Pitfalls in forensic toxicology

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INTRODUCTION

The word ‘forensic’ simply means ‘to do with courts of law’ and, as far as toxicology and biochemistry laboratories are concerned, embraces everything which has or might have legal implications. Some of the work in which the biochemistry department becomes involved is obviously ‘forensic’ but this may not be apparent at the time of receipt of the request; this is the first pitfall.

Toseland highlighted the dangers of ‘...an increasing tendency to carry out clinical analytical toxicology with the use of kits, usually in the form of rapid immunoassay – a system that makes the user believe that such testing is really very simple and that he (or she) cannot understand what all the fuss is about’. Toseland also highlighted the problem of ‘...the increasing referral of what are considered to be simple Coroner’s cases to the nearest hospital or Local Authority, in the hope that a quick and simple answer will be forthcoming. The dangers of applying screening immunoassay tests to post-mortem or even overdose cases that are admitted to hospital can lead to some quite horrendous errors’.

Coroners and others will naturally want their toxicology analyses to be performed by a competent laboratory, and accreditation may be a useful indicator of competence. The demands of such a service and the dangers of its complex nature to the unwary are discussed by de Zeeuw.

There are many pitfalls in forensic toxicology that need to be assessed and managed, and this review, by examining some of the problems raised by the requests received, the specimens submitted, performance of the analyses, interpretation of the results and the writing of the report, attempts to highlight the more important ones.

THE REQUEST

Physicians, pathologists, the police and others making the request often have unrealistic expectations regarding the range, sensitivity, specificity, speed and availability of thorough toxicological analysis. A senior member of the laboratory staff should appraise their expectations and explain what the laboratory will be able to achieve, the time-scale required and any likely limitations of the tests to be carried out.

Drugs-of-abuse screening can be a particularly difficult area if full information is not supplied. Some laboratories would prefer not to analyse specimens if this will involve them in the legal process. Examples include employment and pre-employment drug screening, drug screening for suspected poisoning in infants and children, and screening as part of investigation of suspected brain-stem death. In analysis of autopsy material, knowing the circumstances in which the subject died or in which the body was found is essential when deciding how to approach the analytical problem and make a correct interpretation. It is also important to have a full history, including drug therapy, on those subjects who have been found in a collapsed condition, brought to hospital and who subsequently die, perhaps a week or so after admission.

In addition to the usual patient identification protocols, essential information is detailed in Table 1.

SPECIMENS

Chain of custody
Legal challenges to the veracity of results of toxicological analysis are often successful, not for analytical reasons, but because the specimen could not adequately be identified as belonging to the subject under investigation. Specimens
that have or are likely to acquire a forensic label need to be handled with chain-of-custody provisions, which means that every step in the process of collection, transport, receipt, analysis and storage should be carefully documented. A form for this purpose can be produced by the laboratory with space for patient identification and columns for date and time of receipt and receiver’s name and signature, so that each person into whose possession the specimen passes is recorded. This form should be continued in the laboratory in order to record where the specimen is stored and each occasion on which the specimen is opened for analysis.

The individual steps necessary to verify continuing specimen integrity are summarized in Table 2. A wall chart produced by Syva-Behring UK (Behring Diagnostics UK Ltd, Milton Keynes, UK) is available which summarizes the major points.

**Types of sample**

**Employment/pre-employment screening**

**Urine:** Urine is usually adequate, provided that the volume obtained is sufficient (>60 mL) to allow any necessary confirmatory tests to be performed. The specimen should be divided at collection into two separate containers, each sealed and signed. Only one is processed and the second remains unopened in case independent analysis is required by a third party.

**Hair:** A number of methods have been developed for the detection of drugs in hair.\(^7\)\(^-\)\(^11\) Drugs remain in hair and are not metabolized during the life of the hair, and analysis offers a historical record of drug abuse.\(^12\) Adulteration of the specimen is unlikely although the possibility of surface contamination is recognized.\(^7\)\(^,\)\(^13\) Sample preparation is currently very labour-intensive and no adequate control material is available.

**Saliva:** Saliva has been used for the screening of drugs of abuse although the concentrations are lower than in urine and blood and are detectable in saliva for a shorter period.\(^14\)\(^-\)\(^16\)
Poisoned patients

Urine: For most poisons, urine is the material of choice for initial screening and a few millilitres will often suffice. Screens may be negative, however, if the urine is collected too soon after ingestion or after excretion of the toxic agent is complete. Physicians should be encouraged to submit urine collected on several occasions after admission on all unconscious patients in whom the diagnosis is not immediately obvious. Larger volumes are required for more elaborate analysis and for confirmatory testing.

Blood: A blood specimen is essential for analysis of some common poisons such as salicylate, paracetamol, digoxin, theophylline, iron and antifreeze to aid diagnosis and to direct therapy; it is also worthwhile obtaining a blood sample and/or preserving all blood samples submitted for biochemical or haematological analysis to enable confirmatory tests to be performed when the urine volume is limited. A blood specimen will often allow quantification of the poison in support of the clinical findings.

Gastric contents: Gastric contents, often containing whole tablets, tablet debris or an abnormally coloured fluid, are frequently useful to identify toxic agents recently ingested and should always be submitted to the laboratory when available. A volume of at least 60 mL is preferable.

Meconium: Meconium from newborn infants has also been shown to be useful material on which to screen for drugs of abuse.

Tables and other materials: Casualty departments and wards should be encouraged to submit all tablets, powders and fluids found on or brought in with the patient, even if these materials are in labelled containers. Analyses or other means of identifying a tablet, e.g. with TICTAC (http://www.tictac.org), can often aid diagnosis and occasionally show the container labelling to be incorrect.

Autopsy specimens

The specimen requirements from poisoned patients apply equally to post-mortem investigations. Forrest describes acceptable sampling protocols for various materials (e.g. liver, gastric contents, bile), in order to minimize the risk of contamination.

Blood and urine: At least 40 mL blood and all available urine should be submitted for analysis. It has been shown that the fluoride concentration in the fluoride tubes used clinically for collecting blood for glucose determination is insufficient to inhibit the changes in ethanol concentrations that occur in autopsy specimens after sampling. A sodium fluoride concentration of 1.5% in the blood is required. The changes that have occurred before sampling cannot, of course, be reversed.

Gastric contents: A small volume (60 mL) should be submitted, although ligation, removal and submission of the whole stomach and contents is practised by some pathologists.

Vitreous humour: Vitreous humour, which is in a protected position behind the lens of the eye, can usually be obtained intact even if a corpse has been extensively burnt or damaged, and does not suffer from the extensive post-mortem changes of blood. It is likely to be free from microorganisms, which can raise blood ethanol concentrations by up to 1500 mg/L and may be useful for determining some drug concentrations when a satisfactory specimen of blood is not available or when only limited examination of the body is possible. It is essential, however, that the specimen is collected correctly. Excessive suction during sampling can cause a significant change in the concentration of several analytes.

Liver and bile: A small (60 g) representative specimen of liver, not in formalin, will occasionally assist in post-mortem diagnosis. Bile (60 mL) may also be useful.

Lungs: For the diagnosis of death by inhalation of toxic gases and vapours, some pathologists submit entire lungs. Detection of common gases and solvents can readily be performed on blood collected in glass.

Brain: Many laboratories quantify centrally active drugs in brain tissue (60 g).

Containers, syringes and gas canisters: Containers found at the scene are frequently useful for indicating the nature of any substance injected, ingested or inhaled. In some instances, however, substances found at the scene are not implicated in the fatality, highlighting the danger in relying entirely on circumstantial evidence without analysis of body fluids.

Containers, anticoagulants and preservatives

Containers

Urine: New, unused containers for urine can usually be regarded as being drug-free and generally cause few problems. Some laboratories verify this by rinsing new containers with 1 ml of methanol and subjecting the rinse to analysis by gas chromatography (GC).
Blood: Some anticoagulants can interfere with certain drug assays, the obvious example being collection of blood for lithium determination into a specimen tube containing lithium heparin anticoagulant. In the author’s experience, serum generally gives cleaner traces in many high-performance liquid chromatography (HPLC) assays and avoids the interfering peaks sometimes produced by plasma samples. Fluoride has been reported to cause problems in the determination of ethanol concentration by headspace GC, but this has subsequently been disputed. Blood collection tubes per se have been reported to cause problems with some assays, notably with antidepressants and lignocaine. The author’s laboratory has found the tubes supplied by RossLab (RossLab, Macclesfield, UK) to be trouble-free.

