrigan, 2008).***

Blood specimens are also useful in cases of attempted or accidental poisoning by gases and volatile organic compounds ***(Stimpfl et al., 2007).***

Antemortem blood is collected by venepuncture, typically from the antecubital region of the arm, using a syringe or evacuated container (e.g. Vacutainer, Venoject) ***(Karinen et al., 2010).***

In Post-mortem, whole blood is the specimen of choice for detecting, quantifying, and interpreting xenobiotic (XB) concentrations ***(Leikin and Watson, 2003; Baselt, 2008).***

Urine represents one of the major routes to eliminate XBs from the body.

It is mostly used as a screening specimen (thought it is not always available), e.g. in death related to drugs of abuse and prescribed medication as well as in apparent accidental death where impairment is suspected ***(Drummer and Gerostamoulos, 2002).***

In antemortem settings, a mid-stream urine sample is usually collected into a plastic container containing sodium

fluoride as preservative ***(Dinis-Oliveira et al., 2010).***

In postmortem settings, urine is collected by insertion using a hypodermic syringe directly into the bladder under visualization. Urine is a valuable specimen for both antemortem and postmortem drug testing because it is a relatively uncomplicated matrix ***(Kerrigan, 2008)***.

Stomach contents may include vomit, gastric aspirate and stomach washings it is important to obtain the first sample of washings, since later samples may be very dilute. A volume of at least 20 ml is required to carry out a wide range of tests. This can be a very variable sample and additional procedures such as homogenization followed by filtration and/or centrifugation may be required to produce a fluid amenable to analysis. If obtained soon after ingestion, large amounts of poison may be present while metabolites, which may complicate some tests, are usually absent. It may be possible to identify tablets or capsules simply by inspection. ***(WHO, 1995).***

Sampling of gastric content is usually performed during autopsy. The stomach should be opened away from other specimens and tissues to avoid contamination of other viscera ***(Helper and Isenschmid, 2008).***

Vitreous humor is obtained by direct gentle aspiration from each eye using a 5–10-mL syringe and a 20-gauge needle. The needle should be inserted through the outer corner (just above the junction between the upper and lower eyelids), until its tip is placed centrally in the globe (***(Helper and Isenschmid, 2008).***

2–3 mL of fluid can be removed from each eye in an adult, while up to ~ 1 mL of specimen may be removed from a newborn ***(Coe, 1993).***

Specimens obtained from both eyes are usually combined in one properly labelled specimen container, but different opinions on this procedure exist ***(Helper and Isenschmid, 2008).***

Bile is generally aspirated from the gallbladder using a hypodermic syringe. It may be necessary to tie off the gallbladder prior to collection if contamination appears to be an issue. Bile should be collected prior to the liver specimen to avoid contamination ***(Kerrigan, 2008).***

Cerebrospinal fluid (CSF) may also be useful as an alternative material in cases of multiple traumas where an adequate blood sample is not available ***(Tominaga et al., 2015).***

Cerebrospinal fluid (CSF) can be collected either by lumbar puncture at the base of the spine using a hypodermic syringe or by withdrawal of cisternal fluid by puncturing the base of the neck ***(Kerrigan, 2008) .***

A variety of methods are available for oral fluid collection, including spitting, draining, suction, and collection on various types of absorbent swabs ***(Gallardo and Queiroz, 2008).***

Mostly all tissue Samples are only post-mortem except for keratinized tissues. The liver has been ranked as the primary solid tissue for use in *post-mortem* toxicology, and often the XB analysis, resulting from this tissue, complements the blood toxicology data ***(Gronewold et al., 2009).***

High concentrations of XBs are frequently found in lung tissue, especially after poisoning by intravenous or inhalatory routes. Depending on the properties of a XB, concentrations in lung tissue can be higher than in liver ***(Dinis-Oliveira et al., 2010).***

The specimen should be placed in a glass specimen jar or nylon bag (volatile substance abuse-related deaths) and deep- frozen prior to

transport to the laboratory ***(Flangan et al., 2005).***

A kidney specimen can be a useful sample for XB identification since most XBs and metabolites are excreted into urine and therefore will pass through the kidneys. It is particularly important in cases of heavy metal poisonings due to its capacity of accumulation of these XBs ***(Triunfante et al., 2009).***

Brain tissue is lipid rich and has a tendency to concentrate some drugs, particularly lipophilic analytes, narcotics and halogenated hydrocarbons ***(Skopp, 2004).***

Hair is a unique material for the retrospective investigation of chronic exposure since it provides a longer window of detection compared to blood or urine, and is less invasive to collect. Long scalp hair may provide retrospective information of the previous 5–7 years ***(Daniel et al., 2004).***

