Central Valley Toxicology

GUIDELINES FOR OBTAINING SPECIMENS AT POST-MORTEM FOR ANALYTICAL TOXICOLOGY

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These guidelines have been written in order to assist pathologists in the selection of appropriate specimens of body fluids and tissues for post-mortem biochemical and toxicological analysis. They are adapted, with the kind permission of the copyright holders, from those developed by the NHS Regional Laboratory for Toxicology in Birmingham, U.K. [http://www.toxlab.co.uk/]. More detailed guidelines for pathologists have been published elsewhere (Forrest 1993; Knight, 1996). It is particularly important to obtain samples from the anatomical sites stated in the following guidelines. This will assist in the interpretation of the results by reducing the problems caused by **post mortem drug redistribution**. In addition to indicating the exact site of specimen collection, it is also important to provide any relevant available details regarding the medical and drug history of the deceased as well as the circumstances of the death. The **CVT Toxicology Requisition** should be completed insofar as possible (see Documentation).

POST MORTEM REDISTRIBUTION OF DRUGS

Following death there can be rapid changes in cellular biochemistry as autolysis proceeds, and drugs and other poisons may be released from their binding sites in tissues and major organs; unabsorbed drug may also diffuse from the stomach. Special care should always be taken in the selection of blood and tissue sampling site(s), the method of collection of samples, and the labeling of sample containers. 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 (Pounder and Jones 1990; Pounder 1993). It is common to observe tenfold differences in the concentration of certain drugs and some chemical poisons in post-mortem blood taken from different sites. Specimens taken from "central" sites, *e.g.* heart, tend to give particularly "high" values for most analytes. Moreover, certain commonly used "peripheral" sites, such as subclavian, may sometimes give results closer to "central" sites such as the heart. The most consistent quantitative findings are obtained in blood taken from the **femoral vein**, which is the recommended site of specimen collection. It is also possible to observe differences in the concentration of certain drugs obtained from different tissue sampling sites for liver and lung.

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TRANSPORT OF SPECIMENS

For general post mortem toxicological analysis at Central Valley Toxicology the submission of **blood**, **urine**, and/or **vitreous humor** samples is strongly recommended, should they be available. Appropriate **tissue** (liver *etc*.) and stomach contents should be collected at post mortem but **will not normally be required** by the Laboratory unless special investigations are required; however, they should be retained. It is preferable to send specimens to the Laboratory via a reputable courier service (provided in certain localities by Central Valley Toxicology), or by Federal Express Overnight Service, Monday through Thursday. (The Laboratory is not regularly staffed on weekends.) This is important not only to preserve the physical integrity of the specimens, but also to maintain the chain of custody.

Specimens should be tightly sealed in their respective containers, placed in plastic biohazard bags and securely packaged. Refrigerant is generally advisable; it can be **very** hot in the San Joaquin Valley during the summer, and post mortem samples (particularly those from decomposed bodies) may ferment in transit at any time of year.

SPECIMEN CONTAINERS

Central Valley Toxicology is able to provide appropriate containers for the collection of specimens at post mortem, where cases are submitted to CVT for investigation. Please contact the Laboratory if this service is required. In general, use containers appropriate to the specimen volumes with secure closures. Evacuated blood collection tubes with rubber stoppers are generally satisfactory *as long as they are not overfilled*. Plastic screw-cap containers such as those used for urine or tissue samples in clinical and histology laboratories are often inadequate for long-distance transport.

LABELING AND STORAGE OF SAMPLES

All specimen containers should be clearly labeled with the full name of the deceased, date of collection, the type of specimen and post mortem or reference number. The standard labels affixed to antemortem samples collected in hospitals are normally adequate, but it may be necessary to peel back one or two labels to reveal all the information; please do not affix additional coroner's labels on top of the hospital labels. In the case of post mortem blood specimens, the specific site of sampling should always be given.

All specimens should be stored at 4^{o}C before and during transport to the Laboratory. Each specimen container should be securely sealed to prevent leakage, and packaged in sealed plastic biohazard bags to contain any leakage that might occur. Inclusion of an absorbent inside the biohazard bag is strongly recommended.

