

Toxicology

Forensic toxicology testing is requested in many situations. These situations routinely involve testing for foreign substances (drugs and toxins) in biological specimens. Since there is no single test that can detect all drugs and toxins, a systematic approach is used in each laboratory to focus on the substances deemed appropriate for that particular case.

Some areas of forensic toxicology are highly standardized (e.g., blood alcohol and employment urine drug testing) and others less so. Because each laboratory is set up a little differently and pharmaceutical companies are constantly adding new drugs to the long list of available compounds, the investigator is encouraged to inquire about their toxicology laboratory's ability to detect a particular drug or toxin of interest.

Caution: Observe protocols relating to the collection of evidence.

(See Chapter 1, Evidence Integrity and Appendix A, Bloodborne Pathogens.)

I. Choice of Specimens

Blood and urine are the most commonly analyzed specimens in toxicology. More information regarding drug and alcohol levels can be found about these two specimens than any others. Toxicology results from these specimens provide the strongest basis for the interpretation of effects and exposure.

NOTE: In living individuals the concentrations of drugs in these fluids are constantly changing depending upon the relationship between the time of dosing and the time of sample collection.

Blood Samples

Blood/serum/plasma samples are the specimen of choice for investigations dealing with the effects of a drug on an individual's **functioning and behavior**. The primary example of this is a blood sample drawn for alcohol determination.

Doctors and toxicologists measure blood drug concentrations because they show the best correlation to the effects on an individual. Drug companies analyze these samples as part of their studies to determine “therapeutic” levels prior to releasing new drugs to market. These published studies by the drug companies along with those cataloging blood levels associated with toxicity and death are of prime importance for a toxicologist’s interpretations of a drug’s effects.

Even when an accurate blood level of a drug is obtained, the interpretation of its effects – the impairment and toxicity of the drug on a particular individual should be done with caution. Factors involved in such interpretations are:

- A. Tolerance levels
- B. Multiple drug interactions
- C. Biological variation
- D. Postmortem redistribution

Urine Samples

Drugs in urine samples can be detectable for days and concentrations can be many times higher than those in associated blood samples.

Because of this longer detection window and higher drug concentrations, urine samples are a better choice than blood samples for investigations seeking to answer the question of drug use or exposure. Examples of investigations where urine samples should be collected are cases involving drug use (e.g., bail jumping cases) and drug facilitated sexual assaults.

Urine drug levels show poor correlation to drug effects on an individual, and, therefore, published therapeutic/toxic ranges for urine are rare. Urine alcohol levels are a notable exception, because, if properly collected, they can be related back to the associated blood alcohol levels.

Detection Period

One of the most common questions asked of a toxicologist is “how long can drug X be detected in a person’s system?” This depends on a number of factors including but not limited to:

- A. The size of the dose
- B. The size of the individual

- C. The natural biochemistry of that individual
- D. The analytical capabilities of the laboratory (detection limits)
- E. The specimen being analyzed

As a general rule, most laboratories will not detect drugs in blood if the blood is collected more than 24 hours after the last ingestion/exposure. Of course, there are exceptions to this rule with some drugs/metabolites exceeding this limit and others becoming undetectable prior to this limit.

See Table 28-1 for comparison of **detection windows** for selected drugs/metabolites in blood versus urine. In general terms, drugs are detected in blood on the scale of hours while they are detected in urine on the scale of days.

Table 28-1

Estimated Detection Periods for Selected Drugs in Blood and Urine

DRUG/METABOLITE	BLOOD	URINE
Cocaine	2 – 8 hours	0.5 – 1 day
Cocaine Metabolite (Benzoyllecgonine)	20 – 36 hours	1 – 3 days
Heroin Metabolite (Morphine)	4 – 12 hours	2 – 4 days
Marijuana active chemical (THC)	2 – 6 hours (infrequent user) Longer for heavy users	≤ 1 day (if detectable @ all)
Marijuana Metabolite (11-nor-9-Carboxy-THC)	12 – 72 hours	2 – 7 days (casual use) 28 days (heavy use)
Methamphetamine	4 – 24 hours	2 – 4 days
GHB	≤ 6 hours	≤ 12 hours
Rohypnol Metabolite (7-AminoFlunitrazepam)	≤ 8 hours	2 – 3 days
Ketamine	12 – 24 hours	1 – 2 days

NOTE: *These detection periods will vary depending on an individual's drug use pattern, the specific user and the analytical capabilities/detection limits of the laboratory.*

II. Collection of Specimens – Living Individuals

Blood Samples

Blood samples from living individuals must be drawn by a licensed physician, nurse or medical technician. The directions given below are directed primarily toward these medical professionals, but law enforcement personnel can also benefit from this information.