For the analysis of volatile poisons (e.g. solvents and fuel gases) from acutely poisoned patients and autopsy subjects, plastic tubes are unsatisfactory, principally because solvents present in the specimen will be removed by dissolution into the plastic wall of the container or into the rubber or plastic cap liner of a glass tube. Gross contamination by toluene, 1-butanol, ethylbenzene and xylene has also been reported from blood collection tubes containing gel separators. In addition, plastic or rubber materials in contact with the specimen may introduce contaminants into the specimen and thereby produce spurious additional peaks in the chromatogram. These problems can be avoided if the rubber cap liner is wrapped in aluminium foil.

**Sampling of blood**

**The poisoned patient**
The site of sampling of blood from a patient is generally not critical and needs only to follow the normal rules for common clinical chemistry assays – avoid venous stasis, haemolysis and sampling from veins into which fluids are being infused. It will usually be necessary to note the time of sampling with respect to time of ingestion as dictated by the normal rules of pharmacology.

**The autopsy**
Blood that has been scooped out of a body cavity is an unsatisfactory specimen for toxicological analysis. Cardiac blood is similarly regarded as unsuitable for analysis: ethanol, for example, can diffuse out of the stomach to raise the cardiac blood concentration.

Post-mortem specimens obtained from a patient who has died in hospital several days after a self-poisoning episode are likely to be negative, depending upon the drug involved. It is essential to perform toxicology investigations on the specimens of blood and urine which were obtained on or soon after admission. In the author’s laboratory, the practice of holding specimens from suspected poisoning cases for about 1 month after admission has proved to be useful in a number of instances.

**Sources of contamination**

**On the ward**
Blood samples collected from the same limb as an intravenous fluid infusion are likely to be contaminated with the infusion fluid and with any therapeutic agent added to that fluid. Occasionally, blood collected into the wrong anticoagulant tube will be poured immediately into the correct tube to save performing another venepuncture. The blood is thus contaminated with the wrong anticoagulant. Alcohol swabs may also contaminate blood samples being collected from patients. Such swabs, however, usually contain propan-2-ol, readily identified by any GC method for alcohols and volatiles. Urine samples are often contaminated by local anaesthetics (e.g. lignocaine gel) which are used on catheters.

**In the autopsy room**
Gastric contents containing high concentrations of ethanol or drugs may cause contamination during an autopsy by being splashed into containers to be used for other fluids. Formaldehyde, used in autopsy rooms to preserve tissues for histological examination, is a reactive material which may have an effect on drugs present in blood and tissues. Embalming fluid also usually contains formaldehyde and alcohols and the conversion of nortriptyline to amitriptyline has been described in embalmed bodies.

**In the laboratory**
Accidental contamination of specimens with drugs from stock solutions of calibrators, which usually have very high concentrations, is always a possibility. Splashes, drops and the re-use of pipette tips are all potential means of contamination and should be guarded against. It is preferable that preparation and storage of calibrator solutions should take place in a...
specific area not used for the preparation of specimens for analysis.

The use of plastic containers, and especially disposable plastic pipette tips, should be avoided because they commonly add plasticizers, in the form of phthalates, to specimens. These can be a source of interference on GC and, although the added contaminants are usually readily identifiable using GC–mass spectrometry, increase the work of interpreting an otherwise straightforward chromatogram.

Storage and stability
Determinations available in the local laboratory usually present few stability problems because most analyses were performed soon after collection. More critical are those specimens that have to be forwarded to an outside laboratory or which need to be kept for several months.

Redistribution between cells and plasma/serum
The movement of intracellular digoxin in a way analogous to the movement of potassium and the redistribution of lithium between serum and cells are common illustrations of the need to separate plasma/serum from cells.

Most drugs and poisons are probably stable in biological material for many months, particularly if frozen, although there are some notable exceptions. For some analyses, special arrangements need to be made to prevent loss of analyte by degradation or other processes.

A number of commonly measured drugs are very unstable in blood, notably cocaine (in blood)28,29 and its metabolite (in urine)30 and benzodiazepines, e.g. nitrazepam.31 For a study of the stability of six drugs and one metabolite in blood, see Giorgi and Meeker.32

Temperature
Several studies have addressed the effect of temperature on stability. One study33 found that 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid was lost from urine on freezing below −16°C although benzoylcegonine, phencyclidine, codeine, morphine, amphetamine, metamphetamine and lysergic acid diethylamide were unaffected.33–35

Other factors
Ethanol is lost from fluoride-free specimens or under-filled containers and on exposing the specimen to air. Other solvents (e.g. toluene) are subject to similar problems.36 An advanced stage of decomposition of the body or prolonged immersion in water may change the concentrations of some common poisons such as ethanol,37 cyanide and carbon monoxide.38 Some drugs and metabolites undergo further decomposition due, for example, to enzymes in body fluids or exposure to light.31

ANALYSES
Introduction
The provision of a toxicology service in its broadest sense is very demanding on resources, very instrument-dependent and there is no single technique that will provide all the analyses required for a comprehensive service. For some commonly occurring analytes, e.g. ethanol, an enzymatic or GC technique can be applied directly. For most drugs and poisons, it is usual to employ two-stage testing (i.e. a screening test followed by confirmatory analysis). In the UK, there is no legislation defining how analytical toxicology is to be carried out. In the USA, however, the American Association of Forensic Sciences/Society of Forensic Toxicologists (AAFS/SOFT) laboratory guidelines have defined the requirements for analytical procedures, the controlling principle of which is that two tests should be performed for each analyte detected.4

Sample handling
Viscous samples are frequently encountered in autopsy work and require the use of positive-displacement pipettes, e.g. Transferpettors (Merck, Poole, UK), which are commonly used for sampling whole blood. Tissue samples and blood that yields no fluid material will require the weighing of a small sample followed by homogenization, enzymatic digestion and filtration before yielding a material which can be pipetted. Appropriate dilution factors need to be applied.

Methods in current use
Screening tests: Most analytical toxicology commences with a screen for the presence of unknown substances and would commonly employ one of the many available homogeneous immunoassay techniques that can be performed directly on urine without any sample preparation and are quick, convenient and relatively specific in most circumstances.

Chromatography can also be used for screening and is, in its various forms, the major method employed in toxicological analyses because of its flexibility and relatively low
Preliminary sample processing is usually necessary to remove matrix components and to concentrate the analyte into a small volume of an appropriate solvent for analysis. There is no single extraction system that will work for all drugs and therefore the selection of buffer, its pH and ionic strength, and optimization of the solvent system or solid-phase extraction system are critical for maximum recovery of the drug group under investigation. The very comprehensive reviews of Braithwaite et al.39 and Simpson et al.40 are essential up-to-date sources of information.

Confirmatory testing: All positive results obtained by a screening test need to be confirmed and such confirmatory testing, as defined by the AAFS/SOFT protocol, should have the properties summarized in Table 3.

Quantification of drug and metabolites: Adequate investigation of the poisoned patient and poison-related deaths can no longer rely solely on urine screens for a diagnosis.31 Neither is it sufficient in most instances to quantify only the parent drug and ignore the presence and the potential effects of metabolites, many of which are pharmacologically active and may be more toxic than the parent compound. Some drugs are metabolized very rapidly or are otherwise unstable in blood (e.g. cocaine) and the metabolite may be the only evidence that the drug was ingested. It is now recognized that determination of metabolite as well as parent drug concentrations in blood and other tissues is essential for the adequate interpretation of the data, particularly in post-mortem toxicology.

Range and versatility: New drugs are continually being developed and appear on the market almost weekly. Some are developments of existing drugs, often with a potency greater than their predecessors, e.g. the newer benzodiazepines. Others are new drugs, e.g. the selective serotonin re-uptake inhibitors.

The range of important metabolites continues to expand and their usefulness in interpretation is being recognized. For these reasons, the laboratory faces a continuously expanding range of compounds that it is required to identify and quantify. Analytical methods employed need to be sufficiently versatile to cope with this varying repertoire of analytes over widely diverse concentration ranges and in a variety of materials submitted for analysis.

Specificity and sensitivity: The purpose of an assay will determine the requirements for specificity and sensitivity. Limitations of specificity may be caused by a number of factors, for example, the structural and conformational similarity of many drugs and the non-specific nature of the analytical system employed. Not all ‘interferences’ or ‘cross-reactivities’ are undesirable. Screening tests are required to have adequate sensitivity but do not necessarily need high specificity. Indeed, the ability to detect all members of the same drug group is essential for screening purposes. Immunoassays fulfil this role admirably and usually have higher sensitivity than thin-layer chromatography (TLC). Many workers today choose to have a low specificity assay as a screen, which is then followed by a more specific confirmatory test.

Specificity of non-separation techniques (e.g. UV and fluorescence spectrometry) is invariably inadequate since, in such techniques where drugs and metabolites cannot be separated, each will add to the concentration of the other. Published data based on these techniques should be disregarded.

