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Guidelines for Obtaining Specimens for Post mortem Toxicological Analysis

The following guidelines have been written to assist pathologists and anatomical pathology technicians in selecting the appropriate specimens for toxicological analysis. If it is unclear which specimens are required for a particular analysis please contact the laboratory to discuss specimen requirements before collection.

Ante mortem Samples

Where individuals have died several hours or days after hospitalisation, there may be ante mortem specimens available. These are the ideal specimens to submit. It is important to ensure that the blood is pre transfusion and has not been collected from indwelling catheters. If only small volumes are available, post mortem specimens should also be submitted for screening purposes (Depending on the duration of hospital admission) and the ante mortem specimens will be retained for quantification.

Blood

10-20mL blood should, preferably, be collected from the femoral or subclavian vessels, so that the effects of post mortem redistribution are minimal. The most consistent quantitative findings are derived from samples collected from these sites. If this is not possible, other sites for collection include the root of the aorta, the pulmonary artery, the superior vena cava or the heart. All blood is assumed to be femoral unless otherwise stated. An additional 5mL blood should be collected into a separate tube containing 2% sodium fluoride. This is to inhibit bacterial production of analytes such as alcohol, GHB and cyanide, and to prevent degradation of labile drugs.

Urine

10-20mL un-preserved and 5mL 2% sodium fluoride preserved urine should be submitted to the laboratory. Urine is useful for qualitative analysis and confirmation of blood results. As the bladder is a confined compartment, largely free from bacteria, it is of particular value to quantify alcohol, where the validity of the blood result is in question. Drugs and their metabolites are usually present in urine in much higher concentrations than in blood and are detectable for much longer periods following ingestion. Depending on the half-life of the drug and the sensitivity of the assay, it may be possible to detect the drug in urine for a few days or even a week after ingestion. However, this also means that the presence of the drug in the urine does not necessarily suggest the drug was in the blood and exerting a pharmacological effect. Analysis of drugs in urine is easier than blood as it is a much cleaner specimen, there

is no protein binding to hinder extraction, and it is often free from putrefaction products that can interfere with analytical methods.

Vitreous Humour

All vitreous humour should be collected into tubes containing 2% sodium fluoride preservative. As it is collected from a relatively contained compartment, vitreous humour offers a number of advantages over more commonly submitted specimens, particularly when some putrefaction of the body has occurred. Vitreous humour is particularly advantageous for confirmation of blood alcohol concentrations as it is well protected from bacterial infiltration and is, therefore, less prone to post mortem fermentation observed with blood. This sample matrix is also useful for biochemical tests such as glucose and lactate. Vitreous humour should be submitted in all cases of suspected heroin overdose. The effects of post mortem redistribution in vitreous humour are believed to be minimal and as it is largely free of protein and other complex substances, analysis is relatively straightforward. Currently, drug concentrations are difficult to interpret due to lack of reference data.

Liver

This is available from almost all cases and can be collected easily. As it is the primary metabolic organ, drugs are often found in higher concentrations than in the blood. Liver should be submitted in cases in which little or no blood can be obtained. Approximately 10g of wet, unfixed, tissue should be placed in a plastic or glass container. Data suggest that concentrations can vary depending on the site from which the liver specimen is collected. It is recommended that the right lobe be sampled to reduce contamination from bile and redistribution artefacts from the stomach contents.

Other Tissues

Other solid tissues may be used for drug analysis. However there are few data on the significance of these results. Muscle may be submitted in the absence of liver while other tissues can be useful in investigating deaths involving volatile substance abuse (see below).

Stomach Contents

10-20mL stomach contents should be collected into a plastic or glass container without preservative. This matrix is useful in cases of oral overdose in which large amounts of unabsorbed drug remain in the stomach, as concentrations are substantially higher than in any other fluid. If recognisable tablets or capsules are found, these should be removed and placed in separate containers for identification.

Larvae

Entomotoxicology is still a relatively novel area of toxicology. It involves the study of drugs and poisons in insects feeding on decomposing bodies, and offers a number of technical advantages over putrefied human remains. The extraction of drugs from larvae is the same as that from tissue but, usually, no emulsion is formed, whereas this is not always the case with human tissue. There is also less contamination

observed from endogenous substances, which is particularly problematic with putrefied human remains. It is possible that secondary bioaccumulation of drugs in larvae may also occur. Larvae are usually present in abundance on decomposed bodies and sampling is often a relatively straight forward procedure. The larvae should be placed directly into as many plastic tubes as is necessary. To date, most investigators have found no correlation between drug concentrations in larvae and those in human tissues. However, the full range of drugs that can be identified in larvae and the potential qualitative application of entomotoxicology have still to be fully documented.

Syringes

Syringes **MUST** be packed with care and any needles protected by a suitable plastic shield to prevent injury. Please advise the laboratory if you intend to submit sharps.

Specialist Analyses

Volatile Substances (Solvents)

In addition to the nominal specimens, submit a minimum of 5mL blood in a glass tube with minimal head space. Brain, adipose tissue, lung and kidney are useful for the investigation volatile substance deaths. 10g of tissue should be collected and placed in separate glass jars or nylon bags.

Insulin or Diabetes Related Deaths

The analysis of vitreous humour for glucose, lactate, acetone, insulin and C-peptide and blood for HbA1c (glycated haemoglobin) can provide an indication of ante mortem glycaemic control. Please submit 2mL vitreous humour in 2% sodium fluoride tube in addition to the nominal samples.

The volumes indicated above are the ideal, to ensure optimal sensitivity and to enable repeat analysis if required. Reduced sensitivity and scope of screening may result from smaller volumes. However, smaller volumes may be sufficient and should always be submitted.

Please ensure that all specimens are clearly labelled with the full name of the deceased, if known, the post-mortem date, sample type, mortuary and name of pathologist. In the case of blood specimens, please indicate the exact site of collection if not femoral blood. The specimens should be placed in sample bags that separate any paperwork from the specimens. **DO NOT** send more than one case in the same sample bag.

Please submit a 'Pathologist Analysis Request Form' and 'Coroner's Officer Report' with each case referred to the service.

Pathologist Analysis Request Forms can be downloaded from our web site: www.forensic-toxicology.org