TOXICOLOGY

Contents

Overview

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Introduction

Toxicology is the science of poisons, and, when applied to forensic and legal medicine, the terms forensic toxicology or analytical toxicology are often applied. A forensic toxicologist is concerned with the detection of drugs or poisons in samples and is capable of defending his/her result in a court of law. This distinction from an ordinary analytical toxicologist is important, since a conventional toxicologist is mainly concerned with the detection of substances, and may not understand the specific medicolegal requirements in forensic cases.

The process of conducting toxicology is similar to other analytical disciplines, in that sufficiently suitable analytical techniques need to be employed that are appropriately validated for use in case work. The conduct of suitable quality assurance is important to assure the analyst and clients of the quality of the result. These issues are discussed in this overview, while in other articles specific issues of techniques, specimens, and interpretation are further discussed.

Applications of Forensic Toxicology

Forensic toxicology has a number of applications. Traditionally, it is used in death investigations. It provides physicians and pathologists with information of a possible drug taken in overdose, or authorities investigating a sudden death, or poisoning, of the possible substances(s) used. Ultimately toxicology testing results will assist the client in establishing the evidence of drug use, or refuting the use of relevant drugs.

Toxicology testing is also important in victims of crime, or in persons apprehended for a crime. Drugs may have been given by the assailant to reduce consciousness of the victim, such as in rape cases. These drugs include the benzodiazepines (e.g., clorazepam,

flunitrazepam, diazepam), antihistaminics, and gamma-hydroxybutyrate (GHB). Toxicology also establishes if the victim used any drug which may have affected consciousness or behavior. Defendants arrested shortly after allegedly committing a violent crime may be under the influence of drugs. It is vital, therefore, that toxicology testing is conducted (on relevant specimens) to establish the extent of drug use, since allegations of drug use and its effect on intent or clinical state may be raised in legal proceedings. Driving under the influence of drugs is one of the main uses of toxicology testing.

Forensic toxicology is also used in employment drug testing and in human performance testing. The former category relates to the detection of drugs of abuse in persons in a place of employment, prior to being hired by an employer, or even a person in detention, such as in a prison. Human performance testing relates to the detection of drugs that may have increased (usually) performance in athletic events or may mask the use of performance-enhancing drugs. This may even apply to animals such as horses. Specimens used in these cases are usually urine, although hair is increasingly used to provide a longer window of opportunity.

Initial Tests and Confirmation

The foremost goal in forensic toxicology is the need to provide a substantial proof of the presence of a substance(s). The use of conventional gas chromatography (GC), thin-layer chromatography (TLC), or high-performance liquid chromatography (HPLC) would not normally be sufficient to accept unequivocal proof of the presence of a chemical substance. Two or more independent tests are normally required, or the use of a more powerful analytical test, such as mass spectrometry (MS) is usually preferred. Because of the need to perform a rigorous analysis, the analytical schema is often broken up into two steps. The identification stage is termed the screening or initial test, while the second analytical test is the confirmation process. The confirmation process often also provides a quantitative measure of how much substance was present in the sample; otherwise a separate test is required to quantify the amount of substance present in the specimen. In all processes it is important that no analytical inconsistency appears, or a result may be invalidated (Figure 1).

For example, in the identification of codeine in a blood specimen, an immunoassay positive to opiates is expected to be positive for codeine in the confirmation assay. The apparent detection of a drug in one analytical assay but not in another means that the drug was not confirmed, providing both assays are capable of detecting this drug. Table 1 provides a listing of common techniques used in screening and confirmation assays.

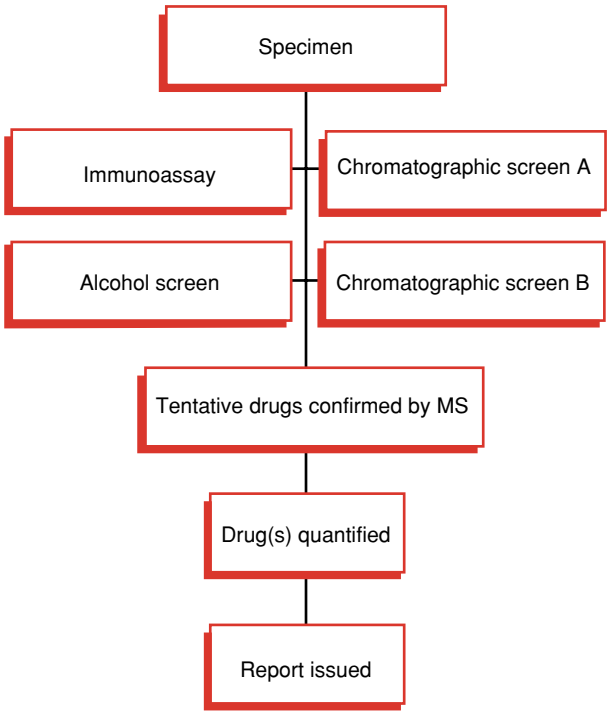

Figure 1 Schema showing identification, confirmation, and quantification processes in forensic toxicology. MS, mass spectrometry. Reproduced from Drummer OH. Toxicology: Overview. In: *Encyclopedia of Forensic Sciences*. Edited by Jay A Siegel, Pekka J Saukko and Geoffrey C Knupfer. Academic Press: London. © 2000. With permission from Elsevier.

Table 1 Screening and confirmation techniques

Screening tests	*Confirmation tests*
Immunoassays	MS (LC, GC, CE)
Spectroscopy (UV, F, etc.)	Second chromatographic test
HPLC (UV, F, ECD, CD)	HPLC (DAD)
GC (FID, NPD, TD)	AAS
CE (UV, F)	ICP-MS
AAS, colorimetric tests	

MS, mass spectroscopy; LC, liquid chromatography; GC, gas chromatography; CE, capillary electrophoresis; UV, ultraviolet; F, fluorescence; HPLC, high-performance liquid chromatography; ECD, electrochemical; CD, conductivity detection; DAD, photodiode array detector; FID, flame ionization detector; NPD, nitrogen phosphorus detector; TD, thermionic detector; AAS, atomic absorption spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry. Reproduced from Drummer OH. Toxicology: Overview. In: *Encyclopedia of Forensic Sciences*. Edited by Jay A Siegel, Pekka J Saukko and Geoffrey C Knupfer. Academic Press: London. © 2000. With permission from Elsevier.

While MS is the preferred technique for confirmation of drugs and poisons, some substances display poor mass spectral definition. Compounds with base ions at mass/charge ratios of less than 100, or with common ions such as m/z 105 and with little or no ions in the higher mass range, are not recommended

for confirmation by MS alone. Derivatization of a functional group to produce improved mass spectral properties can often be successful. Common derivatives include perfluoroacyl esters, trimethylsilyl ethers, etc. Alternatively, reliance on other chromatographic procedures can provide adequate confirmation. It is important when using any chromatographic procedure (such as HPLC, GC, or capillary electrophoresis (CE)), that the retention time of the substance being identified matches with that of an authentic standard.

Some apparent analytical inconsistencies may provide important forensic information. For example, if a result for opiates is negative in urine, but positive in blood, it is possible that heroin was administered shortly before death, and therefore metabolites had not yet been excreted (heroin (diacetylmorphine) is rapidly metabolized to morphine through 6-acetylmorphine). This situation is often found in heroin users dying from an acute sudden death in which substantial urinary excretion has not yet occurred.

Common Drugs and Poisons

The most common drugs and poisons are clearly the initial targets of any forensic toxicological analysis, particularly if no specific information is available to direct the investigation. The most common substances can be categorized as fitting into four classes: (1) alcohol (ethanol); (2) illicit drugs; (3) licit (ethical) drugs; and (4) the nondrug poisons. An example of the distribution of drugs in various types of coroners' cases is shown in Table 2. These data are likely to be similar throughout developed countries.

Alcohol is the most frequent detection in many countries, and, when detected, can play an important role in any investigation because of its ability to depress the central nervous system (CNS). At best, alcohol will modify behavior, causing disinhibition and possible aggression; at worst it can cause death, either by itself, or in combination with another drug.

Illicit drugs include the amphetamines, barbiturates, cocaine, heroin, and other opiates, cannabis, phencyclidine, designer fentanyls, GHB, and lysergic acid diethylamide (LSD). It should be borne in mind that some illicit drugs also have medical uses in some countries. Cocaine is used in some forms of facial and nasal surgery, amphetamine is used to treat narcolepsy and attention deficit hyperactivity disorder (ADHD), and cannabis is used (among other indications) to reduce nausea following chemotherapy.

Ethical drugs include the whole range of prescription and over-the-counter drugs used in the treatment of minor to major ailments. Those of most interest include the antidepressants, major tranquilizers, narcotics and other forms of pain relievers, and anticonvulsants. Since these drugs are widely prescribed, this is by far the most common drug category encountered in toxicology. Each country will have its own list of registered drugs, hence laboratories will need to consider these as a matter of priority over other members of a particular class available elsewhere. For example, most countries only have a relatively small number of benzodiazepines registered for medical use, whereas over 35 are available throughout the world. From time to time laboratories will be required to consider drugs not legally available in their countries because of illicit supplies or through tourists visiting their country.