Calibrants

Commercial kits of reagents are usually supplied with calibrators and sometimes even control samples. Drug analysis not involving the use of commercial kits often presents a major problem in obtaining a supply of the drug for calibrators. Some drugs are readily available in pure form, often with certification of source and purity, but most are not and must be obtained from the drug manufacturer. Supplies of some drugs and metabolites, including certificated solutions and deuterium-labelled compounds for mass
spectrometry, are available from the sources listed in Table 4.

Confirming the purity of a product is not easy because good quality spectral and other data are not readily available. In most instances, purity has to be assumed. Few metabolites are available as pure material with which to prepare calibrators, even from drug companies and reagent kit manufacturers. Individual published methods, and occasionally the manufacturers, are often the only sources of data on the stability of calibration solutions. Source of material, batch number and date of preparation must be recorded. It is usual to calibrate assays in mass units of base material (not salts) per litre of solution.

**Quality control and quality assurance**

*Internal quality control programmes*

Material for internal quality assurance is available (see Table 4) in the form of freeze-dried urine and serum to which a limited range of common drugs and metabolites has been added. For the control of assays of less common drugs, it is necessary to add measured amounts of drug and/or metabolite to urine which has been shown to be drug-free. A similar situation exists for serum control material. After addition of the analytes, prolonged mixing is usually required to ensure dissolution and equilibration of protein binding, etc., before the material is aliquoted and stored at −20 or −70°C, at which temperatures most drugs and metabolites are usually quite stable. After thawing at room temperature, each aliquot should be thoroughly mixed before analysis.

*External quality assurance programmes*

Cardiff Bioanalytical Services Ltd organize the United Kingdom National External Quality Assurance Scheme (UKNEQAS) programme in which over 120 laboratories currently participate. A large amount of useful data are supplied including comparison of methods and laboratories. Table 5 gives some of the external quality assurance programmes available.

**Methodology**

*Immunoassay*

*Range:* For most laboratories, the range of immunoassays available is still very much in the hands of the reagent manufacturers. Immunoassay, however, has made drug

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<tr>
<td>Alltech Associates</td>
<td>Applied Science Ltd, Units 6–7, Kellet Road Industrial Estate, Carnforth, Lancs LA5 9XP, UK</td>
<td>01524 734451</td>
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<tr>
<td>Laboratory of the Government Chemist</td>
<td>Queens Road, Teddington, Middlesex TW11 0LY, UK</td>
<td>01943 7000</td>
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<tr>
<td>Promochem Ltd</td>
<td>PO Box 300, Welwyn Garden City, Herts AL7 1SS, UK</td>
<td>01707 396677</td>
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<tr>
<td>Radian Corporation</td>
<td>8501 N. Mopac Blvd, PO Box 201088, Austin, TX 78720-1088, USA</td>
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<tr>
<td>Sigma Chemical Company</td>
<td>Fancy Road, Poole, Dorset, BH17 7NH, UK</td>
<td>0800 373731</td>
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<tr>
<td>Ultrafine Chemicals and Research Ltd</td>
<td>Synergy House, Guildhall Close, Manchester Science Park, Manchester, M15 6SY, UK</td>
<td>0161 226 8774</td>
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<tr>
<td>Bio-Rad</td>
<td>Bio-Rad Laboratories Ltd, Bio-Rad House, Maylands Avenue, Hemel Hempstead, Herts</td>
<td>01442 232552</td>
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<tr>
<td>Bio-stat Ltd</td>
<td>Bio-stat House, Pepper Road, Hazel Grove, Stockport SK7 5BW, UK</td>
<td>0161 483 5884</td>
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<tr>
<td>DPC</td>
<td>DPL Division, Euro/DPC Ltd, Glyn Rhonwy, Llanberis, Gwynedd LL55 4EL, Wales, UK</td>
<td>01286 871872</td>
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**Table 5. External quality assurance programmes**

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<tr>
<td>Birmingham EQA</td>
<td>Dr R Braithwaite, Regional Laboratory for Toxicology, City Hospital NHS Trust, Birmingham B18 7QH, UK</td>
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<tr>
<td>UKNEQAS</td>
<td>Cardiff Bioanalytical Services Ltd, 16 Mount Stewart Square, Cardiff CF1 6DP, UK</td>
</tr>
<tr>
<td>Oslo EQA</td>
<td>Dr Grete Wethe, National Institute for Forensic Toxicology, PO Box 495 Sentrum, N-0105 Oslo, Norway</td>
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concentration determination in plasma a realistic and convenient possibility for most hospital laboratories and immunoassay may also be applied, with due caution and proper controls, to other fluids, e.g. vitreous fluid.42

Limitations: The limitations of immunoassays are most obvious in the screening of urine for drugs of abuse, although in the quantitative determination of drugs, too, one should be aware of potential problems.43

Interferents: Interferences may be due to other drugs or non-drug compounds present accidently or, as sometimes occurs with specimens from drug-abuse clinics, added deliberately. The use of many common adulterants by drug abusers anxious to avoid detection has passed into laboratory folk-lore, e.g. bleach, washing-up liquid etc.44–46 Other adulterants are less obvious.47 Homogeneous immunoassays are not the only assays affected and the development of ‘designer drugs’ (i.e. analogues of existing drugs) compounds the problem.48 ‘UrinAid’, a solution of glutaraldehyde, was originally developed and marketed by Byrd Laboratories (Topanga CA, USA) to ensure false negative results for cannabinoid assays and was later found to interfere with all immunoassays.49 This particular product is understood to be unavailable in this country but solutions of glutaraldehyde can be bought under various guises as cleaning agents or wart treatment. Concentrations as low as 1–2% produce false negative results and are undetectable by colour or smell. Urine samples adulterated with glutaraldehyde will usually show reaction rates lower than those obtained on a negative, non-adulterated sample.

Microparticle agglutination inhibition immunoassays are less affected by interferents or are affected in such a way that false positive rather than false negative results are obtained.50–52 This has the obvious advantage of highlighting the possibility of interferents being present and must, of course, be set against the disadvantage of the extra costs of running confirmatory tests.

Sensitivity: Sensitivity, the subject of SAMHSA (Substance Abuse and Mental Health Services Administration, previously NIDA: National Institute on Drug Abuse) guidelines in the USA, is a major issue and seems to be affected not only by the concentration chosen as the ‘cut-off’ but also by the nature of the calibrator and the presence of surfactant.44,53–55 Sensitivity of the assay is a major determinant of how long the urine of a particular patient will remain positive.

<table>
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<tr>
<th>Benzodiazepine compound</th>
<th>EMIT dau* current reagents (ng/ml)</th>
<th>EMIT dau* new reagents (ng/ml)</th>
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<tr>
<td>Alprazolam</td>
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<td>Triazolam</td>
<td>170</td>
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*EMIT dau: EMIT reagents for drugs of abuse in urine.

Manufacturers undoubtedly put a great deal of effort into ensuring reproducibility of performance of reagents between batches. None-the-less, there is variability39,40,56 between batches regarding sensitivity and cross-reactivity, as shown by the continuous updating of pack inserts,57 an example of which is shown in Table 6.

Specificity: A major pitfall with immunoassays is that, while being simple and quick to perform, they have not lived up to the claims of specificity predicted for them when they first appeared. Whilst a degree of cross-reactivity between members of the same drug group is essential for screening tests, the cross-reactivity with members of other drug groups can frequently cause problems. This limitation of specificity may be due to a number of factors, for example: (a) the structural and conformational similarity of many drugs, allowing antibody binding to occur with members of different drug groups; (b) the non-specific nature of the indicator reaction used with homogeneous immunoassay due to inhibition of enzyme activity or consumption of co-enzyme; (c) the presence or generation of a high background absorbance preventing measurement; or (d) other mechanisms.

In screening specimens obtained in self-poisoning cases, and especially in fatalities, it is important to refer frequently to the cross-reactivity tables produced by the manufacturer. The reported cross-reactivities, while favourable for drugs-of-abuse specimens, are likely to lead to misinterpretation in specimens from self-poisoning cases and fatalities, in which a variety of drugs may have been ingested in excessive quantities. Table 7 gives examples of cross-reactivity of immunoassays with various drugs. Some non-abused drugs produce metabolites...
which are structurally similar to abused drugs and therefore give positive immunoassay screens. Some of these are also included in Table 7.

For more information on cross-reactivity, particularly on potential mechanisms, consult one of the very useful reviews of the limitations of immunoassays.39,40,56

Matrix effects and parallelism: When using immunoassays in the quantitation of drugs and metabolites it is essential to demonstrate by recovery experiments and a range of dilutions that the assay has satisfactory parallelism and is not affected by matrix effects in the fluid under analysis.

