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Review Article

Collection, preservation and forwarding of biolgical samples for toxicological analysis in medicolegal autopsy cases : A review

Tabin Millo, A. K Jaiswa**& C. Behera****

Abstract

Collection of proper autopsy specimen is an essential step in the process of toxicology case work[1]. Improper collection of these specimens can greatly alter or negate chemical and toxicological analysis. This article is an update about the standard methods of biological specimen collection procedures for toxicological analysis which will be helpful for the forensic pathologist and forensic scientists.

Keywords: *Sampling, preservation, body fluids, poison, tissues*

Introduction

In handling the Medicolegal autopsy cases, certain standard guidelines are necessary to be laid down to assist in the selection of appropriate specimens of the body fluids and tissue for postmortem biochemical and toxicological analysis.After death there is a rapid change in the cellular level biochemistry due to autolysis. The drugs and other poisons may be released from the binding sites in tissues and major organs.

The unabsorbed drug may diffuse from stomach, care should be taken in selection of blood and tissue sampling sites. Many a times the autopsy is conducted before all the circumstantial evidences are collected and investigated. Hence, it is vital to preserve all the necessary samples at the time of autopsy. Ideally the samples for toxicological or biochemical analysis should be collected before the postmortem. However it may not be possible for all the samples and there may be dificulty in sampling without opening the body.

Biological fluids

1. Blood

In all medicolegal investigation cases a blood specimen should be obtained when blood is available. It is used as a reference sample for identification in unidentified cases and also for toxicological analysis. Peripheral blood concentration have been shown to be more reliable for toxicological analysis than the conventional heart blood. Therefore, in all suspected poisoning deaths or in all cases of unknown causes of death a femoral blood specimen should be collected. Before autopsy it can be collected by inserting the needle at about two finger breadth below the inguinal ligament at middle point marked between the the anterior superior iliac spine and the symphysis. But it is best obtained by punturing the femoral vein using a 30 ml syringe with wide bore needle after exposing the vein by dissection and clamping or ligating it proximal to the collection site.

Usually 20 ml of blood[4] is sufficient and it has to be preserved in sodium fluoride of 10mg/ml and potassium oxalate, 30 mg/10 ml of blood concentration in a fresh wide mouthed glass container of 30 ml with screw cap1 (universal container). The glass container should be made of amber glass to inhibit photodegeneration.The rubber or cork caps should be avoided. Sodium fluoride protects should be avoided. Sodium fluoride protects blood from postmortem changes such as bacterial production of ethanol or other alcohols. It also helps to protect other labile drugs such as cocaine, ntrazepam and clonazepam from degradation[4]. The most satisfactory way of

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obtaining a venous blood sample is venepuncture of the femoral vein by direct punture in the groin before the autopsy begins.

2. Urine

Urine specimen is of great value even in small amount especially in screening of unknown drug or poison, particularly substance of abuse since the concentrations are generally higher than in blood and a number of metabolites may also be present. Urine specimen are also valuable in the quantitative analysis of alcohol, where there is uncertainity over the validity of a blood specimen. Before conducting the autopsy, urine can be collected by catheter or suprapubic puncture with 5-10 ml syringe and needle (22 gauge 3 inch). With the body in supine position, patpate the bladder and identify the insertion site at midline and 2 cm cephalad to the pubic bone. At the insertion site, introduce the 22 gauge 3 inch needle attached to the 10 ml syringe. Direct the needle caudad (the bladder is a peritoneal organ in adults) at a 10° to 20° angle from the perpendicular at midline. Gently aspirate while introducing the needle. If no urine is aspirated, withdraw the needle to the subcutaneous space and readvance in a slightly different direction, 10° caudad or cephalad and aspirate again. But it can be best obtained during autopsy after exposing the abdomen by puncturing the fundus of the bladder with syringe and needle. It has to be preserved in sodium fluoride (10 mg/ml) in a 30 ml glass container with a screw cap. A sample of 20 ml is sufficient for toxicological analysis.

3. Bile

Bile is helpful in estimating the drugs, which are concentrated by liver and excreted into the gall bladder like opiates and acetaminophen (paracetamol) . It is not routinely preserved, but only in selected cases. It is preserved in 30 ml glass screw capped container. A 20 ml of bile is adequate for toxicological analysis. It can be collected directly by incising the gall bladder into a glass bottle. It is a viscous fluid, which makes it difficult to be sucked by needle and syringe.

