Toxicology. It all started with bows and arrows. Greek bows and arrows. The words toxic, toxin, intoxicated, toxicology, all have their roots in Cupid’s little bow and arrows (Eros’ really; Cupid was a Roman, not a Greek). The Greek word “toxa” means bow as in bows and arrows, archery. “Toxikon,” the word for the poison in which the arrows were dipped, evolved to mean any poison and became our word toxin. So, toxicology became the study of poisons. And toxicology screening is the search for poisons. Since quite a few poisons exist out in the world, the most general use of the term “toxicology screening” has become testing for drugs (prescription, over-the-counter, and illicit) and naturally occurring compounds that people ingest, by accident or on purpose (without the aid of someone shooting arrows at them).

The reasons for wanting to detect drugs in people are many. Healthcare facilities may need screening for emergency-room cases, or for inpatients who may have been given incorrect medications or drugs brought in by visitors. Industries need to monitor workers for exposure to toxic substances. Legal issues fall under the general heading of forensic toxicology. The Society of Forensic Toxicologists gives the following definitions at its website:

Post-mortem forensic toxicology … determines the absence or presence of drugs and their metabolites, chemicals such as ethanol and other volatile substances, carbon monoxide and other gases, metals, and other toxic chemicals in human fluids and tissues, and evaluates their role as a determinant or contributory factor in the cause and manner of death;

Human-performance forensic toxicology … determines the absence or presence of ethanol and other drugs and chemicals in blood, breath, or other appropriate specimen(s), and evaluates their role in modifying human performance or behavior; and

Forensic urine drug testing … determines the absence or presence of drugs and their metabolites in urine to demonstrate prior use or abuse.
Many techniques and methods are used to accomplish this screening for drugs. Each has its own strengths and weaknesses, and so a combination of several techniques is used to accurately determine what drugs are present or absent. As in most fields of endeavor, toxicology has its own terms and jargon. A good dose of “alphabet soup” is involved.

**DRE is more than just a rapper**

Law-enforcement agencies have the duty to enforce drunk-driving laws. One of the first drugs screens performed in this area is the evaluation of the suspect by the drug-recognition expert (DRE) (see “Alphabits” chart on this page for all acronyms). A DRE is an officer who has gone through extensive training to recognize and evaluate the effects that drugs exert on people. In addition to the standard field-sobriety tests, the DRE also looks for changes in eye movement, pupil size, body temperature, heart rate, respiration rate, muscle tone and control, mental competency, and other variables. Using checklists and charts, the DRE is usually able to make a determination as to what class of drug or drugs may be influencing the suspect.

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**Immobilized IA**

For fast point-of-care screening, immunoassay devices are available that use antibodies impregnated onto a support medium that is packaged in a rigid container. Although these devices are simple to use, they are complex in their makeup. They contain antibodies to the specific drugs and drug-conjugate complexes. The sample is introduced and allowed to migrate through the medium. There is competitive binding between any drug in the sample and the drug-conjugates with the antibodies present. For some devices, a line or a band visible at a specified location indicates the presence of the drug. For other devices, the absence of a line at a specific location would indicate a positive result. These devices come in single-drug and multidrug packages and are used in many settings including doctors’ offices, emergency rooms, and law-enforcement agencies. Some urine-collection containers have the detection device built in. The suspect provides the urine sample and, within minutes, the officer can have a reading as to what class of drugs may be present. Some devices work with other fluids as well. One device, for example, requires a swab of the inside of the mouth to obtain oral fluids. Testing can be done at the roadside — no need to find a bathroom.

**Everybody needs a little TLC**

When samples make it to the laboratory, one of the oldest and most versatile screening techniques is TLC: thin-layer chromatography. Many of us first experienced thin-layer chromatography in junior-high-school science labs. Put dots of several colors of ink near the bottom of a piece of filter paper; suspend the paper

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over a solvent so just the bottom edge of the paper is in the solvent. As the solvent migrates up the filter paper by capillary action, the primary colors of the inks are separated and stop migrating at different positions along the filter paper. Commercial TLC systems are available, and some labs still make their own plates using glass slides and coating them with a thin layer of silica gel. Either way, the technique is similar.

