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1 Introduction

1.1 Scope and purpose

The purpose of this document is to outline the collection procedure and documentation requirements for samples submitted to the laboratory for post mortem toxicology analysis.

1.2 Responsibility

This document is to be reviewed by the Head of Toxicology.

1.3 Related documents

BS/CB/TOX/FT/15 Toxicology Request Form

2 Sample collection and requesting procedure for toxicology analysis

2.1 General Considerations

Every sample must be clearly labelled with Name of Deceased, Date of Birth, the date and time of sample collection and the sample site. A post mortem reference number may also be appropriate. Ensure that the request form is fully completed with all relevant details of the deceased, including the circumstances of the death and any relevant clinical history (including details of infectious disease risk) or available drugs. The name of the requesting Pathologist must also be supplied.
All specimens should be stored at 4^{o}C before transporting them to the Laboratory. Each specimen bottle should be securely sealed to prevent leakage.
The Toxicology laboratory at Southmead provides sample collection kits, which include appropriate specimen containers. If further supplies are required or if you have any queries with respect to sample collection please telephone the laboratory on 0117 4148436

2.2 Antemortem samples

If the decedent was hospitalised prior to death, antemortem samples if available, should be submitted for analysis. The relevant hospital pathology department should be contacted as a matter of urgency (as routine blood samples are often only kept for 24-48 hours prior to disposal) and arrangements made to obtain the antemortem samples. This does not negate the need to collect post mortem samples.

All available antemortem samples should be submitted, however, caution may be required with ante-mortem serum samples as the gels used in many serum gel tubes may absorb drugs and thus affect the blood concentration.
Antemortem samples should be labelled with the date and time of collection.

2.3 Blood Samples

Blood samples are the ideal samples if quantitative analysis of drugs is required.
Do not mix blood samples from different sites. There is substantial published evidence to show that for most drugs and poisons, including alcohol, there are important differences in their concentration in blood according to the time of specimen collection after death, choice of sampling site, method of sampling and volume of blood collected
Blood samples are best obtained from the femoral vein by percutaneous puncture, using a 10 mL or 20 mL hypodermic syringe. The leg should not be massaged prior to sampling in order to increase specimen volume. The femoral artery is located in the inguinal canal midway between the superior anterior spine and the pubic tubercle. The femoral vein may be found a centimetre or so medial to the artery at that point. To obtain femoral samples, some toxicologists advocate clamping the vein prior to sampling, (so that the vessel is tied and clamped proximally near to the inguinal ligament before sampling). This prevents drawing down of blood from the inferior vena cava and from the inguinal vein, however clamping the vessel adds time and added incisions to the autopsy procedure. Obtaining a femoral blood by a 'blind stick' or 'stab' technique to the unclamped external iliac vein is also accepted practice and is probably the most practicable in a routine post mortem setting. A third technique involves taking femoral blood by cutting the external iliac vein proximal to the inguinal ligament and draining from the distal cut end into a plain 20mL sterile plastic container.
If the volume drawn is restricted to 5 – 10 mL there is unlikely to be significant redistribution from central sites. Ideally two samples should be taken from distinct sites – eg left and right femoral vein.
Place at least 4-5 millilitres of blood into the tube containing fluoride preservative. Ensure that only the fluoride tubes supplied by the Toxicology Laboratory are used, as they contain a higher concentration of fluoride (1.5% by weight) than standard fluoride blood collection tubes.
Place the remainder of the blood sample into a plain plastic (20 mL) universal.
If analysis for volatile substances eg. Solvents or fuel gas, fill the glass vial provided to the top.
If femoral samples are not available, eg in severe trauma, venous subclavian or jugular blood can be obtained.
It is particularly important that the blood should not be collected by being 'milked' from a limb as this process can cause dynamic changes in drug concentrations in the expressed blood. If there is a need to apply pressure to obtain sufficient sample, this should be gentle pressure only along the line of the vein.
If it is not possible to obtain a peripheral blood sample, then cardiac blood can be submitted, but must be labelled as such. Collection from the right chamber is preferable. Limitations of cardiac

blood include accumulation of drug due to post-mortem redistribution, diffusion and putrefaction. Drug concentrations may also be increased due to autolysis of cardiac tissue or due to trauma. Cardiac blood is of most use for screening purposes.
Blood collected from the body cavity is generally a poor sample for toxicology analysis as it is likely to be contaminated by gut contents, urine, or other body fluids. On occasions, eg following severe trauma, this may be all that is available, so can be submitted, so long as it is clearly labelled as to its origin.

