Forensic Toxicology
An Introduction

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KEYWORDS

• Forensic toxicology • Workplace drug testing • Postmortem toxicology
• Human performance toxicology

KEY POINTS

- The difference in the science behind the fields of forensic toxicology and clinical toxicology is minimal, if any.
- Providing toxicology results to the legal system requires the use of terms and language used in that field, as opposed to strictly medical language.
- There are 3 components of forensic toxicology: workplace drug testing, postmortem toxicology, and human performance toxicology.

Forensics, by definition, is the use of science within the legal system. Forensic toxicology is no different. The difference between clinical toxicology and forensic toxicology is not in the science or the methods. Those are exactly the same. The difference lies in the end use of the results. In clinical toxicology, the end user is a physician who is using the results to treat and care for a patient. In forensic toxicology, the end user can be a physician, or a nonmedical professional such as a lawyer, a human resources employee, or probation officer who is using the results to determine a cause of death, employment eligibility, or compliance with terms of parole.

Forensic toxicology can be generally divided into 3 areas:

- Workplace or preemployment testing
- Human performance
- Postmortem

Workplace toxicology deals with preemployment drug screens or drug screens required by the Department of Transportation. Human performance deals with correlating a person's actions with a drug(s) they ingested. This could be driving under the

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influence of alcohol or drugs, committing a crime while on a drug, or having a crime committed against an individual such as a sexual assault. Postmortem toxicology deals with the toxicology testing on deceased individuals and is a routine part of the autopsy process.

WORKPLACE DRUG TESTING

Workplace drug testing is divided into two areas, regulated and nonregulated testing. Regulated testing is testing that is mandated by the federal government via the Department of Health and Human Services, and is overseen by Substance Abuse and Mental Health Services Administration (SAMHSA). This testing is mandatory for truck drivers who cross state lines, all federal employees, military employees, and for those with many other federal jobs. Nonregulated Workplace drug testing is any testing that is required of a new employee to start a job. The guidelines are not as stringent as regulated testing, although the basic tenants are still adhered to.[1]

Accreditation

Regulated workplace drug testing laboratories are accredited by SAMHSA through the National Laboratory Certification Program. Laboratories are inspected twice a year. They are challenged with proficiency samples 4 times per year, 25 samples per challenge. As of 2016, there were 30 accredited laboratories in the country. It is a very difficult, but prestigious, accreditation to obtain, hence the low number of accredited laboratories. To qualify as a federal drug testing laboratory, the laboratory has to demonstrate and adhere to the most stringent protocols in the world for drug testing. The goal of the National Laboratory Certification Program is to ensure consistency among all certified laboratories. So that regardless of the location where the sample is tested, the same result would be produced. It also creates an environment where split-sample testing can be instituted, and the comparison of results of "A" and "B" samples are made easier. At the time of collection, the specimen is split into 2 separate containers, "A" and "B." Each container is sealed and sent to the testing laboratory. The "A" sample is tested and the results are reported. If those results are disputed by the donor, they have the option to have the "B" sample reconfirmed at a separate laboratory. Additionally, the guidelines that are imposed are designed to protect the laboratory in litigation.

Nonregulated laboratories are accredited by the Collage of American Pathology's Forensic Drug Testing program. Although not as stringent as the SAMHSA program, the same forensic principles are adhered to.

Specimen

The specimen for regulated workplace testing is always urine. It must be collected under direct observation or with measures in place so that tampering with the collection are eliminated. Once a sample is collected, it is split into 2 containers ("A" and "B"). A tamper-evident seal is placed across each lid and is signed by both the donor and collector. A paper requisition must be presented by the donor to the collector before sample collection. This is known as the Custody Control Form. This paperwork will accompany the specimen from the time it is collected until final results are recorded.

The specimen for nonregulated workplace testing is also urine. The collection may or may not be observed, and the use of a tamper-evident seal is also optional, although many establishments do use it. There is usually a paper requisition that accompanies the specimen; however, the results are usually not reported on it.