For blood alcohol determinations, the skin at the collection site should be cleaned with techniques that avoid solutions containing alcohol. Modern analytical techniques can distinguish among the different alcohols, but it is highly recommended to use antiseptic towelettes (e.g., Zephiran® or benzalkonium chloride) that do not contain any alcohols for this sterilization step.

Fifteen to twenty milliliters of blood should be collected in gray-topped tubes (see Appendix B, Collection Kits). These tubes contain sodium fluoride as a preservative and potassium oxalate as an anticoagulant. Lavender-topped EDTA tubes can be substituted if necessary. After collection, gently invert the tubes for a few minutes to mix the additives into the blood. Each collection tube should be labeled with the following:

- A. The name of the individual whose blood was drawn
- B. The date and time of the blood draw
- C. The initials of the person drawing the blood

The tubes and the primary container should be sealed to prevent unauthorized opening. This can be accomplished with the use of evidence tape placed over the top of the closed tube and around the primary container and initialing across the boundary between the tape and the tube/container. Best practice is to seal **both** the individual tubes **and** the primary container.

Urine Samples

When urine samples are collected for forensic purposes, the donor may be motivated to submit a false (clean) sample. In this situation, the urination should be observed by authorized personnel. Common ways to falsify a urine sample include dilution with tap or toilet water or by substituting clean, purchased urine for one's own urine. This can even be done through the use of an anatomically correct apparatus.

Twenty or more milliliters of urine should be collected in plastic cups that are manufactured for this purpose (see your local hospital) or in a pristine glass screw-top jar. After the urine is collected, the lid should be tightened and the container sealed with evidence tape. The collector/witness should place their initials across the boundary between the evidence tape and the container. The container should be labeled with the following information:

- A. The subject's name
- B. The date/time of collection
- C. The collector/witness's initials

If the urine sample is to be transported, the primary container should be sealed in a plastic bag to retain any liquid that might leak. If done properly, this plastic bag can be a secondary evidence seal which will allow any leaked urine to still be analyzed if necessary. The person sealing this plastic bag should initial the seal. Do not place any paperwork inside this plastic bag in the event of a leak.

Other Toxicology Samples

For other types of toxicology samples, follow the directions above for urine samples.

III. Collection of Specimens – Deceased Individuals

For deceased individuals, an autopsy is often warranted (see Chapter 25, *Autopsy*) at which time samples for toxicology should be collected. The selection of toxicology samples in postmortem cases can vary; however, some samples are of primary importance. A tiered approach is used here.

Tier One: Primary Specimens

Blood: Collect at least 20 milliliters in gray-topped tubes. As with living persons, blood is the primary specimen for postmortem toxicology. Blood collected at autopsy is routinely from the heart. This cardiac blood is usually plentiful and readily available. The collection of an additional blood sample from a peripheral site is a valuable adjunct to the cardiac blood because of the phenomenon known as postmortem redistribution

(see below). Samples to substitute for blood can include spleen, bile and liver (see Tier Two: Replacement/Additional Specimens).

Urine: Collect 15 or more milliliters of urine in a clean glass jar or a urine cup. Urine is a valuable specimen for the same reasons as discussed for living people. Urine alcohol levels can also be useful in decomposition cases to interpret the neo-formation of ethanol. Samples to substitute for urine include bile, liver and kidney (see Tier Two: Replacement/Additional Specimens).

Peripheral Blood: Collect 10 or more milliliters of blood from a peripheral location in gray or lavender-topped tube(s). The preferred peripheral location is the femoral vein followed by a subclavian vein. The vein should be tied off nearest the central cavity to prevent pulling blood from this area. Drug levels in blood can elevate over time following death as drugs diffuse from blood-rich organs (heart/liver/lungs) into the blood contained in nearby blood vessels or cavities. This phenomenon, known as **postmortem redistribution**, is less pronounced in peripheral blood which is further from these major organs. Samples to substitute for peripheral blood include vitreous humor and liver.