The nondrug poisons include most commonly organophosphates and other pesticides, carbon monoxide, hydrogen cyanide and cyanide salts, and volatile substances (petrol, lower-molecular-weight hydrocarbons, and kerosene). Carbon monoxide and hydrogen cyanide are gases emitted by fires and are therefore frequent detections in fire victims. Other poisons include heavy metals (arsenic, mercury, thallium), plant-derived poisons (hyoscine from *Belladonna*

Table 2 Incidence of drugs in various types of death (%)[a]

Type of death	*Ethanol*	*Opioids*[b]	*Benzodiazepines*	*Stimulants*[c]	*Cannabis*	*Antipsychotics*
Natural death	15	13	9.4	1.4	2.3	2.6
Homicides	38	11	11	4.0	16	0
Drivers of motor vehicles	27	6.2	4.3	4.3	16	<1
Nondrug-related suicides	33	10	21	2.9	13	2.1
Licit drug deaths	40	41	59	3.2	8.0	13
Illicit drug deaths	35	96	61	7.1	38	5.4
All cases	27	20	20	3.1	12	3.2

Data produced from the toxicology database of the Victorian Institute of Forensic Medicine.

[a]Taken from 2000 cases.

[b]Includes codeine and propoxyphene.

[c]Includes legal stimulants, amphetamines, and cocaine.

Table 3 Incidence of poisons in coroners' cases in Australia

Poison	*Incidence*[a]
Organophosphates	18
Butane and other hydrocarbons	10
Other pesticides/herbicides	12
Solvents (methanol, chloroform, etc.)	8
Strychnine	6
Potassium cyanide	5
Plant-derived poisons	5
Ethylene glycol	2
Heavy metals	2
Potassium	2
Others	14

[a]Taken from over 24 000 Victorian coroners cases from 1989 to 2000.

species, coniine from hemlock), strychnine, and toxins such as venoms. Performance-enhancing drugs such as the anabolic steroids may also be considered in some instances. This list is necessarily limited due to space. Practitioners should always consider the availability of drugs and poisons in certain occupational groups since this can give clues to the nature of a usual substance.

In a review of 12 years of forensic cases from Victoria, Australia, the variety of unusual poisons is shown in Table 3. Obviously, the variety of drugs and poisons will vary from country to country.

Scope of Testing Protocols

As the previous sections indicate, cases may involve a variety of ethical and illicit drugs, or unusual poisons. Worldwide experience also shows that forensic cases often involve more than one drug substance. Surveys of drug-related cases show that three or more drugs are present in more than 70% of cases. High rates of multiple drug use are also found in perpetrators and victims of violent crimes, suicides, and often also in accidents and road crashes.

It is also well known by forensic toxicologists that the information provided to the laboratory concerning possible drug use may not accord with what is actually present. It is therefore strongly recommended that laboratories provide a systematic approach to their toxicology cases and include as wide a range of common ethical and illicit drugs as feasible. This approach is termed systematic toxicology analysis (STA). A laboratory using this approach would normally include a range of screening methods, often incorporating both chromatographic and immunological techniques. Drug classes such as alcohol, analgesics, opioid and nonopioid narcotics, amphetamines, antidepressants, benzodiazepines, barbiturates, cannabis, cocaine, major tranquilizers (antipsychotic drugs), and other CNS-depressant drugs would be included.

The incorporation of a reasonably complete range of drugs in any testing protocol is important since many of these drugs are mood-altering, and can therefore affect behavior, as well as affecting the health status of an individual. Persons using benzodiazepines, for example, will be further adversely affected by cocaine and amphetamine use, and the use of other CNS-depressant drugs. The toxic concentrations of drugs are also influenced by the presence of other potentially toxic drugs. For example, the fatal dose for heroin is affected by the concomitant use of alcohol and other CNS-depressant drugs, since heroin effects are potentiated.

Specimens

It is essential that the relevant specimens are taken whenever possible, since re-collection is rarely possible. The preferred specimens collected in forensic toxicology will of course depend on the nature of the case. In general, a blood specimen is a minimum requirement, although specimens such as urine can be useful for laboratories as a screening specimen, and to check for the use of drugs 2 days or more before sampling.

Hair can provide an even longer memory of drug intake, lasting up to several months, depending on the length of hair. Drugs are usually incorporated into the growing root and appear as a band in the hair shaft when it externalizes from the skin. This process can therefore provide a history of when exposure to a drug or poison has occurred. Most drugs and poisons are incorporated into hair, although the extent will depend on the physiochemical properties of the substance. Basic drugs are often found in higher concentrations than acidic drugs, and invariably the parent drug is present rather than metabolites. For example, cocaine and the heroin metabolite 6-acetylmorphine are more likely to be found in hair of cocaine and heroin users than their corresponding metabolites found in blood and urine (benzoylecgonine and morphine, respectively). Drug will be absorbed into the hair from skin secretions adjacent to the hair follicles, and may even be incorporated from external contamination. Care and treatment of hair, such as washing and the use of dyes and bleaches will also affect the concentration of drug in hair. Consequently, any interpretation of drug content in hair needs to factor in these considerations.

Courts and other legal processes usually require proof that the laboratory has taken all reasonable precautions against unwanted tampering or alteration of the evidence. This applies to specimens and

to physical exhibits used by the laboratory in its toxicology investigations (the term exhibit applies to both specimens and to physical items, such as tablets and syringes). Consequently, it is essential that the correct identifying details are recorded on the exhibit or specimen container, and an adequate record is kept of persons in possession of the exhibit(s). Alternatively, when couriers are used to transport exhibits, the exhibit must be adequately sealed to prevent unauthorized tampering and therefore show continuity of the exhibit. This is called the chain of evidence. Procedures are available to assist laboratories in establishing suitable chain of custody.

General Techniques

The ranges of techniques available to detect drugs in specimens are very similar through the range of applications. These range from commercial kit-based immunoassays (enzyme-multiplied immunoassay technique (EMIT), enzyme-linked immunosorbent assay (ELISA), fluorescence polarization immunoassay (FPIA), cloned enzyme donor immunoassay (CEDIA), radioimmunoassay (RIA)), traditional TLC, to instrumental separation techniques such as HPLC, GC, and CE. MS is the definitive technique used to establish proof of structure of an unknown substance, and can be linked to GC, HPLC, and CE.

The use of appropriate extraction techniques is critical to all analytical methods. Three main types of extraction are used: liquid–liquid, solid-phase, and direct injection. Traditionally, liquid techniques have been favored in which a blood or urine specimen is treated with a buffer of an appropriate pH followed by a solvent capable of partitioning the drug out of the matrix. Solvents used include chloroform, diethylether, ethylacetate, toluene, hexane, various alcohols and butyl chloride, and mixtures thereof. The solvent is then isolated from the mixture and either cleaned up by another extraction process or evaporated to dryness.

Solid-phase techniques are becoming increasingly favored, since mixed phases offer the ability to extract substances of widely deferring polarity more readily than with liquid techniques. Often less solvent is used, or simple hydroalcoholic systems can be employed, rather than potentially volatile or inflammable solvents.

Direct injection techniques into either GC or HPLC instruments bypass the extraction step, and can offer a very rapid analytical process. In GC, solid-phase microextraction (SPME) can be used, whilst HPLC tends to require use of precolumns which are backflushed with use of column-switching valves.

Quality Assurance and Validation

An essential part of any form of toxicological testing is validation and the conduct of quality assurance. It is important that the method used is appropriately validated, i.e., it has been shown to detect accurately and precisely the substance(s) detectable, there is little or no interference (from other drugs and from the matrix) with the specimens used, and a useful detection limit has been established. Moreover, it is essential that the method is rugged and will allow any suitably trained analyst to conduct the procedure and achieve the same results as another analyst. To achieve these aims, it will be necessary to trial the method in the laboratory over several assays with varying specimen quality before claiming a full validation has been conducted.

It is strongly recommended to include internal quality controls with each batch of samples to enable an internal check of the reliability of each assay. These controls contain known drugs at known concentrations. Suitable acceptance criteria are required for these controls before results of unknown cases can be accepted and released to a client. Acceptance criteria vary depending on the analyte and application. For example, blood alcohol estimations often have acceptance criteria less than 5%, while postmortem blood procedures may be 10–20%. (Normally the coefficient of variation of the mean is calculated as a standard deviation divided by the mean of the result.)

An important feature of analytical assays in forensic toxicology is the use of internal standards. These are drugs of similar chemical and physical characteristics as the drug(s) being analyzed and, when added at the start of the extraction procedure, provide an ability to negate the effects of variable or low recoveries from the matrix. Hence, even when recoveries are low, the ratios of analyte and drug are essentially the same as for situations of higher recovery. An ideal recovery marker is when the internal standard is a deuterated analog of the analyte. When deuterated internal standards are used, it may not be necessary to match the calibration standards with the same matrix as the unknown samples, providing the laboratory has verified that no significant matrix effects occur. It is important, however, that absolute recoveries are reasonable, i.e., at least over 30%. This ensures less variability between samples and optimizes the detection limit.

