TIAFT – The International Association of Forensic Toxicologists

Committee of Systematic Toxicological Analysis

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Recommendations on Sample Collection

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ISSUE

Sampling is of the utmost importance for a successful systematic toxicological analysis (STA). The reliability and accuracy of any toxicological result is usually determined by the nature and integrity of the specimen(s) provided for analysis. Furthermore, proper specimen selection and collection is of paramount importance for the analytical results to be accurately interpreted with scientific validity, particularly when the results are to be used in the judicial system. These guidelines address the issue of sampling for forensic toxicological analyses by providing recommendations of specimen types, amounts that should be collected and submitted to laboratories expected to perform STA, and criteria for ensuring quality assurance in sample collection.

BACKGROUND

STA is the application of an adequate analytical strategy for the identification of potentially toxic compounds and their metabolites in biological samples. Generally speaking, this involves the identification of a "general unknown", as opposed to the detection of common drugs or metabolites from a finite list.

As early as 1922, Jansch recognized that in cases of suspected poisoning the collection of insufficient amounts of appropriate tissue specimens and the use of unsuitable containers would lead to unreliable results (1). This remains the case today.

There are thousands of potentially toxic or lethal drugs and chemicals available for abuse or poisoning. Therefore, it is important that relevant case histories and the best available specimens be provided to the toxicologist in cases involving both living subjects and decedents when a case requires STA.

In forensic toxicology, the purpose of sampling is to provide a representative part of the whole that is suitable for screens and confirmations, affords reliable interpretation, and, when possible, allows for subsequent reanalysis, if required. Given this, it should be recognized that sampling is case-dependent (2). The recommendations that follow are specific to cases in which STA is to be performed. This occurs when the case details suggest a non-routine drug or poison may be involved, requiring a thorough toxicological work-up to be employed.

RECOMMENDATIONS

1.0 - SPECIMENS FROM LIVING SUBJECTS

STA is typically required on specimens from living subjects in cases of drug-facilitated crimes or attempted poisonings. The limitations in the variety of specimens available from living subjects can challenge the toxicologist attempting to identify an unknown substance in these cases. In all situations, samples should be collected as quickly as possible and properly labeled with a means of readily tracking the specimen (e.g., full name of the victim, date/time of collection, initials of the person collecting the sample). The best containers for liquid samples are disposable hard plastic or glass tubes. When a preservative is needed, sodium fluoride should be used at a concentration of approximately 2% weight by volume. Unless otherwise noted, specimens must be maintained at temperatures not greater than 4ºC to ensure sample integrity.

1.1 Urine.

The best specimen for comprehensive drug and poison screening is urine (3). The accumulation of drugs, poisons, and metabolites in urine will often result in high concentrations that facilitate their detection. A minimum of a 30-ml sample of urine is required for thorough screening of this specimen (4). The addition of fluoride as a preservative is encouraged, but not always required.

1.2 Blood.

The specimen of choice for quantifying and interpreting concentrations of drugs and their metabolites is blood. At a minimum, a 10 to 15-ml sample preserved with sodium fluoride is recommended. An additional 5-ml blood sample placed into a collection tube containing a clot activator and K2EDTA may also prove useful in cases of suspected fluoride poisoning and for obtaining a plasma sample (after centrifugation), if so desired. In addition to confirmation and quantitation of drugs, poisons, and metabolites initially detected in the urine sample, blood specimens are also useful in cases of attempted or accidental poisoning by gases and volatile organic compounds. Maintaining a frozen fraction of blood may help ensure better analyte stability in later re-analyses.

1.3 Hair.

When there has been a significant delay between suspected exposure to a drug or poison and reporting to law enforcement, one of the most useful specimens for STA is a sample of hair3. Approximately 100-200 mg of hair should be collected from the vertex posterior on the back of the head by cutting as close to the scalp as possible, ensuring that it is clearly marked which end is closest to the scalp and appropriately securing the hair into a bundle with a rubber band, twist tie, or string. The hair sample may then be placed into aluminum foil, an envelope, or plastic collection tube and stored at room temperature until analysis.