Chromatography
Chromatography has been the mainstay of toxicological analysis for many years, principally because the techniques are sufficiently diverse and flexible to allow the separation and identification of most compounds of toxicological interest. In principle it is simple, but a high degree of skill is required to keep the instrumentation working well, to obtain reliable results and to exploit its capabilities to the full. Chromatography remains, however, an essentially non-specific technique and in its various forms and throughout its history has been modified to improve its specificity by the use of a variety of extraction techniques, different stationary phases, derivatization and a variety of detectors. The presence of non-drug compounds in the matrix has been shown to affect retention data, to present as discrete peaks in TLC,58,59 GC60 and HPLC61 and/or to affect absorbance spectra of drugs. This latter reference contains a useful review of this aspect of chromatography. These problems may be reduced by the use of certain detectors, e.g. a diode array detector, but positive identification in any form of chromatography is achieved only by mass spectrometry.

Sample preparation: Some form of sample preparation to separate the compounds of interest from the matrix is required for all forms of chromatography. Traditionally, this has been achieved by liquid–liquid extraction after mixing the sample with a buffer at a selected pH and ionic strength. Extraction at around pH 8–9 will isolate many unconjugated drugs, the so-called ‘basic drug screen’, including narcotic analgesics, benzodiazepines, methadone, local anaesthetics and phenothiazine tranquillisers. Amphetamines require a pH of 10 or more. Barbiturates are extracted with other acid and neutral drugs in the ‘acid drug screen’ at a pH between 5 and 6. The use of bromocresol purple as an ion-pairing reagent has allowed the extraction of acid, neutral and basic drugs at pH 6.62

A wide variety of solvents has been employed with the principal aim of maximizing recovery while leaving most matrix compounds, especially lipids, behind. Popular solvents have included chloroform, dichloromethane (barbiturates, benzodiazepines and methadone) and mixtures of these with each other and with propan-2-ol or ethanol (cocaine) or diethylether, di-isopropyl ether or hexane for amphetamines. N-butyl acetate has been used for a wide-ranging basic drug screen.63

Water-soluble conjugates are not extracted by solvents of low polarity and require hydrolysis by boiling with a strong mineral acid or by incubating with β-glucuronidase before extraction. Acid hydrolysis will also form the benzo-phenones from some benzodiazepines and these can subsequently be identified.

Solid-phase extraction columns and discs in a wide range of sizes and materials are now available for sample preparation; they will cope

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<td>KIMS Opiate</td>
<td>Rifampicin148</td>
<td></td>
</tr>
<tr>
<td>EMIT Amphetamine</td>
<td>Ranitidine49</td>
<td></td>
</tr>
<tr>
<td>DPC coat-a-count Metamphetamine</td>
<td>Ephedrine and pseudoephedrine50</td>
<td></td>
</tr>
<tr>
<td>TDx Metamphetamine</td>
<td>Selegiline151</td>
<td></td>
</tr>
<tr>
<td>TDX Amphetamine</td>
<td>Clobenorex152</td>
<td></td>
</tr>
<tr>
<td>Abuscreen Amphetamine</td>
<td>Amphetamine analogues48</td>
<td></td>
</tr>
<tr>
<td>TDX Cannabinoid</td>
<td>Various compounds153</td>
<td></td>
</tr>
<tr>
<td>EMIT Opiate</td>
<td>Amitriptyline154</td>
<td></td>
</tr>
<tr>
<td>EMIT Methadone</td>
<td>Diphenhydramine155</td>
<td></td>
</tr>
<tr>
<td>EMIT Methadone</td>
<td>Doxylamine147</td>
<td></td>
</tr>
<tr>
<td>Synchro Methadone</td>
<td>Diphenhydramine156</td>
<td></td>
</tr>
<tr>
<td>Synchro Methadone</td>
<td>Verapamil156</td>
<td></td>
</tr>
<tr>
<td>Synchro Methadone</td>
<td>Sertraline156</td>
<td></td>
</tr>
</tbody>
</table>
with whole blood and tissue homogenates as well as urine, and will allow a degree of automation using such instruments as the Gilson ASPEC or ASTED (Anachem Ltd, Luton, UK).

The material extracted by liquid–liquid- or solid-phase needs to be dissolved in a solvent compatible with the system on which the intended separation will take place. This may involve evaporating away the final extractant in order to dissolve the analyte in a smaller volume of a suitable solvent. Some drugs are unstable in air or are very volatile, e.g. amphetamines, and need to be converted to their acid salts before drying; alternatively, a method can be employed which avoids this evaporation step.64

Thin-layer chromatography
Thin-layer chromatography methods provide a single system which will detect a wide range of drugs. Most basic drugs will give a positive reaction with Dragendorff’s reagent whilst barbiturates require reaction with mercurious nitrate. Generally, TLC has poor sensitivity unless large volumes of sample are extracted, has low specificity, is slow and cumbersome, requires considerable time to learn the skill of interpreting the chromatograms, and cannot produce a quantitative answer. Some of these limitations have been partially overcome by the use of ‘high-performance’ TLC (HPTLC) plates, separation on twin systems, use of more than one locating agent for one group of drugs or the application of complex locating agents which produce fluorescent derivatives (e.g. amphetamines).

Toxi-Lab: The widely used commercial system Toxi-Lab (Microgen Bioproducts Ltd, Camberley, UK) provides a relatively simple and comprehensive system for detecting the presence of approximately 200 drugs and metabolites. There are two types of prepared extraction tubes containing a solvent mixture and buffer salts for basic drugs (Toxi-tubes A), acidic drugs (Toxi-tubes B) and cannabis tubes. After centrifugation, the extraction solvent is evaporated so that the drugs are concentrated onto 3-mm discs, which are then pressed into holes at one end of a thin-layer plate (the Toxi-gram). After development, the plates are dried and stained by a succession of reagents and viewed between each step. Visible spots are identified by comparison with photographs of each stage of the locating process.

Toxi-Lab’s principal disadvantages are as listed above for TLC. In addition, it performs poorly for opiate detection compared with other methods,65–68 in common with all TLC, it performs poorly for amphetamine detection according to UKNEQAS data,68,69 and it has low sensitivity for cannabis (25 μg/L)70 although it probably performs better than most other TLC systems. Toxi-Lab separates cocaine and benzoylecgonine in addition to methadone and its EDDP (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine) metabolite, but is considerably more expensive and generally less sensitive than in-house systems. Badcock and Zanetti71 describe modifications to the Toxi-Lab system for use in paediatric toxicology.

Gas chromatography
Packed-column GC is robust and generally easy to use, although frequent re-packing of the head of the column is usually necessary, particularly when used for post-mortem samples. Capillary column GC is now very popular and relatively easy to use, although the columns are still rather expensive. It is common practice to have a sacrificial guard column before the main analytical column and to maintain performance by cutting off the first metre of the guard column whenever deterioration occurs. With temperature programming and a range of detectors, a flexible system can be operated to cover most types of screening and quantitation for drugs and poisons.

Many drugs, including the amphetamines, the cocaine metabolite benzoylecgonine and a number of benzodiazepines, are unstable at GC temperatures or produce marked peak tailing and therefore derivatives are usually prepared. Commonly, trimethylsilyl, trichloroacetyl, trifluoroacetyl, heptafluorobutyryl, pentafluorobenzoyl and propyl derivatives are used.72

The retention time (Rt) or retention index (Ri) of a derivative or the difference in Rt or Ri between drug and derivative can increase the specificity of GC, e.g. the ‘acetone shift’ commonly employed to identify amphetamine. Two columns, often in the same chromatograph oven but with different retention characteristics, can also be used to improve specificity. The availability of a mass spectrometric detector on the gas chromatograph enables more positive identification to be made. The major disadvantages of GC remain the large capital outlay and the degree of skill required to operate the instrument and interpret the resultant chromatograms. Legally-defensible identification of detected components will usually require the
specification which only a mass-selective detector is able to provide.

**High-performance liquid chromatography**

High-performance liquid chromatography has probably overtaken GC in popularity for drugs determination and can provide a high degree of flexibility with reduced risk of compound degradation during analysis. Purchase of the equipment demands large capital outlay and a skill level similar to GC is required. HPLC is suitable for screening and quantitation of most drugs and the running costs are usually modest.

There are many published methods – reverse-phase, normal-phase, ion-exchange – for all groups of drugs but no single method is able to detect all drugs. Barbiturates and a wide range of basic drugs, including methadone and cocaine and their metabolites, are readily separated using an isocratic system. A variety of methods is available for the quantitation of benzodiazepines and amphetamines, usually requiring gradient elution to separate the widest range.

Detectors are not equally sensitive to all drugs, even drugs within the same class, and there is often no single UV wavelength suitable for all members of one group, e.g. benzodiazepines. The wavelength of the UV detector can be changed to tune in or tune out compounds at will. At certain wavelengths some compounds will be invisible to the detector and, therefore, the measuring wavelength chosen is usually a compromise to enable detection of all the members of a particular group of compounds. Similar adjustments will be required with fluorescence and electrochemical detectors. Diode-array detectors, because of the ability to perform a spectral scan while the peak is being drawn, have become increasingly common and enhance the specificity of the technique over a conventional UV detector. The spectrum of each peak can be compared with a library of spectra held on hard disc in an associated computer and a high grade match will indicate some degree of identity with the library entry.