From time to time, it will be important to run unknown samples prepared by another laboratory, or a person not directly involved in laboratory work, to establish proficiency. These are known as proficiency programs or quality assurance programs. These trials are often conducted with many other

laboratories conducting similar work, and provide an independent assessment of the proficiency of the laboratory to detect (and quantify) specific drugs. The performance of the laboratory should be regularly assessed from these results, and any corrective action implemented, if appropriate. This process provides a measure of continuous improvement, an essential characteristic of any laboratory. There are a number of collaborative programs available throughout the world. The College of American Pathologists (CAP) organizes an excellent series of proficiency programs in forensic toxicology.

The international (The International Association of Forensic Toxicologists (TIAFT)) and American (Society of Forensic Toxicologists) societies of forensic toxicology provide guidelines on the conduct of analytical assays and in quality assurance aspects of analyses.

Postmortem Artifacts in Analysis

The process of death imparts a number of special processes that affect the collection and analysis of specimens obtained at autopsy.

These include postmortem redistribution, in which the concentration of a drug in blood has been affected by diffusion of drug from neighboring tissue sites and organs, such as the stomach contents. This is minimized, but not arrested, using peripheral blood from the femoral region. Even liver concentrations may be affected by diffusion from intestinal contents or from incomplete circulation and distribution within the liver. Some drugs are metabolized after death, i.e., nitrazepam, flunitrazepam, heroin, aspirin. Bacterial processes in decomposing bodies may even produce substances such as ethanol and cyanide.

Estimation of Dose

A common request from legal counsels and police is to estimate a dose from a blood or tissue concentration. This may relate to determining likely intent, or simply to rationalize the circumstances to specific amounts of drugs used.

Dose can be estimated from knowledge of the volume of distribution (V_d) of drug (available from several sources, including the books edited by Baselt and Moffatt). The calculation multiplies the blood concentration by the volume of distribution corrected for the body weight of the person. Unfortunately, this calculation assumes one V_d for all persons, and importantly, assumes equilibrium has been established at the time of drug ingestion. This is rarely the case in toxicology cases, since recent drug ingestion is common. The calculation also fails to account for unabsorbed drug (and excreted drug) and may be severely affected by postmortem processes.

The variation in blood concentration at a specified time from a standard dose of drug is well known in clinical pharmacology, even in controlled situations. Therefore, it is not recommended to estimate dose, unless these factors are considered, and a range of doses is computed. Occasionally, it may be possible to compare blood (and tissue) concentrations to other cases in which doses were known, or by measuring the body burden in several tissues, including muscle and fat. Analysis of gastric and intestinal drug content will assist in this process, and also provide information on the route and time of ingestion.

Interpretation of Toxicological Results

Interpretation of any toxicological result is complex. Consideration must be given to the circumstances of the case, and in particular what significance may be drawn from the toxicology. For example, the finding of a drug in potentially toxic concentrations in a person killed by a gunshot wound to the head cannot reasonably lead to the conclusion that drugs caused the death. Alternatively, the absence of an obvious anatomical cause of death will lead investigators to consider the role of any drug use. Considerations must include the chronicity of drug use, the age of the person, the health of the person (presence of heart, liver, kidney disease), the use of multiple substances, and even genetic factors that may lead to a reduced metabolism.

Problems in Court Testimony

Forensic toxicologists and other analysts called to give evidence in court should consider that much of their technical evidence is beyond the ready comprehension of lay people in juries, legal counsel, and judges. Restricting their evidence to understandable language and simple concepts is highly recommended.

A further problem relates to an assumption often made by legal counsel (and indeed other parties) that a toxicological investigation was exhaustive and all drugs and poisons were excluded in the testing processes. Most toxicology performed is restricted to a few analytical tests for a range of "common drugs and poisons," unless the client (e.g., pathologist or police officer) has made a request to examine for (additional) specific chemicals. Analysts should make courts aware of the actual testing conducted and provide a list of substances incorporated in the investigation. Importantly, advice on any limitations applied to the interpretation of the analytical results should be provided,

e.g., poor-quality specimens and postmortem artifacts. Above all, toxicologists must restrict their evidence to those areas in which they claim expertise. Stretching their expertise in the aim of assisting the court can lead to incorrect or misleading evidence, and damage the reputation of the expert and of toxicology.

See Also

Autopsy, Findings: Organic Toxins; Fire; **Carbon Monoxide Poisoning:** Clinical Findings, Sequelae In Survivors; Incidence and Findings at Postmortem; **Immunoassays, Forensic Applications**; **Sexual Offenses, Adult:** Drug-Facilitated Sexual Assault; **Substance Misuse:** Heroin; Urine Analysis; **Toxicology:** Methods of Analysis, Antemortem; Methods of Analysis, Postmortem

Further Reading

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Methods of Analysis, Antemortem

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Introduction

In the absence of some form of drug screening, drug use cannot be confirmed or eliminated as a reasonable possibility in relevant forensic cases. Toxicology testing also assists the courts in establishing the veracity, or otherwise, of other evidence suggestive of drug use. Ultimately, drug screening assists the investigating authorities in providing forensic scientific information pertaining to relevant cases (Table 1).

Toxicology testing is particularly important in victims of sexual assault, where drugs may have been given by the alleged assailant to reduce consciousness and memory of the victim. Drugs used in these cases are typically one of the benzodiazepines (clonazepam, flunitrazepam, alprazolam, etc.), gamma-hydroxybutyrate (GHB and its precursors such as 1,4-butanediol), or a number of other drugs.

Perpetrators of violent crime may also have consumed alcohol, illicit drugs, or even be under prescribed medication. In practice, drug users committing crimes are likely to be under the influence of two or more drugs. Drivers involved in motor vehicle crashes or those causing traffic infringements are also frequently under the influence of two or more drugs.

Toxicology testing on specimens taken soon after the investigated incident is more likely to assist in establishing any drug-induced behaviors of persons than when a specimen is obtained much later. For this reason it is more appropriate to test antemortem specimens from persons taken shortly after admission to hospital, rather than those taken later or at postmortem. This also reduces the interpretation problems associated with postmortem artifacts. Postmortem processes can change blood concentrations, complicating any interpretation of postmortem toxicology. These include redistribution, fermentation (for alcohol), and bioconversion.

Since the great majority of cases (>70%) involve more than one drug, it is advisable to conduct a broad drug screening to include most of the common drugs of abuse, rather than target the analysis to one or a limited range of drugs suggested by the circumstances. This is termed systematic toxicological analysis.

Specimens

Specimens collected antemortem are most often whole blood or the plasma/serum portions, or urine. However, alternative specimens such as hair, sweat, and saliva have also been used to assess drug use and can be a valuable additional specimen (Table 2).

Blood and Plasma

Whole blood, or plasma/serum derived from blood, is the most useful specimen that can be collected since drugs present in this fluid can best be related to a physiological effect and can be used to assess the likelihood of recent drug use or exposure to chemicals. Blood contains predominantly red blood cells, white blood cells, and plasma. Plasma is obtained from

Table 1 Reasons for drug testing in forensic cases

Assisting death investigations to establish cause and mode of death
Establishing drug use in alleged offenders of crimes
Establishing drug use in victims of sexual and physical assaults
Establishing drug use in drivers of motor vehicles and in pedestrians
Establishing drug use in persons involved in workplace accidents
Establishing workplace or environmental exposure of workers
Assisting investigators with estimation of timing of drug use

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Table 2 Most common specimens collected antemortem or in clinical cases

Specimen	*Examples of use*	*Most common detection window*[a]
Blood and plasma (or serum)	Detecting drug impairment, compliance, or drug testing in overdoses	Hours to day
Urine	Workplace or correctional drug detection, sexual assault victims when delay to reporting has occurred	1–3 days
Hair	Preemployment testing, detecting past exposure	Weeks to months
Saliva	On-site drug testing for recent exposure (e.g., drivers)	Hours

[a]Only approximate and will vary somewhat from drug to drug.

nonclotted blood by removal of the cells by centrifugation; serum is the liquid phase remaining after blood is allowed to clot. In this article blood, plasma, and serum are considered one specimen, unless otherwise differentiated. In forensic cases, and particularly postmortem cases, whole blood preserved with sodium fluoride (1%) is most often used, while in clinical cases plasma treated with some kind of anticoagulant, or serum, is most often used. Therapeutic drug-monitoring programs are frequently conducted in clinical toxicological laboratories in plasma and form the basis of therapeutic drug compliance and help to optimize drug doses. Typically, immunoassays are used in drug monitoring and screening, although high-performance liquid chromatography (HPLC), gas chromatography (GC), and mass spectrometry (MS) techniques are equally well suited.

Urine

This is a frequently collected specimen since concentrations of drugs and metabolites of drugs are usually much higher than for blood. Urine can be treated with sodium fluoride (1%) to prevent fermentation for alcohol detection; otherwise it should be kept at about 4 °C for use within a few days or if required beyond a few days it should be frozen. Urine provides a valuable specimen to assess drug use over the previous day or two. Relatively large volumes (50 ml or more) can be collected, allowing sufficient specimen even for less sensitive techniques. However, drug presence in urine does not necessarily imply recent drug use, let alone assist in predicting possible drug effects. For this reason it is advisable to include blood testing if an assessment of possible drug effects is required.

Hair

Hair has long been used to test for exposure to heavy metals such as arsenic, mercury, and lead and has also proven to be a useful specimen for the analysis of drugs. It is particularly useful to establish drug use many weeks to months prior to collection.