The Department of Health and Human Services has proposed guidelines as to the use of oral fluid and hair as acceptable samples for regulated testing.[2] Oral fluid is

becoming more routine as a testing specimen. The ease of collection makes this an ideal specimen to collect where a restroom is not available, such as at the scene of a traffic accident. Because oral fluid is a hyperfiltrate of blood, parent compounds are detected opposed to metabolites. Detection lengths are thus shorter than urine, 1 to 2 days compared with 2 to 5 days with urine.[3,4] Hair is also another sample type that can be used for drug testing. The main advantage is the length of detection in hair is 3 months. However, environmental contamination is a significant concern with hair testing, so laboratories must take special concern during the specimen preparation steps to ensure as much environmental contamination is removed.[3,4]

Testing

Initial testing

Initial testing of the specimen, also known as screening or screen testing, is done by immunoassay. For regulated testing, the cutoffs to determine negative from nonnegative are established by SAMHSA. Nonregulated testing can have any cutoff, although many laboratories use SAMHSA values. Any value greater than or equal to the cutoff is considered "nonnegative" (note that the term *positive* can only be used with the confirmatory testing because of the possibility of false-positive screening test). Screening is for a specific class of drugs as shown in **Table 1**.

If all screening tests are negative, the results are released and there is no additional testing. If any of the results are greater than or equal to the cutoff value, a new aliquot

Table 1
Screening for a specific class of drugs

Initial Test Analyte	Initial Test Cutoff (ng/mL)	Confirmatory Test Analyte	Confirmatory Test Cutoff (ng/mL)
Marijuana (THCA)	50	THCA	15
Benzolecogonine	150	Benzoylecgonine	100
Codeine/morphine	2000[a]	Codeine Morphine	2000 2000
Hydrocodone/ hydromorphone[b]	300[a]	Hydrocodone Hydromorphone	100 100
Oxycodone/ oxymorphone[b]	100[a]	Oxycodone Oxymorphone	50 50
6-Acetylmorphine	10	6-Acetylmorphine	10
Phencyclidine	25	Phencyclidine	25
Amphetamine/ methamphetamine	500[a]	Amphetamine Methamphetamine	250 250
MDMA/MDA/MDEA	500[a]	MDMA MDA MDEA	250 250 250

Abbreviations: MDA, methylenedioxyamphetamine; MDEA, methylenedioxyethylamphetamine; MDMA, methylenedioxymethamphetamine; THCA, Δ-9-tetrahydrocannabinol-9-carboxylic acid.

[a] *Immunoassay*: The test must be calibrated with one analyte from the group identified as the target analyte. The cross-reactivity of the immunoassay to the other analyte(s) within the group must be ≥80%. If not, separate immunoassays must be used for the analytes within the group.

[b] Proposed analytes source.

Data from Drug Enforcement Administration, Department of Justice. Schedules of controlled substances: extension of temporary placement of UR-144, XLR11, and AKB48 in schedule I of the Controlled Substances Act. Final order. Fed Reg 2015;80(94)27854–6.

is taken from the original specimen and is subjected to confirmatory testing. Results are not released until all testing is complete.

Another part of the screening process is specimen validity testing. This portion of the testing determines if the specimen has been tampered with in any way. The specimen is tested for creatinine, specific gravity, pH, and oxidants (nitrites). When specimen validity testing falls out of the specified ranges of what is considered normal, it is labeled with 1 of 4 categories: dilute, substituted, adulterated, or invalid.

Dilute A specimen will be reported as dilute when:

- The creatinine concentration is greater than 5 mg/dL and less than 20 mg/dL; and
- The specific gravity is greater than 1.0010 and less than 1.0030.

Substituted Substituted means the donor has submitted a nonhuman specimen for testing. A specimen will be reported as substituted when:

- The creatinine concentration is less than 2 mg/dL; and
- The specific gravity is less than or equal to 1.0010 or greater than or equal to 1.0200.