Please note that antemortem blood specimens collected from hospital laboratories may lack the original rubber stoppers; they may have plastic snap caps (with or without plastic cups in the top), serum filter tubes, or in the case of hematology specimens metal capillaries with plastic feet designed to make preparation of slides convenient. Such "closures" are ***not*** satisfactory for transport; they almost always leak, and application of Parafilm® hardly helps. In order to prevent loss of specimen, they must be removed, and the tube closed with a rubber stopper; the cost of discarding a new evacuated tube to get the stopper is trivial, especially when compared to the value of an irreplaceable specimen. If there is serum in a serum separator tube or a cup in the top of the original tube, pour it back into the specimen tube before inserting the stopper.

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SUMMARY OF PREFERRED SPECIMEN CONTAINERS

BLOOD: Two 6- or 7-ml fluoride/oxalate (gray-top) tubes. Additional blood or blood collected without preservative may be submitted, but will not often be required.

URINE: A 6- or 7-ml tube of urine, with or without preservative, will generally be more than enough. Only a few rare tests (*e.g.* anabolic steroids) will require more. A good screw-cap container is satisfactory if the cap is screwed down **hard**.

VITREOUS HUMOR: Place in an appropriate size of vial. Do not add preservative; vitreous humor is among the last body fluids to be contaminated by microbiota, and preservatives interfere with tests for electrolytes and lithium. If there is sufficient specimen it may be divided between two smaller containers, one with preservative for alcohol testing and one without.

TISSUES: Use a good screw-cap container and screw the cap down **hard.** If available, liver is preferred because more interpretive information is available for liver than for any other solid tissue. Do not fill the container; fermentation can cause the container to break, and a piece of tissue the size of a walnut should be more than enough for all testing.

SPECIMENS	Central Valley Toxicology

BLOOD Specimens (Ideally free-flowing blood)

Importance of site and method of sampling

The sites and methods of blood sampling are important; care taken at this stage will often be rewarded by more reliable and confident interpretation of toxicological findings. The concentrations of some drugs, notably common compounds such as tricyclic antidepressants and opiates, are much more likely to be “falsely” elevated in blood from thoracic and abdominal vessels than in "peripheral" vessels such as femoral veins. Moreover, even blood from these sites is easily contaminated by blood drawn from contiguous, more central vessels if large specimens are taken without ligation. The procedure recommended below for the collection of femoral venous blood should minimize these effects.

Our current recommendation for an **ideal** set of blood specimens for toxicological investigations is as follows:

Blood should be obtained from a peripheral site, preferably a femoral vein, taken with care so as not to draw a large volume containing blood from more central vessels. **The sampling site must be indicated on the label if more than one site is used.** Femoral blood can be taken by cutting the external iliac vein proximal to the inguinal ligament and milking the distal cut end into a plain disposable container, from which it can be transferred into two (volume permitting) fluoride-oxalate tubes. The balance may be retained or submitted to the lab. If femoral blood is not available or is not available in sufficient volume, the order of preference is subclavian, heart, and cavity blood. A total blood volume of at least 12 ml is desired, divided into at least two (appropriately sized) containers lest one should break in transit. (Breakage is now unusual because of the general change from glass to plastic evacuated blood collection tubes, but the possibility of a stopper coming out due to overfilling or fermentation remains.)

URINE

Advances in blood testing have rendered urine specimens far less important for post mortem drug testing than they once were, but if available they should be submitted. Occasionally urine specimens will be useful for glucose or drug testing, but their principal importance is now as a complementary specimen in the quantitative analysis of alcohol. If other specimens are **very** limited in amount, screening may sometimes be done on urine to spare blood or vitreous humor for such quantitative analyses as may be indicated. Urine specimens may be submitted in fluoride-oxalate tubes or in plain containers.

VITREOUS HUMOR

Advances in analytical methodology have made a sample of vitreous humor so potentially valuable that it should be collected and forwarded to the Laboratory whenever possible. This specimen is especially useful for certain biochemical tests such as urea, creatinine, glucose, acetone and electrolytes; for verification of blood alcohol test results; and for investigation of high levels of drugs in blood that may result from postmortem redistribution. More information on the collection of vitreous humor can be found in the recent reviews of Forrest (1993) and Knight (1995, 2002).