Drug entry into hair is complicated and involves a number of processes. Incorporation by entrapment from the blood bathing the growing follicle is a major mechanism, although incorporation through direct contact of mature hair with sweat and/or sebaceous secretions is also a significant source of drug entry.

Because of the ability of hair to absorb drug directly, contamination of hair by direct environmental exposure should also be reasonably excluded, if hair results are to be used. For example, nicotine is found in the hair of nonsmokers and cocaine is found in the hair of children of cocaine users. This is a major limitation of this specimen.

In contrast to urine, the target analytes in hair are predominantly the parent drugs. Cocaine, Δ^9-tetrahydrocannabinol (THC), heroin and its first metabolite 6-acetylmorphine, and benzodiazepines are found in higher concentrations than their corresponding metabolites.

There are a number of factors that influence retention of drugs into hair. Hair color is well known to affect retention of drugs to hair. Hair color is a factor to consider when binding and retention of drugs are concerned. Pigmented hair has higher levels of cocaine than weakly pigmented hair. This is likely to be true for all basic drugs, which bind to melanin, the major pigment in hair. Acidic drugs tend to have lower concentrations than basic drugs. Bleaching and the excessive use of shampoo and conditioners can also reduce the concentration of drugs in hair. For this reason, and the various routes of drug intake into the hair, quantitative results in hair are rarely useful.

Notwithstanding these issues, hair has become a particularly useful specimen to monitor drug use over months for persons seeking new employment,

in individuals under corrective services ordered to abstain from illicit drug use, and in custodial matters requiring proof of abstinence of drug use. Segmental analysis of 1–2-cm sections can also provide some picture of changing drug use over a longer period of time.

Sweat

Sweating is a physiological process providing a mechanism to reduce body temperature. Sweat is produced by eccrine glands located in the transdermal layer of most skin surfaces, and apocrine glands located in axillary and pubic regions. Approximately 40% of all sweat is produced by the trunk, 25% by the legs, and 35% by the head and upper extremities. Sweat is approximately 99% water, the remainder being sodium chloride. A rate of sweating of over 20 ml h^{-1} can occur in stressed situations. Sweat glands are often associated with hair follicles and therefore it is sometimes difficult to differentiate the presence of drugs in hair and sweat.

Sweat is normally collected using suitable absorbent devices such as sweat patches. Contact time may vary from a simple swipe over a portion of skin to days for a sweat patch to absorb accumulated sweat. The device used and collection time will affect the ability to detect excreted drugs. In some devices, local heating facilitates sweating, accelerating the detectability of drugs.

Modern sweat patches have a low incidence of allergic reactions; however inadvertent or deliberate contamination can limit its usability. Drugs detected in sweat include alcohol (ethanol), amphetamines, cocaine, benzodiazepines, barbiturates, opioids, and phencyclidine.

Saliva

Saliva is primarily excreted by three glands: the parotid, submaxillary, and sublingual, and by other small glands such as labial, buccal, and palatal gland. Mixed saliva used for drug analysis consists of approximately 65% from the submandibular, 23% parotid, 4% sublingual; the remaining 8% is from the other three glands.

The daily flow of saliva in an adult ranges from 500 to 1500 ml. Saliva flow is mediated by a number of physiological factors, particularly emotional factors, as well as age, gender, and food intake.

Saliva is not an ultrafiltrate of blood, rather a complex fluid formed by different mechanisms against a concentration gradient, by pinocytosis, by ultrafiltration through pores in the membrane and by active transport. Passive diffusion is a dominant mechanism.

Saliva is best collected by absorption on to an absorbent material or a device that stimulates production of saliva. A number of such devices are available to facilitate the collection process. It is also essential that collection of saliva takes place at least 30 min after a meal, or consumption of a beverage or drug, and the oral cavity is free from food material and other objects before collection.

The main disadvantage is that the saliva volumes are usually small, hence there will be limited ability to repeat analyses. Additionally, not all subjects will be able to provide saliva on demand. Certain drugs can "dry" the mouth and a number of physiological mechanisms can markedly reduce salivation.

Interpretation of saliva drug concentrations is more difficult than blood since saliva concentrations are subject to more variables than blood, such as degree of protein binding and pK_a of drug and pH of saliva. For some drugs saliva concentrations (e.g., benzodiazepines) are much lower than for blood, whereas for others (e.g., amphetamines) concentrations are higher.

This specimen is being investigated as a possible "on-site" specimen to establish drug use at a workplace or roadside. Also, it does not require specialist medical or paramedical experience (e.g., for blood collection) or special collection facilities (e.g., urine collection).

Techniques

A range of techniques are available to detect drugs in specimens collected antemortem. These range from commercial kit-based immunoassays, traditional thin-layer chromatography (TLC), to sophisticated instrumental separation techniques such as HPLC, GC, and capillary electrophoresis (CE). MS is the definitive technique to establish proof of structure of an unknown substance, although a number of other detectors can be used to identify the presence of unknown substances in biological specimens.

Immunoassays

A number of different immunoassay methods are available for drugs of abuse. Numerous commercial kits now exist for this purpose. These include enzyme immunoassay (EIA) (e.g., enzyme-multiplied immunoassay technique (EMIT)) and enzyme-linked inmmunosorbent assays (ELISA), fluorescence polarization immunoassay (FPIA) (e.g., Abbott TDx and ADx), agglutination or kinetic interaction of microparticle immunoassays (e.g., Triage® and Online®), cloned enzyme donor immunoassay (CEDIA), and various radioimmunoassays (RIA). These kits also include devices for rapid on-site testing on blood, saliva, urine, and sweat without the need for biochemical analyzers.

These tests have the advantage of recognizing more than one member of a class of drugs, e.g., amphetamines, benzodiazepines, and opioids. However, not all members are detected with equal sensitivity. For this reason the sensitivity will not only be dependent on the cross-reactivities of the antibodies to the benzodiazepines, but also to the profile of metabolites present in the specimen, and the amount of the target drug ingested. Different batches of antibody will also influence the sensitivity and selectivity to benzodiazepines and their metabolites.

The overall sensitivity in urine can also be increased by prior hydrolysis of urine to convert glucuronide and sulfate conjugates to substances that are detectable by the kit, although reducing recommended cutoff concentrations can accommodate most of the loss of sensitivity. This technique is particularly useful for cannabis, morphine, and the benzodiazepines that are metabolized to hydrolyzable conjugates.

Urine-based kits, modified appropriately, can be used for all the specimens listed in Table 2. Precipitation of blood proteins by treatment with methanol, acetonitrile, dimethylformamide, or acetone, and direct analysis of the supernatant are frequently used techniques; however, the high-potency drugs will not always be detected. Prior extraction of blood with a solvent (e.g., butylchloride) provides improved detectability since a concentration step can be employed and most interference can be removed. With all these techniques, not all drugs are extracted. Individual validation must be conducted to ensure adequate detectability.

For nonurine specimens it is recommended to use ELISA or DNA technology. In most cases this technique allows direct analysis without the need for specimen treatment.

False-positive results with immunoassays occur, from structurally related drugs, from metabolites of other drugs which are recognized by the antibodies, or occasionally by artifacts such as adulterants affecting pH, detergents, and other surfactants. For this reason any positive result must be confirmed by an alternative technique, preferably chromatography with MS identification.

Thin-Layer Chromatography

This is the oldest of the chromatographic techniques and is still used in some clinical and forensic laboratories as a screening technique. The movement of an organic-based solvent on a plate containing an absorbent material is based on the separation of drugs (and their metabolites). The stationary absorbent phase is typically silica, although other supports are used. Chromatography is usually rapid (taking less than an hour) and a number of samples can be run simultaneously with little cost. Drugs are identified by visualization under ultraviolet (UV) light (as a dark spot), or by spraying with one of a number of reagents which are directed to specific chemical moieties (as a colored spot), or to organic compounds generally.

The retention factor (R_f) is calculated by dividing the distance moved from the origin over the distance moved by the solvent front. Characteristic colors of the spots, the presence of metabolite patterns, and the R_f values provide a good means of identifying drugs in biological specimens. Unfortunately, the technique is relatively insensitive and is usually limited to urine analysis, although analysis of gastric contents and liver extracts (in postmortem analysis) is also possible. Densitometry of TLC plates can provide some quantitation of the amount of drug present in an extract. Detection limits of 500 ng ml^{-1} are possible from 5 ml of urine.

The use of high-performance TLC (HPTLC) plates has been shown to provide higher sensitivity and can detect some drugs at levels of 100 ng ml^{-1} from 1 ml of blood. Since specificity is not very high, it is advisable to confirm any positive result by an alternative technique, preferably MS identification.

Gas Chromatography

GC is based on the principle of partitioning a substance in a gaseous phase from a stationary liquid phase. The stationary phase is typically a polymeric liquid, which is either coated on to silica, or chemically coated on to the glass surface of the column itself. The nature of the functional groups and polarity of the polymer and the temperature of the column provide the means of varying the separation conditions.