Adulterated Adulterated indicates that the donor has added a substance to the specimen after it has been collected. A specimen will be reported as adulterated when 1 of the following criteria is met:

- pH of less than 3
- pH of 11 or greater
- Nitrite of 500 μg/mL or greater
- Chromium (VI) is present
- A halogen (eg, bleach, iodine, fluoride) is present
- Glutaraldehyde is present
- Pryidine is present
- A surfactant is present

Invalid A specimen will be reported as invalid when 1 of the following criteria is met:

1. Creatinine concentration and specific gravity results are discrepant:
   - Creatinine of less than 2 mg/dL and specific gravity of greater than 1.0010 and less than 1.0200
   - Creatinine is 2 mg/dL or greater and specific gravity is 1.0010 or less.
2. pH is outside the acceptable range:
   - pH of 3 or greater and less than 4.5
   - pH of 9 or greater and less than 11
3. Nitrite is 200 μg/mL or greater and less than 500 μg/mL.

Urine that falls into 1 of these 4 categories is considered to have failed the drug test, even if the tests for drugs are all negative.[5]

Confirmatory testing

Confirmation testing is performed by mass spectrometry, coupled either to gas chromatography or liquid chromatography. Confirmatory testing is specific to a unique drug analyte. The testing occurs on a fresh aliquot from the original sample, just in case there was a mix up with the initial screening aliquot. There are specific confirmation tests for each of the classes of drugs that are screened. The confirmatory testing result is definitive, and when performed correctly, is indisputable. Part of this assurance is based in the fact that the confirmed analyte is defined by multiple parameters.

When confirming using mass spectrometry–gas chromatography operating in single ion monitoring mode, the analyte is defined by the retention time compared with the calibrators, and the ratios of multiple ions within the ionic spectra that are unique to the specific analyte. Typically, the most abundant ion is called the "quantitation ion." The next 2 most abundant ions are called the first and second "qualifier ions". The ratios of the quantitation ion to the first and second qualifiers are used to positively identify the analyte. If they fall within a specified range of the same ratios from the calibrators, the analyte is identified positively. When using mass spectrometry–liquid chromatography, the use of 2 separate ion transitions is considered unique and is sufficient for identification, where the first and most abundant transition is called the quantitation transition, and the second transition is the qualifier transition.[2] The quantitation of the drug is greater than or equal to the confirmation cutoff value. This cutoff value is less than the screening cutoff but greater than the limit of quantitation. If developed correctly and properly maintained through proficiency testing, the confirmation test is considered to be definitive and unquestionable.

Resulting

Reporting of the results occurs after a second review of all results by someone within the laboratory that was not part of the testing process. If all results are in order, the results are certified and released either to the client or to a medical review officer. A medical review officer is a physician who acts an intermediary between the testing facility and the client who requested the test. The medical review officer is trained specifically to explain to the client and/or donor the results of the testing. They are often required to confront a donor whose specimen is positive and determine if the confirmed drug was taken in accordance to a physician's orders or recreationally. If the donor feels there is compelling evidence that a possible mistake was made in the laboratory, they can request the "B" sample be retested. In this case, the original testing laboratory sends the unopened "B" sample to another certified laboratory for testing of the contested analyte.

POSTMORTEM TESTING

Toxicology testing is a routine component of the autopsy process. When death occurs, metabolism of drugs and other substances stop. If an autopsy is performed within a reasonable amount of time, and the body has not been exposed to harsh environmental conditions, the results of toxicology testing are a snapshot of what was in the body at the time of death. Quantitation of these drugs can indicate if an overdose occurred, a subtherapeutic level of drug was present, or a combination of multiple substances contributed to the cause of death.[6]

Accreditation

With the National Academy of Sciences report on the State of Forensic Sciences, laboratory accreditation was one of the recommendations to standardize the field.[7] This can be overseen by the American Board of Forensic Toxicology, which also has testing programs for individuals to become certified.