The hair should be collected from the posterior vertex region of the scalp or the back of the skull, where the average hair growth rate is fairly constant ***(Henderson, 1993).***

Post-mortem, it is strongly recommended to take a hair sample prior to autopsy. The hair sample should be firmly tight together and tied with cotton, before being cut as close as possible to the scalp, making sure the scissors are leveled with the scalp ***(Flanagan et al., 2007).***

Drug detection in different biological samples:

Drug detection in urine

a) Screening Methods:

- **Preliminary tests:**

The immunoassay drug tests, which are designed to classify substances as either present or absent according to a predetermined cutoff threshold, remain the most common methods used in clinical care. Immunoassays are based on the principle of competitive binding, and use antibodies to detect the presence of a particular drug or metabolite or class of drugs or metabolites in a urine sample ***(HHS. MRO, 2004).***

- **Confirmatory Tests:**

Generally, a more definitive laboratory-based procedure (eg, GC/MS, LC/MS) to identify specific drugs and/or their metabolites is needed in 3 instances:

(1) to specifically identify the drug where class alone is insufficient; for example, that it actually is prescribed morphine that is accounting for the positive immunoassay class response (rather than some other opioid or cross-reacting substance);

(2) to identify drugs not otherwise included in other tests; and

(3) when results are disputed by the patient (ie, contested results) **(ASAM, 2013 and Pesce et al., 2012).**

b) Quantitative Methods

Positive results must be confirmed by a more specific method **(HHS, 2010)**.

1- Gas Chromatography/ Mass Spectrophotometry (GC/MS) (all substances).

2- Gas Chromatography/ Nitrogen Phosphorous Detector (GC/NPD) (e.g., methadone, cotinine).

3- Gas Chromatography/ Flame Ionization Detector (GC/FID) (Alcohol).

4- Capillary electrophoresis with UltraViolet detection (CE-UV) .

5- High-Performance Liquid Chromatography with Diode-Array Detection (HPLC- DAD) (e.g., benzodiazepines).

6- High-Performance Liquid Chromatography with Mass Spectrometric Detection HPLC-MS (all substances) ***(SCDAT, 2012).***

Interpretation Of Urine Drug Testing (UDT) Results:

UDT in clinical practice, like any other medical test, should be

performed to improve patient care ***(Gourlay and Heit, 2009).***

Consultation with an individual knowledgeable in UDT interpretation (eg, laboratory director or toxicologist) is strongly encouraged, especially when unexpected test results are obtained ***(Casavant, 2002).***

Drug detection in blood/serum

Where forensic investigations are concerned, generally it does not suffice to test urine for drugs by means of an immunoassay, a quantitative determination of the drugs in blood/serum must be performed following qualitative urine analysis ***(SCDAT, 2012).***

Drug detection in gastric contents

Qualitative drug screening of gastric contents may prove helpful in carefully selected cases involving relatively acute ingestion of unknown agents ***(Kellermann et al., 1988).***

The HPLC method appears to be useful in the screening of gastric material ***(Politi et al., 2004).***

Drug detection in vitreous humor

The analysis of drugs in vitreous humor is similar to the analysis in other postmortem fluids. Detection systems, such as gas chromatography, gas chromatography/mass spectrometry, liquid chromatography, and liquid chromatography/mass spectrometry have all been used to identify and quantify drugs in vitreous humor **(Levine and Jufer ,2008).**

Toxicological report forwarding:

Standard practices before reporting:

1. Validation.
2. Quality assurance and quality control.

Validation

Validation is the process of performing a set of experiments that reliably estimates the efficacy and reliability of an analytical method or modification to a previously validated method ***(SWGTOX, 2013).***

The principal elements of validation according to Cooper et al. (2010),

a. Selectivity;
b. Calibration model (Linearity);
c. Precision and Accuracy;
d. Upper limit of quantification;
e. Limit of detection.
f. Stability ;
g. Recovery;
h. Ruggedness (robustness);
i. Matrix effect.

Quality assurance and quality control:

Quality assurance encompasses all aspects of the analytical process, from specimen collection and reception through analysis, data review and reporting of results. It includes, but should not be limited to, quality control of each analysis and proficiency testing of the laboratory. One purpose of a quality assurance program is to detect error, whether random or systematic, and to initiate appropriate remedial action ***(SOFT / AAFS Forensic Laboratory Guidelines , 2006).***

Quality control refers to step taken to ensure and monitor precision and accuracy of test results. Quality control practices include the analysis of quality control samples with each set of samples. These include calibration standards, certified reference materials, spiked samples, duplicate sample analysis, and blanks. ***(UC Davis analytical laboratory, 2010).***

Reporting of results

The purpose of the laboratory is to produce the results of examinations in reports that are correct, timely, unambiguous and clinically useful.