Typically, columns are flexible capillaries made of fused silica with internal diameters of 0.1–0.5 mm, and that are coated with heat-resistant polymers to promote flexibility. A large range of columns is available to provide analysts with a sufficient flexibility to optimize separation conditions. The type of columns range from low-polarity dimethylpolysiloxane, 14% cyanopropylphenyl, 5% diphenylmethylpolysiloxane, to the polar trifluoropropylpolysiloxane, and to 50% diphenyl methylpolysiloxane phases. The use of cyanopropylphenyl or 5% phenylmethylsilicone stationary phases can give better separation of a number of moderately polar compounds than a 100% methylsilicone phase. Due to the wide polarity differences of drugs, temperature programming is necessary for assays involving detection of a number of drugs.

A range of detectors is available for GC. Flame ionization detectors are workhorse detectors for any compounds containing carbon, whereas a number of detectors are available for specific functional groups. The nitrogen phosphorus detector (NPD) selectively detects compounds with either nitrogen or phosphorus, while the electron capture detector (ECD) relies on the ability of a compound to capture electrons when passing through an electric field. ECD detectors give the best detection limits ($\leq 1\,\mathrm{ng\,ml^{-1}}$) from 1.0 ml plasma, although NPD provided detection limits down to $5\,\mathrm{ng\,ml^{-1}}$ for nitrogenous substances and better than $1\,\mathrm{ng\,ml^{-1}}$ for phosphorus-containing substances (e.g., organophosphate pesticides) (Table 3). Poisonous and other gases can be detected using thermal conductivity detectors which do not rely on the presence of carbon or nitrogen.

For drugs to be amenable to GC, they must be thermally stable to enable volatilization into an inert gas (e.g., helium and nitrogen). In many cases compounds can be derivatized to improve their thermal stability, or to alter their retention characteristics and thus enable a separation to occur (Table 4).

Solid-phase microextraction is a relatively recent technique to enable rapid analysis of drugs without requiring extensive sample cleanup and concentration. Direct online injection using a dialysis technique involving a copolymer precolumn for absorption has also been reported on small sample volumes.

High-Performance Liquid Chromatography

HPLC is a commonly used chromatographic system which involves the separation of compounds by partitioning between a pressurized moving liquid phase and a solid support containing very fine silica (4–10-μm diameter particles) or bonded silica. The bonded ligand acts as a pseudoliquid phase. Bonded groups include C2, C8, C18, CN-alkyl, and phenyl-alkyl chains. The physiochemical properties of the bonded phase and the moving phase determine the separation process.

Moving phases are often hydroalcoholic solvent systems such as acetonitrile or methanol/unbuffered water to solvent/buffered phosphate solutions, the base modifier triethylamine, and ion-pairing reagents such as methane sulfonic acid, tetramethyl ammonium hydrogen sulfate, and tetrabutyl ammonium bromide. Gradient programming in which the composition of solvent is altered with time provides an ability to separate compounds of widely differing polarity. Normal-phase chromatography on a CN-, OH-bonded column or a silica column function in a similar way to TLC, except that resolution and sensitivity are far greater.

Table 3 Examples of detection systems used in gas chromatography analysis of selected drugs

Drug class	*Detector*
Alcohol and other volatiles	FID
Amphetamines	NPD, EI MS, NCI (as derivative)
Antidepressants	NPD, EI MS
Antipsychotics	NPD, EI MS
Benzodiazepines	NPD, ECD, NCI
Cannabinoids (THC, carboxy-THC, etc.)	EI MS, NCI (as derivative)
Carbon monoxide, and other gases	TCD
Cocaine and metabolites	NPD, EI MS (as derivative of BE)
Heroin, morphine, and other opioids	NPD, EI MS (as derivative of morphine)
Organophosphate pesticides	NPD, EI MS

FID, flame ionization detector; NPD, nitrogen phosphorus detector; EI MS, electron impact mass spectrometry; NCI, negative ion chemical ionization mass spectrometry; ECD, electron capture detector; THC, Δ^9-tetrahydrocannabinol; TCD, thermal conductivity detector; BE, benzoylecgonine. Adapted from Drummer OH. Toxicology: Methods of Analysis – Ante Mortem. In: *Encyclopedia of Forensic Sciences*. Edited by Jay A Siegel, Pekka J Saukko and Geoffrey C Knupfer. Academic Press: London. © 2000. With permission from Elsevier.

Table 4 Examples of detection systems used in high-performance liquid chromatography analysis of selected drugs

Drug class	*Detector*
Amphetamines, including ecstasy	UV and F (of derivitized drug), MS
Analgesics (acetaminophen (paracetamol), salicylate)	UV and photodiode array
Anions (bromide, chloride, azide, etc.)	Ion conductivity
Antidepressants	UV and photodiode array, MS
Benzodiazepines	UV and photodiode array, MS
Buprenorphine	MS
Cannabinoids (THC, carboxy-THC, etc.)	EC and photodiode array, MS
Catecholamines (epinephrine (adrenaline), dopamine, etc.)	ECD
Cocaine and metabolites	UV and photodiode array, MS
Morphine/codeine	EC, F and UV, MS
Nonsteroidal antiinflammatory drugs	UV, DAD

UV, ultraviolet; F, fluorescence; MS, mass spectrometry; THC, Δ^9-tetrahydrocannabinol; EC, electrochemical; ECD, electron capture detector; DAD, diode array or multiwavelength detector.

Detection of the sample is most often by UV spectrophotometry at or near the maximum absorption wavelength. Alternatively, other physiochemical properties of the compound(s) can be exploited. These include infrared (IR), fluorescence (F), phosphorescence, electrochemical (EC) properties, and

conductivity (for ionically charged substances). Compounds with functional groups can be reacted with reagents to impart greater detectability with one or more detectors, or to allow resolution of stereoisomers (Table 4).

Photodiode array or multiwavelength detection (to supplement UV detection) offers real advantages to analysts in identifying peaks and assisting in establishing peak purity. Photodiode array detection can be a very useful technique if MS instrumentation is not readily available, or if absolute proof of structure is not required.

Detection limits around 10–50 ng ml^{-1} are expected for most compounds by HPLC, depending on the physiochemical properties of the drug, the volume of specimen extracted, and the method used. Lower detection limits are possible if larger amounts of sample are extracted and when a concentration step is employed.

Solid-phase extraction (SPE) using small columns to absorb drug selectively from the matrix (e.g., Extrelut, Sep-Pak, Bond-Elut, etc.) provides an excellent alternative to conventional liquid–liquid extraction techniques. Solid-phase techniques have been published for most analytes and tend to be quick, and often provide clean extracts. These SPE procedures can also be readily automated to improve throughput.

Narrow-bore columns (1–2 mm internal diameter) require less specimen and can easily be interfaced with MS.

Capillary Electrophoresis

A powerful emerging technique showing widespread application in forensic science is that of CE. Capillary electrophoresis is actually a number of related techniques, including capillary zone electrophoresis (CZE), micellar electrokinetic capillary chromatography (MECC), capillary electrochromatography, capillary isotachophoresis, capillary gel electrophoresis, and capillary isoelectric focusing, and is complementary to HPLC with high separation power.

Capillary electrophoresis consists in its most simple form of a separation capillary of 20–100 μm internal diameter and up to 100 cm long, a high-voltage source, electrodes, an injection system, and a detector. The capillary is often fused silica coated with plastic polyimide to confer elasticity. The capillary ends are dipped in buffer and are held at a potential of up to 30 kV. The separation is based on migration of charged drug molecules against an electric field and electroosmosis caused by the osmotic migration of cations and water to the cathode because of ionization of the silylhydroxyl groups on the fused silica. The electroosmosis factor (EOF) can be altered by changing the pH of the buffer, ionic strength of buffer, modifiers added to buffer, and type of capillary internal wall coating.

Electrokinetic micellar chromatography is capable of analyzing illicit drugs in urine and in plasma. It is also used in screening seized powders for the presence of illicit drugs. This is a powerful technique since it can separate a large range of compounds with high sensitivity and has the ability to separate compounds of widely differing polarity in one run.

Multiwavelength UV detection can be used to provide an added degree of confirmation. The sensitivity is adequate for routine confirmatory analyses of presumptive positive urines for drugs of abuse. CE linked to mass spectrometers is an emerging versatile and sensitive analytical technique.

The amount of sample or biological extract applied to CE is in the nanogram scale allowing for trace analysis with adequate sensitivity for most applications. It can operate in both qualitative and quantitative modes.

Mass Spectrometry

MS is the definitive technique if unequivocal identification of unknown compounds is required for forensic purposes. MS is usually linked directly to a chromatographic separation process such as CE, HPLC, or GC, or even to another MS (MS–MS).

Mass spectrometers can be operated under full scan mode, i.e., from m/z 50 to m/z 550 or even higher depending on the molecular weight of the molecules and the size of fragment ions. For MS–MS, fragmentation of one or more ions formed in the primary spectrum can also be produced under various "reaction modes." Full scan MS provides optimum spectral information (abundance of ions at their respective m/z ratios). Mass spectrometers can also operate in a selected ion mode or equivalent. In this mode only a few ions are normally monitored. This is most commonly used to improve sensitivity for quantifications at lower concentrations or to confirm commonly observed drugs that have already been presumptively identified by other techniques.