4. Vitreous Humor

The vitreous humor specimen is particularly useful for alcohols, or in diabetes and insulin related deaths. It is also very useful where the body has decomposed. The fluid in the eye resists putrefaction longer than other body fluids as it is sterile and remains well protected in eye. It is useful for certain biochemical tests such as urea, creatinie, glucose, lactose and alcohol. Vitreous humor must be collected from both eyes in separate vials of 10 ml. It is preserved with sodium fluoride (10 mg/ml). A punture should be made through the sclera at the outer canthus with a fine 19 gauge needle in 5 ml syringe. It should be placed laterally as far as possible, pulling the lid out, so that when released, it returns to cover of the puncture mark for cosmetic reasons. The sclera should be puntured at a lattitude of about 60° taking the pupil as the north pole. The needle should be directed towards the centre of the eyeball. The fluid comes out slowly because of its viscosity. Gentle aspiration will usually yield 2-3 ml of vitreous humour. Once the sample has been collected the syringe should be detached from the needle, leaving the needle in place. A volume of water or physiological saline equal to the amount of vitreous humor removed should be slowly injected into the eye to achieve cosmetic restoration.The preservative used is sodium fluoride.

5. Cerebrospinal fluid

The cerebrospinal fluid sample is rarely required for toxicological analysis. If needed it should be collected by cisternal puncture.It is difficult to collect CSF at medicolegal autopsy by conventioal lumbar punture. It is relatively easier to obtain by cisternal punture. With the neck flexed, palpate the atlanto- occipital membrane in the midline and, using a needle and syringe, gently introduce a disposable spinal needle through the skin at that point, directing the needle towards the bridge of the nose. As the atlanto occipital membrane is puntured at a depth of approximately 2 cm, loss of resistance will be felt following which CSF can be aspirated. It should be collected in a 30 ml screw capped plastic or glass container. The CSF sample has to be preserved in sodium fluoride**.**

6. Other body fluids

In cases where blood and urine are not available other available body fluids like pericardial[14] and synovial[15] fluids can be used for toxicological analysis like alcohol.

BIOLOGICAL TISSUES

1. Liver

Body tissues are often used for toxicological analysis. Liver is the most important tissue because it concentrates many substances. It can contain large amount of drugs and metabolites and may in some difficult cases help establish whether acute or chronic toxicity has occurred. Ideally the part of the liver retained should be fresh unfixed, taken from the periphery of right lobe, away from the stomach,major vessels and gall bladder. A minimum of 100 gm is sufficient for toxicological analysis.

2. Stomach contents

The other routinely preserved viscera are stomach and small intestine with its contents and kidney. The sample is useful when drugs have been taken orally as the concentrations will be many times higher than in other fluids. It can also be helpful to determine the amount of drug present in stomach if blood concentration is difficult to interpret. The stomach should be ligated on both ends (oesophagus and pylorus) and dissected out. Then the greater curvature should be opened up, so that, the contents directly pour onto the wide mouthed jar.About 30 cm of small intestine are preserved with the contents. One half of each kidney is preserved. The stomach and intestine with its contents are preserved in one bottle.

3. Other tissues

Other tissue samples may be useful for investigating deaths where volatile substances e.g solvents or gases, are implicated. Brain, fat tissue, lung and kidney are the most useful. Ideally a wet unfixed tissue should be collected into separate glass containers. In case of lung, the sample has to be collected from the apex of the lung. The whole lung may have to be preserved in case of solvent abuse or volatile substance poisoning. After opening the thorax the lung is mobilized and the main bronchus tied off tightly with a string ligature. The hilum is then divided and the lung placed immediately into a nylon bag (prevents the volatile in the sample from escaping) which is sealed and sent as soon as possible to the laboratory.

4. Bone and Muscle tissue

In case of decomposed, exhumed, burnt or skeletonized body it becomes difficult and challenging due to absence of blood or scarcity of solid tissues.But ,whatever remains are available we have to collect all the relevant samples though it may not be the routine sample.If bones[10] are available the whole long bone should to be collected and preserved. It has to be dried in normal temperature and sealed in plastic bag. Bone marrow samples may be useful in drug identification (qualitative and also quantitative) in cases where all soft tissue has degenerated. The skeletal muscle is also useful for toxicological analysis. A 100 gm muscle tissue (preferably quadricep muscle) has to be preserved in saturated solution of common salt in a plastic or glass container.