The following recommendations are made in the report according to ***Cooper et al. (2010),***

1) Name and/or identification number;
2) Laboratory identification number;
3) Name of submitting agency and/or individual;

4) Date submitted;
5) Date of specimen collection;
6) Details transcribed from any ante-mortem sample(s);
7) Date of report;
8) Specimens tested;
9) Test results;
10) Signature of approving individual; and
11) A phrase such as 'Unless the laboratory is informed otherwise in writing, samples will be disposed.

Conclusion

In conclusion, There are several important issues when considering the suitability of a sampling approach to fulfill the needs of toxicity testing . Those relate to the challenge of collecting sufficient sample amounts. In addition, the preservative used for each poison that doesn't interact or influence the analyte. However much useful information can be obtained by the thoughtful analysis of samples obtained at examination and the interpretation of results obtained. Great advances that have been made regarding the methodology for analytical toxicology, laboratory accreditation, the training and certification of analytical toxicology laboratory staff, and in the knowledge of factors influencing the interpretation of analytical results. Adequate written procedures must be in place and appropriate training provided.

References

1. ***A White Paper of the American Society of Addiction Medicine (ASAM) (2013):*** Drug testing in addiction treatment. Current Applications of Drug Testing and Promising New Opportunities. Vol.IV:46-55.

2. ***Baselt, R.C. (2008):*** Peripherally-acting drugs and common toxic chemicals. Disposition of Drugs and Toxic Chemicals in Man. 8th ed. Vol.2. Foster City, CA: Biomedical Publications: 560-1690.

3. ***Casavant, M.J.(2002):*** Urine drug screening in adolescents. Pediatr. Clin. North Am.,49: 317-327.

4. ***Coe, J.L. (1993):*** Postmortem chemistry update. Emphasis on forensic application. Am. J. Forensic Med. Pathol., 14:91–117.

5. ***Cooper, G.A.A.; Sue Paterson and Osselton, M.D.(2010):*** The United Kingdom and Ireland Association of Forensic Toxicologists . Forensic toxicology laboratory guidelines. Science and Justice. 50:166–176.

6. ***Daniel, C.R.; Piraccini, B.M. and Tosti, A. (2004):*** The nail and hair in forensic science. J. Am. Acad. Dermatol., 50:258–261.

7. ***Dinis-Oliveira, R.J.; Carvalho, F.; Duarte, J. A.; Remião, F., Marques, A.; Santos, A. and Magalhães, T. (2010):*** Collection of biological samples in forensic toxicology, Toxicology Mechanisms and Methods, 20 (7): 363-414.

8. ***Drummer, O.H. and Gerostamoulos, J. (2002):*** Postmortem drug analysis: analytical and toxicological aspects. Ther Drug Monit., 24:199–209.

9. ***Flanagan, R.J. and Connally G. (2005):*** Interpretation of analytical toxicology results in life and at postmortem. J. Toxicol Rev., 24(1):63-71.

10. ***Flanagan, R.J.; Taylor, A.; Watson, I.D. and Whelpton, R. (2007):*** Sample collection, transport and storage. Fundamentals of Analytical Toxicology.Ch.2, West Sussex: John Wiley & Sons Ltd: 21-47.

11. ***Gallardo, E. and Queiroz, J.A. (2008):*** The role of alternative specimens in toxicological analysis. Biomed. Chromatogr., 22:795–821.

12. ***Gourlay, D. L. and Heit, H. A. (2009):*** Commentary on unexpected urine drug testing results in a hospice

patient on high dose morphine therapy. Clinical Chemistry, 55(10), 1767-1769.

13. ***Gronewold, A.; Dettling, A.; Haffner, H.T. and Skopp, G. (2009):*** Doxepin and nordoxepin concentrations in body fluids and tissues in doxepin associated deaths. Forensic Sci. Int., 190:74–79.

14. ***Health and Human Service /Medical Review Officer (HHS. MRO.), (2004):*** Urine drug testing. Medical Review Officer Manual for Federal Agency Workplace Drug Testing Programs. Ch.3. 6-19.

15. ***_Helper, B.R. and Isenschmid, D.S. (2008):*** Specimen selection,collection,preservation and security. In: Karch, S.B. Editor. Postmortem Toxicology of Abused Drugs. 1st edition.Ch.2, Boca Raton, FL: CRC Press:13-31.