Compounds do not always show characteristic spectral detail (e.g., amphetamines). Consequently, it is recommended to prepare derivatives for such compounds, or for substances that show poor chromatographic properties (Table 5). One of the most

Table 5 Examples of derivatives used in gas chromatography–mass spectrometry analysis of selected drugs

Drug class	*Derivatives*
Amphetamines	AA, HFBA, methyl chloroformate
Barbiturates	None, or iodomethane in TMAH
Benzodiazepines	*t*-butyl-DMS, TMS, PC/PI
Cannabinoids (THC, carboxy-THC, etc.)	TFAA, TMS, PFPA/PFP, *t*-butyl-DMS
Cocaine and metabolites	*t*-butyl-DMS, PFPA/PFP, TMS
Morphine	PFPA/PFP, TMS

AA, acetic anhydride; HFBA, heptafluorobutyric anhydride; TMAH, tetramethylammonium hydroxide; *t*-butyl-DMS, *t*-butyl dimethylsilyl; TMS, trimethylsilyl; PC, propionyl chloride; PI, propyl iodide; THC, Δ^9-tetrahydrocannabinol; TFAA, trifluoracetic anhydride; PFPA, pentafluoropropionic anhydride; PFP, pentafluoropropan-2-ol. Adapted from Drummer OH. Toxicology: Methods of Analysis – Ante Mortem. In: *Encyclopedia of Forensic Sciences*. Edited by Jay A Siegel, Pekka J Saukko and Geoffrey C Knupfer. Academic Press: London. © 2000. With permission from Elsevier.

frequent derivatives described is the trimethylsilyl ether for amines, hydroxyl-, and carboxyl-containing substances. Alternatively, other silylethers such as *t*-butyl are used, and fluorinated acylanhydrides (e.g., pentafluoropropionic anhydride) are widely used for amines and hydroxy compounds, and a combination of a perfluorinated alcohol with a perfluorinated acylanhydride for carboxy-, hydroxy-, and amine-containing substances. Other derivatives are also known.

Positive-ion chemical ionization produces a much higher-intensity molecular ion, and is often used to reduce fragmentation and to provide evidence of the molecular weight of the compound. In this mode reagent gases, such as methane, and ammonia are used to produce different ion–molecule collisions in the ion chamber (source).

The use of negative-ion chemical ionization (NCI) affords a greatly enhanced detection limit for certain compounds compared to electron impact mass spectrometry (EI MS). In this NCI mode a single ion cluster is often observed and can provide for some drugs (e.g., benzodiazepines and derivatized THC) a detection limit of 0.1 ng ml^{-1}.

The use of deuterated internal standards provides an ideal way of monitoring changes in chromatographic performance, and most importantly, essentially eliminating matrix effects caused by poor recoveries of drug. While recoveries of drug may vary from one matrix to another, and even from calibrators, the deuterated internal standard will correct for this. For this reason, assays involving MS should use deuterated internal standards wherever possible.

The combination of HPLC with MS (LC-MS) and tandem or ion-trap MS (LC-MS-MS) provides good examples of the separation power of HPLC with the sensitivity and specificity of MS. Detection limits range from 10 pg on-column, resulting in detection limits of better than 1 ng ml^{-1} for many compounds using a thermospray or electrospray interface. This technique has become a desired technique in forensic chemical procedures because it can separate substances that are not normally amenable to GC, such as higher-molecular-weight substances or polar compounds that require derivatization. Examples of its use include anabolic and other steroids, diuretics, benzodiazepines, buprenorphine, and other opioids.

See Also

Alcohol: Breath Alcohol Analysis; Blood and Body Fluid Analysis; Acute and Chronic Use, Postmortem Findings; **Autopsy, Findings:** Drug Deaths; Organic Toxins; **Carbon Monoxide Poisoning:** Incidence and Findings at Postmortem; **Toxicology:** Methods of Analysis, Postmortem

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Methods of Analysis, Postmortem

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Introduction

In postmortem cases, as with other forensic cases, toxicology assists the investigating authorities in the investigation of a case (Table 1). Ultimately toxicology testing results assist the coroner, medical examiner or the procurator fiscal (a legal officer in Scotland whose function is to investigate cases of sudden death, amongst other duties, whereas in other jurisdictions based on common law various combinations of coroner and/or medical examiner systems apply), or equivalent judicial officer in other legal systems in establishing the evidence of any drug use. In cases where toxicology fails to detect foreign substances, it allows the investigating pathologist to turn his attention to other relevant factors, since a pathological examination often does not show indicia suggestive of drug use. Drug use can only be confirmed by appropriate toxicology testing procedures.

Toxicology testing is particularly important in victims of homicide in which drugs may have been given by the assailant to reduce consciousness of the victim and in cases in which drugs were used by the victim. In the latter scenario, modification of behavior and/or the state of mind by drug use may be important in criminal trials, not necessarily to mitigate the intent of the accused, but primarily to reconstruct, as far as possible, the events that led to the act. Such reconstruction may involve corroboration of witness accounts of drug-using behavior.

Typical drugs used in these cases are alcohol, amphetamines, cocaine, or one of the benzodiazepines (alprazolam, diazepam, flunitrazepam, etc.). Victims or perpetrators of violent crime may also have consumed medication to treat a psychiatric problem or a host of other medical conditions. The presence of drugs may therefore allude to such treatment, or at least confirm that the person concerned has taken their medication. In some cases, these medications may even have contributed to behavioral problems.

In practice, it has been observed that deceased persons have often consumed two or more drugs, and in many cases the investigating authority (pathologist, coroner, etc.) is not aware of all the drugs used. Since the great majority of cases (>70%) involve more than one drug, it is advisable to conduct a broad drug screening to include most of the common drugs, rather than target the analysis to one or a limited range of drugs suggested by the circumstances. This also allows experts to determine whether any adverse drug interactions have occurred.

Table 1 Reasons for drug testing in postmortem cases

Eliminating involvement of drugs in cases
Establishing drug use in victims of homicide
Establishing drug use in drivers of motor vehicles
Establishing drug use in persons involved in workplace accidents
Establishing drug use in other cases of sudden and unexpected death
Assisting investigators with estimation of timing of drug use

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Specimens

The preferred specimens collected at postmortem will depend on the type of case. Typically one or more blood specimens and urine are collected, although as Table 2 illustrates, a number of other specimens should be taken in certain case types. A useful forensic technical procedure in the autopsy suite is to take a "full" set of specimens, in all but the most obvious natural-cause investigations. This will avoid the embarrassment of insufficient or inappropriate specimens collected in a case and give the toxicologist the best chance to satisfactorily complete the analytical investigation. Against this may have to be balanced the legal and cultural sensitivities surrounding the collection and retention of tissue specimens at postmortem.

Table 2 Recommended minimum specimens collected postmortem

Type of case	*Recommended specimens collected*
All cases	Peripheral blood (2 × 10 ml), one tube preserved with fluoride to at least 1% w/v Urine (10 ml) Vitreous humor (2–5 ml)
Homicides and suspicious cases	Plus liver, hair
Drug-related cases	Plus gastric contents, liver, hair
Volatile substance abuse cases	Plus lung fluid or tied-off lung, liver
Biochemical abnormalities (insulin, etc.)	Plus serum
Heavy metal poisoning	Plus liver, hair, kidney

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Blood

Blood is the most useful specimen that can be collected since drugs present in this fluid can best be related to a physiological effect and can be used to assess the likelihood of recent drug use or exposure to chemicals. A number of problems are associated with the collection of this fluid in cadavers. Two 10-ml samples of blood are recommended, one to be used for blood alcohol analyses and the other for blood toxicology. The splitting of the two blood specimens reduces the possibility of contamination in the laboratory and enables the blood alcohol specimen to be retained separately to the other blood specimen. Forensic technicians or pathologists should be aware that the collection of peripheral blood reduces the possibility of postmortem artifacts frustrating interpretation of any positive results. The preferred collection site is the femoral vein (leg). However, failure to collect the specimen distal to a ligature or clamp applied to the femoral vein may allow blood to be drawn down from the inferior vena cava in the abdomen, where the concentration of drugs may be significantly higher. Similarly, the blood should be collected before the body is eviscerated, to avoid contamination.

Autopsy procedures should therefore accommodate the need to obtain optimal blood specimens for toxicological purposes.

Urine

Urine is the second most important specimen collected. Since concentrations of drugs and metabolites of drugs are usually much higher than in blood, urine provides a valuable specimen to assess drug use over the previous day or two. Urine can be collected after opening of the abdomen, or by direct puncture of the bladder. An autopsy is therefore not necessary to collect this specimen. Blood and vitreous humor can also be taken by direct puncture of the relevant anatomical region. When blood is obtained by direct puncture, the site of collection should be specified on the specimen tube.

Vitreous Humor

Vitreous humor is an ideal fluid to accompany positive blood-alcohol cases, since the alcohol content of vitreous is very similar to that of blood and can prove useful to exclude putrefactive formation of alcohol in blood, and visceral contamination. Vitreous humor is also a useful fluid for a range of drugs including digoxin and antidepressants, as well as a number of biochemical markers. Since vitreous humor can easily be collected, it is strongly recommended that this specimen should be included in a routine sudden-death investigation. In pediatric cases, where the eyes may need to be examined histologically for evidence of shaking, vitreous humor should only be taken after careful consideration and procedures such as retinal photography have been completed.