2.0 - POSTMORTEM SPECIMENS

More so than in any other investigations, cases of mysterious deaths tend to call upon the forensic toxicologist to perform an extensive STA. The availability of autopsy specimens in postmortem toxicology allow for a more flexible analytical approach to the analysis, although some specimens have more value than others when specific drugs or poisons are involved in the death. However, it should be noted that the autolysis and putrefactive

processes that occur after death, as well as postmortem redistribution, can have a profound effect on drugs and poisons that are present in the body prior to death.

Postmortem specimens should be collected as quickly as possible (2). All samples must be collected in separate containers. For most specimens, disposable hard plastic or glass tubes are recommended.5 Each sample must be labeled with the full name of the deceased, specimen type, collection site, date/time of collection, and initials of the individual collecting the sample. Samples should be stored at a maximum of 4ºC when analyzed promptly after autopsy, otherwise at -20ºC. When liquid specimens are to be frozen, it is recommended to leave a small (10-20%) headspace in the specimen tubes (5).

2.1 Blood.

In most postmortem cases, blood remains the single most important specimen to analyze. When possible, at least two blood specimens should be collected: a) 30 ml of central blood (from the right atrium of the heart, inferior vena cava, or another convenient large vessel) for qualitative analysis and b) 10 ml peripheral blood (preferably from the left and right femoral veins) by direct vascular access (not "milking" the vessels once the abdominal cavity has been opened) for quantitative analysis (4, 5). Both samples should be preserved with sodium fluoride/ potassium oxalate, unless suspicion of poisoning with fluoride or a fluoride-producing compound exists.

2.2 Urine.

The advantages of urine in postmortem cases is similar to its advantage in specimens collected from living patients - many drugs and metabolites are present in higher concentrations in urine than in blood and they remain in urine for days or longer.

A disadvantage of urine occurs in instances in which death occurs very rapidly after exposure to a drug or poison. In these cases, the urine specimen may be negative for the causative agent, so caution must be used in evaluating results from this specimen. In most cases, all available urine should be collected in postmortem cases (3). The use of fluoride as a preservative is encouraged.

2.3 Vitreous Humor.

The fact that vitreous humor resides in an anatomically isolated and protected area of the body (behind the lens of the eye), coupled with its good stability as a biological fluid, makes this specimen more resistant to putrefactive changes than other postmortem specimens (6). All available vitreous fluid from each eye should be collected separately (5).

2.3 Gastric Contents.

Oral ingestion remains the most popular means of exposure to drugs and poisons. Therefore, gastric contents are essential for screening tests. All of the available sample should be collected without the addition of a preservative. Undigested pills and tablets should be separated and placed into plastic pillboxes for analysis (3).

2.5 Bile.

In postmortem cases in which urine samples are not available, bile may be substituted. Many drugs and poisons have been shown to concentrate in bile (e.g. narcotics, benzodiazepines, heavy metals). When it is collected, all available bile should be removed from the gall bladder and preserved with sodium fluoride (2% w/v). To avoid fermentation of this specimen, it should be stored at temperatures of at least -20ºC (5).

2.6 Cerebrospinal Fluid.

Cerebrospinal fluid is a useful specimen for the screening of drugs with their site of action in the central nervous system. It is also less likely to be contaminated or have bacterial infiltration due to its protected environment. While there are only a few published references to assist in the interpretation of drug concentrations in cerebrospinal fluid, reference values are documented for urea and creatinine. It is also a useful specimen for the detection of glucose and lactate in cases of hypoglycemia.

2.7 Tissues.

Tissue samples collected in postmortem investigations generally provide supplemental information to the toxicologist to assist in interpretation of their results. In STA, analysis of the correct tissue specimen may be vital to the identification or confirmation of an unknown causative agent. All tissue specimens should be submitted as unfixed samples and frozen until analysis (2).

2.7.1 - Liver.