The drugs are extracted from the sample using a suitable solvent and concentrated by evaporating the solvent. The concentrated sample is put on or in the thin-layer chromatogram and developed by allowing a solvent to migrate up the chromatogram. When the solvent almost reaches the top of the “gram,” it is removed from the solvent and dried. By using a variety of chemical dips and sprays, and viewing under UV light, drugs will show up at predictable heights and present predictable color changes to the various reagents. Many of the drug metabolites will also be visible. Spots of pure drug standards are often included on the chromatogram for comparison against the unknown sample. Almost any drug can be detected using TLC.

The method is very versatile but labor intensive. Not all drugs extract in the same solvents, so several extractions are necessary for a comprehensive screen. Many drugs may migrate to the same height and interfere with the interpretation. A drug in high concentration may mask the presence of other drugs by spreading out across a large area of the chromatogram. To be able to read the results with competency takes substantial training and practice. Good toxicologists with years of experience can almost read chromatogram results with their eyes closed — just a taste of old-fashioned chemistry full of solvent fumes, measuring and mixing of reagents, shaking tubes, and a rainbow of spots before your eyes — and a very colorful technique.

Alphabits

The most widely used screening techniques are the immunoassays (IA). These assays are known by a variety of acronyms, some of which trademarked: RIA, EMIT, ELISA, FPIA, MEIA, and CEDIA, to mention a few. Some are labeled as homogeneous (when the reactions and readings take place in the same reaction mixture); and some are heterogeneous (when the antibodies are attached to the reaction vessel or precipitated out of the reaction mixture, the excess reaction mixture is then discarded before introducing the detection reagents). Most are read in some sort of spectrophotometer, and even the direction of the reactions vary. In some assays, higher optical-density readings equate to higher drug concentration, while in others, a high optical-density reading means less or no drug present. Many of the immunoassays are available for use on routine chemistry analyzers as well as on analyzers dedicated for use as drug-detection systems. They can be run in microtiter plates that have been coated with antibodies. These microtiter-plate methods can even be automated by using robotic pipetters, automated plate washers, and high-speed spectrophotometric readers.

Whichever specific method is used, all of them follow the same general pattern. There is an antibody for the drug or class of drugs in question, the sample, and a drug-conjugate reagent (which is the drug in question attached to an entity that possesses some physical means for detection, i.e., radioactive iodine, an enzyme, a latex particle, an immunoglobulin, a molecule that fluoresces). These are mixed together. The drug in the sample competes with the drug-conjugate for binding sites on the antibodies. After an incubation period, the drug-conjugate or absence of drug-conjugate is measured.

For a specific example, the simplest immunoassay might be a coated tube radioimmunoassay, an RIA. Let us use an LSD kit. In this assay, the manufacturer has coated the insides of polyethylene test tubes with the LSD antibodies. The sample is added along with the LSD-conjugate reagent. Radioactive iodine, I-125, is used as the conjugate for most RIA tests. After incubation, the reaction mixture is decanted from the tubes, and the radioactive disintegration of the iodine remaining in the tubes is measured in a scintillation counter. A high number of counts per minute for a tube indicates a large quantity of LSD-conjugate is bound to the
antibodies, which means a low or no concentration of LSD in the sample. Conversely, tubes with low counts per minute indicate a lesser quantity of LSD-conjugate in the tube because the sample had a high concentration of LSD, which bound to most of the antibody binding sites.

Immunoassays can also be divided into two additional categories: those that test for specific drugs (fentanyl, propoxyphene, and acetaminophen, for example) and those that test for classes of drugs (opiates, benzodiazepines, tricyclic antidepressants, and others). The specific drug varieties may have cross-reactivity with metabolites of the drug, may react only with free unbound drug molecules, or may be designed to detect all forms of the drug. The kits designed to detect classes of drugs have to have antibodies that will react to as many of the drugs in that class as possible but not react with other compounds of similar size or structure. For instance, approximately 30 benzodiazepines are on the market. Most immunoassay kits are geared to detect the benzodiazepines similar to diazepam, temazepam, and oxazepam. They have only limited cross-reactivity with alprazolam and clonazepam. Most opiate kits are geared toward morphine and have limited cross-reactivity to oxycodone. An amphetamine kit may detect amphetamine (MDA), and several over-the-counter decongestants but not detect methamphetamine and MDMA. Depending upon how comprehensive the required screening, several kits for the same class of drugs may have to be used.