Stability of analytes during chromatography is not usually a problem with HPLC and therefore derivatization is used mainly to enhance specificity or sensitivity. The main disadvantages of HPLC are as for GC (i.e. capital outlay, skill required in operation and interpretation and the lack of positive identification without the presence of a mass-spectrometric detector). Some problems of performing drug analysis by HPLC are discussed by Lurie.

**Mass spectrometry**

Small, ‘bench-top’ mass spectrometers have been available for a long time as detectors in GC and have recently become available for HPLC. The limitations of chromatography per se still apply to GC–MS (mass spectrometry) and HPLC–MS, but the limitations of the other detectors are removed. GC–MS usually requires preparation of derivatives which will give a unique spectral pattern for each compound and thereby allow members of the same drug class to be easily distinguished (see Table 8). The technique is essential for providing unequivocal and legally defensible identification but is not without its own limitations in the determination of, for example, cannabinoids, amphetamine and metamphetamine, and morphine and codeine, and there are numerous other examples in the literature.

The purchase price of gas chromatograph–mass spectrometers has been falling in recent years but is still a considerable capital outlay. Annual maintenance of the instrument is usually about 10% of the capital cost, and routine operation and maintenance are very demanding of operator time and skill but essential to produce reliable results. The components that need periodic replacement (e.g. septa, ‘Vespel’ ferrules, injection port liners and capillary columns) are expensive, as is the high-grade (99.999% pure) helium and the purest solvents (e.g. Pesticide Grade; BDH Laboratory

<table>
<thead>
<tr>
<th>Derivative</th>
<th>Drug class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pentafluoropropionic anhydride</td>
<td>Amphetamines</td>
</tr>
<tr>
<td></td>
<td>Tricyclic antidepressants</td>
</tr>
<tr>
<td></td>
<td>Opiates</td>
</tr>
<tr>
<td>BSTFA/TMCS*</td>
<td>Opiates</td>
</tr>
<tr>
<td></td>
<td>Cannabinoids</td>
</tr>
<tr>
<td></td>
<td>Benzodiazepines</td>
</tr>
<tr>
<td>BSTFA/TMS**</td>
<td>Opiates</td>
</tr>
<tr>
<td></td>
<td>Cannabinoids</td>
</tr>
<tr>
<td></td>
<td>Benzodiazepines</td>
</tr>
<tr>
<td>Acetic anhydride/pyridine</td>
<td>Opiates</td>
</tr>
</tbody>
</table>

*Bis-(trimethylsilyl)trifluoroacetamide/trimethylchlorosilane; **bis-(trimethylsilyl)trifluoroacetamide/trimethylsilane.
A number of specialist ‘mass spectrometry laboratories’ are often prepared to chromatograph samples which have already been extracted by a non-specialist laboratory.

Ultimately, the choice of technique depends on many factors including available resources, the type of service a laboratory is required to provide and the nature of the work involved. A novel statistical approach to selecting methodology for drugs-of-abuse testing has been described by Ferrara et al.

Selected analytes

Ethanol

Specimen-related problems: Ethanol is the most commonly analysed poison in autopsy toxicological examination. In life, a peripheral blood sample will give a reliable indication of a patient’s state of inebriation, and in the post-absorptive state the urine concentration is approximately 1.3 times the blood alcohol concentration. These simple facts rarely hold true after death.

Ethanol is distributed throughout body water and therefore plasma ethanol concentrations are 10–15% higher than whole blood concentrations. Blood specimens obtained at autopsy vary greatly in red cell and protein content owing to the unevenness with which blood clots and clot lysis occur. Pathologists, by avoiding clots when they sample blood at autopsy, produce specimens that are serum-rich and are therefore more likely to have higher ethanol concentrations than a true whole blood specimen. The water content of whole blood, however, decreases after death such that by 10 h the mean water content is only 80% of its former level. This has the effect of lowering the ethanol content of the blood.

Microorganisms in cadavers produce alcohol during putrefaction. Bacteria from the gut escape via the lymphatics and the portal venous system and can generate ethanol in blood, brain, liver and other tissues within a few hours of death. Blood ethanol concentrations can reach 1500 mg/L due to putrefaction at room temperature within a few days. Physical disruption of the body has also been shown to enhance post-mortem alcohol production. Post-mortem urine and vitreous fluid are largely free of this effect and may be analysed to corroborate the blood ethanol concentration and to highlight when the latter is artefactually raised, although some workers would disagree.

It is now well recognized that alcohol diffuses from the stomach, small bowel and even airways contaminated with gastric material into the blood of large central vessels if death has occurred soon after ingestion of alcohol. This passive diffusion can create a difference in concentration between central and peripheral vessels of up to 400%. For this reason, blood for alcohol determination, in common with other toxicological analyses, should be obtained from a peripheral vessel.

Alcohol dehydrogenase methods: Ethanol can be determined in body fluids by a variety of methods. In clinical situations involving the analysis of blood and urine from patients, most analytical methods are satisfactory. Assays using alcohol dehydrogenase are simple and easy to use, can be automated and provide an accurate answer in a very short time. The measured parameter is the change in absorbance due to the production of NADH.

It has been shown that lactate and lactate dehydrogenase are present at high concentration in the blood of most cadavers. Falsely high blood ethanol concentrations are obtained when the enzymatic method is used on cadaver blood due to the production of NADH by lactate dehydrogenase when converting lactate to pyruvate.

Other alcohols have been shown to interfere with the alcohol dehydrogenase method for determination of blood alcohol. Their presence in cadaver blood cannot be distinguished from ethanol by the enzymatic method and can only be revealed by GC.

Alcohol dehydrogenase is also inhibited by all the putrefactive bases – cadaverine, putrescine and phenethylamine – which occur in the blood of putrefying corpses (Ivison F, personal communication). This results in falsely low and even falsely negative ethanol concentrations in autopsy specimens. The enzymatic method is therefore unlikely to produce reliable results on autopsy specimens and should not be used.

GC methods: Determination of ethanol by GC involves addition of a suitable internal standard and precipitation of proteins, followed by either injection of clear supernatant or evaporation of the ethanol in a sealed vessel and injection of the vapour phase (head-space analysis). Available methods usually allow separation and identification of other alcohols, e.g. methanol, n-propanol and isopropanol; a head-space technique has been described for the determination of higher solvents such as toluene from cases of...
glue-sniffing, and fuel gases such as propane and butane from inhalation of lighter fuel etc.\textsuperscript{99}

**Carbon monoxide**

*Sources*: Carbon monoxide (CO) is produced endogenously in the body from the breakdown of haem pigments\textsuperscript{100} and accounts for the small proportion (<0-7\%) of oxyhaemoglobin (OxyHb) present as carboxyhaemoglobin (COHb) in healthy, non-smoking human adults.\textsuperscript{101} Smoking of tobacco in any form causes an increase in this proportion to around 4\%,\textsuperscript{102} and the presence of high concentrations of CO in the atmosphere from motor vehicles and industrial and domestic heaters may raise the blood COHb concentrations of city dwellers even higher.

The most common source of CO in the UK is the petrol engine, closely followed by poorly maintained or inadequately ventilated domestic gas appliances. Many fatalities due to CO are deliberately caused by either trapping the exhaust gases of a car in a closed garage or routing the exhaust gases into the cab by means of a hose.

Victims of house fires, if they were breathing when the fire started, usually have very high concentrations (>80\%) of COHb in their blood. Part of this is due to the fact that production of CO in fires is directly proportional to temperature; a higher concentration ratio of CO to carbon dioxide (CO\textsubscript{2}) is produced at higher temperatures.

Dichloromethane, a common solvent in the home and industry as a component in paint stripper and metal cleansing solutions, is, after inhalation or ingestion, metabolized (via oxidative dehalogenation by the P\textsubscript{450}-dependent mixed-function oxidase system or by the glutathione-S-transferase pathway) to formaldehyde, formic acid, inorganic chloride and CO.\textsuperscript{103} Users of this solvent in a poorly ventilated work place are thus at risk.

**Specimen-related problems**: Kojima et al.\textsuperscript{104} determined the concentrations of CO, total haemoglobin (THb) and COHb in blood and red-coloured body cavity fluids of cadavers which had not been exposed to fire. They concluded that there was endogenous production of CO in cadavers found on land and in fresh or salt water. Although this had no significant effect on the results for blood, results for cavity fluids were significantly affected. Kojima et al. recommended that cavity fluids should not therefore be used for CO or COHb measurements.