DEATHS IN HOSPITAL

For deaths which have occurred in hospital, the hospital pathology laboratory should be contacted as soon as possible to see if any antemortem specimens of urine, blood, serum, or plasma are available, and these should also be sent for analysis. Unless advised to the contrary, analysts will assume that the earliest hospital specimens are the most significant.

The standard labels affixed to antemortem samples collected in hospitals are normally adequate, but it may be necessary to peel back one or two labels to reveal all the information; please do not affix additional coroner's labels on top of the hospital labels.

Please note that antemortem blood specimens collected from hospital laboratories may lack the original rubber stoppers; they may have plastic snap caps (with or without plastic cups in the top), serum filter tubes, or in the case of hematology specimens metal capillaries with plastic feet designed to make preparation of slides convenient. Such "closures" are ***not*** satisfactory for transport; they almost always leak, and application of Parafilm® hardly helps. In order to prevent loss of specimen, they must be removed, and the tube closed with a rubber stopper; the cost of discarding a new evacuated tube to get the stopper is trivial, especially when compared to the value of an irreplaceable specimen. If there is serum in a serum filter tube or a cup in the top of the original tube, pour it back into the specimen tube before inserting the stopper.

COLLECTION OF OTHER SPECIMENS

Other types of specimen will not normally be required, but they may be valuable in the investigation of certain cases and should be retained. All retained tissues should be placed in separate, clearly labeled, sample containers to remove any chance of cross-contamination. A preservative such as formalin must not be used on specimens for potential toxicological evaluation. Refrigeration, or freezing for long-term storage, is recommended.

Liver

This tissue may be useful in certain complex poisoning cases or when satisfactory samples of body fluids are not available. It is usual to take a portion of the **right lobe** of liver since it should be uncontaminated with bile and less affected by drug diffusion from the stomach; a portion the size of a walnut will be more than sufficient for analytical purposes. The particular value of liver is that more interpretive data are available for liver than for any other solid tissue.

Gastric contents

These materials may be useful in the investigation of oral cyanide poisoning, or in cases of rapid death where relatively large amounts of unabsorbed drug may be found in the stomach. In such cases a representative sample of the gastric contents should be retained, and the total volume recorded. If distinct tablets or capsules are observed in the stomach contents, these should be carefully extracted and put into a separate container. Identification of such material may be carried out by reference to a database of pharmaceutical products, or by direct analysis at the Laboratory.

Brain, lung, spleen, muscle, bile, etc.

These specimens do not generally add any useful information, and are only indicated when more desirable specimens (blood, urine, vitreous humor, liver) are not available. Occasionally decomposition fluids may be little but oil; these are not satisfactory for testing.

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INVESTIGATION OF SPECIFIC DRUGS AND POISONS

Alcohol (ethanol)

Where there has been putrefaction or extensive injury to the body or several days between death and autopsy, it is advisable to take both blood and urine specimens for alcohol analysis. It is also advisable to take a specimen of vitreous humor if available, unless the body has been immersed in water for an extended period of time. Ethanol can sometimes be lost or generated from blood specimens if they have become contaminated by bacteria or fungi. It is the policy of Central Valley Toxicology to test an alternative fluid (if available) whenever alcohol is detected in a post mortem blood sample; there is no need to request this precaution. While fluoride preservative is very strongly recommended for blood samples, it is discouraged for vitreous humor samples unless there is sufficient sample to divide into two portions, one with preservative and one without. Acetone is detected in the course of alcohol tests by gas chromatography (the only method used at CVT), and if more than traces are noted in blood it will be quantitated; it will also be measured in vitreous humor or urine if available. If indicated, further testing will be done for glucose and yeast. (If the subject's history or the circumstances indicate, it is still best to specifically request testing for glucose and ketones in vitreous humor.) In cases where the decedent suffered a blow to the head (*e.g.,* from a fall) with subsequent formation of a **subdural hematoma**, the alcohol level in the subdural blood (or clot) may more accurately reflect the alcohol level at the time of the blow than would any other specimen, particularly if death did not come for some time.

Carbon Monoxide (CO)

Carbon monoxide concentrations tend to decrease with time; blood specimens should be submitted promptly and in containers without excessive headspace; fluoride preservative is recommended. Please note that specimens other than whole blood are of very little or no utility for carbon monoxide testing. If blood is not available carbon monoxide poisoning must be diagnosed by the appearance of the body and the circumstances of death.