The strengths of immunoassays are good specificity and sensitivity, little or no sample preparation, and speed, and they can be highly automated. IAs are excellent choices for high-volume, fast turnaround-time situations, as well as useful as the single screen on a shift for the emergency room. Because of their sensitivity, they are valuable for ruling out the use of drugs.

One final note about immunoassays: While they may have excellent specificity and sensitivity for the drug or drugs they are designed to detect — and the package inserts tell what compounds will or will not cross-react with the assay — always be aware of certain issues. A drug in extremely high concentration in a sample may overwhelm the antibodies of other assays. Cross-reacting may take place simply because there is so much of the drug and, thus, the result may appear as multidrug positive when, in reality, it is only a single-drug overdose. A manufacturer may have tested 10 other drugs of similar structure and found no cross-reactivity to its kit, but the person whose sample is being screened may have taken that eleventh drug that never got tested, which does cross-react.

Rock, paper, scissors

Some screening methods use more sophisticated instrumentation than is normally found in clinical settings. These include HPLC, GC, GC-MS, and LC-MS, to name a few. These techniques have in common a feature, like TLC, wherein the drugs must be extracted out of the sample before they can be analyzed. Two main extraction methods are used: liquid-liquid extractions and solid-phase extractions (SPEs). In liquid-liquid extractions, the aqueous sample is mixed with a non-aqueous solvent. Buffers, acids, or bases may be added to adjust the pH so that the suspect drugs will be driven into the non-aqueous solvent. Several extrac-
tions — back into water and back into the non-aqueous phase — may be necessary to remove proteins and other biological compounds in order to get a clean sample to introduce into the analyzer. Solid-phase extraction uses small columns of resin or small discs of filter paper impregnated with resins that have active sites that will bind the drugs. Usually before the sample is loaded onto the solid phase, there is a pre-treatment step. After loading, the solid phase can be rinsed with buffers and solvents to remove interfering substances before the drugs are eluted off of it.

**Because there are so many options and so many drugs, combined with the fact that the prevalence of the use of certain drugs varies across the country, a forensic lab in California may have a different approach to drug screening than a lab in Maine.**

With the sample now prepared, a separation technique must be chosen: GC, LC, and CE, among others. The whole point of the chromatography is to take the mixed molecules of drugs and compounds, and separate them into nice tight little bundles, one compound per bundle. When these bundles reach the end of the column, they have to be detected. Several detection methods from which to choose include FID, NPD, ECD, PDA, and the most important detection device, the mass spectrometer (MS).

The mass spectrometer is unique because it allows identification of drugs in the sample. As the bundles of drugs enter the mass spectrometer, the drug molecules are broken apart into ionized fragments, and the amounts of fragments of specific masses are counted. These total-ion-count (TIC) patterns are practically unique for each drug. Using the retention time of the drug in the GC and the total ion count from the mass spectrometer, an exact identification of the drug in question is reasonably assured.

**Reality TV?**

On television crime shows, forensic experts commonly use the mass spectrometer to magically get the results they are after. When a laboratorian sits at a computer and calls up a file to view, the total ion vs. time graph is first displayed and looks like any chromatograph with its peaks and valleys. By selecting a peak of interest, the TIC is displayed in lines (much like a bar graph), with each line corresponding to a specific mass and with the height of each line corresponding to the total count of the fragments of that mass. The more counts, the taller the line. A library search through the collection of mass-spectral data on hundreds of drugs and compounds for that TIC can be done.