<table>
<thead>
<tr>
<th>Methodology</th>
<th>Number of laboratories</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL282/482</td>
<td>25</td>
</tr>
<tr>
<td>Radiometer OSM or ABL520</td>
<td>25</td>
</tr>
<tr>
<td>Corning 270 or 2500</td>
<td>19</td>
</tr>
<tr>
<td>AVL 912</td>
<td>6</td>
</tr>
<tr>
<td>Spectro direct</td>
<td>18</td>
</tr>
<tr>
<td>Spectro + dithionite</td>
<td>28</td>
</tr>
<tr>
<td>GC</td>
<td>1</td>
</tr>
<tr>
<td>Whitehead and Worthington (heat denaturation)</td>
<td>7</td>
</tr>
</tbody>
</table>

GC, gas chromatography.

The COHb concentration in sampled peripheral blood will continue to rise unless a sufficient concentration of fluoride (1\%) is present to inhibit the process.

**Analysis**: Spectrophotometric methods for COHb are affected by temperature. Absorbances of COHb, OxyHb and haemoglobin decrease linearly with increasing temperature over the range 20–40\°C. Instruments should therefore have accurate and precise temperature control of the measuring cuvette. CO-oximeter methods predominate in the UK (see Table 9). However, post-mortem blood samples frequently have high concentrations of methaemoglobin and sulphaemoglobin, due to the production of H\textsubscript{2}S during decomposition, and a high concentration of reduced haemoglobin. These high concentrations frequently exceed the capacity of the analyser to correct for their presence and therefore unreliable results are generated. Some blood gas analysers will not print out a result if the measured parameters are outside the ranges compatible with life.

In autopsy samples, the presence of a high concentration of denatured haemoglobin is likely to prevent the preparation of an adequate 100\% (CO-saturated) standard because the reduced Hb and sulphaemoglobin cannot adequately take up CO. Homogenized muscle, liver and lung have all been used in attempts to avoid these problems. For this reason, the common methods for determining COHb in life are likely to produce misleading results when applied to autopsy specimens.

The problems described above are avoided in post-mortem blood by determining CO concentration using GC. Conversion to %COHb is rarely possible because an accurate haemoglobin
concentration is unlikely to be obtained. Available methods are reviewed by Mayes.105

INTERPRETATION
Problems in living patients
The urine drug screen
The interpretation of the urine drug screen, from whatever source, is relatively straightforward provided one has available the relevant data listed in Table 1, justifiable confidence in the analyst who performed the screen and detailed information on the current performance and limitations, i.e. sensitivity and cross-reactivity, of the analytical technique employed. The availability of confirmatory data helps enormously and is essential if the results are likely to be challenged.

The poisoned patient
Plasma concentration data are usually available in addition to the results of screening tests. For adequate interpretation, reliable, up-to-date reference ranges for therapeutic and toxic concentrations are essential, together with the appropriate information detailed in Table 1. Interpretation can occasionally be complex and require an advanced knowledge of pharmacology.

<table>
<thead>
<tr>
<th>Table 10. Factors likely to contribute to the uncertainty of reference ranges for drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Involvement of different preparations of the drug</td>
</tr>
<tr>
<td>Pharmacokinetics of the drug</td>
</tr>
<tr>
<td>Age (neonates, children, adults, elderly)</td>
</tr>
<tr>
<td>Variations in elapsed time between drug administration and sampling</td>
</tr>
<tr>
<td>Distribution of the drug</td>
</tr>
<tr>
<td>Genetic variation in drug metabolism</td>
</tr>
<tr>
<td>Idiosyncratic reaction to the drug</td>
</tr>
<tr>
<td>Development of tolerance</td>
</tr>
<tr>
<td>Presence of ethanol or other drugs</td>
</tr>
<tr>
<td>Post-sampling redistribution of drug between serum and cells</td>
</tr>
<tr>
<td>Post-sampling instability of drug</td>
</tr>
<tr>
<td>Differences in analytical methods employed</td>
</tr>
<tr>
<td>Poor specificity or poor precision of analytical methods</td>
</tr>
<tr>
<td>Presence of concomitant disease</td>
</tr>
<tr>
<td>Insufficient experimental or case data</td>
</tr>
<tr>
<td>Additional factors</td>
</tr>
<tr>
<td>Variations in sampling site</td>
</tr>
<tr>
<td>Variations in elapsed time between administration of the drug and death</td>
</tr>
<tr>
<td>Post-mortem redistribution of the drug</td>
</tr>
<tr>
<td>Other, unknown factors</td>
</tr>
</tbody>
</table>

Reference ranges
There is a large overlap between the various ‘therapeutic’, ‘toxic’ or ‘fatal’ concentration ranges quoted in a number of texts. Some of the reasons for this are listed in Table 10. Data in older text books may be unreliable. Flanagan106 describes a website (http://www.leeds.ac.uk/acb/annals/Netwise) designed to assist in the interpretation of analytical toxicology results, details data sources available and includes several tables that may be useful.

Special problems of post-mortem samples
The provision of a diagnosis is the role of the pathologist, and in helping the pathologist decide to what degree drugs or poisons contributed to the death of the deceased, the problem of reference ranges is more complex because of the additional factors shown in Table 10.

Site and time dependence of post-mortem blood sampling
It is now clear that there are site- and time-dependent drug concentration changes after death. Various studies have shown significant differences in drug concentrations between arterial and venous blood, between blood vessels in different parts of the body and between plasma and cells.107 These differences may or may not pertain after clotting and clot lysis has occurred post-mortem. Post-mortem blood clots unevenly and sediments, and it is frequently not possible to obtain clear serum; the analyst usually has to process partially lysed blood containing an unknown proportion of cell contents. The haemoglobin concentration as an indicator of such processes as haemodilution or lysis does not correlate well with drug concentrations in post-mortem blood. In some instances, a specimen of what appears to be clotted blood never yields any fluid material even after prolonged centrifugation. Some differences may be due to these effects. Some post-mortem concentration differences in the literature may also be caused by lack of care in identifying the site of sampling at autopsy.

In one recent study, paracetamol concentrations were found to approximately double in peripheral (femoral) blood and to increase more than sixfold in central (heart and inferior vena cava) blood over the 12-h period after death.108 For the redistribution of drug to the central compartment, these investigators offered several possible explanations, which included:
(a) diffusion from specific tissue sites of higher concentration post-ingestion (such as liver or mesenteric portal vessels) to central vessels in close proximity; (b) diffusion of unabsorbed drug in the stomach to the heart and the inferior vena cava; and (c) collection of blood from the corpse over time may cause the blood to ‘circulate’, i.e. suction with a syringe moves blood or blood fluid along central vessels from regions of higher concentration. Peripheral vessels may be relatively protected by distance from this effect.

Other studies have suggested different mechanisms of post-mortem redistribution of drug concentrations, such as depletion of energy-dependent processes that may concentrate drug in specific tissues, cell death, changes in drug-binding proteins, changes in the permeability of inter-tissue barriers after death, post-mortem changes in pH and ionic strength of intra- and extracellular fluids, which would allow drugs to redistribute down concentration gradients, route of administration, acute versus chronic dosage prior to death. One study reported that in severely decomposed bodies, the movement of blood through the vasculature was caused by gas formation and may be the major mechanism that enables a drug from different sites to mix and re-equilibrate over time. Since enzymes are readily released from tissues following death, it is hardly surprising that drugs, which are much smaller molecules, are also released.

If intravascular drug concentrations are higher than the concentrations in surrounding tissues at the point of death, i.e. before the distribution phase is complete, then, theoretically, blood concentrations could decrease with time due to the redistribution of intravascular drug into the surrounding tissues. Conversely, drugs with a large volume of distribution (Vd) are found at highest concentration in solid organs (e.g. liver) and the concentration in the blood of associated vessels may rise. Post-mortem catabolism of unstable drugs such as cocaine is also a likely mechanism to explain the decrease in blood drug concentrations. The twin processes of redistribution, which may increase drug concentration, and degradation, which decreases drug concentration, may obviously occur together and at different rates.

The mechanisms whereby these post-mortem changes come about are not fully understood and are likely to be complex. Pounder, in a very thorough review, discusses these mechanisms in the light of a large number of published reports and concludes: ‘For interpretive purposes, the ideal toxicological sample is a peripheral blood specimen obtained from a ligated vessel immediately after death. All autopsy specimens fall short of this ideal, but the more they do so, the more contentious will be the interpretation of the analytical results. The problem is made more difficult because an awareness of the phenomenon of post-mortem drug redistribution has undermined the reference value of databases of drug concentrations in post-mortem blood where the site of the sample is unknown.’

A great deal of work on post-mortem distribution has been reported in recent years and has included investigation of the drugs shown in Table 11.