Opiates

The standard screening test for opiates will readily detect morphine, codeine, and hydrocodone; it *may* detect oxycodone, but the latter will be more reliably detected by a specific immunoassay. For this reason it is important to advise the Laboratory if oxycodone was available to the decedent or is otherwise suspected. It is the custom of CVT to measure and report *total* morphine, because that is normally sufficient, and because far more interpretive information is available for total than for free morphine. In some cases it is appropriate to test for both total and free morphine in blood, and in rare cases it may even be necessary to measure free and total morphine in vitreous humor. 6-monoacetylmorphine (6-MAM) indicates heroin use, but it is rapidly metabolized and is unstable in blood. If seen it is reported.

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Cocaine

Cocaine is relatively unstable in blood due to the presence of viable metabolically active enzymes even in post mortem blood. Therefore it is recommended that in cases where cocaine ingestion is suspected, blood specimens should be placed in fluoride/oxalate tubes. It is also advisable to store the specimens at 4ºC immediately after collection and to deliver them to the Laboratory promptly. Cocaethylene, formed in the body when cocaine and ethanol are taken together, is routinely tested for as part of the cocaine confirmation procedure, and if detected it is noted on the report.

Amphetamines

Methamphetamine and amphetamine are routinely screened for, and while the screening tests will also detect MDMA and its metabolite, MDA, it is helpful to advise the Laboratory if MDMA is suspected. Post mortem blood samples not infrequently give false positive immunoassay screening tests for amphetamines due to beta-phenethylamine, and if the history does not seem consistent with methamphetamine use specimens will be further screened by LCMS before proceeding to confirmation and quantitation by GCMS. CVT routinely differentiates between *d-* and *l-* methamphetamine by stereospecific GCMS and reports the *d-* isomer; there is no need to specially request the differential analysis.

BIOCHEMICAL INVESTIGATIONS

Biochemical investigations carried out on post mortem blood are generally of limited value. Biochemical analysis of vitreous humor can sometimes be useful but the interpretation of potassium and sodium concentrations is complex. The presence of **acetone** in blood and vitreous humor or urine may be an indication of alcoholic or diabetic ketoacidosis. Low **glucose** levels in post mortem vitreous humor are common due to continued cellular metabolism after death, and are not an indicator of perimortem hypoglycemia. If the injection of an **insulin** overdose is suspected, post mortem blood and even vitreous humor are not suitable for analysis of insulin and C-peptide. Due to the instability of these products and the lack of any comparative data, the interpretation of the findings would be very difficult, even if a clinical laboratory were found that would agree to do the test(s). For cases where **lithium** may be involved (*i.e.,* the patient had been prescribed lithium), antemortem serum or unpreserved vitreous humor are preferred because very high potassium levels interfere with measurement by the ion selective electrode method used at CVT; in case of such interference samples will be referred for measurement by atomic absorption.

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ANALYTICAL METHODS

The Laboratory uses a wide range of modern methods to analyze biological specimens for the presence of drugs and poisons, including:

- Enzyme immunoassay (EIA)
- Gas chromatography (GC)
- Gas chromatography-mass spectrometry (GCMS)
- Liquid chromatography-mass spectrometry (LCMS)
- Liquid chromatography-tandem mass spectrometry (LCMS-MS)

GENERAL ANALYTICAL WORK FLOW

Limited requests for drug and alcohol testing will be handled in an appropriately simplified manner; the general procedure for coroners' "complete" drug screens is of course more extensive. Specimens arriving in the morning (generally by 10:00 AM) will be tested beginning the same day; specimens received later may be processed on the following business day. Appropriate immunoassays and alcohol tests are performed on the first day; extracts for general drug screens by LCMS are prepared simultaneously and chromatography begun in the afternoon so that the data can be reviewed the following business day. Individual report templates are prepared by the clerical section with appropriate specimen descriptions, billing and reporting information on the first day of testing. On the second business day samples are distributed for appropriate further testing as indicated in the original request or by the results already obtained. Many drugs are satisfactorily identified and quantitated by the original LCMS procedure; some are better or more efficiently confirmed and quantitated by specific chromatographic tests. Most such specific chromatographic tests are performed at least once a week; some low volume tests may be performed less frequently in order to consolidate several samples into a single run. Rare tests (*e.g.* psylocin, kavain) will be done on an as-needed basis because there is little chance of having more than one sample in two weeks. When no further testing is indicated, the job order is delivered to the clerical section for completion of written reports. Reports are proofread and checked against the original data by at least one Laboratory Director before they are signed and released.