Vitreous humor (eye fluid): Collect 2-5 milliliters in a gray-topped tube. Vitreous fluid is useful for the interpretation of blood alcohol in cases of decomposition or trauma and can be related back to the associated blood alcohol level. This fluid can also be used to some degree as a replacement for peripheral blood.

Stomach contents: Collect the entire contents or a **measured** portion in a clean glass jar. Stomach contents can be used to determine the amount of drug taken by an individual just prior to death. This analysis can be helpful in determining suicidal intention. For accurate analysis, the toxicologist must be informed if only a portion of the stomach contents is submitted (e.g., “1/2 of the total stomach contents submitted”).

Ante mortem blood/serum/urine samples collected upon admission to the hospital: These samples are very important in cases where the individual survived for a significant period of time in a hospital prior to death and can be used in lieu of postmortem specimens.

Tier Two: Replacement/Additional Specimens

Spleen samples: A representative sample (approximately 10-20 grams) in a clean glass jar. The spleen is a blood rich organ which can be used in lieu of liquid blood.

Bile samples: 5-10 milliliters in a gray-topped tube. Bile samples contain high drug concentrations and can be used in lieu of urine samples to screen for toxic substances.

Liver samples: A representative sample (approximately 50 grams) in a clean glass jar. Liver samples can be useful in cases of postmortem redistribution (see peripheral blood) and extreme decomposition.

Kidney samples: A representative sample (approximately 50 grams) in a clean glass jar. Kidney tissue or kidney fluid can be used instead of urine if bile and liver are unavailable.

Tier Three: Specialty Samples

Hair samples: Collect a large sample (at least one-half inch diameter lock of 3-inch long head hair) for toxicology work. Hair samples show historic drug use but are not useful for acute poisoning cases. The Wisconsin State Crime Laboratory does not currently analyze hair samples for drugs.

Lungs: Lungs can be collected in an airtight container (clean, unlined paint can) if solvent inhalation is suspected immediately prior to death. The gaseous headspace above the organ can be sampled for analysis of volatiles.

IV. Specimen Storage and Shipping

All toxicology specimens should be stored according to the following recommendations:

- A.** Short term (weeks/months) – refrigerated storage
- B.** Long term (months/years) – frozen storage

NOTE: For frozen storage of liquid samples, ensure there is enough airspace (about 10% of the volume) in the container to allow for expansion of the liquid as it freezes.

If specimens are to be sent through the mail, follow the U. S. Postal Service regulations regarding shipment of biohazardous evidence. Biological samples sent through the mail must be packaged in the following manner:

- A. The sample must be **triple packaged** in a primary leak-proof receptacle, a secondary container and a rigid outer shipping container.
- B. The two innermost (primary and secondary) containers must each be sealed and marked with a biohazard sticker.
- C. The primary container must be wrapped in enough absorbent material to retain the sample should the container leak or break.

For the most current information regarding packaging, see U. S. Postal Service, Domestic Mail Manual at www.USPS.gov or IATA Packing Instruction 650.

V. Toxicology Testing

Toxicology testing can be as routine as a single blood alcohol test or as complex as the quantitation of numerous drugs in multiple samples. The level of testing required generally depends upon the type of case and the charges pending. The Wisconsin State Crime Laboratory provides the following toxicology testing services.

Blood Alcohol Only

Felony operating while intoxicated cases (OWI), crimes of violence such as domestic abuse, reckless use of a weapon and endangering safety often involve only alcohol testing. For the majority of these cases, if the blood alcohol level is high enough to prove impairment/intoxication (e.g., at 0.08% and above), no further drug testing will be done unless other arrangements are made. Generally, alcohol has the predominant effect over other drugs that may be present and is well suited to support these charges.

Probation and parole violations where the initial charge is a felony will be accepted and testing will follow the dictates of these release programs.

Blood Alcohol and Immunoassay Screening with Confirmation

Alcohol testing plus immunoassay screening for a routine panel of drugs is the next level of testing. Any immunoassay positives are followed by gas chromatography/mass spectrometry (GC/MS) confirmation. This level of testing is often used for felony suspects in controlled substance cases and homicide victims where the cause of death (e.g., shootings or stabbings) is known. The current standard immunoassay panel includes:

- A. Amphetamine
- B. Methamphetamine
- C. Cocaine/metabolite
- D. Opiates (e.g., codeine, morphine, hydrocodone, hydromorphone, oxycodone, heroin metabolite)
- E. Benzodiazepines (e.g., diazepam, alprazolam, clonazepam, lorazepam, flunitrazepam)
- F. Phencyclidine (PCP)
- G. Barbiturates
- H. Cannabinoids (marijuana)

Blood Alcohol, Immunoassay Screening and General Drug Screening with Confirmation/Quantitation

This level of testing adds a general drug screen. This drug screen detects a broad spectrum of acidic, basic and neutral drugs including over 150 pharmaceutical compounds, designer drugs and poisons.