The changes caused by post-mortem redistribution are less likely to cause problems with interpretation when the concentrations obtained by analysis are extremely high, well outside any area of controversy and demonstrated in blood from more than one site. Since the phenomenon

<table>
<thead>
<tr>
<th>Drug</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>amiodarone</td>
<td>157</td>
</tr>
<tr>
<td>cimetidine</td>
<td>109</td>
</tr>
<tr>
<td>clozapine</td>
<td>158</td>
</tr>
<tr>
<td>digoxin</td>
<td>157</td>
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<tr>
<td>disopyramide</td>
<td>159</td>
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<tr>
<td>dothiepin</td>
<td>160</td>
</tr>
<tr>
<td>doxorubicin</td>
<td>161</td>
</tr>
<tr>
<td>flecainide</td>
<td>157</td>
</tr>
<tr>
<td>fluoride</td>
<td>162</td>
</tr>
<tr>
<td>heroin</td>
<td>163</td>
</tr>
<tr>
<td>ibuprofen</td>
<td>164</td>
</tr>
<tr>
<td>imipramine</td>
<td>107</td>
</tr>
<tr>
<td>ketamine</td>
<td>165</td>
</tr>
<tr>
<td>lignocaine</td>
<td>159</td>
</tr>
<tr>
<td>MDMA</td>
<td>166</td>
</tr>
<tr>
<td>melloquine</td>
<td>167</td>
</tr>
<tr>
<td>methamphetamine</td>
<td>168</td>
</tr>
<tr>
<td>metoprolol</td>
<td>169</td>
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<tr>
<td>mexiletine</td>
<td>170</td>
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<tr>
<td>midazolam</td>
<td>171</td>
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<tr>
<td>phenol</td>
<td>172</td>
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<tr>
<td>sertralin</td>
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</tr>
<tr>
<td>sotalol</td>
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<tr>
<td>sufentanil</td>
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<tr>
<td>thiopental</td>
<td>174</td>
</tr>
<tr>
<td>tricyclic antidepressants</td>
<td>175</td>
</tr>
<tr>
<td>zimelidine</td>
<td>176</td>
</tr>
<tr>
<td>zopiclone</td>
<td>177</td>
</tr>
</tbody>
</table>
of post-mortem redistribution has been recognized, many more results will now fall into areas of controversy over interpretation. The problem is further compounded by the phenomenon of tolerance.

**Pharmacokinetics**

A working knowledge of pharmacokinetics is useful in interpreting drug concentrations in blood. Pharmacokinetic data obtained on adult volunteers administered therapeutic doses are not necessarily helpful in interpreting drug concentrations in blood from decedents who may have ingested excessive amounts. Possibly more helpful is pharmacokinetic data on chronic therapy and data on kinetics in overdose. However, these data should be applied with caution.\(^{112}\) The common subjects of pharmacokinetic reports are the hospitalized, poisoned patients whose airway, cardiac output, hydration status, renal and hepatic function, electrolyte and acid/base status were all well maintained. In these, the distribution, metabolism and elimination of drugs and poisons are likely to be very different from the common subject of the autopsy report who has laid in a cramped position for 24 or more hours before dying with diminished cardiac output, falling blood pressure, decreased tissue perfusion, impaired ventilation, tissue hypoxia, acidosis, progressive dehydration and renal and hepatic failure.

Neither should it be assumed that a decedent has ingested a large amount of a drug as a single dose, i.e. within a short space of time. Large quantities of tablets may take a considerable time to swallow, and the subject may ‘rest’ or even change their mind several times during the process. This prolonged ingestion is likely to make the kinetic picture more complex.

Analytical results must be plausible. If the reported drug concentration would have required the deceased to ingest a kilogram of a particular drug then the result is likely to be in error. Similarly, sufficient time must elapse after ingestion of a drug for significant absorption and distribution to take place in order to produce the concentrations determined in the subject’s blood. Slow-release preparations are likely to make interpretation difficult, apart from the simple observation that a large amount has been ingested.

**Interaction**

Interactions between drugs are common, can cause both enhancement and reduction of effect and arise by a number of mechanisms described in any standard pharmacology text. These should be borne in mind when interpreting toxicology data.\(^{114}\)

The presence of more than one drug of the same class or parent plus a pharmacologically active metabolite will usually result in a summation of effect, which may be very significant, especially in the presence of alcohol. Other combinations exhibit potentiation, where the effects of both drugs together are greater than the summation of the effects of each separately.

Ethanol is known to interact with a number of drugs to enhance their effects by either summation or potentiation. The ethanol concentration should always be determined in blood and urine specimens from autopsies. Patients who die in hospital after a prolonged period may not necessarily be free from ethanol at autopsy.

**Tolerance**

Individuals respond differently to drugs not only from time to time but from other individuals. The different responses may be due to age, race, the presence of disease or other, as yet undetermined, factors. Drug abusers are either unaware of or tend to ignore this and attribute variations in their response to changes in the quality of the drugs they purchase. Tolerance is well-recognized in and by regular users of opiates and other drugs; it builds up over a period of time and is gradually lost during abstinence. It is said to have developed when it becomes necessary to increase the dose of a drug to obtain the effect previously obtained with a lower dose. Most tolerance that concerns the toxicologist is acquired tolerance and may be due to decreased efficacy at receptor sites or increased metabolism due to enzyme induction. Decreased efficacy at receptor sites allows individuals to tolerate much higher blood and tissue concentrations. There is commonly cross-tolerance between drugs with a similar structure, as shown by the substitution of methadone for heroin in the treatment of addicted individuals, and occasionally between drugs whose structures are dissimilar.

The presence of tolerance will usually make interpretation difficult. From the author’s case files, a ‘regular abuser’ who was found dead and in whom the only finding was a very modest blood and tissue concentration of morphine, was later revealed to have been in custody and to have been drug-free for almost a year. At the inquest his friend disclosed that, on release from

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prison, he had injected himself with heroin at a 'pre-custodial' dose and died soon afterwards. No other drugs or alcohol were detected. All tolerance-producing drugs could potentially cause this problem.

**Neonates and infants**

Neonates who die within a few days of birth may still have measurable concentrations of drugs which were administered to the mother before delivery and have crossed the placenta. The infant may also have a measurable concentration of drug ingested, injected or inhaled by the mother post-natally and passed to the infant via breast milk.\(^{115}\) Breast-feeding takes several days to become established and to produce 'full' milk. However, the colostrum is lower in fat than full milk and therefore lipophilic drugs are likely to be at low concentration in this fluid.

Absorption of a drug from the gastrointestinal tract is slower in infants than in older children and adults although the total absorbed is likely to be similar.\(^{116}\) The skin of infants absorbs compounds more readily than adult skin and the blood–brain barrier of infants is also more readily penetrated.\(^{115,117}\)

Enzyme systems involved in drug metabolism are usually present at birth but are functionally immature, leading to prolonged elimination half-times. The oxidation and glucuronidation systems are probably the slowest to reach maturity, requiring several weeks after birth, but the sulphation pathway is probably more mature, which provides some compensation.\(^{118–120}\) Glomerular and tubular function are also poorly developed in neonates, especially pre-term babies, and only reach adult values in relation to body surface area at two to five months.\(^{121,122}\)

These factors may make interpretation of drug concentration in neonates and infants difficult but must be borne in mind. There have been some reports of post-mortem findings, e.g. for digoxin\(^{123}\) and amphetamines\(^{124}\) in these age groups.

**Children**

There are important pharmacokinetic differences between children and adults that influence drug toxicity: (a) children have a smaller lipid compartment than adults, so lipid-soluble drugs (tricyclic antidepressants and most other psychotropics) are not taken up and stored in inactive lipid sites to the same extent as in adults; (b) children have a higher level of unbound, pharmacologically active drug for a given drug concentration than do adults, in whom drug binding to plasma albumin is greater; and (c) the ratio of liver weight to total body weight in children is 50% higher at 2 years and 30% higher at 6 years than it is in the adult.\(^{125,126}\)

Drug biotransformation rates are generally enhanced in children, with the exception of neonates.

**Circumstances**

The context in which the patient/subject was found is often of prime importance in interpreting toxicological data. Victims of fires or vehicle exhaust fume inhalation will usually have a high blood concentration of COHb. However, burning upholstery containing polyurethane foam, whether from domestic furniture or vehicle seating, generates volatile cyanides which will cause high blood cyanide concentrations if inhaled. Cyanide inhalation, therefore, may cause death before COHb concentrations have reached significance.

Haemodilution is likely to have occurred in victims of fresh water drowning but not in those drowned in salt water. The concentrations of alcohol or drugs measured in the blood of victims of fresh water drowning are likely to be falsely low.