Liver

The liver is traditionally a favored tissue for toxicologists since drugs are often found in higher concentrations than blood and the liver can be readily homogenized. All cases of suspected drug use should have a portion of liver collected. A 100-g aliquot is sufficient for most analyses. The right lobe is preferred, since it is least subject to postmortem diffusion of drug from the bowel contents and the mesenteric circulation.

Gastric Contents

Gastric contents are invaluable in cases of suspected poisoning. The aim of using this specimen is to establish the actual content of drug (or poison) remaining in this organ at death and gastric analysis may allow the route of drug administration to be determined. Drug residues can be isolated out by direct extraction with methanol, or another solvent, and analyzed by conventional chromatographic techniques. When little or no fluid is present in the stomach provision for the whole stomach allows the analyst to dissolve any drug adhered to the sides of the walls. Toxicologists should be aware that small quantities of drug will derive from the bile, especially during agonal processes, hence drug content in the stomach must not necessarily imply oral ingestion. Results should be reported in milligrams (total gastric content). If only an aliquot of gastric contents is supplied the results may need to be reported as a concentration. However, gastric contents are rarely homogeneous particularly after meals hence whole contents are preferred wherever possible. Occasionally, pathologists will need to examine the stomach. This should be done prior to collection of any contents.

Lungs

Lung fluid (or tied-off lungs) is (are) recommended in cases of suspected volatile substance abuse. Since quantitative results are rarely interpretable, only "detected" or "not detected" results are usually sufficient (Table 3). In jurisdictions where tissue cannot be collected or retained freely, blood from the pulmonary vein or the left side of the heart can be used in this context.

Other Specimens

Occasionally other specimens can provide valuable information in a case. Hair can provide a history of drug use, or exposure to chemicals if chronic exposure is thought to have occurred. Hair can therefore

Table 3 Particular toxicological usefulness of various tissues

Tissue	*Substances detected*
Blood/urine/liver/hair/ gastric contents	All drugs and poisons
Vitreous humor	Alcohol, digoxin, creatinine, urea, glucose
Bile	Morphine and other narcotics, benzodiazepines, colchicine
Lungs	Volatile substances (toluene and other solvents, butane and other aerosol gases, automobile and aviation fuels)

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provide evidence of drug use for much longer periods of time than urine. The relation between dose and hair concentration is usually poor, although some comparisons can be made as to the extent of drug use, e.g., regularity of heroin use. Bile can sometimes be a useful fluid for detecting morphine or heroin use since biliary concentrations are much higher than those in blood. A number of other drugs are also found in bile in relatively high (and therefore more easily detectable) concentrations including colchicine, other narcotics, benzodiazepines, and glucuronide metabolites. Bile may also occasionally be useful in late-stage paracetamol poisonings.

Samples of brain tissue may be more relevant for some centrally active (the term "central" includes the brain and spinal cord) drugs such as morphine, and skin (with associated subcutaneous tissue) may show large deposits of drugs left behind after an injection. When taking skin for the purpose of determining a likely injection site it is important that a control site be also collected, for example, from the other arm. Results are normally expressed as milligrams per gram wet weight tissue.

Other specimens may be useful in specific circumstances, e.g., cerebrospinal fluid in medical matters involving intrathecally administered drugs.

Specimens from a Putrified Body

In cases of extreme putrefaction, the recommended list of specimens will no longer be appropriate. Muscular tissue, hair, and bone can be useful specimens in this type of case, although the physical state of the body will determine what specimens are available for collection. Body fluids will be present in some putrefied bodies, however this is no longer blood, but rather liquified tissues; however, this fluid can be used to screen for the presence of drugs. Quantitative results are of little use in badly putrefied cases.

General Techniques

The range of techniques available to detect drugs in the specimens collected postmortem are essentially identical to those collected antemortem. These range from commercial kit-based immunoassays (ELISA, EMIT, FPIA, CEDIA, RIA, etc.), traditional thin-layer chromatography (TLC), to instrumental separation techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), and capillary electrophoresis (CE). Mass spectrometry (MS) is the definitive technique to establish proof of structure of an unknown substance and can be linked to GC, HPLC, and CE. Even MS has its limitations, e.g., special techniques may be needed to characterize phenethylamines that do not have sufficiently unique spectra.

The specimens analyzed in postmortem cases are most often blood and liver, rather than urine and serum that are used in antemortem analysis and the other specimens listed earlier. The use of blood and liver, and indeed all other postmortem specimens, require separate validation against those methods used in antemortem analysis. The methods used require modification to ensure a reliable extraction recovery, a low level of interference, and reproducible quantitative results. Special attention to these factors is required on partly or fully putrefied specimens to ensure no interference from endogenous substances. Cutoff values often used in workplace, sports, and drugs-of-abuse testing are no longer appropriate in postmortem cases involving alternative speciments to urine. Even postmortem urine should not normally be tested to cut-off limits used in drugs-of-abuse testing since the presence of a small concentration of drug may be of forensic significance.

The range of immunoassays used in antemortem analysis can also be used in postmortem analysis provided suitable modification in the preparation of the specimen occurs. Urine-based kits can be used for urinalysis, but blood or tissue homogenates require special treatment to remove matrix effects. Urine is often unavailable in postmortem cases. Enzyme-linked immunosorbent assay (ELISA) techniques have become the screening technique of choice for the direct analysis of blood (and other specimens such as hair digests) for drugs of abuse. False-positive results with immunoassays occur, either from structurally related drugs or from metabolites of other drugs that are recognized by the antibodies. While HPLC and GC techniques are more specific than immunoassays, any positive result should be confirmed by mass spectral identification, unless sufficient validation of another method has been conducted to ensure courts of the reliability of the result. Unconfirmed drug results, if reported, should

be flagged as presumptive, or by words of similar meaning. Solid-phase extraction (SPE) using small columns to selectively absorb drug from the matrix (e.g., Extrelut, Sep-Pak, Bond-Elut, etc.) provides an excellent alternative to conventional liquid–liquid extraction techniques. Solid-phase techniques have been published for most analytes, tend to be quick, often provide clean extracts, and can be readily automated. The use of deuterated internal standards provides an ideal way to monitor changes in chromatographic performance, and most importantly, essentially eliminating matrix effects caused by poor recoveries of drug. While recoveries of drug may vary from one matrix to another, and even from calibrators, the deuterated internal standard will correct for this. For this reason, assays involving MS should use deuterated internal standards wherever possible in postmortem analyses.

The analyst should always be on the alert for unusual findings. For example, if a large acetone peak is seen in an alcohol analysis this might suggest undiagnosed diabetes in life, or a peak not recognized as a drug in a library search on the MS may be evidence of an unusual or uncommon substance.

Recommended Techniques for Postmortem Analysis

As indicated before it is important that a drug screen encompasses the widest number of drugs and poisons without seriously compromising the ability of the laboratory to work on sufficient cases. Urinalysis (or blood or another fluid) using one of the commercial immunoassays, or even TLC, is recommended for the main classes of drugs. These usually include amphetamines, barbiturates, benzodiazepines, cannabinoids (cannabis metabolites), cocaine metabolite, and morphine-like opioids.

In addition, a series of other (usually chromatographic) tests are strongly recommended. The schema shown in Figure 1 illustrates a typical analytical profile for routine case screening on blood.

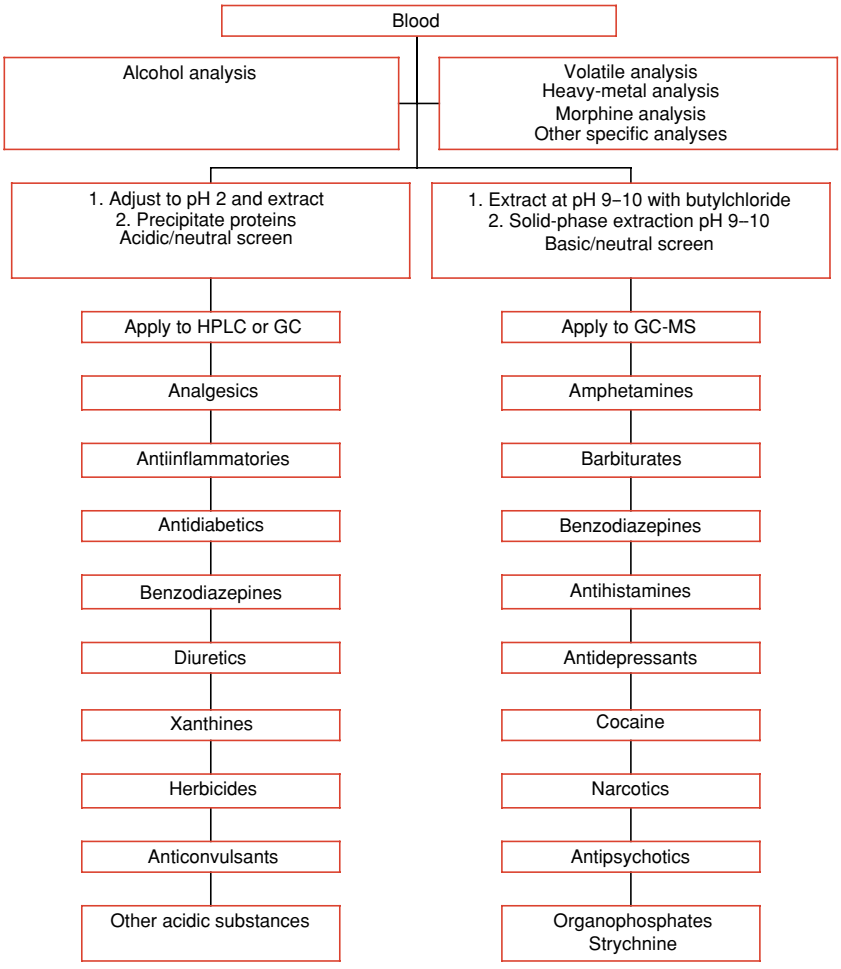

Figure 1 Schematic showing extraction steps for blood analyses and substances classes likely to be detected. Reproduced from Drummer OH. Toxicology: Methods of Analysis – Post Mortem. In: *Encyclopedia of Forensic Sciences*. Edited by Jay A Siegel, Pekka J Saukko and Geoffrey C Knupfer. Academic Press: London. © 2000. With permission from Elsevier.