In most postmortem toxicology cases, a liver sample is usually the most valuable tissue specimen (6). Since most drugs and poisons are metabolized in the liver, both the parent compound and its metabolites may be present in high concentrations in this tissue. Additionally, quantitative analysis of a liver specimen may assist in differentiating acute overdoses from therapeutic use of drugs that have a narrow therapeutic index. To minimize the effect of drug diffusion from the small bowel and stomach on quantitative findings, the sample should be from deep within the right lobe of the liver (5). Approximately 25-50g of tissue should be collected.

2.7.2 - Brain.

Since the brain is the primary site of action of many drugs, it becomes a useful specimen particularly for lipophilic substances such as halogenated hydrocarbons, narcotics, and antidepressants (2). While drug and poison concentrations may have significant variance in different regions of the brain, current data do not suggest that one portion should be collected over another. When collected, a minimum of 25g of unfixed brain should be provided.

2.7.3 - Lung.

The lungs often contain high concentrations of drugs and poisons in cases of inhalation and intravenous exposure. The apex of either lung (25-50g) is the best choice of specimen for STA.

2.7.4 - Kidney.

Since most drug and poison metabolites must pass through the kidney to be excreted in the urine, this specimen can be valuable in STA. The kidney has been found to be particularly useful in heavy metal poisonings. A minimum of a 25g sample of kidney is recommended for submission.

2.7.5 - Subcutaneous Fat and Skeletal Muscle.

In certain cases, subcutaneous fat and skeletal muscle may prove useful to STA. This is particularly true when the postmortem examination reveals apparent injection sites that may still contain a region of concentrated drug or poison. Collection should include the skin and fat layer, in addition to the muscle tissue around the site. Additionally, a control sample from a comparable area away from the suspected injection site should also be collected.

2.8 Hair and Nails.

Keratinized specimens such as nails and hair may be used to test for chronic exposure of heavy metals, drugs, or other poisons. These specimens allow for an assessment of exposure weeks to months prior to collection. The best collection site for hair is from the vertex posterior on the back of the head (7). It is important to label the hair with a rubber band, twist tie, or string to clearly differentiate the end that is closest to the scalp. Strands of hair (approximately 200 mg) should be cut as close to the scalp as possible and placed in aluminum foil, an envelope, or plastic collection tube. Storage at ambient temperature is sufficient. Whole nails should be removed from the fingers and/or toes and stored in a manner similar to hair samples.

2.9 Insect Larvae.

Entomological specimens collected from human remains may be useful for STA; however, it should be noted that the concentrations of drugs and poisons in these specimens is dependent on the tissue that the larvae had fed upon and their developmental stage (2).

2.10 Scene Samples.

In many cases, the evidence found at the scene provides the best guidance to the toxicologist performing STA. Collection of drug paraphernalia, cups or bottles, and suspicious household products will help ensure that a thorough toxicological analysis is performed.

SUMMARY

Specimen acquisition is often the most critical, yet overlooked component of STA. It is usually impossible to acquire an additional sample of equal quality after the first opportunity for collection has passed. Therefore, the Committee of Systematic Toxicological Analysis of The International Association of Forensic Toxicologists strongly endorses the collection of appropriate specimens in sufficient amounts to allow for a thorough and efficient approach to identifying unknown substances in biological specimens.

REFERENCES AND ADDITIONAL READINGS

1. Jansch H (1922) Beitr. Gerichtl. Med. 4:55-88.
2. Skopp G (2004) Forensic Sci. Int. 142:75-100.
3. Levine B (2006) Principles of Forensic Toxicology. American Association for Clinical Chemistry, Washington, DC.
4. Society of Forensic Toxicologists/American Academy of Forensic Sciences. Forensic Toxicology Laboratory Guidelines. 2006. www.soft-tox.org.
5. Flanagan RJ, Connally G, and Evans JM (2005) Toxicol. Rev. 24:63-71.
6. Drummer OH and Gerostamoulos J (2002) Ther. Drug Monit. 24:199-209.
7. Kintz P (2004) Forensic Sci. Int. 142:127-134.