The usual screening procedures at Central Valley Toxicology will generally detect *unsuspected* pharmaceutical and illicit drugs at high therapeutic to toxic levels, but drugs present at low levels, whether because the decedent was noncompliant and the levels declined into the subtherapeutic range (*e.g.,* anticonvulsants), or because the drugs are active at very low levels (*e.g.,* LSD or fentanyl), may be overlooked. For this reason it is vitally important to advise the lab of any drugs that were available or are suspected. Inspection of the chromatographic data for a specific drug can be done to a significantly higher level of sensitivity than for drugs in general.

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ANALYTICAL REPORT AND INTERPRETATION

Median completion time, from specimen receipt to completed report, is five to six calendar days, and even the most complicated cases rarely take more than two weeks. Verbal reports of initial screening and alcohol test results may be available in late afternoon for specimens that arrive by 10:00 AM Monday through Friday, and **will** be available by the following business day.

Completion times of more than two weeks are generally due to follow-on requests for more extensive testing, submission of supplementary samples, or referral of specialty tests (*e.g.,* anabolic steroids or heavy metals) to other laboratories. On occasion it may take a very long time to obtain certified standards for new or highly unusual drugs. In case of unusual delays a preliminary written report will be issued, with the completed report to follow.

Interpretation of findings can present a problem where there is little background information concerning the case, or where specimen collection has been inadequate. It is difficult to provide any valid comment on the significance of quantitative measurements carried out on a single blood specimen from an unknown site. Interpretation of findings can also be difficult in drug abusers where the likely degree of "tolerance" to a drug is unclear because of inadequate history. In addition, much of the published literature on forensic toxicology relating to so-called "fatal" blood concentrations can be misleading in certain circumstances. It is a common misconception that the concentration of a drug (or poison) found in post mortem blood is equivalent to that obtained in the blood or plasma of the deceased at the time of death. Interpretation of findings will always need to take account of possible changes in drug distribution after death. The recent history, age and state of health of the deceased are also important factors to be taken into account in the interpretation of findings. **The Laboratory is always happy to discuss individual cases or give further advice on the interpretation of findings.**

Central Valley Toxicology customarily includes drug level reference ranges on its reports, but some clients prefer that they be omitted in order to avoid misinterpretation by the less knowledgeable. A call to the Laboratory will take care of this.

Reports are most often delivered by fax or E-mail, followed by two hard copies via U.S. Mail or courier. Some clients prefer simpler arrangements, which are easily made by a telephone call.

Toxicology Requisition Forms

The following documentation is desirable in every case:

- Name of the submitting agency.
- Subject's full name, age, sex and date of death.
- Investigator, pathologist, and agency case number.
- Specimens submitted; the specimens received will be compared to those listed on the requisition form.
- The apparent cause of death.
- Drugs and poisons available, if known.
- Current drug therapy, if known.
- Drugs administered by emergency personnel, if any.
- Comments/Circumstances of death.
- Tests requested.

All of the above information is required to enable the Laboratory to provide efficient service and reliable interpretation of results.

Toxicology requisition forms are available from the Laboratory, and if completed in full will provide us with the necessary information. The forms may be customized if desired.

RECOMMENDED READING

Knight, B. (1996),
Forensic Pathology (2nd edition)
Arnold Publishing, London.

Knight, B. (2002),
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Arnold Publishing, London.

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Butterworth, Heinemann, Oxford.

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Butterworth Legal Publishers, Salem, New Hampshire
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Disposition of Toxic Drugs and Chemicals in Man (7th edition)
Biomedical Publications, Foster City, California

Karch, S.B. (2001),
Pathology of Drug Abuse (3rd edition)
CRC Press, Boca Raton, Florida

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