Blood is analyzed for alcohol and is subject to screening techniques aimed at capturing a wide selection of "common" chemical substances. Only GC techniques are recommended for the analysis of alcohol (ethanol).

An acidic screen includes the nonnarcotic analgesics (acetaminophen and aspirin), the nonsteroidal antiinflammatory drugs (celecoxib, naproxen, ketoprofen, ibuprofen, etc.), many of the diuretics (frusemide, hydrochlorothiazide, etc.), the anticonvulsants (carbamazepine, lamotrigine, phenobarbital, phenytoin and valproate), barbiturates and the more potent benzodiazepines, and the xanthines such as theophylline and caffeine. The use of a solvent extraction technique at acidic pH or simple precipitation of blood proteins with acetonitrile enables these substances to be detected by gradient HPLC with multiwavelength or photodiode array detection.

A basic extraction procedure using butyl chloride (preferred solvent, but others are also suitable), or an SPE procedure using octadecyl-bonded cartridges or mixed-phase cartridges will provide a reasonably clean extract from postmortem blood (and other tissues) for analysis by capillary GC. The use of a MS detector is preferred (to allow simultaneous detection and confirmation), although a nitrogen phosphorous detector (NPD) will provide a higher sensitivity for many substances than full-scan MS. Electron capture detectors (ECD) are extremely useful for benzodiazepines. The use of dual detectors (NPD and MS, or NPD and ECD) provides an additional degree of specificity and detection over one detector alone.

These two screening procedures will also enable a number of unusual poisons to be detected. Organophosphates and strychnine are readily detected by GC–NPD, while HPLC of acid extracts enables detection of a number of herbicides and other agricultural chemicals. If circumstances suggest volatile substance abuse, exposure to heavy metals, lysergic acid diethylamide (LSD) and other nonamphetamine hallucinogens, or other noxious substances not covered earlier, specific additional tests need to be performed. It is advisable to perform a blood test for morphine if heroin or morphine use is suspected (or needs to be ruled out) and the urine test for opioid is negative. Heroin deaths have been missed if screening for morphine is restricted to urine since acute deaths in naive users may not show morphine in urine.

Postmortem Artifacts in Analysis

The process of death imparts a number of special processes that affect the collection, analysis, and interpretation of specimens obtained at autopsy.

Redistribution

Foremost is the process of redistribution which affects all analyses in which concentrations of drugs in blood and tissues alter due to disruption of cellular membranes, causing alterations of drug concentrations within tissue elements and diffusion from one tissue to another. This process is particularly significant for drugs with high lipid solubility, since these drugs tend to show concentration differences in tissues and blood. Table 4 shows the extent of these changes for selected drugs when comparisons are made between blood collected from the heart and that collected from the femoral region. The femoral blood is least subject to redistribution after death; however, drugs with much higher concentrations in muscular tissue will still diffuse through the vessel walls and elevate the neighboring blood concentrations. If the femoral vessels are not tied off from the vena cava and aorta then the process of drawing blood can also extract blood from the abdominal cavity that has been contaminated from diffusion of gastric and intestinal contents. It is therefore advisable to reduce these processes by collecting blood specimens as soon as possible after death from the femoral region with blood vessels tied off to reduce contamination. In cases where death has occurred in hospital it is recommended to obtain specimens taken for clinical purposes immediately before death, or on admission to hospital, whichever is more appropriate.

These processes are not limited to blood. Liver and lung tissues show differences in the concentration

Table 4 Likely extent of postmortem redistribution for selected drugs

Drug/drug class	*Likely extent of postmortem redistribution*[a]
Acetaminophen (paracetamol)	Low
Alcohol (ethanol)	Low
Amphetamines	Low to moderate
Antipsychotics	Moderate to high
Barbiturates	Low to moderate
Benzodiazepines	Low to moderate
Cocaine	Low
Digoxin	Very high
Methadone	Moderate
Morphine, codeine	Low
Propoxyphene	Very high
Salicylate	Low
Serotonin reuptake inhibitors	Low to moderate
Tetrahydrocannabinol (THC)	Low to moderate
Tricyclic antidepressants	High

[a]Low, up to 20% elevation; moderate, 21–50%; high, 50–200%; very high, >200%.

of drugs depending on the nature of the drug and whether diffusion of drug has occurred from neighboring tissues or the blood supply. For example, the left lobe of the liver is more likely to exhibit elevated drug concentrations than the right lobe.

Metabolism and Bioconversion

A number of drugs can undergo chemical changes in a body after death. These chemical changes can be either metabolically mediated or caused by spontaneous degradative processes. For example, the metabolism of heroin to morphine occurs in life and in recently deceased persons by the action of blood and liver esterases. For this reason, heroin is rarely detected in cadaveric tissues. 6-Acetylmorphine is detected in urine for a few hours after last use. Morphine is the main target drug for most specimens. Aspirin is converted rapidly to salicylate by hydrolytic mechanisms. Most prodrugs activated by desterification or hydrolysis will be subject to similar processes.

Nitro-containing drugs, such as the benzodiazepines, nitrazepam clonazepam, flunitrazepam, and others are also rapidly biotransformed after death to their respective amino metabolites by the action of certain types of bacteria (obligate anaerobes). Toxicologists must therefore target their analyses to these transformation products rather than the parent drug.

Sulfur-containing drugs such as dothiepin, thiopental, thioridazine, etc., are also subject to bacterial attack during the postmortem interval leading to progressive losses due to putrefaction. Of course, the parallel process of tissue loss will also affect the tissue concentration during putrefaction.

Chemical degradation occurs for a number of drugs and metabolites even when specimens are stored frozen at $-20\,^{\circ}C$. Some benzodiazepines and benzodiazepine metabolites, antipsychotics such as thioridazine, and the beta stimulant fenoterol, show time-dependent losses. For many drugs, complete stability characteristics have not yet been evaluated. Alcohol will be lost by evaporation unless sealed tubes are stored at $-80\,^{\circ}C$; however, alcohol (as ethanol) can also be produced by bacterial action on glucose and other sugars found in blood. The use of potassium fluoride as preservative (minimum 1% w/v) is required to prevent bacterial activity for up to one month after collection, when stored at $4\,^{\circ}C$.

Reports

Once an analysis is complete, a report must be issued to the client(s) that accurately details the analytical findings. These results should indicate the type of tests conducted, the analytical method used (i.e., HPLC, GC–MS, etc.), on which specimens the analyses were conducted, and of course the result(s). The result(s) should be unambiguous using such terms as "detected" or "not detected." The use of the term "not present" should be avoided, since it implies no possibility of the substance being present. A toxicologist can rarely be so definitive and can only indicate that a substance was not detected at a certain threshold concentration. For this reason, a detection limit alongside tests for specific substances should be provided for "not detected" results.

For quantitative results, consistency in units is advised and should not be given with more significant digits than the accuracy will allow. For example, there is no point in reporting a result for blood morphine as $0.162\,mg\,l^{-1}$ when the accuracy and precision of the method is $\pm 20\%$. A result of $0.16\,mg\,l^{-1}$ would suffice.

For drug screening results it is advisable to provide clients with an indication of the range of substances a method is capable of detecting, and some indication of the detection limit, i.e., "at least therapeutic concentrations" or "only supratherapeutic concentrations." Positive immunoassay results should also be reported even if this presumptive detection has not been confirmed. This information can be useful since it may imply (to an expert later investigating the case) that the substance may have been present but at very low concentrations, or that there was another immunoreactive compound which was not excluded in the confirmation assay. To exclude these results could be construed by courts as a deliberate withholding of evidence.

To enable proper interpretation of evidence all reports should indicate the site of blood sampling, and provide where relevant, some comment on the possibility of postmortem artifacts such as redistribution. By incorporating these comments, uninformed persons reading the report are less likely to unwittingly misinterpret the results.

See Also

Toxicology: Methods of Analysis, Antemortem

Further Reading

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Toxicology, Accreditation

See **Accreditation:** Toxicology

Toxicology, History of

See **History of Toxicology**

Toxicology, Internet and

See **Internet:** Toxicology