Specimen

Postmortem testing is not limited to only urine. Specimens can be blood, urine, vitreous humor, gastric contents, liver tissue, hair, fingernails, or bile. This is not a comprehensive list. In addition to testing specimens collected at autopsy, the forensic pathologist may be interested to know the status of the decedent, particularly if

they were seen at a medical facility. Specimens that may have been collected by the hospital or health care facility before death (antemortem specimens) are often tested as well. Last, it is not uncommon for nonhuman items to be found at the time of death, which may have contributed to the death. Items such as unmarked pills, powders, syringes, or liquids may be submitted for analysis as well.

It is important that an accurate description of the sample type and the location of the collection be noted and sent to the laboratory with the samples. Blood can be taken from many different parts of the body, and each area can have a very different concentration of drugs. For example, blood can be taken from the heart, jugular, subclavian, and femoral veins. Blood from the heart is called "central blood," and blood from other sites is called peripheral.[6] Ideally, blood is collected from the central and at least 1 peripheral site, in case one of the sites is contaminated owing to the manner of death. Blood is collected into tubes with sodium oxalate preservative. This is important because specimens are often stored for extended amounts of time. Also, the state of the specimens can be compromised by bacteria. Depending on the manner of death, certain specimens may become contaminated with bacteria, either through exposure to the normal flora or from outside contamination, such as in the example of a body with open wounds that is not found for an extended amount of time and microorganisms have entered the body. The collection of specimens as well as the testing of these samples is always performed under chain of custody.

Testing

Instrumentation ranging from automated chemistry analyzers to manual enzyme-linked immunosorbent assay systems are used routinely for the initial screening. Because of the variety of specimens, both sample type and sample quality, enzyme-linked immunosorbent assay is the most widely used screening methodology. Postmortem blood is difficult to work with as a result of coagulation and/or degradation, and because of the state of the specimen at the time of testing, the small sample probes on automated chemistry analyzers are often unable to aspirate the blood. Enzyme-linked immunosorbent assay also allows the laboratory to institute different cutoffs for the same tests when analyzing different sample types. Confirmation testing is performed by gas chromatography/mass spectrometry or liquid chromatography/mass spectrometry.

HUMAN PERFORMANCE

Human performance testing relates how a person acts when under the influence of a substance or drug. Examples of this type of testing are blood alcohol and drug testing from a suspected drunk/drugged driver, blood testing for drugs from a possible drug facilitated sexual assault, or for cause testing of a worker who is exhibiting strange behavior while at work.[8]

Specimen

The specimen of choice is blood, although oral fluid may begin to be used in the future. Testing of a blood specimen is critical because if a substance is confirmed, it is possible to establish a window as to when the substance was ingested. This is unfortunately not possible if urine is used, because drugs have a longer detection window in urine. Being able to definitively prove the timeframe of when a substance is ingested is critical in human performance testing.

Testing

The testing panel is widely different, and depends on the situation that the specimen is being tested for. An alcohol only panel may be requested when a subject agrees to a field breath alcohol test and the result is positive. If a drug-facilitated sexual assault is suspected, a panel that would include alcohol, benzodiazepines, barbiturates, opiates, Δ-9-Tetrahydrocannabinol-9-carboxylic acid, gamma-hydroxybutyrate, zolpidem, and other depressants would be appropriate.

SUMMARY

Although forensic toxicology and clinical toxicology are defined as separate fields, the difference in the science behind these fields is minimal, if any. However, the differences are with the use of the results, and who is the recipient of those results. Providing toxicology results to the legal system requires the use of terms and language used in that field, as opposed to strictly medical language. For example, a common set of qualitative terms used are "positive" and "negative." However, these terms do not provide any room for the situation of false-positive or false-negative results. More accurate terms instead of *positive* are *presumptive positive* and *confirmed positive*. The results are often an integral part of the case and if one does not use the correct terminology and leaves ambiguity in the report, the many hours of work to generate those results could be for naught.

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