Additional Testing

Additional tests are available and are utilized as needed or requested. These include

- A. GHB (gamma-hydroxybutyrate) which is routine for drug facilitated sexual assault cases
- B. LSD
- C. Carbon monoxide (carboxyhemoglobin)
- D. Cyanide
- E. Salicylates (aspirin)
- F. Acetaminophen
- G. Heavy metals (urine only)

Communication between the Crime Lab and the submitter is important to ensure the proper level of testing.

VI. Laboratory Choices: Hygiene Lab vs. Crime Lab

The State of Wisconsin has two independent FORENSIC TOXICOLOGY laboratory systems. One is the Wisconsin State Crime Laboratory which is part of the Wisconsin Department of Justice and the other is the Wisconsin State Laboratory of Hygiene (WSLH) which is part of the University of Wisconsin System. For animal toxicology cases, contact the Wisconsin Veterinary Diagnostics Laboratory (for contact information, see section VII).

The submitter should select the correct laboratory prior to mailing. **Valuable time is lost and the chain of custody is extended when specimens are sent to the wrong laboratory.** For this reason, if you are using a WSLH kit for a Crime Lab case do not use the mailing label supplied with the WSLH kit; instead use a mailing label for the Crime Laboratory serving your area (Madison covers the Wausau Lab service area for toxicology). It is also important to include a completed Transmittal of Evidence Form when WSLH kits are submitted to the Crime Lab.

Table 28-2 should help you determine which toxicology laboratory to use.

By statute, the Crime Laboratories are only required to accept cases involving a felony or a potential felony. By agreement, however, the Crime Laboratory does analyze misdemeanor intoxicated use-of-a-firearm cases and misdemeanor marijuana cases which have a court date scheduled.

Table 28-2**Submissions: Hygiene Lab vs. Crime Lab**

OFFENSE	HYGIENE LAB	CRIME LAB
Driving Under the Influence (DUI) Impaired by alcohol and/or other drugs. Includes snowmobiles, motorboats, and ATV's.	ALL OFFENSES Including great bodily harm, vehicular homicide.	ONLY FELONIES ACCEPTED
Death Investigations	Suicides & routine death investigations. Submission by coroner or medical examiner only.	Death is suspicious and possible felony CHARGES pending.
Probation and Parole Violations	Initial charge must be a MISDEMEANOR otherwise a FEE is required.	Initial charge must be a FELONY.
Crimes of Violence Domestic Abuse, Drug Facilitated Sexual Assaults, Weapons Charges	NOT ACCEPTED	ACCEPTED
Controlled Substance Cases	NOT ACCEPTED	MUST BE A FELONY Biological samples alone are not sufficient for possession charges.

VII. Toxicology Laboratory Contact Information

The Crime Laboratories in Madison and Milwaukee both have Toxicology Units. The addresses and phone numbers are listed in the Introduction.

The State Lab of Hygiene can be contacted at

Wisconsin State Laboratory of Hygiene (WSLH)

2601 Agriculture Drive, P.O. Box 7996

Madison, WI 53707-7996

(608) 224-6241

<http://www.slh.wisc.edu/ehd/toxicology>

Contact information for the Wisconsin Veterinary Diagnostics Laboratory can be found at <http://www.wvdl.wisc.edu>.

In addition to the state laboratories, there are also independent laboratories which are approved to analyze blood and urine specimens for alcohol under Wisconsin Statute 343.305(6)(a). A list of all approved laboratories can be found at http://dhs.wisconsin.gov/rl_DSL/Labs/LABSintro.htm or by calling the Wisconsin Department of Health and Family Services, Division of Supportive Living, Clinical Laboratory Unit at (608) 267-9862.

VIII. Toxicology Kits

The Wisconsin State Crime Laboratory provides autopsy kits for a fee. See **Appendix B, Collection Kits** or call the Madison Laboratory for details.

The WSLH provides blood and urine collection kits intended for use in driving under the influence (DUI) related offenses and are free of charge for these purposes. See above for their contact information.