Most mass spectrometers have several libraries installed; and, eventually, the computer search through them suggests possible matches, displaying the library TIC, giving a percentage estimate as to how well the particular TIC fits the library data. Sometimes, the library will contain a drawing of the chemical structure of the best-matched compound. Users can zoom in on selected portions of the TIC, enlarge the view, subtract background interference, extract specific masses, and manipulate the TIC with other nifty,
sophisticated features. Using the mass spectrometer is like watching the TV “professionals” manipulate fingerprints to get a closer, better look at the details.

One plus one plus one is three

Many techniques for use in drug screening are available, and each comes with its own sets of advantages and disadvantages. Deciding which system to use depends upon what kind of screening a particular laboratory will be doing. Will most screens be for emergency-room cases? Will large volumes of urines for drug-free workplace clients be screened? Will drug use for rehab clinics be monitored? Or will screening be used for forensic applications? Labs may have to use more than one technique to reach the proper level of confidence for the task at hand.

A positive IA opiate screen is probably sufficient for an emergency-room physician who would then start appropriate life-support treatment and possibly opiate-antagonist therapy. Knowing which specific opiate the patient took would probably not change the course of treatment. The same IA-positive opiate result in a drug-free workplace setting would mean the medical-resource officer would have to interview the sample donor to find out if the opiate came from an over-the-counter or prescribed medication, heroin use, or poppy-seed bagels for breakfast. In a forensic setting, a lab professional would never want to report a positive finding based on a single method. A positive result from a second method, different from the first is desirable (in other words, opiates would be positive on an IA screen and a TLC screen) and, whenever possible, identification of the precise opiate by mass spectrometry.

To pee or not to pee, that is the question

What is the sample? The answer depends on what you are trying to discover. Urine makes a very good sample for most situations. As drugs pass through a person’s system, the parent drug and many of the metabolites end up in the urine. Drugs start collecting in the urine shortly after use. Because it takes several half-lives for a drug to clear the system, most drugs or their metabolites can be detected in the urine for several days after use. Many drugs get deposited in hair, so hair can be a sample, even as it continues growing. Sweat patches can be used to collect sweat over several days to monitor drug use. Oral fluids in connection with DUI enforcement are samples. Breath for alcohol testing is also an important sample. Urine and blood samples together are useful for law-enforcement cases; screen the urine to detect drugs and then screen and quantify the drugs in the blood. For post-mortem toxicology, samples of urine, blood, vitreous fluid, and various organ tissues are routinely used.

Searching for drugs in all the right places

This brief and simplified explanation cites some of the most commonly used techniques for drug screening. There are many options as to method and sample type, but are there any specific guidelines?

Human-performance toxicology as it applies to sporting events is regulated by the different governing agencies for the specific sports. The NHL, NFL, MLB, IOC, NCAA, all have different testing needs and requirements for athletes. Even horses have to pass drug screens.

Workplace drug testing is regulated by the Substance Abuse and Mental Health Services Administration (SAMHSA), which has regulations regarding what types of specimens and what type of assays can be used. SAMHSA also has specific drug-concentration cut-offs and specific confirmation requirements.
The different aspects of forensic toxicology — post-mortem, human-performance, forensic urine drug testing — have numerous choices as well. Each one has to provide results that satisfy state and local statutes and, sometimes, even federal regulations. Because there are so many options and so many drugs, combined with the fact that the prevalence of the use of certain drugs varies across the country, a forensic lab in California may have a different approach to drug screening than a lab in Maine.

In the September 2007 issue of the *Journal of Forensic Sciences*, the official journal of the American Academy of Forensic Sciences, a paper deals with this issue as it pertains to the area of DUID.

The authors state in their abstract: “There is currently no common standard of practice among forensic toxicology laboratories in the United States as to which drugs should be tested for, and at what analytical cutoff. Having some uniformity of practice among laboratories would ensure that drugs most frequently associated with driving impairment were consistently evaluated, that appropriate methods were used to screen and confirm the presence of drugs, and that more accurate data were collected on the extent of drug use among drivers.”

The paper also contains the results of several surveys of laboratories from around the country and proposes specific recommendations for establishing a level of standardization that is currently lacking.

This is an exciting new development to a field of investigation that has its roots in ancient Greece. 

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References