16. ***Henderson, GL. (1993):*** Mechanisms of drug incorporation into hair. Forensic Sci Int., 63:19–29.

17. ***Jickells, S. and Negrusz, A.; Moffat, A.C.; Osselton, M.D.; Widdop, B.; (2008):*** Introduction to forensic toxicology. In: Jickells, S. and Negrusz, A. (eds.). Clarke's Analytical Forensic Toxicology, 3rd. edition. Ch.1. London:Pharmaceutical Press. Vol.1: 1-11.

18. ***Karinen, R.; Oiestad, EL.; Andresen, W.; Wethe, G.; Smith-Kielland, A. and Christophersen, A. (2010):*** Comparison of ethanol and other drugs of abuse concentrations in whole blood stored in Venoject glass and plastic and Venosafe plastic evacuated tubes. J. Anal. Toxicol., 34: 420–428.

19. ***Kellermann, A.L.; Fihn, S.D.; Logerfo,J.P. and Copass, M.K.(1988):*** Utilization and yield of drug screening in the emergency department. American Journal of Emergency Medicine. 6 (1): 14-20.

20. ***Kerrigan, S. (2008) :*** Sampling, storage and stability. In : Jickells, S. and Negrusz, A. (eds.). Clarke's Analytical Forensic Toxicology, 1st edition. vol. 1. ch.13. London , Pharmaceutical Press: 335-356.

21. ***Leikin, JB. and Watson, WA. (2003):*** Post-mortem toxicology: what the dead can and cannot tell us. J. Toxicol. Clin. Toxicol., 41:47–56.

22. ***Levine, B. and Jufer, R. (2008):*** Drugs of abuse testing in vitreous humor. In: Jenkins, A.J. and Caplan, Y.H. (eds.). Drug Testing in Alternate Biological Specimens. ch.7.USA, Humana Press: 117-128.

23. ***Malamud, D. and Tabak, L. (1993):*** Saliva as a diagnostic fluid. Ann. NY. Acad. Sci., 694:11–12.

24. ***Moffat, A.C.; Osselton, M.D. and Widdop, B. (2013):*** Introduction to forensic toxicology. In: Negrusz, A. and Cooper, G. Clarke's Analytical Forensic Toxicology. 2nd edition. vol.1. ch.1. London: Pharmaceutical Press:1-10.

25. ***Pesce, A.; West, C.; Egan, C.K. and Strickland, J.(2012):*** Interpretation of urine drug testing in pain patients. Pain Med.,13:868-885.

26. ***Politi, L. ; Groppi, A.; Polettini, A. and Montagna M.(2004):*** A rapid screening procedure for drugs and poisons in gastric contents by direct injection-HPLC analysis. Forensic Science International, 141 : 115–120.

27. ***Skopp, G.(2004):*** preanalytic aspect in postmortem toxicologicy Forensic Sci. Int.; 142:75-100.

28. ***Society of Forensic Toxicologists/American Academy of Forensic Sciences (SOFT/AAFS) (2006):*** Forensic Toxicology Laboratory Guidelines.1-24 . www.soft-tox.org.

29. ***Stimpfl, T.; Muller, K.; Gergov, M.; LeBeau, M.; Polettini, A.; Sporkert, F. and Weinmann W.(2007):*** Recommendations on Sample

Preparation of Biological Specimens for Systematic Toxicological Analysis. TIAFT – The International Association of Forensic Toxicologists . TIAFT-Bulletin 41 (2): 1-11.

30. ***Swiss Committee for Drugs of Abuse Testing(SCDAT)(2012):*** Guidelines for Drugs of Abuse Testing., 1-62.

31. ***Tominaga, M.; Michiue, T.; Ishikawa, T.; Inamori-Kawamoto, O.; Oritani, Sh. and Maeda, H. (2015):*** Evaluation of postmortem drug concentrations in cerebrospinal fluid compared with blood and pericardial fluid. Forensic Science International, 254 : 118–125.

32. ***Triunfante, P.; Soares, M.E.; Santos, A.; Tavares, S.; Carmo, H. and Bastos Mde, L. (2009):*** Mercury fatal intoxication: two case reports. Forensic Sci. Int., 184:1–6.

33. ***University of California (UC) Davis analytical laboratory(2010):*** Introduction and over view. Quality Manual. Rev.0004.02: 3-18

34. ***World Health Organization (1995):*** International program in chemicalsafty,BasicAnalyticalToxicology,http://www.who.int/ipcs/publications/training_poisons/_toxicology.