2.4 Urine

Urine samples are of value in screening for an unknown drug or poison, particularly drugs of abuse. Preferably at least 10mL of urine should be placed in a plain 20mL sterile plastic container. Boric acid containers should NOT be used.
Urine is best obtained by direct puncture with syringe and needle of the exposed bladder once the abdomen has been opened. It may also be obtained by insertion of a urethral catheter before the start of the autopsy. A common practice is to obtain urine by aspiration with a syringe, without a needle, once the dome of the bladder has been opened in the course of the post mortem examination. If this procedure is adopted then great care should be taken to ensure that the sample is not contaminated by blood or other fluids. If the patient was catheterised shortly before death, ensure that this is stated on the request form. Under these circumstances the urine sample can be contaminated by lignocaine or other local anaesthetics.
Urine samples are also used in the quantitative analysis of alcohol, as there may be uncertainty over the reliability of a blood result.
If the bladder is empty, ensure that vitreous humour samples are obtained.

2.5 Vitreous Humour

Vitreous humour, which occurs behind the lens of the eye, can be extremely helpful in cases of suspected diabetic or alcoholic ketoacidosis where the analysis of β-hydroxybutyrate can be informative in sudden deaths in alcoholics. Analysis for other biochemical analytes for example electrolytes and markers of renal dysfunction can also be performed on this fluid.
Vitreous humour alcohol (ethanol) analysis is helpful, particularly when putrefactive formation is suspected.
All vitreous humour from both eyes should be collected; however, it can be collected into a single container (e.g. a plain 5mL sterile plastic container). Care must be taken during sampling because use of excessive suction can cause significant change in the concentration of several analytes (through contamination with tissue fluid).
Vitreous Humour is obtained by needle puncture of the eyeball. A 17 or 15 gauge needle attached to a 5mL syringe may be used. The eyelid should be retracted laterally and the sclera punctured at a latitude of about 60^{o} taking the pupil as the North Pole, with the needle being directed towards the centre of the eyeball. Once the sample has been obtained, the syringe should be detached from the needle, leaving the needle in place, and a volume of water or

physiological saline equivalent to the amount of vitreous removed should be slowly injected into the eyeball to achieve cosmetic restoration.

2.6 Other Samples

Samples identified with an * should only be submitted for analysis following discussion and agreement with the toxicology laboratory. These samples are not analysed within the department and would be referred to another laboratory.

Liver*

This tissue may be useful in certain complex poisoning cases. It is usual to take a portion from deep within the right lobe of liver since it should be uncontaminated with bile and less affected by drug diffusion from the stomach; 100 grams are sufficient for most analytical purposes.

Bile*

Bile is easily aspirated from the gall bladder following abdominal evisceration. If the patient has undergone cholecystectomy, useful amounts of bile may still be obtained by aspiration of the common bile duct with a needle and syringe. The interpretive value is limited, but this fluid is occasionally of use if no other fluids are available

Stomach Contents*

Gastric contents are rarely of use. If a sample is to be submitted, obtain one 25-30 mL sample without preservative. The sample should be clearly labelled to indicate that it is only a portion of the contents. Record the total volume. Take CARE if cyanides or phospides are suspected to have been taken as highly toxic hydrogen cyanide or phosphine gas may be released because of a reaction with stomach acid.

Remains of undegraded tablets, capsules or other materials of exogenous evidence, usually present if specimens are collected soon after intoxication, should be transferred to a separate container. Identification of such material can be carried out by reference to a computerised database of pharmaceutical products, or by direct analysis at the Laboratory.

Hair*

Hair specimens may be useful in the investigation of death related to drug abuse. Analysis of hair (approximate rate of growth 1 cm per month) is able to provide useful information concerning the chronicity of drug abuse, which is valuable in the interpretation of post mortem drug concentrations. If hair specimens are cut from the head, the proximal end should be clearly identified; the cut end tied with a piece of thread. The hair should be cut from the vertex region of the scalp (additional sample should be pulled from the vertex region). However, the Laboratory does not currently provide a hair analysis service, but in selected cases these can be referred to an alternative laboratory for analysis.

Other*

Other samples that may be considered in specific circumstances include:

- Muscle – can be useful for screening but quantitative data is limited. There is a lack of consensus on which muscle should be sampled, but the psoas is normally used. The site of sampling should be recorded.
- Injection site (skin) – may be useful in determining the type of substance that has been injected e.g. insulin. Rarely required. A control site sample should be sent for comparison. Do not fix the specimen unless histology is required.
- Lung tissue – approximately 2 cm cubed, sealed in an airtight container may be useful.

3 References:

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2) Dinis-Oliveira RJ., Carvalho F. *et al.* Collection of biological samples in forensic toxicology. Toxicology Mechanisms and Methods. 2010; 20(7):363-414
3) Drummer OH. Post Mortem Toxicology of Drugs of Abuse. Forensic Science International 2004; 142:101-113
4) Flanagan RJ., Connally G., Evans JM. Analytical Toxicology: Guidelines for Sample Collection Post Mortem. Toxicological Reviews 2005; 24:63-71.
5) Elliott SP, Stephen DWS, Paterson S. The United Kingdom and Ireland association of forensic toxicologists forensic toxicology laboratory guidelines (2018). Sci Justice. 2018;58(5):335-345.
6) Howard M., Morley S., McCarthy H. The Royal College of Pathologists: Guidelines on autopsy practice: Autopsy when drugs or poisoning may be involved. 2018; G169.