The physical position in which a body is found may have been an important factor in determining the length of the subject’s survival following ingestion of drugs and/or alcohol. For example, the effects of respiratory depressant drugs may be enhanced and death accelerated if the subject’s airway is constricted, for example by lying in a position with the head pressed into the chest. A subject lying head downwards on stairs, for example, may survive longer than one lying head upwards.

**Effects of treatment in hospital**

The collapsed or unconscious patient who is transferred to hospital and survives for a period of time before dying often presents the most difficult investigative and interpretive problem. Treatment with intravenous fluids and other manoeuvres usually renders post-mortem blood and tissues devoid of detectable alcohol and drugs. It is essential to have available as many of the antemortem blood and urine samples as possible in order to demonstrate the presence of a toxic agent. Hospitalization may also complicate the interpretation of drug concentrations in those abusers in whom tolerance has developed.
Many drugs may be administered in the course of diagnosis or treatment of the unconscious patient. Unless a detailed record of all therapy is available to the laboratory, it is often impossible to determine which drug(s) is likely to be the causative agent.127

Occasionally, the question arises of whether a particular dose of an opiate or an anaesthetic has contributed to the demise of a patient. In addition to reliable analysis and confirmation of the drug in question, it is essential to obtain the appropriate drug treatment documentation from the ward. Wide consultation with senior colleagues, including, possibly, hospital managers is essential before a final report is produced.

Screening for drugs in suspected instances of brain death must be performed thoroughly and, if a single drug is not specified, requires a much wider search using blood and urine than a simple immunoassay screen. Reliance should not be placed upon the negative results from a single urine sample, particularly if the patient is in shock or renal failure.41,128

Anaphylaxis
Most classes of drug have been implicated in deaths due to acute anaphylaxis.129 This possibility should be borne in mind when ingestion of excessive amount of drug has been ruled out, so that an appropriate test (e.g. mast-cell tryptase assay) can be performed as early as possible.

Pre-existing medical conditions
Pathologists performing post-mortem examinations are usually aware of pre-existing medical conditions before they commence the autopsy. In some instances, the biochemistry laboratory can assist in demonstrating if these were likely to have contributed to the subject’s demise. The presence of heart, liver and renal disease can change the pharmacokinetics of drugs in life and complicate the interpretation of post-mortem concentration data. Coe and colleagues130–133 have detailed the concentrations in post-mortem blood and vitreous fluid of a range of common clinical chemistry parameters and the activities of common enzymes. These data, if applied carefully, can be useful in determining the degree of hepatic and renal dysfunction as an aid to interpretation of post-mortem drug concentrations.

Diabetes can be associated with death in a number of ways. An elevated HbA1c concentration might indicate poor control or poor compliance in taking the prescribed insulin and, coupled with an elevated level of vitreous glucose and ketones,134 may indicate diabetic ketosis. Insulin excess is much harder to diagnose unless the injection site is known for certain. Hypoglycaemia, the result of ingestion of a number of possible drugs, is usually impossible to demonstrate with certainty.

Renal failure diminishes the ability to eliminate drugs normally cleared in the urine and therefore drugs ingested by a patient shortly before a urine specimen was collected may not yet have been excreted, e.g. in shock or in renal shut-down.128 Diuretics taken at the prescribed dose without potassium supplementation can lead to hypokalaemia. These facts need to be borne in mind when examining post-mortem urine samples.

Epilepsy as a cause of sudden, unexplained death is not readily diagnosable.135 Poor compliance to the drug regimen can sometimes be demonstrated by quantitation of concentrations of the prescribed anticonvulsant(s) in blood. Patients whose epilepsy is well controlled run a higher than normal risk of sudden death, even when the anticonvulsant concentration is within the population therapeutic range.136 Some workers137 have suggested that quantitation of serum prolactin can indicate recent seizure activity and status epilepticus, while others,138 because prolactin is increased in any form of stress, have disputed its usefulness. A number of drugs can lower the seizure threshold (e.g. amitriptyline).

Vitreous analysis may be useful in demonstrating the presence of dehydration and, as suggested recently, hypoxia.139

Calculations
The toxicologist is frequently asked to estimate the amount of drug or alcohol ingested. This has usually been approached either by comparison of the results obtained with literature reports of blood and tissue concentrations of fatalities following a known dose or by the application of simple pharmacokinetic calculations. Clearly, both of these methods are dependent upon whether the measured blood concentration accurately represents the drug concentration at the time of death. Sampling site has a major bearing on this. This limitation will also apply to the published data with which it is intended to make a comparison. So, one is drawn into a circular argument.

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Calculations of the amount of drug or alcohol ingested in overdose situations are limited by the uncertainties regarding pharmacokinetic parameters such as the Vd, elimination half-time, etc, which have usually been determined using small doses administered to healthy individuals under controlled conditions. A ‘population reference range’ is usually quoted for these parameters, e.g. for methadone Vd = 3–5 L/kg, for cocaine, 1–3 L/kg. These data, when applied to post-mortem blood concentrations of drugs, can generate unrealistic estimates of dose.

Estimates of the total amount of drug present in the tissues of a corpse can be obtained by summation of: (a) the total drug concentration in various organs and their respective weights; (b) total drug concentration in gastric contents and its volume; and (c) total drug concentration in blood and its volume. This procedure, practised more by US laboratories than in the UK, involves an enormous amount of work for all concerned. It is argued by some that this is the only satisfactory route to an adequate interpretation.

Parent drug:metabolite concentration ratio

It is common to interpret a high parent drug:metabolite concentration ratio as indicating that death has occurred soon after ingestion and, conversely, a low ratio as indicating that death has been delayed.

The tricyclic antidepressants display a wide individual variation in relative concentrations of parent drug and metabolite in plasma following a therapeutic dose. In patients treated with tricyclic antidepressants, it is common to observe parent drug:metabolite ratios within the range 0.5–1.5 and it is commonly assumed that ratios >2 indicate acute overdose. We have observed several patients with ratios >2, presumably gained by slight chronic overprescribing or non-compliance, and instances where death was probably due to ingestion of an excessive amount of a single tricyclic without alcohol, where the parent:metabolite ratio was <2. A challenging exploration of the whole problem of interpretation of observations and analyses is presented by Nordby.140

REPORTING

The report

When the analyses are complete, it is necessary to produce a report. For those results likely to have forensic implications something more than the regular laboratory report is usually required and it is common to append an interpretation to the data. This type of report, once in the hands of the person making the request (e.g. the Coroner) becomes a legal document. A good analytical service and competent interpretation can easily be let down by inadequate reporting.

The formation of the report proceeds by several stages, which are described by Green,141 who uses the phrase ‘consider, consult, confer’: (a) consider the blood and tissue concentrations in context; (b) consult recent literature and draft a preliminary report; (c) confer with the requesting pathologist, an experienced pharmacist at the Regional Drug Information Unit (if drugs are involved) and medical and/or scientific colleagues; (d) finalize the draft report; (e) reconfer; (f) produce and submit the finished report.

Colleague review can highlight the problems of the wording of reports since phraseology can be a difficult area, and there may also be an experienced toxicologist at another hospital to whom one could turn if necessary. The report will commonly take one of three forms:

- Results only. This is identical to the regular report used to report laboratory results to physicians on the wards and leaves interpretation to the one making the request.
- Simple. This is the form used most commonly and usually comprises part A, containing factual information such as concentrations, and part B, which carries the interpretation and conclusions.
- Statement. This form follows the requirements of the Criminal Justices Act, 1964, and is used most commonly by the Forensic Science Service.

It is usual to send a copy to the requesting pathologist, the investigating Coroner and to keep a copy on file. It is also usual to send to the Coroner, with the report, an invoice for the work done.

ACTING AS AN EXPERT WITNESS

There are many papers and texts covering this topic more than adequately.142,143 In most instances, the toxicologist will not be expected to appear in court. The major points are described briefly below.

The review

As soon as the request to appear in court is received, it is essential to set aside sufficient time...
to review what has previously been written in the Toxicology Report, to review contemporaneous notes and to check data and calculations. It is also an opportunity to have another look at the recent literature and consider other possible interpretations.

The case conference
If the case is to be heard in the Crown Court it is important to request to be present at a conference well before the hearing. This is an opportunity to find out which issues are being addressed, to explain to the lawyers the details of the report and its implications, to raise issues relating to the report that need to be addressed and, in the light of any new evidence, discuss other interpretations that can explain the data. It is unwise and rarely possible to raise new issues or make new points in the witness box.

In court
The courts usually provide a leaflet of guidance for those appearing as witnesses. The texts cited earlier give ample direction on court procedures and expected behaviour.

CONCLUSION
Provision of a forensic toxicology service is technically and intellectually very demanding and the difficulties and pitfalls are not usually appreciated by the naive until it is too late. If this personal view has awakened some, at least, to the sort of dangers which lie in wait for the unwary, it will have served its purpose.

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