5. Hair and Nail

Hair and nails are useful samples for analysing chronic poison (heavy metals) or drug of abuse (opioids). These should be sent if chronic poisoning is suspected, particularly to distinguish between episodic or continuous exposure, or for those poisons which may have already been eliminated from the body by the time of death. Hair should be plucked from the scalp with the entire root, shaft and tip. About 500 ug (20 – 30 hairs) of hair should be collected and laid aligned by rolling into a clean plastic or foil sheet with an indication of the scalp ends on the attached label. The whole nail from one toe or fingers can be lifted and collected in a plastic packet.

6. Maggots

In decomposed body, if maggots are present 20 gms of maggots[9,16] can be collected in a plastic or glass container with saturated common salt as the preservative. If drugs or intoxicants are detected they could only have originated from tissues upon which the larvae were feeding. However the correlations between the level in the larvae and the human has not been established. It only provides qualitative information about drug use.

7. Injection sites or snake bite

In case of death due to injection of drugs or suspected snake bite the sample from the injection site has to be preserved. The skin sample with the underneath muscle tissue around

the injection site area must be preserved along with a control sample of similar composition from the opposite normal site in saturated solution of common salt

8. Tablets, powders and syringes

These samples should be packed with care and any needle protected by a suitable shield to avoid injury. These items may be particularly useful in deaths in medical personal or drug addicts who may use agents which are difficult to detect once they have entered the body.

The use of disposable, hard plastic or glass containers are recommended for preservation. The plastic containers (especially of polypropylene) are increasingly used and have the advantage of not smashing when dropped and also much lighter. The ideal samples are best sent in their original state without adding any preservative in a refrigerated storage (4^0C) within few hours. But generally it is not possible to send in this ideal state due to lack of good autopsy facilities, cold storage facilities, quick transport arrangements, legal formalities and quick forensic laboratory services. It usually gets delayed. Therefore, sample has to be put in ideal preservatives to provide optimal conditions till they reach the laboratory. The specimen are generally preserved at 4°C during the time until they are analysed. For long term storage it has to be kept in freezer (- 10°C) until analysed and disposed off. The most commonly used preservative for viscera tissues are saturated solution of common salt. It is the most easily available, cheap and effective preservative. It is important that the solution should be prepared using pure sodium chloride in distilled water to avoid any contaminants. The other option is rectified spirit (90% ethanol) except in cases of poisoning due to alcohol, chloral hydrate, chloroform, phenol, formaldehyde, ether, and phosphorus. In acid or alkali poisoning rectified spirit is the prescribed preservative. The blood for toxicological analysis has to be preserved in NaF at the concentration of 10 mg/ml of blood and potassium oxalate, 30 mg/10 ml of blood . Fluoride should be added to urine, vitreous humor if alcohol estimations are required.

Forwarding Samples

All samples should be properly sealed and labelled with the deceased's name, postmortem number, nature of sample, collection site, preservative used,date and time of collection. Particular attention should be paid to the packaging of samples to avoid loss during transport, and to comply with health and safety regulations. It should be protected by the use of tamper-evident seals around the lids, and accompanied by an intact chain of custody record. It should be handed over to the investigating officer after obtaining proper receipt.

The following documents should be enclosed along with the samples[4]:

I. Name, address and phone number of forensic pathologist and investigating officer.
II. Circumstances of death and details of drugs thought to be implicated.
III. Past medical history including current or recent prescription medication.
IV. Details of emergency hospital treatment and medication given.
V. Copy of forensic pathologist report if available.

Conclusion

The samples collected during the postmortem may not yield the expected normal results. However much useful information can be obtained by the thoughtful analysis of samples obtained at postmortem examination and the interpretation of results obtained. Most drugs and poisons including alcohol shows variation in concentration[5-8] in blood according to the time of specimen collected after death, choice of specimen site, methods of sampling and the volume of blood collected. The blood specimens taken from central sites e.g. heart tends to give particularly high value for most of the analysts. It is particularly important that blood should not be milked from the limbs as this process can engender significant changes in the concentration of critical analytes in the expressed blood. The most consistent quantitative findings are obtained from blood taken from the femoral vein, which is the recommended site for specimen collection.

Because of the very great variations[12] in the concentration of drugs in blood samples taken from different sites, it is important that sample collection is standardised, so that the results obtained can be meaningfully interpretated by comparision with the databases that are being developed incorporating the results of the analysis of samples of blood collected by a uniform technique at postmortem examinations

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